Standard and sample manipulation for calibration in flame atomic absorption spectrometry

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Standard and Sample

Manipulation for

Calibration in Flame Atomic

Absorption Spectrometry

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A Doctoral Thesis
Submitted in partial fulfilment of the requirements
for the award of Doctor of Philosophy
of the Loughborough University of Technology

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F.R.S.C.

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DEDICATION

M & D
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ABSTRACT

This thesis describes a study of existing calibration methods and a comparison of them, with novel calibration and sample pretreatment methods for flame atomic absorption spectrometry (FAAS) developed by the author.

A comparison of commercially available curve fitting algorithms was carried out to show how concentration errors arise and vary, due to the use of different empirical models for the calibration curve.

Novel online dilution manifolds were designed. Using flow injection and continuous flow techniques, different calibration procedures were developed to allow null methods of calibration to be used and to extend the calibration range.

Methods of sample pretreatment were developed, including online dissolution and species separation, using flow injection analysis techniques.

Members of the atomic spectroscopy group of the Royal Society of Chemistry were surveyed to discover calibration practices used in commercial laboratories. Respondents were asked questions on sample type, treatment and presentation, and data reduction for calibration.

During the research, several papers were published and lectures given on the topics described.
Articles in Primary Scientific Journals


(x)
Submissions to other Scientific Journals


External Lectures, Conference Presentations etc.


6. 7th International SAC/3rd BNASS Conference, Bristol, July 1986.

7. 7th International SAC/3rd BNASS Conference, Bristol, July 1986.


9. Combination Techniques in Analytical Atomic Spectrometry

Other occasions where the work has been presented


CHAPTER 1
Introduction and Aims

1.1 Calibration

Calibration is a necessary step in analysis when any instrumentation is used. Even classical analysis which involves the use of a balance and graduated glassware, relies on accurate calibration of the balance and glassware. The majority of textbooks describing spectrochemical analysis by atomic absorption spectrometry include only brief accounts of this crucial topic and do not describe the principles behind the common strategies employed. The brevity or omission is serious, as the majority of the time spent on an analysis can be spent preparing suitable standards, obtaining the sample in a form suitable for presentation to the instrument and optimising the performance of the instrument. These processes all affect the calibration of the instrument. Samples and standards must be similar in nature to obtain a valid calibration. Variation in instrument optimisation can change the usable range and the shape of the calibration curve. Once sample and standard preparation and instrument optimisation have been completed, the absorbances of the standards and samples are measured and the resultant data processed. Normally, only the measurement of the absorbance of standards and data processing are considered to be the calibration steps.
1.2 Atom production within flames

Many processes occur before the element of interest in a sample solution appears as ground state atoms in the light path of a flame atomic absorption spectrometer. The solution must be drawn up a tube, nebulised to produce an aerosol, which is desolvated to salt particles, vapourised to produce gas molecules and dissociated or reduced to give ground state atoms.

The last three processes occur after the aerosol enters the flame and these processes must occur before the sample element enters the optical path, to enable absorption to take place. This means that the droplets must be small. Large droplets are removed or made smaller by the mist being forced to impinge on, usually, a ball, paddles or baffles, the resulting waste solution being taken away via a drain. The amount and size of aerosol droplets produced will depend on the properties of the solution and the operation of the nebuliser. A high solution surface tension could cause larger droplets to be produced. As the solution has to be drawn up a capillary by suction generated at the nebuliser tip, an increase in sample viscosity will reduce the rate of aerosol production.

Desolvation of the aerosol may present problems if the surface tension or the heat of vapourisation of the solvent is high. Once a salt particle is obtained, it must be vapourised. The rate of vapourisation of the analyte element may be reduced if it is contained within a matrix of less easily vapourised material. The element of
interest may even form a non-volatile compound with matrix components. As the sample passes up the flame, a region of maximum atom population is produced. Its position is dependent on the element itself and sample matrix, flame temperature, gas flow rates and the dimensions of the burner slot. Atoms which could absorb radiation may be depleted in this region by oxidation or association with other material in the flame, non-photonic excitation (collisions) and ionisation. If the atoms are not formed from the sample material because of the formation of a non-volatile compound, they will not be available for absorption of radiation and will pass out of the light path. The depletion by all these processes may be great enough to prevent ground state atoms being detected in the flame. If the solvent used is easily vapourised, less heat will be taken out of the flame leaving more heat available for the atomisation processes. The solvent may even act as a fuel, changing the flame temperature.

All these processes may be dependent on the concentration of the analyte element and could affect the calibration. A classic example is the concave calibration typical of an easily ionised element.

1.3 The optical path

Light of a specific wavelength is produced using a hollow cathode lamp or, less frequently, by an electrodeless discharge lamp. In the case of a hollow cathode lamp, the element of interest is incorporated into a cylindrical cathode contained within a glass envelope.
which is filled with a fill gas at low pressure. When voltage is applied, the atoms of the coating are struck by the fill gas ions and vaporised. The atomic vapour produced can then become excited by further collisions and emit the characteristic radiation of the particular element. This radiation passes through the flame and a specific atomic line is monitored by passing the radiation through a monochromator and onto a photomultiplier tube. The discharge from the lamp is modulated and the output from the photomultiplier is taken through a lock in amplifier to enable the emission from the flame to be distinguished (and subtracted) from the emission of the flame plus lamp. These signals are then converted to absorbance. If the radiation which has not passed through the flame or has passed through the flame but cannot be absorbed by the atom of interest, is allowed to pass through the monochromator and onto the photomultiplier a transmittance of zero will be unattainable and the calibration curve will be bent towards the concentration axis. Unabsorbable or weakly absorbed radiation can arise from radiation generated by the fill gas or from other spectral lines from the element of interest in the lamp, which lie close to the required spectral line. Radiation in the lamp is produced from an atomic vapour. The line profiles produced can be broadened by pressure broadening and by the Doppler effect, where the wavelength of light produced is shifted by the motion of the atoms. Some of the radiation produced is absorbed by the atomic vapour within the lamp, decreasing the intensity of this.
radiation at the desired wavelength.

The broadening processes are more pronounced in flames due to the higher temperatures and pressure. Hence the absorption profile is broader than the lamp emission profile. If this were not the case, more radiation from the lamp would enter the monochromator as stray light.

Salt particles in the flame scatter the incident light deflecting it away from the monochromator, causing an apparent absorption. This can be avoided by reducing the concentration of the salt or by background correction. Background correction enables the differentiation between non-specific absorption and atomic absorption. Background correction can be achieved in three ways. All rely on the measurement of absorption due to the non-atomic species and subtracting this from the absorption due to the non-atomic and atomic species together. The two line method compares the absorbance of a line not absorbed by the element of interest (non-specific absorbance) with the absorbance of a spectral line. The continuum lamp method compares the absorbance from a continuum lamp (only a small amount will be due to the element of interest, the greater part being due to non-atomic species) with the absorbance of a spectral line. A recent modification of this method, the Smith Hieftje method, produces radiation in a normal hollow cathode lamp at wavelengths greater and less than the spectral line, by pulsing the lamp with a high voltage to induce severe self absorption. This radiation is then used instead of continuum radiation. Zeeman background correction is performed by applying a
magnetic field to the atomic vapour of the analyte. This splits the absorbing spectral lines into a number of components at $\langle \pi \rangle$, and above and below $\langle \sigma \rangle$, the normal wavelength of the absorption line. The $\pi$ components of the spectrum line become polarised in a plane parallel to the magnetic field and the $\sigma$ components perpendicular to it. Because of the shift of the $\sigma$ component away from the wavelength of the absorption line, no atomic absorption occurs in this plane, only scatter due to particulate material. In the $\pi$ plane, both atomic absorption and scatter occur. By using a rotating polariser, the two components can be resolved and the scatter subtracted from the atomic absorption signal.

The dependence of the calibration quality on the optical parameters of the components on the optical path is not covered in great detail in any text book.

1.4 Aims

The aims of this thesis are to examine all the processes which may affect the calibration, to study the limitations of the calibration strategies commonly employed and develop novel alternative calibration methods. Any alternative methods that are developed should rationalise the calibration procedure or extend the capabilities of the method of analysis. The study was limited to flame atomic absorption spectrometers because of the popularity of this type of instrument, the specific problems associated with their calibration and, more particularly, the ease with which flow manifolds can be interfaced with these instruments.
CHAPTER 2
Calibration and flame atomic absorption spectrometry:
A Literature Survey

2.1 Calibration

Walsh [11], when introducing the method of analysis using the atomic absorption phenomenon, described the two possible methods of analysis, namely absolute and relative. Absolute analysis is where the absorbance of the analyte atomic vapour is directly related to the concentration of the analyte. Rann [2] attempted to place flame atomic absorption spectrometry on an absolute basis using the Beer-Lambert law, represented by equation 2.1

\[ I_t = I_i \exp(-\alpha N l) \]  

where \( I_t \) and \( I_i \) are the transmitted and incident radiation respectively, \( \alpha \) the atomic extinction coefficient, \( N \) the number of absorbing atoms and \( l \) the length of the absorbing path. Processes which affect the various parameters in equation 2.1 were considered, including absorption and emission line width of absorbing atoms and source respectively, the degree of dissociation of the analyte into an atomic vapour and the effect of this upon the atomic extinction coefficient. de Galan [3] pointed out flaws in this approach, in particular, that the concentration of absorbing atoms in the flame is not simply related to the concentration of atoms in the
analyte solution, but depends on aspiration rate, evaporation of droplets and particles, the dissociation of molecules and the distribution of atoms in the flame. If all the factors which affect equation 2.1 are kept constant by using fixed experimental conditions, the absorbance of a sample solution can be compared with appropriate standards. de Galan stated that this is a remarkably reliable and accurate relative method of analysis. Rann [4] concluded that the absolute absorption measurement of atoms in a flame is experimentally difficult and could give conflicting results.

The relative method of analysis has been established as the usual procedure for flame atomic absorption spectrometry and a number of calibration methods have been shown to be useful. These can be divided into two groups:

Those methods used where there is no interferent in the sample, or where the degree of interference upon the analyte can be reproduced for the standards (2.2); and

Those methods where an unknown interferent acts upon the sample analyte (2.3).

2.2 Calibration for samples without interferences

The use of a working curve generated from a number of standards was envisaged by Walsh [1]. This method is described in most textbooks on analytical spectroscopy (see for example 5). Because the absorbance of a sample does not usually coincide with the absorbance of a standard, the concentration of the sample is calculated by interpolating between standard data points. This
interpolation can give rise to errors in the concentration calculated for the sample [6, 7]. Several standards are required to cover the calibration range for two reasons: If the calibration curve was a straight line, use of one standard would be acceptable if no error in the concentration mixed was possible and the measurements of standard and blank were precise. Use of several standards ensures the averaging of errors in standard concentration. When the calibration curve is curved, several standards must be used to increase the accuracy of interpolation and ensure the curve is followed accurately.

Null point calibration [8, 9] removes the need for interpolation by matching the concentration of a standard to the concentration of the sample. This is achieved by measuring the absorbance of the sample followed by the absorbance of the standard. The concentration of the standard is then varied to produce the same absorbance as that found for the sample. If the conditions during measurements of the sample are the same as those during measurement of the standard, the concentrations of sample and standard will be the same.

Gradient methods [10-12] employ the principles of null calibration and working curve calibration. A solution of increasing concentration is aspirated by the spectrometer, and the response monitored with respect to time. The absorbance of a sample is then measured and matched to an absorbance obtained during aspiration of the gradient. As the change in concentration with respect to time is known for aspiration of the gradient, the time
value associated with the gradient absorbance, which matches the sample absorbance, will yield the sample concentration. If a chart recorder is used to record the gradient \([10,11]\), no interpolation is necessary. However, if a computer is used \([12]\), discrete values of absorbance and time are stored, and time values must be interpolated if a sample absorbance lies between the gradient values.

Flow injection methods produce peaks from which concentration-time relationships can be obtained. These can then be used for normal gradient calibration \([13]\), gradient calibration where peak widths are measured \([14-16]\) or simply for dilution if only the peak height is measured \([17]\).

2.3 Calibration for samples with interference

All the calibration methods described above can be used for calibration where there is an interferent present in the sample, if the concentration of the matrix components in the standard/s and sample are the same (Matrix matching), or the interference completely suppressed.

Three techniques have been developed for analysis of samples where the nature and degree of interference is unknown. These are the methods of standard additions, successive dilution and changing parameter.

Standard additions \([5,18-20]\) employs an extrapolation of a calibration graph. Increasing amounts of standard are added to equal portions of the sample and these mixtures made up to the same volume. The absorbances of
these solutions are measured and plotted against the concentration of added standard in the final solutions. The calibration curve is extrapolated and intercepts the axis at a point where there is no analyte either from the sample or standard. The distance of this point (in concentration terms) from the origin, is the concentration of the analyte in the final solutions due to the sample (Fig. 2.1).

![Graph: Standard Additions Calibration]

**Fig. 2.1**

Standard Additions Calibration
For this method to work, any interference must act equally on all the analyte in the solutions analysed: If the interference only acts upon the analyte due to the sample, or acts upon a decreasing proportion of the added standard, then the slope of the calibration will be different from that expected, and the amount of analyte calculated to be in the sample will be incorrect [21]. Usually [5], standard additions calibrations require linear calibration curves. These can easily be extrapolated using a ruler or using a least squares procedure. Hosking et al [22] pointed out the dangers of extrapolation when analysing cement samples for calcium. They showed that although the absorbances from the addition solutions may lie on a straight line the region of the calibration which is extrapolated may be curved. It was shown that the degree of this curvature varied with flame conditions and the type of interference. If, as stated earlier, the interference was constant over the whole calibration curve an accurate figure for the concentration of the analyte may be found. This is often difficult to achieve. Hosking et al suggest using matrix matching or interference suppression in preference to a standard additions method. A calibration curve of the element with no interferent present may also be non-linear, as will be discussed later, and this non-linearity will be reflected in a standard additions curve. Favretto et al [23] fitted a curve to the standard additions calibration points and extrapolated this to find the concentration of analyte in the sample. They justified
this procedure by comparing the results obtained using their technique with the accepted concentrations for standard samples. They stated that 'extrapolation of nonlinear curves is risky..... This procedure may not apply in all cases'.

If the standard additions curve is the only calibration curve available, the source of curvature, extent of interference and therefore the validity of using the standard additions technique will be unknown.

Gilbert [24] developed the technique of successive dilutions for the determination of cadmium by flame photometry. A number of dilutions of the sample are made and the response measured. The apparent concentration in the undiluted sample is then calculated using a working curve obtained from pure standards, and the dilutions of the sample. These values are then plotted against the relative concentration of the diluted sample to the original sample concentration. The linear graph obtained (fig. 2.2) is then extrapolated to zero relative concentration. This point represents a solution with the interferent infinitely diluted.

The method assumes that the effect of the interferent decreases as the solution is diluted until there is no interferent for an infinitely diluted solution and hence the intercept of the calibration with the apparent concentration axis will yield the concentration of the analyte in the sample. Shatkay [25] has pointed out that the successive dilutions calibration curve may not be a straight line as the interference may not decrease
linearly with increasing dilution. The method appeared to give the expected result only if the working curve was prepared using standards containing similar concentration of interferent to the sample. This being the case a matrix matching technique would be more satisfactory as the number of operations is less.

Shatkay [25] also developed the method of changing parameter. In the paper, the method is not clearly...
explained and there is no logical description of how to perform a calibration using the changing parameter technique. However, the method described is based on measurement of sample solutions with different concentrations of interferent added or different concentrations of analyte. Shatkay compared this method with the working curve method and the standard additions method and concluded that although the method is labourious it can produce greater accuracy where strong interference occurs.

Cardone [26] conducted a survey of more than 50 experienced analytical chemists and showed the lack of agreement between them on the calculations which should be performed when using a working curve and the standard additions method. These discrepancies were due to disagreement as to when to subtract the absorbance obtained using a method blank from the absorbance of sample and standard solutions and what the significance of the difference in slopes for the standard additions curve and working curve was. Thirty three different types of calculated approaches were submitted by fifty one correspondents! The correct solution to the problem was provided [27] and in this paper Cardone describes methods to calculate sample blanks from the sample and the use of these blank values in the working curve and standard additions methods. Only linear calibrations were considered and as such, the application of the methods described is limited.
2.4 Curvature

Since the early sixties various authors have produced papers describing the main causes of curvature of calibration curves in atomic absorption spectrometry. Light which enters the monochromator but is not available for absorption by analyte atoms (stray light) is a major cause of non-linearities in calibration curves [28-33] and can come from a variety of sources. Rubeska and Svoboda [28] have pointed out that the finite line width of the source, which is normally considered to be negligible compared with the width of the absorbance profile will cause significant bending of the calibration curve, especially for hollow cathode lamps operated at high currents. Where it occurs, the hyperfine structure of the resonance line from the source will also cause the calibration to be bent, the degree of curvature depending on the separation of lines within the hyperfine structure. They also discussed the possibility that the calibration curves would be bent if the absorption line width was increased with increasing concentration of analyte, by resonance broadening. A shift of the absorption line maximum with increasing concentration of analyte, would also cause curvature. De Galan and Samaey [29] considered the degree of curvature caused by a monochromator passing several spectral lines. Firstly they considered the case for manganese, where the most sensitive absorption line at 279.48 nm is actually a triplet. When these are resolved using a high resolution monochromator (bandpass 0.04 nm), linear calibrations are obtained using each separated line.
within the triplet. When the spectral bandwidth is increased to 1.6 nm (approximately that used for atomic absorption analysis), the incomplete separation of the multiplet reduces the sensitivity and produces curvature of the calibration curves at higher concentrations. They then considered the curved calibrations obtained for antimony where the normal bandpass of a monochromator allows wavelengths which cannot be absorbed by the element in the flame to reach the detector. This unabsorbable radiation may be continuum radiation, stray light, or produced by the fill gas in the source and may be resolved by decreasing the bandpass of the monochromator. The subsequent loss of precision can increase the limit of detection.

Van Gelder [30] agreed that the most prominent curvature was caused by stray light and non-absorbed spectral lines and that hyperfine structure of the line was also important. Source line profiles broadened by self absorption and the Doppler effect were calculated and shown to contribute to the curvature of calibration. Curvature was also shown to be due to the non-uniform concentration distribution in the absorption cell (the flame) and the variation in the pathlength of the incident light beam (the light beam is often focussed on the centre of the flame and the beam 'crosses over' at this point).

The degree of curvature which is caused by non-monochromatic light was considered by Agterdenbos and co-workers [31-33] for spectrophotometric measurements. Different line profile shapes for the absorbance cell and
incident beam were considered and the error in the absorbance observed, calculated.

Curvature is not only caused by optical effects (i.e. those produced by the incident light, the absorbance characteristics of the element or from stray light). The volatilisation of the element in the flame can vary with concentration and give rise to non-linearity of the calibration curve [34]. Roos [35] showed how the amount of element in the observed part of the flame varied with observation height and flame temperature and that different degrees of curvature of the calibration could be produced by changing these variables. The population of strontium in the flame was measured from the emission of strontium hydroxide (SrOH) to eliminate the optical effects upon the curvature of the calibration as described earlier and self absorption of the atomic emission. How the concentration of the hydroxide in the flame relates to the concentration of ground state atoms is not discussed. It was concluded that curvature due to incomplete volatilisation was less significant than that caused by optical effects.

Ionisation of the analyte will also cause curvature [5]. If an analyte is easily ionised at the flame temperature, the degree of ionisation will be greater at low concentrations: If the partial pressure of electrons in the flame is fixed the amount of ionised analyte which produces these electrons will be fixed and, as the analyte concentration is increased, the proportion that is ionised will decrease. As ions do not absorb radiation of the
atomic spectral wavelength, ionisation will lead to depression of the signal. This effect produces a 'concave' calibration curve (fig. 2.3).

Fig. 2.3
Concave calibration curve caused by ionisation

Thompson [36] observed calibration curves for chromium (III) solutions where minima and maxima were produced. These effects were observed particularly when solutions were analysed using a luminous, fuel rich flame.
quite often recommended for the analysis of chromium [5]). These perturbations were less marked for chromium (VI) solutions than for chromium (III) solutions and were removed altogether if a fuel lean flame was used (with subsequent loss of sensitivity).

The reduction of curvature by minimising the causes has been discussed by Park [37] but, as the causes of curvature are complex and some cannot be removed, methods of curve fitting were recommended.

2.5 Curve correction and fitting

Three methods of straightening a calibration where the curvature has been produced by stray light, have been developed. de Galan and Samaey [29] described a method where the stray light is subtracted from the transmitted light. A concentrated solution of the element (in their case 1000 mg l\(^{-1}\) antimony) is aspirated and the transmittance set to zero: Any light reaching the detector is assumed to be stray light. The blank is then aspirated and the transmittance set to 100%: The light reaching the detector is the transmitted intensity but now the response due to the stray light has been subtracted. The values of transmittance obtained when samples are aspirated are then converted to absorbances. This linearised the calibration for antimony where the bandwidth was 3 nm and the calibration followed the line obtained using a bandwidth of 0.16 nm. This method is recommended by Pye Unicam [38] using a switch on the instrument to convert the transmittance to absorbance.
A more modern instrument [39] is supplied with a circular slide rule for calculating the degree of curvature and the amount that must be 'dialled in' using the knob on the instrument.

Kindsvater and O'Haver [40] developed a model (equations 2.2 - 2.4) of the transmittance/wavelength profile obtained when atoms from a solution absorb radiation from a continuum source. Experimental profiles were compared with those from the model and the value for the intrinsic absorbance in the model was changed until a match between the profiles was obtained.

\[
A = \frac{A_I}{1 + (\lambda - \lambda_0)/\delta \lambda}^2 \\
I' = I 10^{-A} \\
I = I' S(\lambda) + I_s
\]

where \( A \) is the absorbance on the profile at wavelength \( \lambda \),
\( A_I \) is the intrinsic absorbance (the absorbance at the line centre \( \lambda_0 \), measurable only with infinitely great resolution),
\( \delta \lambda \) is the absorbance line width,
\( I' \) is the true transmission intensity,
\( I \) is the observed intensity i.e. \( I' \) convoluted by the slit function \( S(\lambda) \) with addition of stray light \( I_s \).

The values of intrinsic absorbance were then plotted.
against concentration to produce nearly linear calibration curves.

As suggested by Park [37], once it is found that curvature cannot be corrected by changing the operating conditions, and if the curvature cannot be accounted for by considering stray light, a method of fitting a suitable curve must be employed to enable concentrations to be interpolated from the calibration. Before the introduction of microcomputer data handling facilities the only quick way to fit a curve to calibration data was to plot the points on graph paper and draw the line of best fit estimated by eye using a "flexicurve" or similar device. This method is described in most text books on analysis by atomic absorption spectrometry [see for example 5]. If the curve fitting is to be performed by microcomputer, the curve fitting method must be suitable. Some of the methods of curve fitting which have been suggested are least squares methods in various forms [41-49], Bayesian calibration [48,49], minmax and maximum likelihood [48]. Not all these methods are applicable to curves and, as will be discussed later, the most common approach is the use of a least squares method. The assumptions made and required for the method of least squares are discussed by Agterdenbos and co-workers [51,52]. Some functions are described which are commonly applied to calibration curves in analytical chemistry, but in the case of atomic absorption spectrometry, the reasons for curvature are complex and therefore, a universal function has not been developed. Many of these functions
express concentration as a function of absorbance when in fact absorbance is a function of the concentration of the analyte in the absorption cell. This leads to minimisation of errors in the concentration values and an assumption that no error exists in the absorbance values, when a least squares method of curve fitting is used. In fact, for calibration, it should be assumed that the concentration of the standards is known (no error) and the errors in the absorbances are to be minimised. Anderson and Moser [53] showed that satisfactory fits can be obtained when concentration is expressed as a function of intensity for emission spectrography. It appears that this assumption has been extended to atomic absorption spectroscopy.

The curve fitting operations can be divided into two groups:
a) The fitting of a single function to all the calibration points and the fitting of several functions, or
b) the fitting of same function several times, to different subsets of the calibration data.

A quadratic function, equation 2.5,

\[ A = a + bC + cC^2 \]  \hspace{1cm} 2.5

(in this and all subsequent equations in this section, A is absorbance, C concentration and a, b, c and d the parameters to be determined during the fitting process) has been suggested by Wendt [54], Limbek and Rowe [55] and
Adams et al [56] as suitable. Willmott and Mackenzie [57] describe the use of a quadratic function but do not state if the quadratic is of the form above or the form of equation 2.6.

\[ C = a + bA + cA^2 \]  

This quadratic gives a more satisfactory fit [55] and was adapted by Baird Atomic (now Spex Industries (UK) Ltd.) in their commercial systems [58, 59]. Another method of improving the fit of a curve to calibration data is to increase the number of terms in the equation e.g. equation 2.7,

\[ A = a + bC + cC^2 + dC^3 \]

as was suggested by Wendt [54], or fix the number of terms and change the power to which a variable is raised as suggested by Schwartz [60].

A cubic function is used by Thermo Electron (formerly Instrumentation Laboratory) [61], though whether the cubic is in absorbance or concentration is not stated. The constant term (e.g., a in equation 2.7) was removed from the equation to force the curve through the origin.

The number of terms can, of course, be further increased to produce a polynomial of any degree. These have been suggested for calibration by various authors [61, 62-70]. If the calibration data is fitted to
functions with increasing numbers of coefficients, the degree of polynomial which gives best fit must be sought. All these polynomial methods employ a goodness of fit parameter calculated to enable the best curve to be chosen. These are described later.

Exponential functions of the type represented by equation 2.8 have been suggested by Agterdenbos [51] and were investigated by Limbek and Rowe [55] for calibration in atomic absorption analysis.

\[ C = a + b \log A \]  \hspace{1cm} 2.8

A number of 'rational' or 'hyperbolic' functions have been used and are commercially available. Hohmann and Lockhart [71] showed how a hyperbola can be made to fit many sets of chemical data. Baird Atomic [59] now use a rational function, equation 2.9 in the AlphaStar system.

\[ \frac{A}{C} = a + bA + cA^2 \]  \hspace{1cm} 2.9

Perkin Elmer [72,73] use a rational function, equation 2.10 but in two forms,

\[ \frac{C}{bA-1} = aA^2 + cA \]  \hspace{1cm} 2.10

For four standards, the equation is used as written.
If two standards are used, c is set to zero. When three standards are used, the algorithm predicts what the concentration of the third standard should be from the calibration data using the previous (lower) two standards and the equation with c set to zero. If the predicted concentration is within 15% of the actual concentration of the third standard, c is kept at zero. If not, the value of c is calculated by least squares.

Watson (74) has used a similar function, equation 2.11 fitted by the method of least squares.

\[
A = \frac{a + bC}{1 + cC} \tag{2.11}
\]

All of these rational functions pass through the origin. Favretto et al (23) modified the Perkin Elmer type two coefficient function to equation 2.12, to enable non-linear standard additions calibrations to be performed.

\[
(C+Q) = \frac{-aA}{bA-1} \tag{2.12}
\]

Q is the concentration of the sample and is found by the fitting procedure. Straight line fitting by least squares for standard additions calibrations has been described by Malakoff et al (75).

A function based on a stray light equation (76) equation 2.13 has been developed by Whiteside et al and Stockdale (77). This equation was originally developed by Roos (78) for the calibrations of cobalt, iron and nickel.
\[ C = \log \frac{1-B}{\alpha \beta} \quad \text{(2.13)} \]

\( \alpha \) is the absorptivity of the species
\( \beta \) is the pathlength of the absorbing cell
\( B \) the proportion of light which cannot be absorbed (i.e. the stray light)
\( T \) is the transmittance

The transmittance of two standards of known concentration are measured and equation 2.13 solved to find \( 1/\alpha \beta \) and \( B \). The resulting equation is then fitted to all the standards. \( T \) can be replaced, in equation 2.13, by \( 10^{-A} \) to convert absorbance to transmittance. This function will only correct for curvature caused by stray light or for curvature which is similar in shape to that caused by stray light. Kleijburg and Pijpers (79J) developed a calibration function (equation 2.14) based on the modification of the Beer-Lambert Law by Rayleigh scattering of light by clusters of atoms.

\[ A = A_{\infty}[1 - \exp(-kC)] \quad \text{(2.14)} \]

\( A_{\infty} \) is the limiting absorbance due to Rayleigh scattering and \( k \) is a constant to be found during curve fitting. The authors state that more experiments need to be performed before the relationship between \( A_{\infty} \) and Rayleigh scattering can be confirmed. Even then, the function will only be useful where curvature due to Rayleigh scattering predominates.
A method involving three standards has been proposed by Satsmadjis and Voutsinou-Taliadouri [80]. If the concentration of the standards are in order of increasing concentration $c_3$, $c_2$, $c_1$ with associated absorbances $A_3$, $A_2$, and $A_1$ respectively, the function generated is given in equation 2.15.

$$C = C_2(h/A_2) + \log(h/A_2)$$  \hspace{1cm} (2.15)

where

$$g_2 = g_3 - \left[ \frac{(g_3 - g_1)}{\log A_3/A_1} \right] \cdot \log A_3/A_2$$  \hspace{1cm} (2.16)

$$a = \frac{(g_3 - g_1)}{\log A_3/A_1}$$  \hspace{1cm} (2.17)

$$g_1 = \frac{(\log C_1/C_2)}{(\log A_1/A_2)}$$  \hspace{1cm} (2.18)

$$g_3 = \frac{(\log C_3/C_2)}{(\log A_3/A_2)}$$  \hspace{1cm} (2.19)

and

$h$ is the absorbance of a sample, unknown concentration $C$.

If the absorbance of the sample is less than $A_3$, the concentration is found using equation 2.20.

$$C = C_3 \left( \frac{1 + (g_3 - 1)}{h/A_3} \right)$$  \hspace{1cm} (2.20)

No reason is given as to why such a complex function is
used or how it was derived. The authors just give the function as an 'empirical formula'.

A complex procedure investigated by Whiteside et al [76] and Stockdale [77] involved the use of a pair of related parametric cubic functions equations 2.21 and 2.22.

\[ C = a + bt + ct^2 + dt^3 \]  \hspace{1cm} 2.21

\[ A = e + ft + gt^2 + ht^3 \]  \hspace{1cm} 2.22

t relates the two functions and parameters a to h are to be found during curve fitting. The fitting process is difficult and unreliable [77].

The simplest, multiple curve method is the linear segment (or linear interpolation) method [60]. This involves the simple process of solving equation 2.23 for each pair of calibration data points.

\[ C = a + bA \]  \hspace{1cm} 2.23

A more complex multiple curve method has been developed by Mitchel et al [70]. The regression order is chosen by the operator and a number of curves of this order are fitted to different groups of standards. Confidence bands are then calculated for each curve. When a sample absorbance is measured, the concentration is estimated using all the curves but only the value estimated with the smallest
confidence interval is taken as the actual concentration value. Malakoff et al [75] and Ramirez-Munoz et al [81] describe a multiple curve method where a linear portion is fitted to the calibration data close to the blank level and a quadratic or quadratics are fitted to the curved region of the calibration. The range covered by each function is specified by the operator. Butler et al [82] developed a two-curve method for electrothermal atomic absorption spectrometry. The straight line, equation 2.24 where $m$ is the slope of the line, was fitted to the data

$$A = mC$$  \hspace{1cm}  \text{(2.24)}$$

close to the blank level where calibration curves actually give a straight line. This line was then used to predict the absorbances of the standards on the curved portion of the calibration. The difference, $\Delta A$, between the predicted absorbance and the observed absorbance $A_s$ was used to find the unknown constants $p$, $q$ and $r$ in the quadratic, equation 2.25, for three standards. When a

$$A_s = p(\log \Delta A)^2 + q(\log \Delta A) + r$$  \hspace{1cm}  \text{(2.25)}$$

sample, absorbance $A_s$ is aspirated, the parameter $X$, equation 2.26 is calculated and if negative, the concentration is calculated from equation 2.24

$$X = q - 4p(r - A_s)$$  \hspace{1cm}  \text{(2.26)}$$
If X is positive $\Delta A$ is calculated from equation 2.27 and the concentration, therefore, from equation 2.28

$$\log \Delta A = -\frac{q \pm q^2 - 4p(r - A_s)}{2p}$$  \hspace{1cm} 2.27

$$C = \frac{1}{m} (A_s + \Delta A)$$  \hspace{1cm} 2.28

Fitting of splines has been examined by deGalan et al [83]. This curve fitting method involves plotting different polynomials between each calibration point such that the slopes of the lines are equal at the calibration point. This method of curve fitting tends to allow the calibration curve to meander between the calibration points.

Whiteside et al [76] have developed a multiple curve fitting method. The system is similar to spline fitting in that curves are fitted between calibration points, but the slopes at each point do not have to be equal. A straight line is fitted between the origin (blank level) and the first calibration point. The slopes of this line and the line joining the first calibration point to the second are averaged. A third data point is then calculated. This point has the concentration value calculated from the mean absorbance of the first two points using the line with averaged slopes (Fig. 2.4). A quadratic in absorbance equation 2.29 is then solved for these three points
Calibration curve fitting used by Pye-Unicam

1. First calibration point
2. Second calibration point
3. Third point with mean absorbance of points 1 and 2 lying on line L of mean slope of lines A and B

\[ C = a + bA + cA^2 \] 2.29

The calculation of a third point is then repeated for the
next pair of calibration points (points 2 and 3) and equation 2.29 solved. The maximum number of calibration points that can be used with this system is 5 and therefore equation 2.29 is solved five times. This system is used by Pye Unicam [39] in the SP9 system.

Varian have adopted a multiple curve method developed by Limbek and Rowe [55]. Equation 2.30 (Baird Atomic have also adopted this function in their AlphaStar system),

\[
A = \frac{a+bA+cA^2}{C} \quad 2.30
\]

is solved for every three calibration points to generate a 'family' of overlapping curves (Fig. 2.5). Where there is a choice of curve, the curve which is generated using standards of lower concentration is used for calculation of sample concentration during analysis.

Methods which solve equations, force the calibration curve through the calibration points. The method of least squares reduces to the solving of simultaneous equations when the number of data points equals the number of terms to be found. When a curve is forced through the calibration points, no curve smoothing is employed. This therefore assumes the data points are infinitely precise. Some smoothing is desirable to allow for imprecise solution concentration (due to imprecisions in dilution) and absorbance (due to imprecisions in the absorbance signal). Weighted least squares has been suggested [44] as an improved method of least squares fitting as the
The Family of Curve type curve fitting as used by Varian curves produced fit the calibration at the blank level where large errors can occur using normal least squares procedures. These procedures use the standard deviations of the calibration points absorbance values, measured several times to calculate a weighting factor. When the standard deviation is large relative to the absorbance value, the resultant weighted fit produces a line which
does not pass as close to the point as the line produced by a normal least squares procedure. As the calibration points are the only information available with which to predict the true calibration curve, the fitted function must be capable of passing through or as close as possible to these points. If the function chosen is incapable of following the true curve, no least squares method, weighted or otherwise, will produce an accurate calibration. The ideal calibration algorithm therefore, must use a function which is capable of following the true calibration curve and use a weighted least squares fitting procedure to smooth the imprecisions in the calibration points.

A number of authors [6,7,83-85] have compared some of the curve fitting algorithms that are commercially available. Miller-Ihli et al [85] compared the curve fitting algorithms when used for extending the calibration range.

The most promising calibration function appears [6,7] to be the 'rational' function as used by Baird Atomic and Varian, equation 2.30.

Greater accuracy when using this function may be possible if the function were to be fitted using a weighted least squares method.

2.6 Measures of "Goodness of fit"

If a calibration algorithm allows a choice to be made between a number of functions that can be fitted to the
data, a test which indicates how well a function fits the data must be generated to allow such a decision to be made. A measure of 'goodness' of fit must also be generated if curve fitting algorithms and functions are to be compared.

The simplest method of comparing the fit of a number of curves to calibration data, is a visual examination of a graphical plot. The calibration function can then be chosen. This is a very 'operator dependent' method as both the shape of the plot of the calibration function and the proximity of the line to the calibration points must be taken into account. Statistical tests which are independent of the operator have been developed to allow an unbiased choice to be made. A common statistic that is used [62-65] is the correlation coefficient [86]. This statistic is unity for a perfect fit. It is close to unity if the fit is good at high values of concentration and absorbance, even if the fit is poor close to the blank level. This statistic is therefore biased. Gottschalk [87], describes the use of another statistical test for choosing a calibration function but the paper is in German and therefore not readily understood.

Mitchel et al [70] chose the curve from which the concentration is to be calculated by calculating confidence intervals about the concentration values estimated from each curve available: The curve which gives the smallest confidence interval is then used to calculate the concentration. This process is repeated for all absorbance values.
Use of an 'F test' has been described by Wentworth [41]. Here a value of 'F' is calculated as the ratio of the sum of squares of the residuals to the degrees of freedom (number of calibration points minus the number of terms in the function being fitted) divided by the variance of the absorbance equation 2.31.

\[ F = \frac{\sigma_{\text{EXT}}^2}{\sigma_{\text{Int}}^2} \]

where \( \sigma_{\text{EXT}}^2 \) = sum of the squares of residuals per degree of freedom
and \( \sigma_{\text{Int}}^2 \) = variance of absorbance

If the value for F is significant when compared with values in the statistical tables, the fit is unsatisfactory.

Barnett [73] in the method adopted by Perkin Elmer [72] chose between the two functions they used, by calculating the concentration of the third and highest concentration standard, from the function fitted to the first two standards. If this was within 15% of the known concentration of the standard, the two coefficient function was used, otherwise the three coefficient function was used. This is a measure of the 'Goodness of fit' of the two coefficient function.

These measures of 'Goodness of fit' cannot be used to compare all the curve fitting functions available. Most of those described above can only be used for functions fitted by the method of least squares. When a calibration
function is forced through the calibration points, as is the case for splines and where an equation is solved exactly, these statistics will show that a perfect fit has been obtained: The function may not follow the calibration curve between the calibration points. Barnett's method of choosing a curve is specific to the Perkin Elmer algorithm and cannot be used to compare other functions.

Miller-Ihli et al [85] developed a method of comparison of curve fitting functions based on the 'root mean square of the percent deviations' (RMSPD). They fitted the functions to a number of calibration points and measured the difference between the known concentration of the standards and that predicted by the calibration function. They not only used the calibration points in the calculation but data points from concentrations between the calibration standards. This enabled the RMSPD, equation 2.32, to be calculated even in cases where the curve passed through each calibration point.

\[
\text{RMSPD} = \left( \frac{\text{SSPD}}{N} \right)^{\frac{1}{2}} \quad 2.32
\]

where \(N\) is the number of data points used in the test and the SSPD (The sum of squares of the percent deviations) is calculated using equation 2.33.
SSPD = \sum_{i=1}^{N} \left( \frac{C^i_c - C^i_k}{C^i_k} \times 100 \right)^2  \tag{2.33}

where \( C^i_c \) is the \( i \)th concentration calculated from the calibration function and \( C^i_k \) is the \( i \)th known concentration.

The RMSPD represents the mean percentage error in concentration that can be expected when the calibration function is used over the entire calibration range.

This function was used by Bysouth and Tyson \([6, 7]\) to compare all the commercially available curve fitting functions. This work is described in detail in section 3.3. They also devised a statistic SC equation 2.34 to enable the significance in the difference in the RMSPD's calculated for different functions, to be estimated.

\[
SD_{\text{RMSPD}} = \left( \frac{1}{N} \right)^{\frac{1}{2}} \frac{SD_{\text{SSPD}}}{2(\text{SSPD})^{\frac{1}{2}}} \tag{2.34}
\]

where

\[
SD_{\text{SSPD}} = 10^4 \left\{ \sum_{i=1}^{N} \left[ 0.02C^i_c (C^i_c - C^i_k) \left( \frac{C^i_c}{\langle C^i_c \rangle^2} \right) \right] \right\}^{\frac{1}{2}} \tag{2.35}
\]

De Galan et al \([83]\), used a similar statistic QC, equation 2.36, to that of Miller-Ihli et al, devised by Knegt and Stork \([88]\) but this was based on the errors in
absorbances.

\[ QC = 100 \left( \frac{\sum_{i=1}^{N} [1 - \left( \frac{A_i}{A_i^*} \right)^2]}{N - 1} \right)^{1/2} \]

where \( A_i \) is the absorbance of the \( i \)th standard
\( A_i^* \) is the absorbance of the \( i \)th standard predicted from the fitted curve.
and \( N \) is the number of calibration points.

Although this is a useful statistic it does not predict the error in concentration that can be obtained using a particular function. In this respect, the RMSPD value is more easily understood.

DeKreuk et al (89J have described how the inclusion of a calibration point of erroneous concentration value (defined by the operator) will lead to a calibration curve which will not follow the true calibration curve. Systems which do not produce a visual display of the data points and calibration curve are particularly prone to this type of mistake. A number of authors (56,69,79,87,90J have described ways of finding and excluding 'outliers' or points which do not conform to the expected calibration curve shape. The validity of excluding such points depends on how the non-conformity of the point arises. If the concentration that has been given is correct, then the point is a genuine calibration point and should be included. If the concentration of the standard has been entered into the system wrongly the point should be excluded. This can only be seen if the absorbance of a redilution of the stock solution is measured or if the
'wayward' standard is diluted to the concentration of one of the other calibration standards and the absorbance compared.

2.7 Noise and precision

Flame atomic absorption spectrometry is inherently noisy: The absorbance of atoms generated from an aerosol in a flame, which is subject to drafts is measured using optical devices, the resultant signal being converted to absorbance via electronic circuitry. Each stage of atom production measurement and signal conversion will have some noise associated with it. This produces a varying absorbance signal and hence an uncertainty in the concentration of the sample. Some of these sources of noise depend on the concentration of atoms in the flame or the size of the generated signal (which in itself is dependent on the concentration of atoms in the flame).

Alkemade et al [91,92] and Boutilier et al [93], derived expressions for signal to noise values for the different sources of noise. The sources of noise discussed were shot noise generated because of the quantum nature of measured light, whistle noise caused by oscillations in the flame burner system and flicker noise caused by random drift of light sources, analyte production and detection. Ramsey [94] pointed out that analyte flicker noise could cause bending of the calibration curve, basing his argument on a model of absorbance where the optical beam crosses over in the centre of the absorption cell. He concluded that high levels of analyte flicker noise are
improbable and that this cause of bending the calibration curve is unlikely to be significant.

Ingle [95] developed a model which predicted the changes in noise for an increasing absorbance signal. Relative standard deviations were used (one hundred times the inverse of a signal to noise ratio). Different curves were generated for instances where different sources of noise predominated. Bower and Ingle [96-98] used this model and compared theoretical curves with the curves produced from measurements made on a number of elements. Different curves were obtained by altering the operating parameters of the instrument. The sources of noise were correlated to the curves through the model. Ringbom [99] and Ayres [100] used plots of 'percent absorptancy' (1 - Transmittancy, where Transmittancy is the transmittance expressed as a fraction) versus the log of concentration to find the optimum range over which a colorimeter and photometer respectively could be used. The range over which the greatest accuracy can be obtained is the portion of such a graph which has the greatest slope. These plots are now known as Ringbom-Ayres plots. This method of finding the range over which greatest accuracy can be obtained, was applied to atomic absorption spectrometry by Ramirez-Muncz et al [101,102], but, as had been pointed out by Crawford [103] such a procedure is invalid when the precision is not constant with increasing measurand. Ringbom [99] and Ayres [100] also showed that when the relative error (equivalent to relative standard deviation) is plotted against the measured quantity a U shaped curve
is produced Fig 2.6, which yields the range of the measured quantity over which a chosen relative error will not be exceeded. These curves are known as Ringbom plots.

Roos [104, 105] produced similar curves using error functions which can be applied to atomic absorption data. As stated earlier, Ingle [95] used the same approach to
find the sources of noise under different operating conditions. Roos, on the other hand, used error functions to find the range of optimum precision of absorbance for a calibration curve which deviates from Beers Law. Van Dalen and de Galan [106] produced Ringbom plots for lead by flame atomic absorption spectrometry but showed that although the coefficient of variation (relative standard deviation) in absorbance may not exceed 1.5% for absorbances greater than 0.2, the coefficient of variation may exceed this level when converted to concentration via a non-linear calibration curve (fig. 2.7).

Non-linearities of the calibration curve will therefore decrease the range over which precise measurements can be made. It was also pointed out that for a certain level of precision the linear portion of a calibration may be unusable and interpolation may only be valid over a non-linear portion of the calibration curve.

Long term drift in sensitivity (i.e. a significant change in absorbance over the analysis time) will make a calibration curve generated at the start of an analysis, invalid by the time the last sample is analysed. This drift is a form of low frequency noise which cannot be compensated for using integration of the signal, as is used for noise of higher frequencies. Sotera et al [107] showed that the change in sensitivity due to changing operating parameters could be corrected by 'resloping' the calibration curve using one standard. They generated a calibration curve using several standards, changed an operating parameter to produce a change in sensitivity and
Fig. 2.7

Ringbom plots showing the difference in useful range calculated using variance in absorbance and variance in concentration.

reaspirated one of the standards. The difference in absorbance of this standard when aspirated before and
after the change of conditions was used to calculate the expected absorbance of the other standards, i.e. the calibration was 'resloped'. The absorbances of these standards was then measured and converted to concentrations using the resloped calibration. The concentration found compared favourably with the original concentrations.

Once a calibration curve has been constructed and the optimum range in terms of precision has been found from a Ringbom plot, the uncertainty in concentration found from the calibration curves used can either be found from the Ringbom plot, by using probability distributions or by constructing confidence intervals about the curve. The Ringbom plot does not give any information about how the varying, noisy concentrations or absorbances are distributed about their mean values. Schwartz [69] showed that the transformation of a normal distribution of absorbances through a non-linear calibration curve produce skewed distributions in concentrations (fig. 2.8). A computer program was written to calculate uncertainties in concentration for non-linear calibration curves which took account of this skewness. He demonstrated later [60] that confidence intervals (fig. 2.9) can be constructed about calibration curves using the standard deviations of the calibration points, produce an estimate of the uncertainty in a sample concentration.

Confidence intervals, based on terms calculated during a least squares method of line fitting to a standard additions calibration, have been used [108] to estimate
Fig. 2.8
Distortion of a normal absorbance distribution to a skewed concentration distribution by a curved calibration

the uncertainty in the result of an analysis by the standard additions method.

The precision of flow-injection atomic absorption spectrometry (FIAAS) will depend on the precision that can be obtained from the spectrometer and the precision of operation of the flow-injection equipment. Harnly and Beecher [109] and Brown and Ruzicka [110] showed that precision is degraded by introducing a sample using an FIA system when compared with conventional nebulisation. This
Calculation of uncertainty from confidence levels is due to the introduction of further sources of imprecision (i.e. sample injection and transport) and the decrease in sensitivity due to sample dilution in the manifold. If the conditions are carefully controlled this degradation of precision is minimised.

2.8 Interference

Walsh [1] indicated when proposing the use of the
phenomenon of atomic absorption for analysis, that interelement effects will be significant when the method is applied to complex matrices containing the analyte.

The methods of calibration to account or correct for interferences have been described (section 2.3). Of the elements used in this study, chromium and calcium are particularly prone to interference. The other elements used, namely magnesium and nickel, are less prone to interference. Cresser and MacLeod [111] have shown that sulphate can interfere with magnesium, nickel and cobalt when analysing high concentration in the presence of sulphate. The depression of the signal was thought to be due to the atomisation process proceeding via an oxide of the metal. When chlorides of the metal at the same concentrations were analysed, no depression of the absorbance was observed: The atomisation of the chloride did not proceed via the relatively involatile metal oxide. Cresser and MacLeod concluded that for analyses of solution of these metals with concentrations within the normal analytical range the interference of sulphate would be negligible.

Interference can arise from (a) effects caused by the physical properties of the solution [112] such as different viscosities and surface tensions due to interferents, (b) from processes in the flame such as catalytic removal of free radicals [113] which changes the proportion of ground state atoms in the flame (c) from formation of stable compounds [see for example reference 5] and (d) from molecular absorption spectra overlapping.
the atomic absorption spectrum [114].

In the specific case of interferences upon calcium in air/acetylene flames several authors [84, 115-118] have shown the dependence of the extent of the interference on the concentration of the interferent and analyte. The depression of some interferents e.g. phosphate becomes constant, after an initial decline in absorbance, with increasing concentration of phosphate [84, 115-117], indicating the formation of a stable compound which is not completely volatilised by the flame [84]. There is no further depression upon further addition of phosphate, once all the calcium is incorporated into the stable compound. The depression of the absorbance of calcium does not become constant with increasing aluminium concentration, but asymptotically approaches complete suppression of the calcium signal [84, 116]. In this case, not only is the formation of a stable compound probable, but this compound is not vapourised at all by the air/acetylene flame.

Tyson [84], pointed out that the standard additions procedure was valid only if the added analyte from the standard was depressed to the same extent as the sample analyte. If the level of interferent is less than that which gives constant depression with increasing interferent concentration, further addition of analyte from the standard will decrease the depression of the sample analyte. This will change the slope of the calibration curve or cause non-linearities in the calibration. This causes inaccuracies in determinations
of calcium in the presence of an interferent by the standard additions method [116]. It may be necessary to add interferent to the sample [84] so that sufficient depression capacity is assured. In the cases of aluminium type depression, a level of constant depression cannot be achieved and the standard additions procedure will not be applicable.

The effect of the different oxidation states of chromium on the calibration curve [36] has already been discussed (section 2.4). Aggett and O'Brien [118] demonstrated that different absorbances would occur if the chromium VI was present as different ionic species in the solution. The species present are influenced by the pH of the solution [119, 120] and hence when solutions containing chromium VI ions are to be analysed, care should be exercised to ensure that the pH of solutions are equivalent. Aggett and O'Brien also described the depression of chromium absorbance by alkali metal chlorides and sulphates [121]. Both the interference from these compounds and the difference in absorbance obtained for different species containing chromium VI was thought to be due to the formation of solids within the flame. These had different thermal properties from the solids formed from solutions of pure analyte. The depression was noted to be greatest in the part of the flame which gave the greatest sensitivity.

Roos and Price [122] showed how iron with chromium formed a less volatile alloy than chromium alone. This caused the characteristic depression of chromium by iron.
Only when iron is in great excess, does an increase in iron concentration give no change in the depression of the chromium signal. The converse was shown to be true for the effect of chromium on the iron signal. In this case the iron chromium alloy is more volatile than pure iron and enhancement of the absorbance of iron occurs.

2.9 Online Dilution and Calibration

Online dilution and mixing of solutions has developed from a desire to speed up the process of solution preparation, to save reagents and improve the precision of dilution techniques. Dilution normally involves the transferring of a known volume of a solution into a volumetric flask and making the volume up to the mark using water or an appropriate reagent.

Koscielniak [123] produced successive dilutions from one solution by aspirating a set volume directly from a volumetric flask, making the solution back up to the original volume with diluent, re-aspirating the set volume and repeating the dilution. This is a rather complicated procedure, not easily modified for automation. Already, simpler methods had been developed. T- or Y-shaped capillary connections have been employed [124, 125] to add reagents or dilute samples. Addition of different concentrations of standard, allows the production of standard additions calibrations. These authors did not control the uptake rate of solution by each branch of the system. Cresser and Edwards [126] pointed out the danger that if the solutions were aspirated from different
heights, different heads of solution (and hence flowrates) were produced. This is important if known dilutions are required or if a repeatable composition of the aspirated solution is required as the flowrates in each tube should be known. Ramsey and Thompson [127] overcame this problem by pumping the two lines leading into the T-piece at constant flowrates. They also employed a small mixing tube containing a coiled filament, to ensure complete mixing between the two streams. A further development of pump controlled mixing is the use of a variable speed pump. Thompson and Ramsey [128] used one fixed speed pump to keep the flowrate of sample into and out of a mixing point constant. In this case the mixed samples were used to investigate interference of varying matrix concentrations on the inductively coupled plasma but constant sample flowrate is also required for flame atomic absorption spectrometry. Diluent or matrix was pumped into the mixing point via a variable speed pump, to produce different concentration, excess flow was taken to waste. This is illustrated in figure 2.10. The stream pumped to the instrument will always contain some sample. A similar arrangement [9,129] for calibration, is described in this thesis (section 4.3.3) but the arrangement of pumps allows the flow delivered from each stream to vary from 0 to 100%.

Drake [130] noted that sample zones can become mixed with the surrounding material when a discrete sample boundary flows through tubing or a flow cell, but it was not until Ruzicka and Hansen [131] introduced the ideas of
controlled dispersion in flow injection analysis that this mixing process was studied as a method of dilution. In flow injection analysis, a sample slug is interjected into the flow of a carrier solution, flowing towards a detector. During passage of the slug, mixing occurs with the carrier, diluting the sample zone but (if a reagent is present) forming a product. When spectrophotometric detection is used it is often this product that is monitored. For atomic spectrometry this is not possible, but the sample dilution can be utilised. By using
different lengths of tubing, Tyson et al [17] produced different dilutions of samples and standards. A single length of tubing was chosen by switching two valves placed between the injection valve and the nebuliser of a flame atomic absorption spectrophotometer (fig. 2.11). Once the dispersion produced by each length of tubing had been characterised, the system was used to extend the range of concentrations that could be analysed. Ruzicka et al [132] and Fernández et al [133] used systems in which the sample slug was split into two and allowed to pass down
different lengths of tubing before being merged before a spectrophotometric detector. The different lengths of tubing produce different resistances to flow which, coupled with the difference in distance that the split slugs must travel, causes them to arrive at the merging point at different times. The difference in flowrates down each tube divides the sample slug unequally and this coupled with the different dispersions produced by each tube gives two dilutions of the sample. These arrive at the detector at different times to produce two overlapping peaks of different heights. The application of this procedure to flame atomic absorption spectrometry but with increased numbers of tubes [129], is described in section 4.4.4 of this thesis. Not only were the peak heights used for calibration, but the troughs between the peaks were used.

Concentration gradients have been used by various authors [10-16, 134-138] for producing solutions of various concentrations for analysis and calibration. Poste and Lakatos [134] used well stirred mixing chambers to produce gradients for continuously varying an interferent concentration in the aspirated solution. When a 'small' chamber was used, exponential gradients were produced according to the relationship in equation 2.37,

$$C_2 = C_1 (1 - e^{-(Ft/V)})$$  \hspace{1cm} 2.37

where $C_2$ is the concentration in the aspirated stream, $C_1$ is the concentration in the stream flowing into the
chamber, $F$ the flowrate in and out of the chamber, $t$ the time from allowing the mixing to take place and $V$ the volume of the mixing chamber. The volumes of the mixing chambers were not fixed, but controlled by the volume of solution which they contained i.e. as mixing only takes place within the liquid, transferring a known volume of solution into a vessel which is then closed produces a mixing chamber of the pipetted volume, even if the volume of the vessel is greater. Two volumes of solution were used: 5 ml were used to produce exponential gradients and 50 ml were used to produce pseudo-linear gradients, using the initial portion of the experimental gradient as an approximation to a linear gradient. An unnecessary feature of their system was different sized vessels feeding the mixing chamber for the two types of gradient. As the flowrate was controlled by the nebuliser, a constant head feed vessel would have provided a constant flowrate and as can be seen from equation 2.37, the concentration of the effluent stream is not dependent on the volume of any other vessels connected to the system.

Tyson and co-workers [10, 11, 135, 136] have developed a system using a closed mixing chamber of fixed volume to produce concentration gradients according to equation 2.37 for calibration. Flowrate was found to be an important factor in producing accurate calibrations and was therefore controlled by a peristaltic pump.

Dispersion of an injected solution in a flow injection manifold produces concentration gradients which produces the rise and fall curves of the resultant peaks.
Olsen et al [13] used the gradient on the fall curve of the peak from a spectrophotometer in two ways. If absorbances on the fall curve were measured after a number of fixed times had passed from injection several dilutions could be produced which were then used to obtain calibrations of varying slope and sensitivities fig. 2.12.

---

**Fig. 2.12**

**Flow injection gradient calibration**

- Calibration points of different sensitivity which are equivalent to
- calibration points of different concentrations
This was developed in the same paper to allow several calibration points to be obtained from a single injection using times from the peak maximum which gave known dispersions (dilutions). The dispersions were obtained by characterising the system, rather than developing a model for the gradients produced by flow injection. This technique was applied to the method of standard additions by Araújo et al [137]. They injected a standard into carrier which was merged with water to enable the concentrations at points on the fall curve to be found. The carrier was then merged with samples and the standard reinjected. The concentration of sample was constant. the points of known concentrations on the fall curve from the injected standard superimposed on the constant sample level fig. 2.13 were used to obtain a standard additions calibration. Giné et al [138] produced standard additions calibrations in a similar manner using zone sampling. They allowed the standard to disperse after injection and then reinjected dispersed portions of the standard into the sample stream fig. 2.14. Tyson demonstrated [15] that the gradients produced by flow injection (as peaks) could be used to produce calibrations with extended ranges. By considering the nebuliser of a flame atomic absorption spectrophotometer to act as a well stirred mixing chamber and injecting a sample slug directly into the nebuliser an equation for the peak width was developed, equation 2.38,
Gradient flow injection standard additions calibration

\[ t' = \frac{V}{u} \ln \left( \frac{C_m}{C'} \right) - 1 \]  - \( V/u \) \ln \left( D - 1 \right) \hspace{1cm} 2.38

where \( t' \) is the peak width, \( u \) the flowrate, \( V \) the theoretical volume of the mixing chamber, \( C_m \) the concentration injected, \( C' \) the concentration level on the rise and fall gradients at which the width is measured and \( D \) is the dispersion of the system. Under fixed conditions \( V, u \) and \( D \) are constant. This allows peak width calibration curves to be constructed to cover several orders of magnitude of concentration. Because the concentration at which the peak widths are measured \( (C') \) is required and the response of an atomic absorption instrument is non-linear, a conventional calibration curve
Dispersed standard peak  Reinjected standard and merged sample

Reinjected zones of dispersed standard

Absorbance  time

Fig. 2.14

Generation of standard additions solutions by zone sampling

is needed to relate the measured absorbance values to values of $C'$. $V$ is the theoretical volume of the mixing chamber, as the nebuliser chamber contains the solution as an aerosol rather than being filled with liquid. Stewart and Rosenfeld [14] used real mixing chambers for extended calibration in FIA with spectrophotometric detection. They based their calibration equation on the models developed by Pardue and Fields [139] to produce
calibrations where peak widths were proportional to the logarithm of the concentration injected. This is a simplification of equation 2.38 and may be unnecessary. Work using both the full equation (16,133) and the simplified equation (129) is discussed in section 4.4.6 of this thesis.

Riley et al [140], produced peaks of different heights from a single solution by injecting different volumes into a flow injection manifold. They achieved this by stopping the pump delivering the carrier solution to the detector, changing the solution feeding the pump from carrier solution to the sample, re-starting the pump to aspirate the required volume, stopping the pump, changing the solution back to carrier and finally starting the pump again. These steps were controlled by a microcomputer which enabled reasonable precision to be obtained, even though the sample slug had to pass through the peristaltic pump. Use of a valve to produce variable volume injection is discussed in section 4.4.5 of this thesis.

The reproducible mixing of an injected solution with the carrier solution has been utilised by Tyson and co-workers [11,135,136,141] to produce a flow injection standard addition method for flame atomic absorption spectrometry. This method has become known as 'reverse FIA' as the sample stream is used as the carrier and standards are injected into it. When a standard which has an analyte concentration less than that in the sample is injected, the sample carrier is diluted resulting in a
decrease in absorbance producing negative peaks. When a standard which has an analyte concentration greater than that in the sample is injected, positive peaks result fig. 2.15.

![Graph showing absorbance over time with peaks and a sample level marker.]

**Fig. 2.15**

_reverse flow injection standard additions peaks_

Plotting the change in absorbance against the concentration injected allows the concentration of analyte in the sample to be interpolated fig. 2.16. Interpolation is a more accurate procedure than the extrapolation used in conventional standard additions calibrations. Several standards were injected to enable the FIA standard additions calibration curve to be drawn accurately. Israel and Barnes [142] used the same procedure for
inductively coupled plasma spectrometry but injected only two standards, one of greater and one of lesser concentration of analyte than the sample. The typical reverse FIA calibration curve illustrated by Tyson and Idris [141] is not linear and hence use of two standards may not be valid for the procedure.

2.10 Flow injection for sample concentration. separation and speciation

Although flame atomic absorption spectrometry is a sensitive and reasonably interference free technique, the requirement for detection of increasingly lower levels of analyte concentration is reducing the popularity of this
technique in favour of furnace atomic absorption spectrometry. Inductively coupled plasmas are increasingly being used for elements which are difficult to atomise or suffer severe interference in the flame or furnace. This technique is expensive when compared to flame atomic absorption spectrometry. Olsen and co-workers [143, 144], Malamas et al [145] and Fang et al [146] incorporated small cation exchange columns in flow injection manifolds. This allowed the analyte to be concentrated on the column, which was achieved by injecting a large volume of sample from which the analyte was absorbed onto the column. When a small volume of acid was injected, this eluted the analyte, the analyte being concentrated by a factor depending on the difference between the volume of the sample injected and the much smaller volume of acid injected for elution. Olsen et al [143] used stream switching to switch from the carrier stream into which the samples were injected, to a continuous stream of eluent. This allowed elution to be performed in the opposite direction to loading the column. In each of these cases, the volume of sample injected was about 2 ml. Malamas et al [145] used timed injection (or variable volume injection) to vary the amount of sample from which the analyte was concentrated. They also used an immobilised chelating agent as the immobilised complexes were more stable than those obtained using conventional ion-exchange resins. Both Olsen et al [143] and Malamas et al [145] point out that preconcentration on an immobilised reagent enables the removal of the analyte
from an interfering matrix. The matrix can be allowed to pass through the detector or to waste, whilst the analyte remains on the column. When the analyte is eluted, it will pass to the detector in an interference free eluent matrix.

Kamson and Townshend [117] separated interferents from injected analyte slugs by passing the slug through an anion exchange column. Phosphate and sulphate interference could be completely removed for the analysis of calcium. Although the removal of silicate interference was attempted, the resin used did not enable silicate interference to be removed completely. Once the analyte had been detected, the interferent was eluted from the column.

If complete interference removal is achieved and the sample can be concentrated to a sufficient degree, the combination of flame atomic absorption spectrometry with flow-injection analysis will be as sensitive as furnace atomic absorption spectrometry, with the convenience of the relatively interference free inductively coupled plasma atom source.

As Milosavljevic et al [147] demonstrated if analyte and interferent species can be separated on the basis of charge, then, if different species of the analyte have opposite charge, they can be separated. They separated copper II ions from copper complexed with EDTA (which forms a negative divalent ion) by passing a slug of the mixed species through a cation exchange column. Once the copper/EDTA complex had been detected, the column was
eluted with a slug of nitric acid to allow the copper II ions to pass to the detector. Hill and Ebdon [148] have connected the output of an HPLC column to a flame atomic absorption spectrophotometer in order to separate organometallic species. Flame atomic absorption spectrophotometers have also been used for detection of total analyte after one species has been detected spectrometrically using a UV/vis spectrometer connected in series [149,150]. The sample was injected, merged with an appropriate reagent and the slug passed through the UV/vis spectrometer where one of the species was detected. The slug then passed to the nebuliser of the atomic absorption spectrophotometer where all the analyte species were detected.

2.11 Conclusion

The application of flow-injection and related methods of solution handling to flame atomic absorption spectrometry increases the versatility of the technique by allowing separation, concentration and dilution of analyte in a convenient and reproducible manner. These three uses of the technique could be applied to calibration to allow simple, automated procedures to be developed.
3.1 Introduction

Conventional calibration involves the dilution of a stock standard to give a number of calibration solutions which are then presented to the spectrometer. When aspirated, the response for each solution is noted. The values of absorbance and concentration are then plotted on a graph, or more recently entered into a computer, and a relationship between absorbance and concentration obtained. This is usually referred to as "curve fitting". These relationships, either a manually drawn curve or a mathematical model, enable the analyst to interpolate between the calibration points. Without these relationships, an infinite number of standards would be required to produce a smooth concentration function.

The response of the spectrometer is different for each element and under different conditions. This will change the ability of the methods of curve fitting to accurately follow the absorbance concentration relationship.

Automated standard production and curve fitting are investigated with data for different elements under various conditions in the following section.
3.2 Preliminary experiments

3.2.1 Parameter optimisation and generation of calibration data under various conditions

The purpose of this work was to collect calibration data under different conditions for use in curve fitting experiments, examine the shapes of calibration curves, find the optimum operating parameters for the instruments and gain experience in the operation of a variety of spectrometers. It was also intended that the performances of the different instruments would be compared.

Apparatus

The AA spectrometers used were a Shandon Southern A3300 and a Pye Unicam SP90A Series 2. Accurate solutions were made by serial dilution of 1000 mg l⁻¹ stock solutions. Three elements which have characteristically different curve shapes were investigated: magnesium, nickel and chromium.

Procedure

Before each parameter was varied, the other parameters were optimised by manually varying the settings for maximum response when a solution was aspirated. If optimum values had been obtained in previous experiments, these settings were used. Solutions were aspirated for at least five seconds after steady state absorbance was achieved. Response was recorded on a chart recorder enabling the measurement of absorbance and noise. Noise was estimated as the mean fluctuation about the absorbance.
level.

Results

From the values of absorbance and noise obtained from the chart recordings calibration curves were plotted and signal to noise ratios calculated. This enabled the quality of the calibration curve to be assessed on sensitivity (the reciprocal concentration that would give an absorbance of 0.0044), linear range (estimated by eye) and signal quality (from the signal to noise ratios).

Lamp Current

Both magnesium and nickel show similar changes in calibration shape when the lamp current is varied. As the current is increased the linear range and sensitivity improve to a peak value, then deteriorate. For chromium the sensitivity again has a peak value but the linear range is unchanged by varying the lamp current. The signal to noise ratios of chromium and magnesium are not affected by varying the lamp current but for nickel there is an optimum setting.

Burner height (observation height)

The relationship between burner height and fuel flow rate for chromium was found to be complex and further work was carried out (section 3.2.2). Magnesium shows an increase in linear range but deteriorating sensitivity, as the burner height is increased. For an optimum calibration, these effects would have to be balanced. The
sensitivity for nickel shows an optimum, but the linear range does not change for increasing burner height. Signal to noise ratios for magnesium are only dependent on burner position at low concentrations but for nickel and chromium there is an optimum position for all concentrations.

**Slit width**

When magnesium calibrations were produced using large slit widths, the curves became sigmoidal. This did not occur with nickel calibrations. Both of these elements showed a deterioration in sensitivity with increasing slit width. The sensitivity of the chromium calibrations were unaffected. For magnesium and chromium, slit width did not affect the linear range but the linear range for nickel calibrations increased if the slit widths were reduced below 0.1 mm. Signal to noise ratios showed a maximum for increasing slit widths.

**Fuel/Oxidant mixture**

Magnesium calibrations showed little effect with changing fuel/oxidant mixture. The sensitivity for nickel calibrations improved with increasing fuel flow to a maximum but at high fuel flows the absorbance meandered with increasing concentration (Fig. 3.1). Sensitivity sharply increased to a maximum for a moderately fuel lean flame. Fuel/Oxidant mixture did not greatly affect signal to noise ratios or linear ranges. Chromium showed marked changes in calibration curves linked with burner height.
Nickel calibration under fuel rich conditions

and further work was carried out (section 3.2.2). Linear range decreased whilst sensitivity improved for increasing fuel flows. At moderate fuel flow rates the curve appeared to be made up from two linear segments.

Conclusion

Calibration curve shape

Calibration curve shapes for magnesium are robust when subjected to changes in the operating parameters. The parameter which had the greatest effect was the burner
height. This is probably due to there being an area in
the flame where most magnesium atoms are formed and any
movement from this position is a move away from the
optimum. Magnesium calibrations are sensitive and
relatively linear compared to nickel and chromium.

The curve shapes for nickel calibrations are mainly
dependent on slit width and lamp current, due to the
presence of emission lines from the lamp which are close
to the principal resonance line. The wider the slit or
the higher the lamp current the more unabsorbed light from
these lines is allowed to pass into the monochromator
increasing the stray light. The "waves" produced in the
calibration when a fuel rich flame is used is probably a
chemical effect (though I cannot think of any reasonable
explanation).

Chromium calibration curve shapes are independent of
slit width and lamp current but dependent on fuel/oxidant
mixture and burner height. The number of atoms produced
in the flame must depend on flame temperature and
chemistry: A hot reducing flame produces greater
sensitivity. As the stray light produces a limiting
absorbance, the linear range decreases with sensitivity;
a problem if the standard additions method is to be used.
Further work on the dependence of absorbance on
fuel/oxidant mixture and burner height was carried out
(section 3.2.2).

Noise

Noise on magnesium absorbance signals is more
dependent on concentration than on the operating parameters. Noise on absorbance signals for nickel on the other hand, are dependent on burner position, lamp current and slit width as well as concentration. This shows that nickel absorbance is prone to flame noise and noise from the hollow cathode lamp. Noise on the chromium signal is dependent on fuel/oxidant mixture, burner height and concentration, indicating again, that chromium absorbance is dependent on flame effects.

The calibration data which was suitable for use in comparing calibration curve fitting algorithms section 3.2.2 is presented in table 3.1 for nickel and chromium. The spacing of calibration points for magnesium in these experiments was not suitable for the algorithm comparison experiments. New data was generated which produced better spacing of the data points, using conditions comparable to those found to be the optimum from these experiments. These are also presented in table 3.1. Table 3.2 shows the conditions under which these calibrations were obtained.

Because of the limits of time and availability of each of the instruments, it was not possible to compare two instruments under the same conditions.
Table 3.1
Optimum calibration data

<table>
<thead>
<tr>
<th>Element</th>
<th>Lamp</th>
<th>Observation/</th>
<th>Wavelength</th>
<th>slit</th>
<th>fuel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>current</td>
<td>Burner height (cm)</td>
<td>nm</td>
<td>nm</td>
<td>l min⁻¹</td>
</tr>
<tr>
<td>Mg</td>
<td>4</td>
<td>0.95</td>
<td>285.2</td>
<td>0.1</td>
<td>1.8</td>
</tr>
<tr>
<td>Ni</td>
<td>5.5</td>
<td>0.7</td>
<td>232.0</td>
<td>0.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Cr</td>
<td>6.1</td>
<td>0.7</td>
<td>357.9</td>
<td>0.01</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Table 3.2
Conditions for optimum calibrations

Conclusion
The calibration curves obtained for various elements for flame atomic absorption spectrometers, vary greatly in shape. Not only does this shape depend on element but on the operating conditions used. The calibration curve shapes for different elements are affected by different operating parameters.
3.2.2 Chromium absorbance contours for varying fuel flow and burner height

During the previous experiments it was realised that the absorbance increase obtained with fuel flow increase may be due to the region of highest analyte atom concentration in the flame, moving with respect to the light path, and that for maximum sensitivity fuel flow and burner height (observation height) were interdependent. This experiment was designed therefore to find the optimum fuel flow and burner height by varying both parameters.

Procedure

The instrument (SP90A) was optimised and operated under the following conditions (Table 3.3).

<table>
<thead>
<tr>
<th>Table 3.3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lamp current</strong></td>
</tr>
<tr>
<td><strong>Air flow</strong></td>
</tr>
<tr>
<td><strong>$\lambda$</strong></td>
</tr>
<tr>
<td><strong>Slit</strong></td>
</tr>
<tr>
<td><strong>Fuel</strong></td>
</tr>
</tbody>
</table>

A 20 mg l$^{-1}$ solution of chromium was aspirated for each flow rate setting, interspersed with water, to enable zeroing of the absorbance to account for drift. Set absorbance values were chosen and the burner height adjusted for each fuel flow rate setting to give these values, read directly from the meter.
Results and Discussion

The absorbance values were plotted directly onto the contour map Fig. 3.2. No optimum fuel flow rate was achieved before the flame became very sooty. The optimum burner height is 0.5 if the fuel flow is kept below 2.4 l min\(^{-1}\), the absorbance drops off rapidly for burner heights lower than this, but more slowly for increasing burner heights. Above 2.4 l min\(^{-1}\) of fuel the optimum burner height is >0.5. This increase in optimum height is probably due to the increase in the velocity of gases escaping from the burner slot, moving the region of highest atom population up the flame, as well as the flame changing from a convection flame to a diffusion flame. Conclusion

When optimising for sensitivity with chromium it is best to chose an appropriate fuel flow rate (there being no limit) and adjust the burner to give maximum absorbance, then increase the observation height slightly. This will allow for slight changes in fuel flow-rate without too greatly affecting the absorbance.

3.2.3 Investigation of noise/concentration curves

As pointed out in section 3.2.1, the noise produced on aspiration of a solution into the spectrometer, not only depends on the operational parameters of the instrument, but also the absorbance of the solution. Schwartz [69] demonstrated that when this absorbance and its associated noise is transformed through the calibration to concentration values, the noise as a
Fig. 3.2

Absorbance contour map for chromium at different observation heights and fuel flowrates

Absorbances
- 0.1
- 0.2
- 0.3
- 0.4
- 0.5
- 0.6
- 0.7
percentage of the signal and its distribution will be changed (Fig. 2.8).

Van Dalen and de Galan [106] showed that plots of noise against absorbance would show a rapid decrease from infinity at the origin but, when noise is plotted against concentration this rapid decrease would be followed, in the case of a curved calibration curve, by an increase. If the calibration curve was to become rapidly asymptotic this increase would be marked.

Procedure

Several experiments were performed with calcium, chromium and nickel as the test elements to try to produce noise/concentration Ringbom plots. These elements show curvature at higher concentrations. Nickel gives a curved calibration for all concentration ranges. The Pye-Unicam SP9 system used can convert absorbances to concentration using a built in calibration algorithm. Relative standard deviations, (RSD), which are a measure of noise, can be printed out for either absorbances or concentrations.

A number of experiments were carried out under a variety of conditions. The concentration ranges are summarised in Table 3.4.
<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration range mg l⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>0 - 50</td>
</tr>
<tr>
<td>Chromium</td>
<td>0 - 50</td>
</tr>
<tr>
<td>Nickel</td>
<td>0 - 350</td>
</tr>
</tbody>
</table>

Table 3.4

Results and Discussion

Many of the experiments took several hours to perform allowing the sensitivity of the instrument to drift. This meant that the system had to be recalibrated or the operating parameters changed during the experiment, changing the noise characteristics and the concentration values obtained during the experiment. Although integration times were changed to try to obtain reproducible R.S.D. values this was not possible. The expected initial decrease in noise with increasing concentration was observed but only a slight increase in the noise with concentration was shown for these elements which was marred by an increase in scatter of the points about the expected curve. This could be due to an operating difficulty with this instrument i.e. when calibrated, the system accepts three significant figures. For a calibration range of 5 to 500 mg l⁻¹, Inputting the 500 mg l⁻¹ standard as the top standard specifies that the
5 mg l\(^{-1}\) standard is read with only one significant figure. The noise for this solution will therefore be lowered as a concentration variation of 4.5 to 5.4 will not be registered, but a variation in the 500 mg l\(^{-1}\) solution concentration of 450 to 540 will be registered.

**Conclusion**

Although some slight increase in concentration noise with increasing concentration and curvature of the calibrations, was shown by these experiments, the operation of the system is not ideal for these experiments. Drift in sensitivity itself is a form of noise but usually analyses are performed quickly and the system is frequently recalibrated during a run. Concentration noise levels are higher than absorbance noise levels but can remain at less than 1% R.S.D. for the normal concentration ranges used. This means that for these elements, even when the calibration is asymptotic, its use is still valid.

3.3 Conventional calibration procedures

3.3.1 Instrumental curve correction

A method of curve correction is described in the SP90A manual [38] which involves the aspiration of a concentrated solution which gives an absorbance >3 and adjustment of the transmittance to zero. This is to subtract any unabsorbed stray light. When the calibration solutions are aspirated the stray light transmitted is subtracted giving higher absorbances and greater
linearity.

**Procedure**

Nickel calibration solutions up to 70 mg l\(^{-1}\) were aspirated with SP90A optimised and the absorbances measured with and without the instrument adjusted for curve correction. A 100 mg l\(^{-1}\) solution was used for the stray light subtraction.

**Results and Discussion**

The results are presented as calibrations in Fig. 3.3. This form of curve correction has changed the shape of the nickel calibration. Before correction the calibration is curved towards the concentration axis along its whole length and asymptotic to absorbance 0.8. After correction the slope is increased over the whole calibration and, at the high concentration end of the calibration, the curve now bends away from the concentration axis with no evidence of an asymptotic trend.
Fig. 3.3
Nickel calibration curves
- without stray light subtraction
- with stray light subtraction

**Conclusion**
Although the method has improved the sensitivity of the analysis and increased the slope of the calibration,
the calibration is not a straight line. This indicates either that the correction procedure has not corrected for all stray light or that stray light is not the only cause of curvature in the nickel atomic absorption system. Because of this imperfect correction, an analysis using this method will still require a calibration plot generated using several standards: a straight line cannot be assumed. The advantage is the improved resolution of solutions with a higher absorbance, due to the increased slope.

3.3.2 Comparison of curve fitting algorithms

With the introduction of automatic data handling techniques, curve fitting can now be performed using a microcomputer. Many different models [84] for the calibration curve have been used as the processes which cause it to bend are numerous [5]. Some of these models have been adopted by manufacturers and incorporated into the software packages used on their instruments. To enable a choice of appropriate instrumentation by analysts, the performance of these algorithms and the errors in concentration that they produce must be known. This experiment was designed, therefore to compare these algorithms, algorithms described by respondents to the questionnaire, Chapter 6, and manual curve fitting methods.
Experimental Algorithms and programs

Programs were written in BASIC for a Sharp MZ700 microcomputer, which incorporates a plotter-printer for the production of graphics and "hard copy" of results. The basic curve fitting programs described by Miller [151] were modified to produce the required algorithms (appendix 2), the ten algorithms are summarised in Table 3.5. These were then compared with programs within manufacturers own instruments (if available) to ensure the 'home-made' algorithms would produce the same results as the commercial instruments. Although the curve fitting algorithm described by Instrumentation Laboratory (now thermo-electron) [61] is described as using a cubic function, it is not clear if the cubic is in absorbance or concentration terms. For this reason, both forms of the cubic were tried and are included in Table 3.5. For the other systems, either the equations produced from the algorithms or the results during an analysis were compared. Some of the results and coefficients in the equation were not the same, but when the different rounding errors of the computer systems are taken into account, along with the probable differences in the programming method (e.g. Language and method of least squares fitting) all the programs compared were equivalent to their commercial counterparts. Only the Pye Unicam SP9 system was not copied successfully because of the lack of information and therefore the actual instrument was used with adjustment of burner height through the light path to
Equations used and method of curve fitting

\[ C = a + bA + cA^2 \]

solved for three data points or fitted by least squares for more data points. Coefficients reduced for less than three data points.

\[ A/C = a + bA + cA^2 \]

fitted as above

or \[ A = aC + bC^2 + cC^3 \]

\[ C = \frac{k_1A - k_2A^2}{k_2A - 1}, \]

if the top standard absorbance is within 15% of that predicted by the bottom standard, \( k_3 \) is set to zero. When the number of standards is three or less, the equation is solved with the appropriate number of coefficients. Otherwise the equation is fitted by least squares.

\[ A/C = a + bA + cA^2 \]

solved for each set of three calibration points.

\[ C = a + bA + cA^2 \]

A straight line is calculated between the blank and lowest standard. A quadratic is then applied between the next two data points, a third point being calculated using extrapolated slopes.

\[ C = aA + bA^2.5 + C(e^{1.4A} - 1) \]

\[ a = \frac{a - bC}{1 + cC} \]

\[ C = a + bA, \]

solved for every two points.

---

**Table 3.5**

Summary of Algorithms

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>PE 1 Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE 2 Coefficient</td>
<td></td>
</tr>
<tr>
<td>Varian Rational</td>
<td></td>
</tr>
<tr>
<td>Varian Associates</td>
<td></td>
</tr>
<tr>
<td>Fysh Unicam</td>
<td></td>
</tr>
<tr>
<td>From Questionnaire</td>
<td></td>
</tr>
<tr>
<td>From Questionnaire</td>
<td></td>
</tr>
<tr>
<td>Linear Interpolations</td>
<td></td>
</tr>
</tbody>
</table>

Manufacturers who have adopted Name assigned to algorithms the algorithm

- Baird Atomic (Data-comp system)
- Baird Atomic (Alpha-star system)
- Instrumentation Laboratory
- Instrumentation Laboratory
- IL Cubic 1
- IL Cubic 2
- Pye Unicam
- From Questionnaire
- From Questionnaire
- Linear Interpolations

C is concentration, \( A \) is absorbance and \( a, b, c, k_1, k_2 \) and \( k_3 \) are coefficients to be found during the fitting procedure.
give the appropriate absorbances (This algorithm has now been reproduced (Appendix A2.1.7)).

**Calibration Data**

Calibration data from section 3.2.1 table 3.1, was used to test the algorithms by obtaining four point and five point calibrations and entering all the absorbance data to produce eight concentration values.

**Goodness of fit parameters**

The parameters suggested by Miller-Ihli et al [85], namely the sum of the squares of the percentage concentration deviations (SSPD), equation 3.1 and its root mean square, equation 3.2 were used

\[
SSPD = \sum_{i=1}^{N} \left( \frac{C_i^c - C_i^k}{C_i^k} \times 100 \right)^2
\]  

\[
RMSPD = \left( \frac{SSPD}{N} \right)^{\frac{1}{2}}
\]

where \( C_i^c \) is the concentration calculated by the algorithm, \( C_i^k \) is the ith actual concentration and \( N \) is the number of data points tested.

As the percentage deviation for a blank \( (C_i^k = 0) \) is infinite, only seven data points \( (N = 7) \) were used in the tests.

To give an indication of any significant difference between algorithms, calculations of the standard
deviations (SD) of the parameters were made using equations (3.3) and (3.4) (derived (appendix A1.1) from the rules for the propagation of random errors [86]), assuming 1% relative standard deviation in $c_i^j$ and 0% relative standard deviation in $c_k^i$. 

\[ SD_{SSPD} = 10^4 \left\{ \sum_{i=1}^{N} \left[ \frac{0.02 \ c_i^j (c_i^j - c_k^i)}{(c_k^i)^2} \right] \right\}^{1/4} \]  

\[ SD_{RMSPD} = \left\{ \frac{1}{N} \right\}^{1/4} \frac{SD_{SSPD}}{2(SSPD)^{1/4}} \]  

Results and Discussion

The RMSPD values and their standard deviations are presented in table 3.6. Values for the associated SSPD's are not given as they can be calculated from the RMSPD values. The large value for the Perkin-Elmer three-coefficient fit to the four point magnesium calibration is due to the curve becoming discontinuous and asymptotic to absorbance 0.6, as shown in Fig. 3.4. In this instance, the commercial algorithm [72, 73] would choose the two-coefficient version of the function.

Clearly, a quadratic function (Baird Quadratic) does not provide a satisfactory model for calibration curves, except where the curves are virtually linear. A parabola can produce the required asymptote but will also give curvature at the blank level where, in practice, the calibration is often linear.
1.2

Absorbance

0.6

Concentration mg l⁻¹

0 0.65 1.3

Fig. 3.4

The calibration curve resulting from the Perkin Elmer 3 coefficient equation being fitted to the 4 point magnesium calibration data

Although making absorbance a function of concentration is more correct, when a cubic function is used (IL1, IL2) making concentration a function of absorbance produces an improved fit. Even so, the use of a cubic function does not produce a satisfactory fit to all the calibration curves, producing significantly high values of RMSPD for the curved calibrations, showing the model is not ideal. The algorithms described in the questionnaire (QUEST1, QUEST2) perform reasonably well,
<table>
<thead>
<tr>
<th>Algorithm</th>
<th>Nickel 5-point</th>
<th>Nickel 4-point</th>
<th>Chromium 5-point</th>
<th>Chromium 4-point</th>
<th>Magnesium 5-point</th>
<th>Magnesium 4-point</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RMSPD</td>
<td>$SD_{RMSPD}$</td>
<td>RMSPD</td>
<td>$SD_{RMSPD}$</td>
<td>RMSPD</td>
<td>$SD_{RMSPD}$</td>
</tr>
<tr>
<td>Baird Quadratic</td>
<td>28.77</td>
<td>0.170</td>
<td>26.58</td>
<td>0.194</td>
<td>20.47</td>
<td>0.242</td>
</tr>
<tr>
<td>Baird Rational</td>
<td>1.323</td>
<td>0.373</td>
<td>1.344</td>
<td>0.366</td>
<td>2.672</td>
<td>0.392</td>
</tr>
<tr>
<td>IL Cubic 1</td>
<td>4.890</td>
<td>0.341</td>
<td>7.102</td>
<td>0.317</td>
<td>6.270</td>
<td>0.385</td>
</tr>
<tr>
<td>IL Cubic 2</td>
<td>6.254</td>
<td>0.416</td>
<td>15.705</td>
<td>0.466</td>
<td>4.527</td>
<td>0.416</td>
</tr>
<tr>
<td>PE3 Coefficient</td>
<td>1.660</td>
<td>0.373</td>
<td>1.413</td>
<td>0.371</td>
<td>2.630</td>
<td>0.391</td>
</tr>
<tr>
<td>PE2 Coefficient</td>
<td>2.684</td>
<td>0.390</td>
<td>2.769</td>
<td>0.386</td>
<td>4.733</td>
<td>0.367</td>
</tr>
<tr>
<td>Varian Rational</td>
<td>1.134</td>
<td>0.367</td>
<td>1.687</td>
<td>0.368</td>
<td>2.528</td>
<td>0.396</td>
</tr>
<tr>
<td>PU Quadratics</td>
<td>2.110</td>
<td>0.365</td>
<td>4.621</td>
<td>0.381</td>
<td>2.619</td>
<td>0.374</td>
</tr>
<tr>
<td>QUES1</td>
<td>5.375</td>
<td>0.341</td>
<td>2.403</td>
<td>0.375</td>
<td>4.750</td>
<td>0.353</td>
</tr>
<tr>
<td>QUES2</td>
<td>2.259</td>
<td>0.385</td>
<td>2.457</td>
<td>0.382</td>
<td>4.212</td>
<td>0.374</td>
</tr>
<tr>
<td>Linear</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Interpolations</td>
<td>6.335</td>
<td>0.439</td>
<td>14.45</td>
<td>0.485</td>
<td>4.734</td>
<td>0.411</td>
</tr>
<tr>
<td>Manual</td>
<td>5.107</td>
<td>0.403</td>
<td>3.415</td>
<td>0.430</td>
<td>2.880</td>
<td>0.395</td>
</tr>
</tbody>
</table>

**Table 3.6**

Results of calibrations
though the fit produced by QUES1 deteriorates, the more linear the calibration; the highest values of RMSPD are for the magnesium curve. QUES2 uses a function where absorbance is a function of concentration. This is fitted by least squares, though the equation has to be rearranged to equation 3.5 for this purpose. Unlike the cubic function, equation 3.6, where

\[ A = a + bC - cAC \] 3.5

\[ A = aC + bC^2 + cC^3 \] 3.6

Absorbance is a function of concentration, equation 3.5 can be rearranged to enable concentration to be calculated without finding roots (equation 3.7).

\[ C = \frac{A - a}{b - cA} \] 3.7

In each case, a, b and c are found by the method of least squares.

The results for the manual plots are the average RMSPD and SD$_{RMSPD}$ values for the three analysts involved. Although these average values compare favourably with those obtained by using the computer algorithms, individual results varied and no one person was consistent. Linear interpolation only appears to be useful if
curvature is slight, or if many calibration points are employed. The RMSPD values for the Varian rational and Baird rational algorithms (which fit the same function) are consistently low and, overall, produce the best fits.

As might be expected, all the algorithms perform well for the situations in which the calibrations are nearly linear: The coefficients of the parameters which give an element of curvature to the function (e.g. \( A^2, A^3 \)) become less significant. The algorithm, QUES1 is an exception.

There are several ways in which the goodness of fit of a curve to a set of points can be assessed. A commonly used parameter for straight lines is the correlation coefficient, however, this tends to give values close to unity if the fit is good for higher absorbances and concentration even if the fit is poor further down the calibration curve. In addition, it cannot be used for algorithms that solve equations explicitly for a number of data points. The sum of the squares of the percentage deviations and its root mean square are thus more appropriate measures of the goodness of fit as they can be used for all types of algorithm if intermediate data points are used between calibration points. These parameters represent the fit over all of the curve and, being based on percentage deviations in the concentrations, are unbiased towards any part of the curve. The RMSPD represents the likely error in the concentration calculated by the algorithm, and due to function fitted.

An attempt could have been made to fit the functions
to the calibrations with the standards distributed differently along the curve, but in a practical analytical situation detailed knowledge of the calibration curve shape required for this approach, may not be available.

**Conclusion**

It is apparent that the performance of a particular algorithm depends on the shape of the calibration curve. There are situations in which the errors introduced by the poor fit of the calibration function can be quite serious. In such instances, manual plotting of the calibration curve is no better than the computer fitted curves. Given the difficulty of predicting what the shape of a particular element's calibration curve will be for a particular instrument under a particular set of operating conditions, it seems likely that whatever algorithm is used there will always be errors due to the lack of fit. These errors will generally be worse, the fewer the calibration points that are used. As calibration is a non-productive (in the sense of producing results) part of an instrument's operating period, there is naturally a tendency to reduce the number of standards required to a minimum.

Some improvements in the fits obtained with the various functions may be possible if appropriate weighting is given to each calibration point.

Alternative, non-curve fitting methods should be developed for calibration whilst no accurate model for the calibration function is available.
3.3.3 Analysis with interference compensation

Interferences

There are four main forms of interference which either increase or decrease the number of atoms available for absorption of light, from the level available from 'pure' standard nebulisation. Even the 'pure' standard must be in solution, and the counter-ions present could affect the analyte atom production.

Ionisation

The ionisation of the analyte in the flame will reduce the population of analyte atoms. At low concentrations the partial pressure of electrons in the flame will be low and the ionisation will be significant. As the concentration of analyte is increased, the partial pressure of electrons will increase reducing the degree of ionisation. This effect is exhibited as a concave calibration curve (Fig. 2.3). If the samples and standards are of the same composition, they will be ionised to the same extent. If another element is present in the sample, which also ionises, the partial pressure of electrons in the flame will be increased, increasing the concentration of analyte atoms in the flame. The sample response will therefore, no longer lie on the calibration curve obtained from the standards in which there is no interferent. Analysis of easily ionised elements often involves the addition of other easily ionised elements (e.g. K and La) as "ionisation buffers" to increase the sensitivity of analysis.
Chemical interference

There are several types of chemical interference which are produced either in the solution or in the flame. Compounds may react with the element of interest to produce compounds which do not atomise with ease. Examples of this type of interference are the interference of phosphate on calcium where the compound is produced in solution, and the atomisation of so called refractory metals which form strong complexes with oxygen in the flame. Addition of releasing agents such as lanthanum and protecting agents such as EDTA will reduce the amount of analyte compounded with the interferent in solution. Addition of oxygen scavengers such as ferric chloride will help in the atomisation of refractory metals.

If another element is in excess of the analyte element it may form a particle in the flame in which the analyte is contained, making atomisation of the analyte slow. An example of this type of interference is the interference of iron upon chromium. No chemical compound is formed, but alloy particles are formed.

Where a compound is formed with the analyte, the degree of interference will reach a maximum when all of the analyte is compounded with the interferent. Further additions of interferent will not affect the analyte.

Variable analyte oxidation states

The different oxidation states of an analyte can affect the sensitivity of the atomic absorption for a number of elements. If the sample analyte is in a
different oxidation state to the analyte oxidation state in the standards, the concentration calculated from the calibration curve will not be that in the sample. Use of a nitrous oxide/acetylene flame can reduce this effect but a better approach is to ensure that the oxidation state of the analyte in the standards and samples is the same.

Physical interference

The production of aerosol droplets can be affected by a number of physical properties of the solution. Increased viscosity of the solution will reduce the uptake rate of solution into the nebuliser, surface tension will affect the size of the droplets produced.

Both viscosity and surface tension of sample and standards should be matched so that the solutions are nebulised to the same degree.

Methods of correcting interferences

Some of the methods of reducing specific interferences have been described during the discussion of the types of interference.

Four general methods of interference correction are also available to the analyst.

Matrix removal

An obvious method of reducing the interference is to remove the interferent from the sample or extract the analyte from the sample matrix. This can be achieved using solvent extraction and ion exchange techniques.
These techniques are usually slow as they involve a number of operations, and are not applicable to every analyte. Some degree of preconcentration can be obtained with these techniques as the final volume of sample can be much smaller than that of the original sample.

*Use of hotter flames*

Use of hotter flames enables the atomisation of the analyte atoms in cases where it is lessened by chemical interference. For some elements however, the degree of ionisation can be increased and, therefore, the use of a nitrous oxide/acetylene flame often involves the addition of an ionisation buffer.

*Matrix matching*

If there is enough information about the sample available, the major chemically interfering species in the sample can be added to the standards in the same concentrations to produce the same degree of depression of the absorbance of the standards as the sample. The physical interference can also be matched using reagents which change the physical properties of the standards without producing chemical interference e.g. the viscosity of standards could be matched to that of a blood serum sample by addition of glycerol. If reagents are added to the sample to correct for other interferences these should also be added to the standard.
Standard additions

If there is sufficient capacity for depression in the sample to allow addition of standard so the standard absorbance is depressed to the same extent as the sample analyte, the standard additions technique can be employed. Increasing amounts of standard from zero are added to aliquots of sample before they are made up to the same volume. The absorbances of these solutions are then plotted against the concentration of the standard added. These points will lie on a calibration curve for the analyte with interference, but the origin will be moved from the normal zero analyte zero response point, to zero analyte added, zero response (Fig. 3.5).

If the calibration points lie on a straight line, the normal origin can be found by extrapolation of the calibration curve, and will occur at minus the concentration of analyte in the sample. The value will be negative as it will be equivalent to the amount of analyte which must be removed from the sample to give zero response. This is the concentration of analyte in the sample although the dilution of the original sample should be taken into account.

If a curved calibration is found, extrapolation is not recommended, as the unknown nature of the curvature will mean the course of the calibration will be undefined. The difficulty of obtaining a linear calibration except at low concentrations of analyte, and the prerequisite that the sample contains sufficient interferent to act upon the added standard to the same extent as the analyte present
The difference in origins between a normal and a standard additions calibration in the sample, makes the use of the standard addition method limited.

**Steel Analysis**

To test the efficiency of the above methods for overcoming interferences, their use in the analysis of
chromium in steel was studied. The dissolution procedure used involved the addition of acid to the steel and iron samples which were then heated on a hot plate. The dissolved material was then oxidised by addition of other acids and the resultant mixture diluted with water. The acids used for each method are given in the following descriptions of analyses along with references to the original papers from which detailed accounts of the dissolution procedures can be obtained.

**Use of a releasing agent**

The use of ammonium chloride as a releasing agent was employed for the analysis of the British Chemical Standards (BCS) steel number 404. The certificated assay of this steel is given in table 3.7

<table>
<thead>
<tr>
<th>Element</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.5</td>
</tr>
<tr>
<td>Si</td>
<td>0.69</td>
</tr>
<tr>
<td>S</td>
<td>0.012</td>
</tr>
<tr>
<td>P</td>
<td>0.033</td>
</tr>
<tr>
<td>Mn</td>
<td>0.13</td>
</tr>
<tr>
<td>Ni</td>
<td>0.61</td>
</tr>
<tr>
<td>Cr</td>
<td>3.00</td>
</tr>
<tr>
<td>Mo</td>
<td>0.82</td>
</tr>
<tr>
<td>V</td>
<td>0.23</td>
</tr>
<tr>
<td>Cu</td>
<td>0.43</td>
</tr>
<tr>
<td>Fe &amp; others</td>
<td>93.545</td>
</tr>
</tbody>
</table>

**Table 3.7 Assay of BCS Steel No. 404**
The method described by Barnes [152] was used but the instrument used was a Pye Unicam SP90A. The sample was completely dissolved in nitric/hydrochloric acid mixture and diluted to give a chromium concentration less than 15 mg l\(^{-1}\) and 1\% (w/v) ammonium chloride. This solution was analysed against standards, also containing 1\% (w/v) ammonium chloride, using stoichiometric to fuel rich flame conditions.

**Results and discussion**

The percentage chromium in the steel found by this methods was 1.8\% for all flames. This differs greatly from the certified value and demonstrates that the interference of iron on the absorbance of chromium is not corrected by addition of ammonium chloride as a releasing agent.

**Matrix matching**

The method described by Kinson et al [153] was designed for analysis of steels with chromium in the range 0 - 1\%. Three steel samples were analysed: BCS 404, BCS 325 and BCS 241/2. The assay of BCS 404 has been given (table 3.7), the assay of the others is given in table 3.8.

Both these steels were analysed as unknowns. The unknown steel content was estimated as approximately 3\%.

The amount of steel taken for the analysis of BCS 404 and 241/2 was reduced from 1g per 100 ml to 0.1 g, the
Table 3.8 Assays of BCS Steels 325 and 241/2

<table>
<thead>
<tr>
<th>element</th>
<th>%</th>
<th>element</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni</td>
<td>0.23</td>
<td>W</td>
<td>19.9</td>
</tr>
<tr>
<td>Cr</td>
<td>0.22</td>
<td>Cr</td>
<td>5.35</td>
</tr>
<tr>
<td>Mo</td>
<td>0.16</td>
<td>V</td>
<td>1.59</td>
</tr>
<tr>
<td>W</td>
<td>0.12</td>
<td>Mo</td>
<td>0.53</td>
</tr>
<tr>
<td>Ti</td>
<td>0.013</td>
<td>Co</td>
<td>5.7</td>
</tr>
<tr>
<td>As</td>
<td>0.013</td>
<td>C</td>
<td>0.84</td>
</tr>
<tr>
<td>Sn</td>
<td>0.046</td>
<td>Si</td>
<td>0.21</td>
</tr>
<tr>
<td>Sb</td>
<td>0.002</td>
<td>S</td>
<td>0.025</td>
</tr>
<tr>
<td>Al</td>
<td>0.028</td>
<td>P</td>
<td>0.024</td>
</tr>
<tr>
<td>Fe &amp; others</td>
<td>99.168</td>
<td>Mn</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ni</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cu</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sn</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fe &amp; others</td>
<td>65.306</td>
</tr>
</tbody>
</table>

amount of iron added to the standards was reduced accordingly. The iron and steels were dissolved in phosphoric/sulphuric acid with nitric acid added later. BCS 404 and 325 completely dissolved but BCS 241/2 left a residue probably due to the tungsten. BCS 404 was analysed using a range of flame conditions, and the flame conditions for subsequent analyses were chosen on the basis of the accuracy of determination of the chromium content of this steel.

Results and discussion

The percentage chromium in BCS 404 steel calculated using different types of flames, are given in table 3.9. From these results, it can be seen that an analysis
using a stoichiometric flame produces the most accurate result. The other two steels were therefore analysed using a stoichiometric flame. The results for these steels are given in Table 3.10.

<table>
<thead>
<tr>
<th>Flame Type</th>
<th>% Cr found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stoichiometric</td>
<td>3.09</td>
</tr>
<tr>
<td>Slightly luminous</td>
<td>3.24</td>
</tr>
<tr>
<td>Very rich</td>
<td>3.57</td>
</tr>
</tbody>
</table>

**Table 3.9** The effect of flame type upon the apparent chromium concentration in the steel BCS 404.

<table>
<thead>
<tr>
<th>Steel</th>
<th>% Cr found</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCS 325</td>
<td>0.245</td>
</tr>
<tr>
<td>BCS 241/2</td>
<td>5.8</td>
</tr>
</tbody>
</table>

**Table 3.10** The concentration of chromium in steels by the matrix matching technique.

All the results are higher than expected which must be due to an imperfect matrix match. Some of the other components in the steel will enhance or reduce the interference by iron and these components should also be included in the standards. Because steel is not 100% iron, the amount of iron added to the standards will be greater than that present in the sample solution. The amount of iron that should be added is always unknown, as it depends on the result of the analysis.

**Standard Additions**

The dissolution procedure described for the matrix
matching method above was used to obtain sample solutions of 1 g of the steels BCS 325 and BCS 241/2. These were then analysed using the standard additions technique. 5 ml of the sample solutions were added to portions of chromium standard and made up to volume to produce added standard concentrations of up to 8 mg l\(^{-1}\). These solutions were analysed using a stoichiometric flame.

Results

The percentage chromium in the steel samples found using this method are given in table 3.11.

<table>
<thead>
<tr>
<th>Steel</th>
<th>% Chromium found</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCS 325</td>
<td>0.224</td>
</tr>
<tr>
<td>BCS 241/2</td>
<td>5.85</td>
</tr>
</tbody>
</table>

Table 3.11 The concentration of chromium in steels by the Standard Additions technique

Both values are slightly higher than expected. A short study of the standard additions curve shapes and signal precision showed that this overestimate could occur in two ways.

1) A fuel lean flame will produce linear calibration curves but the precision of the absorbance values will be poor. When the line is extrapolated, the uncertainty in the line will produce great uncertainty in the concentration found (Fig. 3.6). Even though the variation
Standard additions calibration with noise on data points

A maximum overestimate of concentration
B maximum underestimate of concentration
C concentration of analyte in sample

of absorbance may be evenly distributed about the sample absorbances, when the calibration curve is extrapolated, there will be a greater tendency to overestimate (represented by distance A-C, Fig. 3.6) than underestimate (represented by distance B-C, Fig. 3.6).

2) A fuel rich flame tends to produce a more sensitive analysis with steeper calibrations but with more curvature. The precision of the calibration points was better than when a fuel lean flame was used. Extrapolation of the calibration curve tends to produce an overestimation of sample concentration, as the points at the higher concentration end of the calibration reduce the
slope of the line (Fig. 3.7).

The overestimates of sample concentration for the stoichiometric flame, tables 3.8 and 3.9 were caused by a combination of these effects.

![Diagram of Absorbance vs. Concentration with annotations](image)

**Fig. 3.7**

Curved standard additions calibration

- a - straight line fitted to all data points
- b - straight line fitted to linear portion of data
- C - true concentration of analyte
- D - overestimate of concentration of analyte

**Matched matrix calibration with a nitrous oxide flame**

Thomerson and Price [154] describe a method where samples and standards are matrix matched and analysed using a nitrous oxide flame with the burner slot at an angle to the light path. This method of analysis was used for the analysis of steel BCS 241/2, the sample (1 g) and
the standards being dissolved in hydrochloric and perchloric acid and made up to 100 ml. The spectrometer used (Baird Atomic 3400) does not have a rotatable burner, so the burner was moved across the light path to produce the required sensitivity. The solutions had to be analysed quickly as the burner tended to block.

Results and Discussion

The amount of chromium in the steel sample was found to be 5.0% by this method. This is lower than expected and this is due to the same reasons the previous matrix matched method is high: In the nitrous oxide flame, iron enhances the absorption of chromium, the excess iron in the standards raises the absorbance, reducing the apparent concentration of the sample.

Standard additions using a nitrous oxide flame

Solutions of the same concentration as used in the previous standard addition experiment using the perchloric acid/hydrochloric acid mixture described by Thomerson and Price [154], were made for the analysis of the steel BCS 241/2. These were analysed using the Baird Atomic A3400 spectrometer with the burner slot in line with the light path.

Results and Discussion

The amount of chromium in the steel was found to be 5.68%. This is high, probably due to the same reasons as described in the previous discussion of the standard additions method.
Conclusion

Obviously, the releasing agent method described by Barnes [152] does not work for the analysis of steel BCS 404. The ammonium chloride does not release all the chromium.

Matrix matching produces reasonable results but appears to overestimate when an air-acetylene flame is used and underestimate when a nitrous oxide-acetylene flame is used. This shows that the matrix of the standard cannot easily be matched with the sample as iron depresses the absorbance of chromium in air-acetylene flames, but enhances the absorbance of chromium in nitrous oxide-acetylene flames.

If the problem of extrapolation errors could be eliminated, the standard additions method could correct for interferences; assuming the interferent is able to act upon the added standards to the same extent as the analyte in the sample. Only one sample dissolution is required thus making this method more convenient than matrix matching.

Use of a nitrous oxide flame will enable the dissociation of stable flame species but in these cases the interference of iron on chromium is changed in nature but not removed. There is potential for improvement in the method of interference correction and standard addition techniques.
4.1 Introduction

Solutions can be diluted "on-line" by merging with a diluent stream or by introducing the sample into a manifold as a plug (Flow injection) and allowing it to disperse as it flows to the detector. This dispersion can be enhanced by incorporating a mixing chamber after the injection point. Dilution produced by merging will be a direct reduction of concentration depending on the concentrations and flowrates of sample and diluent, as in the equation (derived in appendix A1.2)

\[
C_3 = \frac{C_1 F_1 + C_2 F_2}{F_1 + F_2}
\]

Where \(C_1\) and \(C_2\) are the concentrations of the merged streams flowing at flowrates \(F_1\) and \(F_2\) respectively. \(C_3\) is the resultant concentration.

Dilution produced by dispersion in flow injection analysis is a complex function depending on manifold parameters such as flowrates, manifold size and injected volume. The injected plug develops from a well defined sample zone to a dispersed zone, with concentration gradients at each end (Fig. 4.1). These gradients are formed by convection, diffusion and turbulence. This
causes the carrier solution to migrate towards the centre of the sample zone as it flows to the detector. If the manifold produces sufficient dispersion, the carrier will dilute the centre of the sample zone. Various peak shapes can be produced [155] but under usual FIA conditions they can be modelled by the single well stirred tank model. Equation 4.1 is the equation for the rise curve, equation 4.2 is for the fall curve [135, 136].

\[ C = C_m (1 - e^{-ut/V}) \]  

\[ 4.1 \]
\[ C = C_0 e^{-ut/V} \]

\(^{4.2}\)

C is the concentration measured at time \( t \), \( u \) the flowrate through the theoretical tank, volume \( V \), \( C_m \) is the injected concentration. Concentrations on the rise are measured from the moment sample enters the tank and, on the fall, from the peak, where \( C_p \) is the concentration.

These equations show that the concentration of analyte is dependent on the flowrate of the carrier stream, the volume of the theoretical mixing chamber, the time at which the concentration is measured and the original concentration injected.

4.2 Preliminary experiments

These experiments have been described by Tyson et al [155]

4.2.1 Variation of sensitivity of flame atomic absorption spectrometry with nebuliser flowrate

If an atomic absorption instrument is to be linked to a continuous flow analysis system (including FIA), the behaviour of the instrument must be known for different operational parameters of the manifold that affect the AA signal. The flowrate of solution into the nebuliser will change the nebulisation characteristics. This experiment was designed to study how the atomic absorption signal is affected by flowrate both for the methods of continuous analyte flow and flow injection analysis.
Apparatus

An Atomspek atomic absorption spectrometer was used which has a low natural nebuliser consumption rate of 2 ml min\(^{-1}\). The instrument was optimised for calcium determination, a 20 mg l\(^{-1}\) solution of which was used for measurement of absorbance at various flow rates. Four manifolds were used 1-4 (Fig. 4.2) for studies of (1) steady state absorbance versus flowrate, (2) peak height absorbance and peak width versus flowrate, (3) Peak height absorbance and width versus flowrate of confluencing solutions i.e. increasing dilution and flowrate, (4) Peak height absorbance and width versus flowrate of confluencing solution i.e. increasing dilution only, respectively.

Procedure

Flowrate was calculated from the pump speed setting using a pump speed/flowrate calibration. Peak widths were measured at the base of the peaks, chart speed was 20 mm min\(^{-1}\). Each injection was performed at least three times.

Manifold 1

The 20 mg l\(^{-1}\) calcium solution was continuously pumped into the instrument and the absorbance noted for each pump rate.

Manifold 2

The 20 mg l\(^{-1}\) calcium solution was injected into a water carrier which was pumped at various rates, and the
Variable speed pumps
Injection valves: 72.5 μl

Fig. 4.2
Manifolds
peak heights and widths noted.

**Manifold 3**

A 40 mg l⁻¹ calcium solution was injected into a water carrier stream flowing at 4.1 ml min⁻¹ which was merged with a water carrier stream flowing at various flowrates (This produced various total flow rates). Peak heights and widths were noted.

**Manifold 4**

The 40 mg l⁻¹ calcium solution was injected into a water carrier stream flowing at 4.1 ml min⁻¹ and merged with a water carrier flowing at various flowrates. The stream was subsequently divided downstream, and some of the solution pumped to waste at the same flowrate as the merging stream. This kept the flowrate to the instrument constant whilst varying the dilution.

**Results and Discussion**

Graphs of the results are presented in Figs. 4.3-4.5

**Manifolds 1 & 2**

The graphs obtained using manifolds 1 and 2 Fig. 4.3, show that both for flow injection sample introduction and for continuous pumping of sample solution, there are optimum flow rates for maximum absorbance. The flow injection curve drops off sooner than the steady state curve, with increasing flowrate. This is probably due to the time constant of the chart recorder damping the
Fig. 4.3

Steady state, peak absorbance and the resultant dispersion
movement of the pen so that it does not attain the correct absorbance level. As the flowrate is increased the speed at which the plugs enter the nebuliser increases until the pen cannot follow the peak. This is an 'electronic' increase in dispersion. For continuous flow of analyte, no such problem occurs; the pen attains the level generated by a continuous absorbance value. The decrease in the absorbance must be due to the decrease in the number of small analyte mist droplets formed by the nebuliser. This decreases the number of droplets reaching the flame and reduces the signal.

If the chart recorder and measuring system of the spectrometer did not produce any damping the flow injection graph could be expected to show similar behaviour to the continuous analyte curve.

The damping of the flow injection signal produces a maximum in the dispersion curve Fig. 4.3 at about 8.5 ml min$^{-1}$. The slight rise in dispersion up to 7.5 ml min$^{-1}$ is probably due to changes in dispersion in the manifold [156] rather than instrument response.

**Manifolds 3 & 4**

From the results of experiments using manifolds 1 and 2 a carrier flowrate of 4.1 ml min$^{-1}$ is below the optimum both for FIA and SS. When the stream is diluted without a split, manifold 3, the effect of dilution is offset by an increase in instrumental sensitivity Fig. 4.4. Above 6.0 ml min$^{-1}$ the drop in instrument sensitivity and the dilution both cause a decrease in the absorbance. When
Fig. 4.4

Peak heights versus flowrates

Data using manifold 2
Data using manifold 3
Data using manifold 4
the sample plug is merged it occupies a longer section of
tube, but travels proportionally faster. This means that
although the flowrate is increased the time taken for the
plug to enter the nebuliser is the same. Therefore there
is no electronic dilution with increasing flowrate.

Similarly for the system with a split. Flowrate
remains at 4.1 ml min$^{-1}$, just below the optimum flow rate.
Hence when diluting, the absorbance is less than when a
split is used, Fig. 4.4 as the dilution is the same but
the sensitivity is less. The two curves converge and
cross at 11 ml min$^{-1}$ as the drop in sensitivity decreases
the peak height of the unsplit system.

**Peak widths**

As expected, in the undiluted system the peak widths
decrease with the flowrate (or speed at which the sample
slug enters the nebuliser) Fig. 4.5. The decrease in
width will be affected by the increase in sensitivity
which will increase the peak width. This is the probable
cause of the levelling out at x, in the data generated
using manifold 2.

The slight decrease in peak width with increasing
diluent flowrate is due to dilution lowering the peaks;
The baseline is detected earlier and the peaks appear
narrower, although the time the plug takes to enter the
instrument is the same.

**Conclusion**

Any automatic production of calibration solutions
Fig. 4.5

Peak widths versus flowrate

- Data using manifold 2
- Data using manifold 3
- Data using manifold 4
must be carried out at the optimum flowrate so that variations in absorbance due to flowrate are minimised. The increase in the length of tube occupied by a sample when merged does not affect the peak width.

4.2.2 Variation of instrument response with temperature of solution introduced

Nebulisation of solutions depend on their physical properties such as viscosity and surface tension. A change in viscosity will change the rate of uptake of the solution and the pressure at the inner tip of the nebuliser tube [157]. A change in surface tension will change the drop sizes formed. Both these properties will be altered by an increase or decrease in temperature of the nebulised solution.

This experiment was designed to see how the instrument response changes with temperature of solution, and if that change was altered by controlling the rate of nebulisation with a pump.

Conventional Nebulisation

Apparatus

Three solutions were presented to the Atomspek spectrometer

(i) distilled water (blank)
(ii) 20 mg l\(^{-1}\) Calcium at 25°C (room temperature)
(iii) 20 mg l\(^{-1}\) Calcium at various temperatures

Absorbances were measured from a chart recorder.
Procedure

Solution (iii) was aspirated from a round bottomed, narrow necked flask to reduce the concentrating effect of evaporative solution loss. Up to 90°C, the water vapour refluxed on the sides of the flask which was kept swirled. Above 90°C some water vapour was visibly lost. The flask was heated via a water bath (fig. 4.6).

Ice was used to cool the solution below room temperature. The temperature of the solution was measured using a thermometer.

Between every one or two measurements of absorbance at specific temperatures, the blank and room temperature Ca(II) solutions were aspirated. This provided baseline adjustment and a check on sensitivity, allowing compensation for drift.
Fig. 4.6
Apparatus for natural aspiration and temperature investigations

*Pump controlled nebulisation*

Apparatus

The 20 mg l\(^{-1}\) calcium solution was continuously
pumped at 2 ml min$^{-1}$ the natural aspiration rate of the nebuliser, using a Gilson minipuls pump. It then passed through 2 metres of 0.8 mm id tubing suspended in a stirred water bath before reaching the nebuliser (fig. 4.7).

![Diagram of apparatus for pump controlled aspiration and temperature investigations]

**Fig. 4.7**

Apparatus for pump controlled aspiration and temperature investigations

The temperature of the water bath was measured using a thermometer. The absorbance was measured using a chart recorder.

**Procedure**

The absorbance of the solution was measured at
increasing temperatures but between each temperature the coil was removed from the water bath and the absorbance of the solution measured at room temperature. The coil was then replaced in the solution and the system allowed to equilibrate for a few seconds before absorbance measurements were taken.

Results and discussion

Graphs of the percentage increase in absorbance obtained with increasing temperature are presented in fig. 4.8. As the temperature of the solutions increased, the absorbance became more noisy especially when the solution was pumped. This was due to bubbles forming in the tubing.

The increase in absorbance for conventional nebulisation is due to the reduction in viscosity with temperature increasing the rate of solution nebulisation, reduction of surface tension enabling smaller droplet formation and faster desolvation of these droplets due to their elevated temperature. For pump controlled nebulisation, the reduction in viscosity will reduce the pump back pressure raising the flowrate but this increase will be small for a peristaltic pump. This slight increase in flowrate, the reduction of surface tension and the faster desolvation has caused a slight increase in absorbance with increasing temperature. This increase is about one quarter that produced with normal nebulisation.
Fig. 4.8

Change in absorbance with temperature of solution

- Conventional, natural aspiration
- Pumped aspiration
Conclusion

If a warm solution were injected into a carrier at room temperature, it would be cooled whilst passing down the manifold and the pump would experience virtually no drop in back pressure. Therefore it should be possible to analyse hot solutions by comparing the absorbance with a calibration obtained at room temperature, if a flow injection system is used. This is not possible using conventional nebulisation where there is no control of flowrate.

4.3 Continuous analyte flow calibration methods

4.3.1 Principles of construction and operation of the manifold

The dilution and absorbance versus flowrate experiments used a manifold (fig. 4.2) which adjusted the flowrate after a merging point. A similar manifold was designed to operate in a continuous analyte flow mode figure 4.9. This manifold is different from that in figure 4.2 in the following ways.

(i) There is no injection valve for sample introduction, instead a stream switching valve SS, switches between sample (A) and blank (B).

(ii) The split comes before the confluence to allow the concentration of the stream being pumped to the spectrometer to reach the concentration of the standard (C) being pumped by the pump (P2). If the split and confluence were reversed, as in manifold 4 (Fig. 4.2) the standard pumped to the nebuliser would always contain
Fig. 4.9

Two pump manifold

P₁ is a fixed speed pump
P₂ is a variable speed pump
SS₁ is a stream switching valve
A is sample
B is diluent/blank
C standard

Blank.

If the blank were pumped by P₂ and the standard by P₁, to obtain a blank reading all the standard would have to be pumped to waste to obtain a blank reading.

The concentration of the standard pumped to the spectrometer is dependent on the flow rates delivered by the pumps and the concentration of the original standard C
according to equation 4.3 derived in appendix A1.3,

\[ C^N = \frac{C^S \times F^S}{F^N} \quad 4.3 \]

where \( C^N \) is the concentration reaching the spectrometer, \( C^S \) the concentration of the original standard, \( F^S \) the flow of standard delivered by the variable speed pump and \( F^N \) the flowrate to the spectrometer delivered by the fixed speed pump.

The flow rate to the nebuliser \( F^N \) is constant, whatever concentration is being mixed.

**Apparatus**

For these experiments the variable speed pump was an LKB 2132 microperpex pump fitted with the small tubing supplied. The maximum flow rate produced for each channel was approximately 5 ml min\(^{-1}\). The fixed speed pump was an Ismatec 840. The manifold consisted of teflon tubing of various diameters (Radio spares No. 1, Anachem), Y pieces constructed in our workshops and a computer controlled injection valve (P. S. Analytical) configured for stream switching. The solutions produced were monitored by either a Baird Atomic A3400 or a Pye Unicam SP9 atomic absorption spectrometer optimised for the elements used.

4.3.2 Developmental experiments

*Construction of a Magnesium Calibration Curve*

Pump tubing for the Ismatec pump was chosen to bring
the blank/diluent flow rate close to the optimum, found using a variable speed pump. The flow rate produced was 7 ml min\(^{-1}\). A 2 mg l\(^{-1}\) magnesium solution was aspirated via the variable speed pump. The flow rates of the two pumps were calibrated by measuring the aspiration rate from a 10 ml burette. The absorbance for 23 different flow rates delivered by pump \(P_2\) were noted and an absorbance/flow rate calibration curve constructed. A scale expansion of about 2 x was used to give larger differences between each solutions absorbance.

A 0.5 mg l\(^{-1}\) magnesium sample was aspirated to test the validity of the calibration.

**Results and discussion**

A problem observed when mixing low concentration solutions is that the pulsing produced by the variable speed peristaltic pump produces a noisy signal (fig. 4.10). The pulses in the signal are not due to variations in the flow rate of the mixed standard reaching the nebuliser. This flow rate was optimised and a 1 ml min\(^{-1}\) variation about this optimum will only produce a change in absorbance of about 1.5\% (cf fig. 4.3). The variation in absorbance in fig. 4.10 is about 90\% for the lowest speed.

The variable speed pump is driven by a stepper motor which could produce pulses in flow but the frequency of the motor pulses is 50x that found here.

The pulses produced here are caused by each pump roller pushing the stock standard down the tubes creating
Noisy absorbance signals with slow speeds of the variable flowrate pump \( P_2 \): 

- \( a \): 0.1 ml min\(^{-1}\), 
- \( b \): 0.2 ml min\(^{-1}\)

bursts of more concentrated standard. At high speed, the increase in frequency of these pulses and the flexibility of the tubing reduces the pulses to an insignificant level (fig. 4.11). Some electronic damping of the signal was used to decrease this noise. The damper switch on the A3400 was set to 2.

A linear calibration curve, fig. 4.12 was obtained up to a scale expanded absorbance of 0.952 (actual absorbance approximately 0.476). The scale expanded absorbance of the 0.5 mg l\(^{-1}\) sample was 0.774 which corresponds to a standard flow rate of 1.795 ml min\(^{-1}\). Putting all the
The reduction of noise amplitude with increased speed of the variable flowrate pump $P_2$.

- $a$: 0.1 ml min$^{-1}$, $b$: 1 ml min$^{-1}$.

Variables in equation 4.3, gives

Sample concentration = $1.795 \times 2$

$$\frac{7}{7}$$

= 0.513 mg l$^{-1}$
Fig. 4.12 - Magnesium calibration using the two pump manifold

Scale expanded absorbance

Flowrate delivered by variable speed pump ml min⁻¹
The concentration value obtained is 2.6% high.

**Conclusion**

If the effects of pump pulsing can be reduced and the system optimised, the method shows promise for automated calibration. The error in concentration obtained for the 0.5 mg l\(^{-1}\) sample is similar to that expected from a normal, curve fitting type calibration based on making up standard solutions in volumetric glassware (section 3.3.2).

*Reduction of standard concentration pulses*

Various methods were devised which it was hoped would reduce the 'standard concentration pulses'. They were based on three methods:

i) reduction of the pressure pulses produced by the pumps.

ii) physical mixing of the merged streams.

iii) instrumental damping by either damping the output from the spectrometer or integrating the signal.

*Reduction of pressure pulses*

Pulse dampers were constructed (fig 4.13) and positioned as shown in fig. 4.14. Positions A and B were ineffective but position C did remove some of the noise due to the pump rollers. This emphasised the point that the concentration variation is produced by the pulsing of the pump P\(_2\) and is not due to variations in the flow rates to the nebuliser. The pulse damper was not completely effective.
Fig. 4.13

Simple, air column pulse damper

a glass plug
b silicone rubber tube containing air
c glass T, filled with pump fluid
d silicone rubber stoppers
e manifold tubing
Positioning of pulse dampers A, B and C

Mixing

A wide bore T piece (i.d. 3 mm) was used at the confluence point in place of the normal teflon Y piece. This provided some mixing at the confluence point produced by the dead volume and associated turbulence. This did not greatly reduce the pulses.

A stirred mixing chamber was inserted at A (fig. 4.14) internal volume approximately 0.5 ml. This again did not provide sufficient mixing to smooth out the pulses.

The manifold was modified to produce the network in fig. 4.15

There are two ways in which this system can operate. The fluid in tube x could be standard flowing from D to E,
Fig. 4.15

Two pump manifold with network mixing. Tubes x, y and z are 100, 200 and 500 mm long respectively.

in which case the standard would be merging at points E and F, or the fluid in x may be diluent going from E to D, in which case the standard would merge with diluent at D and this dilute solution would again be merged with diluent at F. Because of the resistance of the 300 mm tube, the fluid in tube x is probably standard flowing from D to E.

This system was most effective at reducing the variations in standard concentration. Because the standard merges at two points, and the flow rates of the solutions at the merging points are different, the concentration variations combine destructively and are smoothed out.
**Instrumental damping (smoothing)**

These methods of removing the pulses in the signal do not attempt to remove the actual variations in concentration at the nebuliser.

Damping the output from the instrument was employed as described in the construction of a magnesium calibration experiment described above. Integration of the signal was also tried using the manifold on the SP9. Both methods were effective at reducing the noise on the signal due to the pump pulses. Integration was only effective with an integration time > 8s.

**Mix and Match**

The manifold, fig. 4.9 can produce a continuous stream of standard at any concentration up to the concentration of the stock standard. Though, because the maximum possible flow rate produced by pump $P_2$ is usually less than the flow rate produced by pump $P_1$, the concentration of the standard that can be mixed will be less than that of the stock standard. Normal calibration and analysis using this manifold consists of mixing appropriate concentration standards, measuring their absorbance, fitting a calibration function, measuring the absorbance of the sample and interpolating its concentration from the fitted function.

If the sample is aspirated first, then a standard which gives the same absorbance can be mixed by varying the speed of pump $P_2$ accordingly. This is not possible when mixing standards using conventional glassware: it
would take a great deal of time to mix the solutions to find a match. With this manifold, the solution concentration is varied simply by changing a pump speed.

*Manual Mix and Match*

The manifold in fig. 4.9 was used, the flow rates of the two pumps being calibrated by timed delivery of a known volume from a 10 ml burette. The flowrate delivered by the fixed speed pump was 9.375 ml min$^{-1}$. A 2 mg l$^{-1}$ magnesium stock standard was used and the calibration method tested by aspiration of 0.4 mg l$^{-1}$, 0.3 mg l$^{-1}$ and 0.2 mg l$^{-1}$ samples. A blank measurement was taken followed by the absorbance of a sample. The valve SS, was then returned to blank and the flow rate of the variable speed pump adjusted so a standard was mixed which gave an absorbance closest to that of the sample. The concentration of the sample was then calculated from this flow rate using equation 4.3.

*Results and discussion*

The flow rates and concentration found are presented in table 4.1. The errors obtained with this manual mix and match technique are greater than the 2.6% obtained for the construction of the magnesium calibration described above. This is due to the poor resolution in pump speed obtainable; only increments of 0.1 ml min$^{-1}$ are possible whereas interpolation is possible with a calibration curve.
Sample conc. (mg l⁻¹) & Flowrate P₂ (ml min⁻¹) & Concentration % error (Calculated)  
0.4 & 1.9 & 0.40 & 0.0  
0.3 & 1.3 & 0.28 & -6.7  
0.2 & 1.0 & 0.21 & +5.0  

Table 4.1
Flowrates and respective concentrations

Conclusion
These experiments showed the potential of automated calibration using this manifold by either the production of a calibration curve, or the novel "mix and match" technique. Accuracy may be improved by improving the resolution, and number, of the concentrations of the standards produced. Some method of reducing the effect of pump pulsing is required.

For automated calibration/analysis the system will be linked to a microcomputer. This will provide the facilities of signal integration and improved resolution of pump speed.

4.3.3 Novel methods of automated calibration
The pump/valve control interface used in this section is described in Appendix A3.1. This enabled control of the variable speed pump and valve using the Apple IIe microcomputer, which also monitored the response via an A/D converter (Microcontrol Ltd.).
Calculation of pump flowrates

The fixed speed pump was calibrated using the usual 'measured volume delivered in a measured time interval': Both these values being entered into the computer. But when control of the variable speed pump is given over to a microcomputer, its internal calibration system is disabled. For the computer to control what flowrate the pump is producing, the volume delivered for each computer pulse must be known. The flowrates can then be calculated from the frequency of the pulses. In these programs, the volume delivered for 10000 computer pulses is used, usually about 3.5 ml. The flowrate is then calculated using equation 4.4 derived in appendix A1.4.

\[
\text{Flowrate} = \frac{\text{VOL} \times 6 \times 10^3}{2^{N/16} \times (3.5 + [2 \times (\text{LB} + (256 \times \text{HB}))])} \tag{4.4}
\]

where \( N \) is the value of the dividers (multiples of 16)

- LB - low Byte number
- HB - high Byte number
- VOL - volume delivered by 10000 pulses.

To define the frequency for a given flowrate the LB and HB values are found by rearranging to give equation 4.5

\[
\text{BITS} = \frac{\text{VOL} \times 6 \times 10^3}{2^{N/16} \times \text{FR}} - 3.5 \times 0.5 \tag{4.5}
\]

where BITS = LB + (256 \times HB)

Initially \( N = 0 \) but if the value of HB is greater than 256 the value of \( N \) is increased by 16 and the value of BITS recalculated. When the values of HB and LB are sent to
the appropriate locations, the pump speed changes accordingly. This produces a very fine resolution between the pump speeds for a change in the LB value. The difference in speed is virtually imperceptible.

Data smoothing

As described previously at low concentration the pump rollers cause the solutions to be mixed unevenly resulting in concentration variations, the frequency of which depends on the speed of the variable speed pump, pulsing at 63.9 pulses/ml delivered. Computer control of that pump allows the possibility of smoothing the data over a specific number of concentration variations. For very low concentrations it can take several seconds for the pump head to turn one revolution and if smoothing took place over one complete revolution, the analysis would be rather slow. At high concentrations the pump head is turning very fast but the concentration variations are not apparent. For these reasons the function (equation 4.6) derived in appendix A1.5 was chosen which defines the number of absorbance values read for each standard.

\[
\text{No. of absorbance} = \text{integer} \left( \frac{\text{VOL} \times 30}{\text{Flowrate}} \right) + 200 \quad 4.6
\]

Part (a) is significant at low flowrates (pump speeds) and is the number of absorbance readings read for 2 pump roller cycles. Part (b) becomes more significant at
higher flowrates. For example, if a 2 mg l\(^{-1}\) solution is required, mixed at a nebuliser flowrate of 7 ml min\(^{-1}\) from an 80 mg l\(^{-1}\) standard, the flowrate of standard required from equation 4.3 would be \((7 \times 2)/80 = 0.175\) ml min\(^{-1}\).

This gives, when substituted in equation 4.6 with calibration volume typically 3.5 ml, the number of absorbance values read is \(600 + 200 = 800\). The pulse rate of the pump would be 0.186 pulses per second. Data were read at 45 conversions per second.

*Calibration by curve fitting using mixed standards*

*The Algorithm:*

The flowrates of the two pumps were calibrated as described and the concentration of the top standard calculated. The range from the blank to the top standard concentration is then divided into ten calibration standards. The absorbance of the blank and the ten standards is then measured by operating the appropriate valves and pumps etc.

The calibration function, equation 4.7 is then fitted by

\[
\frac{A}{C} = a + bA + cA^2
\]

"least squares" to this calibration data and a correlation coefficient calculated. This function was found to be the easiest to fit and reasonably accurate according to the comparison of curve fitting algorithms (section 3.3.2).

The absorbance for ten samples is then read before the calibration process is repeated. The program written
which executes the algorithm is presented in appendix A2.2.1.

**Experimental**

**Apparatus**

The Pye Unicam SF9 Spectrometer was optimised for chromium analysis. Chromium was chosen as the test element as it has a curved calibration and was used in the comparison of curve fitting algorithms. A stock standard of 50 mg l\(^{-1}\) was used and sample concentrations of 5, 10, 15 and 25 mg l\(^{-1}\) were aspirated to test the system.

**Results and Discussion**

The volume delivered by the computer controlled pump was 3.24 ml for 10000 computer pulses. The aspiration rate of the fixed speed pump was 6.2 ml min\(^{-1}\). This produced a top standard concentration of approximately 35 mg l\(^{-1}\). The results of the analysis of the test samples is presented in table 4.2 and were generated from four successive calibrations.

Correlation coefficients although biased toward the high end of the calibration give some indication of how well data fits a particular function. For all the calibrations the correlation coefficient was below 0.9960. This indicates that equation 4.7 does not fit the calibration data to as high an accuracy as would be hoped. The errors in calculated concentration were not all that high and are comparable with the errors in concentration expected for curve fitting algorithms. When an RMSPD
<table>
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<th>Mean Concentration (mg l⁻¹)</th>
<th>%Error</th>
<th>Relative Standard Deviation</th>
<th>95% Confidence Interval</th>
<th>Number of Replicates</th>
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</thead>
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</tr>
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<td>0.52</td>
<td>1.7</td>
<td>0.40</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 4.2

Results for curve fitting calibration

value was calculated according to equation 3.2, a value of 1.633 was found. This was calculated using ten calibration points and compares favourably with the values obtained for curve fitting algorithms, though they were calculated using a three and four point calibrations. A blank reading was not taken between each sample as the time taken to wash the solutions through the pumps is high. The increased accuracy of measuring a blank between each sample will be traded off by the drift in the response (calibration) over the sampling time. If the washout times of the pumps were decreased, accuracy could be increased by measuring a blank between each sample. Resloping on one standard, as shown by Sotera et al [107] would also speed up the system and avoid the need for recalibration.
Mix and Match Calibration (I)

(a null method)

The manifold

If the manifold is modified to that in fig. 4.16 the

![Diagram of two pump manifold with sample dilution facility]

Fig. 4.16

Two pump manifold with sample dilution facility.

D is the position of the flow rate device (see text).

All other symbols see Fig. 4.9.

valve SS2 can be switched to allow blank to pass through the variable speed pump. In this way the sample may be diluted. As no automatic valve was available, a Pharmacia valve was used to switch between blank and standard and operated manually.
The Algorithm

Both pumps are calibrated with respect to flowrate as described. The top standard, of known concentration calculated from equation 4.3, is then mixed by running pump P2 at its maximum rate and the absorbance measured. Next, the blank absorbance is measured. Valve SS1 is then switched and the absorbance of a sample is measured. If its absorbance is higher than that of the top standard, the sample is diluted by pumping diluent via pump P2 at its maximum pumping rate. If the sample is still too concentrated, no further dilution is possible and the program informs the operator that the sample is too concentrated.

Once the sample absorbance is on range, an initial estimate of its concentration is calculated by assuming a linear concentration absorbance relationship between the top standard and the blank. A standard of the concentration estimated is then mixed by returning valve SS1 to water and pumping standard at the appropriate rate and its absorbance measured. For a convex calibration curve, the concentration estimated will be found to give too high an absorbance (fig. 4.17) and conversely for a concave calibration curve. Using the values of concentration and absorbance obtained for this new standard as replacements for the values for the top standard, a new estimate of sample concentration is calculated, mixed and measured as before. This process is repeated until the absorbance of the mixed standard and the sample are within 2% of each other. The iteration
Fig. 4.17

Mix and Match calibration procedure

SA is the sample absorbance
TS the top standard
B the blank

$E_1$ the first estimate of concentration of the sample from line a giving absorbance $A_1$

$E_2$ the second estimate of concentration of the sample from line b giving absorbance $A_2$

$A_n$ tends to SA
then stops and the concentration of the standard (equivalent to the concentration of the sample) is displayed. If the sample was automatically diluted, multiplication by the dilution factor occurs to give the concentration of the sample.

To help correct for drift and prevent the mixed standard concentration oscillating about the sample concentration, the number of iterations is limited to 5. If, after 5 iterations, no match is found, the system resamples.

During the iteration procedure a number of standards are mixed and their absorbances are measured. These concentration and absorbance values are collected in an array of 21 pairs of elements. Each pair of elements will accept a concentration value (and its associated absorbance) to cover 1/20 of the calibration range from 0 to the top standard which occupies the 21st element. After a sample has been analysed some values will have been entered into the array, so the initial concentration estimation of subsequent samples is calculated by starting from the entry in the array which has an absorbance 7% or higher than that of the sample. This ensures that the data in the arrays is continuously updated and that a match is not found immediately so the mix and match process continues. If, in subsequent analyses, concentrations are mixed which are within the range covered by an array value already mixed, the old values are replaced with these new values to allow for any drift.

Once a match has been found, the blank is aspirated
and the next sample analysed.

The program is given in appendix A2.2.2

Experimental

Apparatus

A Baird Atomic A3400 was optimised for chromium analysis and solutions over the range 2-200 mg l\(^{-1}\) were analysed using an 80 mg l\(^{-1}\) stock standard. Each sample was aspirated in turn, three times.

Results

The results are presented in Table 4.3.

<table>
<thead>
<tr>
<th>Nom Conc. mg l(^{-1})</th>
<th>Calc. Conc. mg l(^{-1})</th>
<th>Mean Conc. mg l(^{-1})</th>
<th>RSD %</th>
<th>95% CI mg l(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.00</td>
<td>2.11</td>
<td>1.93</td>
<td>1.99</td>
<td>2.01</td>
</tr>
<tr>
<td>5.00</td>
<td>5.03</td>
<td>5.12</td>
<td>5.08</td>
<td>5.08</td>
</tr>
<tr>
<td>10.00</td>
<td>10.46</td>
<td>10.39</td>
<td>10.38</td>
<td>10.41</td>
</tr>
<tr>
<td>15.00</td>
<td>15.00</td>
<td>15.16</td>
<td>15.09</td>
<td>15.08</td>
</tr>
<tr>
<td>20.00</td>
<td>20.21</td>
<td>20.53</td>
<td>20.38</td>
<td>20.37</td>
</tr>
<tr>
<td>30.00</td>
<td>31.73 by dilution</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>40.00</td>
<td>49.82 by dilution</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>100.00</td>
<td>105.8 by dilution</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>200.00</td>
<td>OFF RANGE</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4.3

Two pump chromium calibration analysis results

Three replicate analyses were performed for
concentrations 2–20 mg l\(^{-1}\). Only one analysis each of the 30–200 mg l\(^{-1}\) solutions was performed as the initial measurement step followed by dilution and measurement, consumed all the sample solutions. Dilution of the 200 mg l\(^{-1}\) solution did not bring it on range. For the results without dilution (2–20 mg l\(^{-1}\)) the results are reasonably accurate except perhaps the 10 mg l\(^{-1}\) solution which appears a little high. If an error in flowrate or stock standard concentration were involved, (on which the method depends) this would occur for the other undiluted concentrations. The resolution between pump flowrates is small enough to allow a 10 mg l\(^{-1}\) solution to be mixed. The error may be due to an error in the test sample concentration rather than a real error in the concentration calculated. When the sample is automatically diluted, any error due to flowrate inaccuracies is doubled as the concentration of the diluted sample and the matching mixed standard are dependent on flowrate. This causes the increase in error shown by the 30–100 mg l\(^{-1}\) samples. The precision is probably poorer for the 2 mg l\(^{-1}\) solution because of the pulsed mixing at low variable speed pump flowrates. This may not be fully corrected by the integration of the signal.

The washout time of the pumps, slows the mix and match process down: Washing the sample out of the system to diluting the sample is slow. If these processes were faster less drift could occur between measuring sample and standard. Because of the pulsed mixing and the
integration of the signal, which is longer for lower concentrations, the time taken to analyse low concentration solutions is high, again allowing time for the system to drift.

**Mix and Match Calibration (II)**

A flowrate device (Appendix A3.2) was constructed which converts the pressure difference between the ends of a length of tube through which liquid is flowing to a potential difference. This can be monitored on a chart recorder. This device was placed in the mix and match manifold (fig. 4.16) at D. This position allows both pumps to be calibrated. When pump $P_2$ is off the device responds to the flowrate of $P_1$, with pump $P_2$ running the device responds to the flowrate of $P_1$ minus the flowrate of $P_2$. The Mix and Match algorithm was modified to allow for pump calibration in this manner (appendix A2.2.3).

The system was tested using a 50 mg $l^{-1}$ stock standard and 5, 10 and 25 mg $l^{-1}$ test solutions of chromium.

**Results and discussion**

Because the flowrate device is still in the development stage and could be improved, only initial results are presented in Table 4.4. Even so, the errors are similar to those obtained using "timed delivery from a burette" calibration of pumps.
<table>
<thead>
<tr>
<th>Nominal Conc.</th>
<th>Calculated Conc. (mg/l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>5.3</td>
</tr>
<tr>
<td>10.0</td>
<td>10.45</td>
</tr>
<tr>
<td>25.0</td>
<td>25.3</td>
</tr>
</tbody>
</table>

Table 4.4
Two pump calibration results with flowrate device

**Conclusion**

The two pump manifold described, shows potential as a method of producing standards for calibration. Peristaltic pumps produce pulsed flow and the flowrate varies with time as the tubing wears. Use of flowrate devices could eliminate these problems by monitoring the flowrate produced by each pump and adjusting its speed accordingly. An alternative to this approach is to use more expensive, accurately calibrated, pulse free pumps such as high pressure liquid chromatography pumps.

The use of the two pump system could be speeded up and enhanced by using flow injection sample introduction and standard dilution as described in section 4.4.2.

Speed of recalibration of the 'Autocal' program could be increased by resloping rather than recalibrating.

Null methods of analysis are not possible with normal aspiration techniques. These experiments show that under the right conditions the null method is appropriate. The limitations of the technique used here are the dependence of concentration on flowrate and the loss of accuracy between sampling and finding a match being time dependent: Any drift during this time will decrease the accuracy.
4.4 Flow-injection calibration methods

4.4.1 Principles of flow-injection methods

The normal spectroscopic method of flow injection analysis involves the injection of sample into a reagent carrier and the monitoring of the reaction downstream of the injection point. Either the consumption of reagent or the appearance of reaction product can be used to quantify the sample concentration. When atomic spectroscopic detection is used the absorbance of an element in the sample is measured, so neither the loss of reagent nor the production of a reaction product can be measured. Only when the element being measured becomes a reagent and the change in absorbance on injection of an interferent is monitored does flow injection analysis using atomic spectroscopy approach, in concept, flow injection analysis using other spectroscopic detectors.

Flow injection sample introduction however can provide a useful method of reducing the volume of sample used in an analysis and the dilution of the sample/standard as it passes through the manifold can be used to produce standards of different concentration \([17,132,133]\). Because of the nature of the dilution, the leading part and tail of the sample slug form concentration gradients which can be modelled as if the sample slug had passed through a single well-stirred mixing chamber \([10,11,135,136]\). This gives rise to equations for the concentration of the injected species from the rise curve (equation 4.1), the peak (equation 4.8) and from the fall curve (equation 4.2)
$C_p = C_m (1 - e^{-V_i/V}) \quad 4.8$

$C_p$ is the resultant peak concentration after an injected volume $V_i$ of a concentration $C_m$ has just completely entered a mixing chamber, volume $V$.

### 4.4.2 Flow injection sample introduction into two-pump systems

When samples are pumped into the two pump system (section 4.3.3) they pass through the whole manifold including the pump, before they reach the nebuliser. Injection of a sample into the flowing carrier enables the sample to reach the detector sooner, without passing through the pump. For mix and match analysis steady state concentration must be achieved. Therefore the equation for the peak concentration, equation 4.8 must reduce to $C_p = C_m$ i.e. $e^{-V_i/V} = 0$

For $e^{-V_i/V}$ to be small, $V_i/V$ must be large and $V_i$ should be very much greater than $V$.

For $C_p = 99\% C_m$

$$1 - e^{-V_i/V} = 0.99$$

$$-V_i/V = \ln 0.01$$

$$V_i = 4.6V \quad 4.9$$

For atomic absorption nebulisers investigated in our laboratory their theoretical mixing volume is approximately 40-50 µl [158] so injected volumes calculated from equation 4.9, must be greater than 230 µl.
if the absorbance is to approximate to the steady state absorbance.

Procedure

An injection valve was connected to the two pump manifold fig. 4.18.

Sample loops of dimensions (a) 1.58 mm internal diameter, 388 μl volume, (b) 0.7 mm internal diameter volumes (i) 350 μl and (ii) 200 μl were used.

Standards of 15 mg l⁻¹ magnesium were injected and the peaks recorded on a chart recorder. The standard was then aspirated via pump P1 and the steady state absorbance
Results and discussion

The results are presented in Table 4.5 and show that in every case, steady state absorbance was not achieved. As predicted from equation 4.8, increasing the volume injected does increase peak height.

<table>
<thead>
<tr>
<th>TUBE</th>
<th>Sample vol.</th>
<th>Peak height</th>
<th>Steady State</th>
<th>Peak % of x</th>
<th>Dispersion</th>
</tr>
</thead>
<tbody>
<tr>
<td>µl</td>
<td>Abs</td>
<td>Abs</td>
<td></td>
<td></td>
<td>(x)</td>
</tr>
<tr>
<td>a</td>
<td>388</td>
<td>0.928</td>
<td>0.952</td>
<td>97.5</td>
<td>1.03</td>
</tr>
<tr>
<td>b</td>
<td>350</td>
<td>0.866</td>
<td>0.916</td>
<td>94.5</td>
<td>1.06</td>
</tr>
<tr>
<td>(i)</td>
<td>200</td>
<td>0.781</td>
<td>0.924</td>
<td>84.5</td>
<td>1.18</td>
</tr>
</tbody>
</table>

Table 4.5
Results of injections of large sample volumes

Injecting a larger volume will enable the steady state absorbance to be achieved, but an increase in the sample tube length would increase the back pressure in the manifold when the valve was in the sample position changing the flowrate to the nebuliser. This could be reduced by using large bore tubing but there would then be a danger of localised mixing where the fluid passes from narrow bore manifold tubing into wide bore sample tubing. This mixing would further increase the sample volume required.
The estimation of the theoretical nebuliser mixer volume of 40-50 µl may be low, as a sample volume of 388 µl still does not give 99% steady state absorbance where a volume of 230 µl is predicted by equation 4.9. If the volume of the nebuliser was reduced, samples could be introduced in low volumes and the steady state would be attained.

4.4.3 Dilution of flow-injection peaks

The idea of flow injection sample introduction for reducing the amount of sample required during an analysis and speeding up sampling time (section 4.4.2) can be further extended to the standards in the 2 pump manifold. Both standard and sample can then be diluted if the valve is placed either at A, B or C (fig. 4.19). Because the sample or standards will be diluted by the same method, only reproducible dilution is required and not steady state absorbance.

![Diagram of two pump manifold with position A, B, and C for the injection valve](image)

**Fig. 4.19**

The two pump manifold with position A, B and C for the injection valve, enabling dilution of the injected material.
The positioning of the valve does not affect the concentrations produced as dilution does not occur at the split but at the confluence point. If the valve is positioned at B the peak widths could become excessively large: When P2 is running at high speed, the flow through the valve at B is slow and the sample will remain in the tubing for a long time. Similarly for position C where P2 is slow. When the valve is positioned at A, the flow through the valve is constant and if pump P2 is running fast, most of the sample passes to waste at the split leaving a short plug to merge slowly at the confluence point giving constant peak widths. Both sample and standard can be introduced and diluted using the same valve. Equation 4.10 derived in appendix A1.3 is the formula for the concentration of resultant solutions injected into the manifold fig. 4.19. Dispersion has not been taken into account.

\[ C = \frac{F_1 - F_2}{F_1} \times C_m \]  \hspace{1cm} 4.10

C is the concentration produced by injection of a solution concentration \( C_m \). \( F_1 \) and \( F_2 \) are the flowrates produced by pumps \( P_1 \) and \( P_2 \) respectively.

**Procedure**

The manifold fig. 4.19 with the valve positioned at A was connected to a Baird Atomic A3400 spectrometer. The pumps and valve were operated under manual control and the
flow injection peaks monitored using a chart recorder. The fixed speed Ismatec pump (P1) was operated at 6.49 ml min\(^{-1}\). The LKB, variable speed pump (P2) was operated with the large tubing supplied to give all dilutions required.

Magnesium solutions of 0.5, 1.0 and 2 mg L\(^{-1}\) were injected and the flowrate of the variable speed pump adjusted to try and produce the peak concentration of the lower concentration solutions, according to equation 4.10.

Results

Although dilution of the injected peaks was possible, both the precision and accuracy were very poor. The poor precision was noted from the visible differences in peak heights for the same pump flowrates and concentrations injected. This must be due to the pulsed mixing of solutions as described in section 4.3.2. At the confluence point the flow going to the nebuliser is taken from alternate lines depending on the pulsing due to the variable speed pumps rollers. When continuous flow standards are being mixed fig. 4.10 section 4.3.2 the absorbance from the resultant solutions can be smoothed but when flow injection sample introduction is used the plug may be diluted after one injection but on the following injection, the peak may pass undiluted. This imprecision contributes to the inaccuracy observed. A three point calibration curve was constructed from the injected undiluted standards and the mixed concentrations were interpolated from this (Table 4.6).

-159-
### Table 4.6

Results of flow injection two pump analyses

The diluted solutions are all far too dilute and this error is not proportional to the speed of the variable speed pump. Therefore the error does not arise from miscalculation during flowrate calibration. There must be some extra dilution occurring within the manifold. It has been shown that dispersion (equivalent to dilution) varies with flowrate [159] in a non-linear fashion. Between the split and confluence, the flowrate depends on the speed of pump P2. The dilution due to the dispersion may therefore vary. The operation of this manifold depends on the dilution due to dispersion remaining constant and the dilution at the confluence varying. There may also be some localised mixing generated at the split and confluence points which may change with the flow at these points.

<table>
<thead>
<tr>
<th>Concentration Injected (mg/L)</th>
<th>Concentration Aimed for (mg/L)</th>
<th>Pump Speed P2 (ml/min)</th>
<th>Absolute Error Conc. found (mg/L)</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>2.0</td>
<td>0.00</td>
<td>0.693</td>
<td>2.0</td>
</tr>
<tr>
<td>2.0</td>
<td>1.0</td>
<td>3.24</td>
<td>0.387</td>
<td>0.86</td>
</tr>
<tr>
<td>2.0</td>
<td>0.5</td>
<td>4.87</td>
<td>0.079</td>
<td>0.18</td>
</tr>
<tr>
<td>1.0</td>
<td>1.0</td>
<td>0.00</td>
<td>0.44</td>
<td>1.0</td>
</tr>
<tr>
<td>1.0</td>
<td>0.5</td>
<td>3.24</td>
<td>0.176</td>
<td>0.4</td>
</tr>
<tr>
<td>0.5</td>
<td>0.5</td>
<td>0.00</td>
<td>0.255</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Conclusion

Until a system which produces pulse free mixing has been built, the accuracy cannot be improved, or the reasons for inaccuracy investigated. The requirements are as suggested in the description of the steady state standard production method (section 4.3.2) i.e. pulse free pumps.

4.4.4 Network flow-injection systems

The dilution due to confluence and the dispersion, can be used without incorporating a second pump, to vary the dilution. If the flow is split after the injection point and the plug flows down lines of different lengths, it will split into plugs of different sizes, suffer different dispersions and different flowrates. If the different streams are then merged at a confluence point each dispersed plug will arrive at this point at different times and diluted by the other streams (fig. 4.20).

Fig. 4.20

Multiple splits and confluence manifold (network) for production of many peaks from one injection
A similar result was produced with two lines by Ruzicka et al. [132] and Fernández et al. [133]. If the tube dimensions have been chosen correctly, suitable dilutions of the standard can be obtained for calibration. When $n$ splits are used, $n$ peaks will be produced and between each will be a trough. Both peaks and troughs can be used for calibration (fig. 4.21).

![Diagram showing peaks and troughs](image)

**Fig. 4.21**

Peaks and troughs produced in a network manifold
The highest peak will appear first as this corresponds to the larger part of the injected slug which has passed down the line of least resistance (the shortest line) and arrives at the detector first.

The heights of the peaks can be adjusted by varying the tube lengths and therefore their resistances.

Various experiments using different coil lengths and numbers of splits were tried and included the following.

**Two line network**

**Apparatus and Procedure**

The manifold (fig. 4.22) was constructed of 0.65 mm id tubing to a Baird Atomic A3400. The peaks resulting from injections of 0.1 to 100 mg l\(^{-1}\) magnesium were recorded on a chart recorder. Each solution was injected four times and the mean absorbance of the peaks and trough measured. Y shaped stream dividers were made in our own laboratories.

**Results and discussion**

Examples of the traces produced are presented in fig. 4.23 and these show where the system can present advantages over a normal single line FIA/AA manifold. In trace (a) the second peak is small and the peak is almost equivalent to a trace produced using a single line manifold. As the concentration of the injected solution is increased, the first peak goes off the scale of the instrument trace (b), but the second peak can now be measured. When the concentration of the injected solution is increased so
Fig. 4.22

Two line network manifold

(length in millimetres)

that the second peak is off scale, trace (c) the trough between the peaks can be measured. Table 4.7 shows peak and trough heights obtained for calibration.

<table>
<thead>
<tr>
<th>Conc.</th>
<th>Peak1</th>
<th>Trough</th>
<th>Peak2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.3</td>
<td>0.088</td>
<td>-</td>
<td>0.008</td>
</tr>
<tr>
<td>0.5</td>
<td>0.152</td>
<td>0.004</td>
<td>0.024</td>
</tr>
<tr>
<td>0.7</td>
<td>0.220</td>
<td>0.006</td>
<td>0.038</td>
</tr>
<tr>
<td>1.0</td>
<td>0.306</td>
<td>0.008</td>
<td>0.052</td>
</tr>
<tr>
<td>10.0</td>
<td>-</td>
<td>0.136</td>
<td>0.484</td>
</tr>
<tr>
<td>20.0</td>
<td>-</td>
<td>0.254</td>
<td>0.900</td>
</tr>
<tr>
<td>30.0</td>
<td>-</td>
<td>0.400</td>
<td>1.264</td>
</tr>
<tr>
<td>40.0</td>
<td>-</td>
<td>0.504</td>
<td>-</td>
</tr>
<tr>
<td>50.0</td>
<td>-</td>
<td>0.644</td>
<td>-</td>
</tr>
<tr>
<td>100.0</td>
<td>-</td>
<td>1.208</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4.7

Two line network results
Fig. 4.23

Peaks produced in the 2 line network

Chart speed 3 cm min$^{-1}$
The absorbance from an injection of 100 mg l\(^{-1}\) solution can be measured using the trough. The trough represents a dilution of about 1/30 compared to the first peak. For this system to be useful, a full range of standards must be injected to construct a calibration for each measuring point on the trace.

**Three line Network**

The two line network produced two peaks and three measurement points (2 peaks, one trough) from one injection. When three lines are used five measurement points can be used. If the dilution/dispersion for every line remains constant and if the length of the lines are adjusted appropriately, an injection of a standard could be used to produce five standard concentrations for calibration. If these standards were correctly spaced, the injection of one standard could be used to produce standards for the whole calibration range.

**Day to Day Precision**

**Apparatus**

When there are three lines in a network, the effects of changing a tube length on the flow characteristics of the manifold are complex. Many experiments were performed to find the right combination of tube internal diameter and length. These resulted in the manifold in fig. 4.24. The split/confluence points were Altech (05-40-5108) 0.84 mm id mounted vertically so that the flow of carrier dislodged any air bubbles that could become trapped in the
A 2 mg l\(^{-1}\) magnesium standard was used. This gives an indication of the actual spacing of the measuring points produced, as the calibration for magnesium is virtually linear over the normal working range. This is of course below 2 mg l\(^{-1}\) but the standard is diluted by the manifold. The carrier flowrate was approximately 6 ml min\(^{-1}\). Peaks were recorded on a chart recorder.

**Procedure**

The standard was injected twelve times over two days and the peak and trough heights for each injection measured relative to the first peak. This enabled changes in instrument sensitivity to be discounted.
Results and discussion

A sketch of the peaks obtained is given in fig. 4.25. The results are presented in Table 4.8.

Fig. 4.25

Peak produced upon injection of a 2.5 mg l\(^{-1}\) magnesium solution into the three line manifold

Chart speed 3 cm min\(^{-1}\)
Table 4.8
Variation in results obtained using the three line manifold

(a) is mean relative peak height
(b) is % RSD (a)
(c) is mean relative peak height
(d) is % RSD (c)

The relative peak heights do change from day to day. From day 1 to day 2, peak 1 has decreased in size and the other peaks increased. The separation between the peaks has also changed as shown by the changes in the levels of the troughs: The first two peaks have become closer, the last two further apart raising the level of the first trough and lowering the level of the second. These effects can be accounted for if the flow in each tube is considered. A reduction in the flow rate in the short tube (from which the analyte plug that gives rise to the first peak emerges), will increase the flow in the other two tubes. This increase in flow, increases the amount of
analyte flowing down the longer tubes and increases the associated peak heights. The decrease in separation between the first two peaks is again due to the reduction of flow in the short tube. The sample plug in that tube will arrive at the confluence point later than expected, the other two plugs arriving earlier, causing the first two peaks to overlap more. The converse is true for the last two peaks. Peak 3 shows less of an increase than Peak 2, indicating that the flow in the longest tube has been reduced compared with that in the medium length tube. The plug in the longest tube arrives at the confluence later and the separation increases.

The within day precision is better. Although the value for the % RSD is close to 10%, these figures include noise not only pertaining to the peak listed but to the first peak, being a relative 'peak height'.

It was noticed during the experiment, that if an air bubble lodged in a stream divider of one of the network lines, the flow characteristics of the whole system changed. Once a bubble was lodged in a line, the flow down that line was reduced because of the restriction. This itself made removal of bubbles difficult. Only repeated sharp taps dislodged a bubble. The change in flow characteristics between days as seen in this experiment, may be due to either small air bubbles in the network or particulate material, blocking the lines. Placing the whole network in an ultrasonic bath removed the air bubbles but caused the peaks to become ragged. This is interesting in itself as it shows that the flow in
a manifold can be affected by vibration.

**Conclusion**

Because of the likelihood of the flow characteristics of the manifold changing, the system is not suitable for production of fixed concentration standards. The dispersion/dilution in each line could change, giving an inaccurate calibration. The system may be suitable for production of multiple calibrations by the injection of several standards, as the within day variation of dispersion is acceptable.

**Calibration graphs**

**Apparatus**

The same apparatus was used as in the previous experiment except magnesium standards from 0.2 to 20 mg 1⁻¹ were injected.

**Procedure**

Each standard was injected six times and the results were recorded using a chart recorder.

**Results and discussion**

The results are presented as calibration curves fig. 4.26 constructed using the mean absorbance. The spacing of the troughs and peaks has produced curves which are rather close, so the range is not very large. If the tube lengths were adjusted to give a large difference between peaks and troughs, the calibration curves would cover a
Fig. 4.26 Three line network magnesium calibration

- First peak
- Second peak
- Third peak
- First trough
- Second trough
larger range. Covering a large range produces the problem of the need for a large number of standards. Methods which can dilute the sample to bring it onto a normal calibration curve show more promise.

4.4.5 Timed injection

Another method of achieving dilution in a flow injection manifold is to reduce the volume of sample injected. This can be done in two ways. (1) Change the sample loops or (2) only partially inject the contents of a large sample loop. Option (1) is difficult to achieve within an analysis using a conventional 6 port valve, and is difficult to automate. Option (2) is simpler. The valve is left in the inject position for a set period of time, then returned.

This system is easy to automate and also provides another advantage. When a sample plug is introduced using normal injection the tail of the slug has to travel, not only through the manifold, but also through the length of sample loop, whereas the front of the slug only passes through the manifold. If the single well stirred tank model is being used, section 4.4.1, the extra dispersion of the tail will not be accounted for, unless a new model is developed to allow for dispersion in the length of the sample tubing e.g. the segmented tube model Appendix A3.3. As the tail of the injected peak in timed injection does not flow through an extra length of tubing, the single well stirred tank model can be applied.
Calculation of Volume Injected

The volume injected is proportional to the time the valve is in the inject position i.e. \( V_i = ut \)
where \( u \) is the flowrate to the nebuliser and \( t \) the time the valve is in the inject position.

Calculation of concentration at the Peak

The single well stirred tank model yields the concentration at the top of the peak \( \langle C_p \rangle \) from equation 4.8.

With timed injection, where \( V_i = ut \) the equation becomes the equation of the rise curve (section 4.1) equation 4.11,

\[
C_p = C_m \left( 1 - e^{-ut/V_i} \right)
\]

but with \( C = C_p \).

When a specific concentration is to be mixed, a point on the rise curve could be used. As a peak is easier to find and measure, timed injection is more convenient.

Flowrate Calculation

When a simple single line manifold is connected to a flame atomic absorption instrument, most of the mixing occurs in the nebuliser chamber. Although material in the droplets formed is no longer diluted directly by dispersion, these droplets are mixed with more dilute droplets, which dilute the material entering the flame. When a sample is injected into the manifold, there is a
delay before any analyte appears in the flame. If all the mixing is considered to occur in the nebuliser chamber and the sample suffers plug flow in the manifold, then the delay time before the peak appears is dependent on the flowrate, and calculated from equation 4.12.

\[ t_D = \frac{V_T}{u} \]  

where \( t_D \) = Delay time before the peak appears  
\( u \) = flowrate  
\( V_T \) = Volume of tubing between valve and nebuliser

If flowrate is to be calculated, equation 4.12 can be rearranged.

**Calculation of the mixing volume of the nebuliser chamber**

The mixing volume of the nebuliser chamber is not its physical volume. The way in which it acts is a complex function of gas flows, liquid flow, etc. [160]. It can be measured using equation 4.11 which rearranges to give equation 4.13.

\[ V = \frac{-V_i}{\ln 1 - (C_p/C_m)} \]  

By injecting a known volume of solution \( V_i \) of known concentration \( C_m \) and measuring the peak concentration produced \( C_p \), \( V \) can be calculated.

If the relationship between concentration and
absorbance is linear, the absorbances of the solution can be used i.e. the steady state absorbance is proportional to the concentration injected and the peak height absorbance is proportional to the concentration at the peak. For the majority of elements analysed using atomic absorption, the relationship between absorbance and concentration is not linear. For this reason the timed injection system includes a characterisation step described below.

**Characterisation of the system**

A large volume of a solution which is known to give an absorbance on the linear range for the particular element is injected so the steady state absorbance is achieved. From the time taken for the material to reach the nebuliser, the flowrate is calculated. The sample loop is then refilled and a fraction injected for a known time. Using the values of flowrate, injected volume and the maximum absorbances of the two injections, the volume of the mixing chamber (or mixing volume of the nebuliser) is calculated. Subsequent injections of sample are performed for just enough time for the absorbance to reach steady state. This means the system has the same sensitivity as conventional sample aspiration. Once the system has been characterised, specific peak concentrations of solution can be produced according to equation 4.11.
Matching standard, timed injection

A program (Appendix A2.3) was written based on the principles described above for use with the manifold fig. 4.27.

![Timed injection manifold diagram](image)

**Fig. 4.27**

Timed injection manifold

To test the simple well stirred tank model and the method of timed injection, the method of matching standards was used, similar to the 'mix and match' technique (section 4.3.3). Flowrate was calculated using the time interval for the absorbance to rise to 1.5 times the largest baseline deviation after injection. Peak maxima were calculated from the maximum absorbance value.

Steady states were calculated by considering the slope between each data point and the tenth preceding point. Once this slope was less than 0.002, then the data was considered to be part of the steady state. When the slope decreased to below -0.004, then the steady state
data was considered to have ended.

The valve timing was controlled by the data reading loop which allowed 25 readings per second and thence a valve timing resolution of 0.04 seconds.

**Apparatus**

The manifold fig. 4.26 was set up and the pump set to give a flowrate of between 4 and 5 ml min\(^{-1}\). The system was connected to a Pye Unicam SP9 spectrometer set up for magnesium analysis.

A 0.4 mg l\(^{-1}\) solution was used to characterise the system. 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg l\(^{-1}\) samples were injected and a match was attempted by timed injection of a 1.0 mg l\(^{-1}\) standard.

**Procedure**

The system was characterised and each sample injected in turn. After the injection the computer estimated the peak sample concentration according to the mix and match method, fig. 4.17, and hence calculated the injection time needed to dilute the 1 mg l\(^{-1}\) standard to produce the same absorbance using equation 4.10.

The standard was then injected for this time and the peak absorbance checked against the peak sample absorbance. The time needed to produce a match was then recalculated and the standard reinjected. This process was repeated until a match was obtained.
Results

No match was obtained for any of the samples. This was due to the lack of time resolution provided by the A/D. It has to be accessed at a relatively slow rate because of the time taken to perform a conversion. The effect is demonstrated in fig. 4.28.

![Graph showing concentration over time with available concentrations governed by conversion times.](image)

**Fig. 4.28**

The resolution of available concentrations governed by the conversion times

The spacing of the available standards is uneven and if a
concentration between an available concentration is
required it cannot be mixed: A concentration above or
below is mixed hence the system never finds a match.
Another problem with this system occurred with the use of
a rotary valve. The time of injection was not known
accurately as there is a time lag between sending a valve
movement signal and the valve reaching the inject
position. This causes a delay in the switching in of
sample solution. There is also an error when the valve is
returned as the flow of sample is not immediately cut off.
This was overcome by increasing the time the valve was in
the inject position by the inject error minus the return
error. This was found by trial and error.

The mean flowrate found was 4.24 ml min⁻¹.

The effect of injection is shown in fig. 4.29.
Although set concentrations were not obtained, dilution
was possible.

Conclusion

If the valve timing were made independent of the data
reading cycle by control from an external clock, then fine
resolution could be achieved.

If a valve with a very fast response was used e.g. a
pneumatically switched slider valve, the valve switching
errors could also be reduced.

Until these problems are solved, the well stirred
mixing chamber model cannot be tested by this method.

A better method of timed injection calibration would
be the simpler approach of injection of a standard for set
Fig. 4.29

Peaks obtained with timed injection
a, plug flow
b, timed injection
times and curve fitting to the data assuming the well stirred mixing chamber model, but until it has been proved that the mixing chamber model applies, a real mixing chamber would have to be included. This would mean an even larger volume would have to be injected to achieve steady state for characterisation.

4.4.6 Peak Width Calibration

Introduction

Another method which allows off range concentrations to be found is the peak width method. The feasibility of using such a peak width calibration method to calculate off range concentrations by flow injection of samples close to the nebuliser of an atomic absorption spectrometer has been demonstrated by Tyson [15], who derived an equation relating peak width to the concentration injected, according to the "single well-stirred mixing chamber" model [136]. Stewart and Rosenfeld [14] incorporated a real mixing chamber into a flow injection system and used an equation by Pardue and Fields [139] for flow injection titrations, to provide an extended calibration. The derivation is complex and in its application to calibration, some unnecessary simplifications were made.

This produced an equation where peak width is proportional to the logarithm of the concentration injected, whereas if no approximations are made in deriving the equation [15], this is not the case. Both groups of workers measured peak widths at one
concentration level on the peak profile and produced linear calibration graphs, thus demonstrating the validity of the relationships. Tyson's equation, equation 4.14 can be used at any concentration level, C'.

\[ t' = \frac{V}{u} \ln\left(\frac{C_m}{C'}\right) - 11 - \frac{V}{u} \ln(D - 1) \]  

where \( t' \) is the peak width, \( u \) the flowrate, \( V \) the theoretical volume of the mixing chamber, \( C_m \) the concentration injected, \( C' \) the concentration level on the rise and fall curve at which the width is measured and \( D \) is the dispersion of the system.

Program

At the time when this program was developed, the pump/valve interface Appendix A3.1 was not built. The program (Appendix A2.4.1) was therefore written which flashed an 'inject now' instruction on the screen. This could now be changed to automatically inject the sample.

Data acquisition

Thirty readings were made for all steady states (i.e. baseline and normally aspirated standards), the baseline being read before normal calibration and before injection of standards or samples. Two hundred time and absorbance data points were collected per injection at a rate of 14 conversions per second.

Data processing

The rational function, equation 4.15,
was fitted to the normal calibration data points. This function was found to give consistently good results in the comparison of curve fitting functions, section 3.3.2. Each data point consisted of the mean of the thirty measured absorbance values collected and the corresponding concentration values input from the keyboard. Curve fitting was as described in section 3.3.2.

The 200 data points collected per injection were sorted into those containing peak information and those at the baseline level. Each absorbance on the rise portion of the peak was then matched to the closest absorbance value on the fall curve. The associated time values were then subtracted to give peak width values. The absorbance values were then converted to concentration using the rational calibration function. This process is summarised in fig. 4.30. These values for peak width and concentration were then used, together with the concentration originally injected (entered via the keyboard), to obtain the peak width calibration function.

Initially the equation developed by Tyson (equation 4.14) was fitted by using the least squares procedure (the same routine used to fit the rational function). It was hoped that injection of a single standard would produce a linear calibration covering a large concentration range. A plot of the calibration data (fig. 4.31) obtained during development of the program showed that the relationship was not linear as expected. A cubic function (equation 4.16) suggested by the shape of fig. 4.31 was used
Fig. 4.30

Peak width data conversion
Fig. 4.31

Peak width calibration curve

\[ \ln \left( \frac{C_m}{C'} \right) - 1 \]
instead, fitted using the least squares procedure to data from injection of several standards.

\[ t' = d + eF + fF^2 + gF^3 \]  

\[ F = \ln \left( \frac{C_m}{C'} \right) - 1 \] and variables \( d \) to \( g \) are those found during the fitting procedure.

On injection of an unknown, peak widths are converted to \( F \) values by solving for the root of equation 4.16 using Newton's approximation method \([151]\). The concentration injected is then calculated from the mean of the concentrations calculated from the \( F \) values, and the concentration levels \( (C') \) at which the corresponding widths were measured.

**Apparatus**

Three elements were used to test the system: chromium, nickel and magnesium. These were determined using the Baird Atomic A3400 spectrometer with an air/acetylene flame, optimised for maximum sensitivity for each element. The manifold (fig. 4.32) consisted of a pneumatically driven injection valve, (P.S. Analytical) I, operated by hand, a peristaltic pump (Ismatec 840) P, and a stream switching valve (Pharmacia) SS. The connection between the injection valve and the nebuliser consisted of 2 cm of 0.71 mm id teflon tubing (Anachem). The flowrate produced by the peristaltic pump was 4.7 ml min\(^{-1}\). The volume injected was 82.3 \( \mu l \).
Procedure

The system was calibrated by aspiration of the standards for normal calibration. Valve SS was switched to the standard/sampling position and the concentration of the standard was entered through the keyboard once steady state was achieved. This initiated the reading cycle. The process was repeated for all the standards. Once a normal calibration had been generated, valve SS was switched to the water carrier position and three 'peak width standards' were injected twice in turn, the concentrations being entered to initiate the reading cycle. The concentrations of the five standards used for the normal calibrations covered the ranges 5 to 40 mg l\(^{-1}\) for chromium, 0.1 to 1.0 mg l\(^{-1}\) for magnesium and 5 to 20 mg l\(^{-1}\) for nickel. Fitting the rational function to these
calibrations gave correlation coefficients of 0.9996, 0.9085 and 0.9984 respectively. The peak width standards were then reinjected at least ten times as unknowns in random order, to enable the calibrations to be evaluated.

The values obtained for each unknown were compared using the Q test [86] and outliers rejected before the standard deviation, mean and 95% confidence intervals were calculated.

**Results and discussion**

Peak width calibrations are non linear (fig. 4.31) when the function (equation 4.14), derived from the well stirred mixing chamber model, is used. The nebuliser and the manifold of this particular instrument do not act like a perfect mixing chamber, which distorts the rise and fall curves accordingly. Timed injection, section 4.4.5, could correct the error from the manifold loop. If the segmented tube model (Appendix A3) could be configured to enable the calculation of peak widths, this may provide a linear peak width calibration. The deviation of the peak width calibration from a straight line could also be due to formation of large salt clotlets at high injected concentrations. These would be slower to vapourise giving smaller C' values than expected at the appropriate peak width.

The results of the analyses are presented in Table 4.9. These show high RSD values usually at high concentrations injected. Although the noise on the peak width values may be low, when these are converted to
<table>
<thead>
<tr>
<th>Metal</th>
<th>( r^{(a)} )</th>
<th>Concentrations of metal (mg l(^{-1}))</th>
<th>Injected standards</th>
<th>Calculated from peak-width calibration(^{(b)})</th>
<th>Mean calcld.</th>
<th>RSD (%)</th>
<th>95% CI(^{(c)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>0.9969</td>
<td>1112, 1117, 1047, 1155, 1098</td>
<td>1061, 1194, 1201, 1036, 943.1, 1110</td>
<td>105.9, 96.94, 95.95, 109.7, 106.1, 111.6, 105.1, 105.1, 112.8, 107.4, 110.5</td>
<td>38.96, 38.14, 41.44, 43.09, 37.17, 34.4, 38.10</td>
<td>7.14</td>
<td>56.1</td>
</tr>
<tr>
<td>Mg</td>
<td>0.9981</td>
<td>63.29, 47.06, 59.40, 55.09, 56.5</td>
<td>55.94, 60.22, 75.32, 48.49, 57.41, 61.68</td>
<td>10.89, 10.65, 12.00, 14.75, 11.16, 10.70, 11.00, 11.98, 10.49, 11.66, 11.03</td>
<td>1.0082, 1.146, 1.7426, 1.030, 1.0677, 1.055, 1.050, 1.082, 1.028, 1.081</td>
<td>11.2</td>
<td>4.31</td>
</tr>
<tr>
<td>Ni</td>
<td>0.9978</td>
<td>1156, 909.0, 1118, 890.4, 1021</td>
<td>1278, 938.4, 906.0, 1043, 1014, 955.9</td>
<td>142.8, 123.1, 103.4, 110.6, 119.2, 127.0, 115.6, 130.2, 129.0, 80.93, 121.9</td>
<td>19.18, 41.69, 24.67, 20.51, 20.99, 20.49, 19.36, 21.02, 18.87, 20.91, 23.94</td>
<td>12.6</td>
<td>91.6</td>
</tr>
</tbody>
</table>

Table 4.9

(a) Correlation coefficient, (b) Results which are underlined were rejected by the Q test and not used for subsequent calculation of mean values, RSD or confidence intervals, (c) Confidence interval.
concentrations injected through equation 4.14, the ln function will increase the noise on the concentration values.

The results also show some bias at the 95% confidence interval. This probably arises from errors in converting the absorbances at which widths are measured to concentrations, through the normal calibration function. Both errors in fitting the rational function to the normal calibration and drift during the analyses, will cause bias.

**Conclusion**

This method of peak width calibration has potential as a method of estimating the concentration of a sample. It could be used to save time by removing the need to dilute a sample by trial and error to bring it on the range of a normal calibration. The algorithm is rather complex requiring

(I) a normal calibration curve and the associated curve fitting

(II) a cubic fit to the peak width calibration and solving the same for roots.

**Triggered Peak Width Calibration**

In the light of discoveries made from the previous peak width calibration method some improvements were made to the system. If the equation relating peak width to concentration is simplified, Appendix A1.6 to equation 4.17 and the widths measured at a specific concentration
the 'normal' calibration function is no longer required.

\[ t' = (\frac{V}{u}) \ln \frac{C}{m} - (\frac{V}{u}) \ln [C'(D - 1)] \] 4.17

Although the relationship between concentration and absorbance is usually non-linear, measurement at one absorbance corresponds to measurement at one concentration. According to Appendix A1.6 equation 4.17 should hold as long as the width is measured at a concentration very much less than the concentration injected. Use of a real mixing chamber before the nebuliser will broaden the peaks and reduce the relative noise on the peak widths. This in turn should improve the precision of the calculated concentrations. When a sample of high concentration is injected without using a mixing chamber, the slopes of the rise and fall curves are steep. Use of a mixing chamber reduces the slope enabling a specific absorbance level to be found more accurately. Use of a mixing chamber could also ensure the system acted as if a well-stirred mixing chamber was used.

**Program**

When this program was written, (Appendix A2.4.2) the pump/valve interface had become available (Appendix A3.1), hence the valve could be switched automatically at the appropriate times.
Data acquisition

On the instruction to inject, the computer first measures the baseline level thirty times. These values are then averaged. The valve is then switched and sample or standard injected. Absorbance levels are then continuously read into the computer via the A/D. Once the absorbance reaches that specified by the operator, the clock is read. When the absorbance drops below the specified value, the clock is again read and the peak width calculated. This enables data to be read into the computer at approximately 45 conversions per second.

Data processing

The number of standards is specified by the operator and each is then injected four times and their concentrations entered. The widths are then measured at the absorbance specified by the operator fig. 4.33. A straight line is then fitted to equation 4.18 by linear regression. As the concentration level C' at which the peak width is measured, the volume of the mixing chamber, the flowrate and the dispersion are all constant, equation 4.17 simplifies to equation 4.18.

\[ t' = a \ln C_m + b \]

where a and b are constants to be found during the regression. A simple method of compensating for any curvature of the calibration is to use linear segment interpolation (see section 3.3.2). This was also
Measurement of peak \( t' \) at a trigger absorbance level \( A_t \) incorporated into the program by solving equation 4.18 for \( a \) and \( b \), for each pair of data points. When a sample is subsequently injected, the width is measured and the concentration injected calculated both from the single line obtained using linear regression on the calibration data points, and also from the line between the two calibration data points with widths bracketing the width produced by the sample. Eventually a visual representation of the calibration would enable the analyst to choose which value was correct by examining the degree of curvature of the calibration.
**Apparatus**

This system was investigated using magnesium as the test element. Flame atomic absorption spectrometry shows high sensitivity for this element and therefore the concentration range normally analysed is small. The range used here was 3-1000 mg l$^{-1}$.

The manifold (fig. 4.34) was connected to the Pye-

![Diagram](attachment:image.png)

**Fig. 4.34**

Triggered peak width calibration manifold

Unicam SP9 which has a smaller nebuliser than the Baird Atomic A3400. This reduced the significance of the mixing by the nebuliser compared with the mixing chamber. The flowrate delivered by the pump was approximately 6 ml min$^{-1}$. 

-195-
Procedure

The standards (Table 4.10) were injected in order of increasing concentration and the widths measured at absorbance 0.2. The standards were then reinjected six times and their concentrations calculated from the peak widths produced. Peak widths were then recalculated from the concentrations using the calibration function.

Results and discussion

The calibration produced is presented in fig. 4.35. Compared to peak width calibration using the non-simplified function and without a mixing chamber (fig. 4.31) the calibration is much more linear over a greater concentration range. The correlation coefficient was 0.9994. This improvement in linearity is also shown in the concentration results (Table 4.10). The concentrations obtained using a single line fitted by linear regression are more accurate than those obtained from linear interpolation. This is due to the least squares fitting smoothing the calibration data: Linear interpolation forces the calibration to pass through each calibration point. This being the case, there is some evidence of drift in the system, as reinjection of the standards and calculation by linear interpolation should return the same values of the standard concentrations, but do not. The peak widths show much less noise (low RSD) compared to the concentration noise. Only when the peak heights are close to the absorbance at which the width is measured does the noise on the width increase:
Triggered peak width calibration
### Table 4.10

**Triggered Peak Width Calibration Results**

<table>
<thead>
<tr>
<th>Concentration mg l⁻¹</th>
<th>Least Squares Calibration</th>
<th>Linear Interpolation Calibration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Injected Mean Concentration mg l⁻¹</td>
<td>Relative Standard Deviation %</td>
</tr>
<tr>
<td>3</td>
<td>3.003</td>
<td>7.860</td>
</tr>
<tr>
<td>7</td>
<td>6.248</td>
<td>1.205</td>
</tr>
<tr>
<td>20</td>
<td>18.88</td>
<td>2.051</td>
</tr>
<tr>
<td>50</td>
<td>47.52</td>
<td>2.640</td>
</tr>
<tr>
<td>150</td>
<td>141.1</td>
<td>1.606</td>
</tr>
<tr>
<td>400</td>
<td>400.8</td>
<td>3.010</td>
</tr>
<tr>
<td>1000</td>
<td>1031</td>
<td>3.820</td>
</tr>
</tbody>
</table>

**Confidence Interval**

- Least Squares Calibration: 0.271
- Linear Interpolation Calibration: 0.294

**Logarithm Values**

- Mean Concentration: 3.100
- Deviation %: 8.65x10⁻²
- Confidence Interval: 9.75x10⁻²
for example results for the 3 mg l$^{-1}$ solution. Bias is shown for the concentrations towards the centre of the range examined. Where the relative standard deviation (RSD) is greater than 3.0, at the extremes of the calibration range, no bias is detected. For the peak width method without a mixing chamber, the results tended to be biased on the high side. These results, on the other hand, are biased low. This bias must arise from small errors in interpolating values from the width calibration, these errors being amplified as they are transformed through the logarithmic function (equation 4.18) to their respective concentration values.

**Conclusion**

This peak-width calibration method is accurate and suffers less noise in the resultant concentration values, than the peak width method without using a mixing chamber and the unsimplified peak width function. This method is useful as an analytical technique covering a large concentration range or for finding the dilution required to bring a sample onto the range of a normal calibration. A larger concentration range can be tolerated by this method, using a mixing chamber, than by the method without a mixing chamber. The peak widths are increased and solutions are diluted by the mixing chamber. The simple algorithm speeds up the calibration process and increases the accuracy of the result by reducing the number of operations performed.
4.4.7 Valve and Pump Control program

A program (appendix A2.5) was written to enable normal flow injection sample introduction to be performed using the PS Analytical valve and LKB microperpex pump. The program enables the control of pump and valve, reads the data into the the computer from the spectrometer via the A/D converter and calculates the maximum peak height. Each peak is plotted on screen and can be transferred to paper using the printer. No data smoothing has been incorporated into the program.

4.5 Hybrid Methods

4.5.1 Introduction

These systems are those which utilise both continuous analyte flow and flow injection techniques to produce the desired result. When the sample is flowing continuously to the detector and reagent or standard is injected into it, the technique is known as 'reverse FIA' [16].

4.5.2 A Standard Additions System

The problems of interference can be overcome by the method of standard additions discussed in section 3.3.3. Normally, this involves the addition of a fixed amount of sample to fixed volumes of standard of varying concentrations. Tyson and Idris [136] developed a standard additions procedure where several standards were injected into the sample stream. The concentration of analyte in the sample could be interpolated from the recorded peaks.
In the following experiments, investigations into the possibility of a standards additions procedure where the sample is injected into standards of varying concentration are made. If dispersion characteristics are chosen correctly, a suitable range of carrier standard concentrations is used and the normal standard additions procedure works (see section 3.3.3), the effect of a normal standard additions dilution procedure will be produced. If the dispersion characteristics are not suitable, the injected sample slug will not mix with the carrier standard and the interferant will not act on the standard. The equations for sample concentration derived for this method in appendix A1.7 rely on the standards containing excess interferent i.e. enough interferent to reduce the absorbance of both the analyte from the standard and analyte dispersed into it from the sample.

Apparatus

The manifold (fig. 4.36) was used. During the pulsed mixing experiments (section 4.3.2) it was found that this system, although slow, produced continuous, steady concentrations of standard. Electronic damping is not suitable for flow-injection experiments and the sample must be injected into standards of a uniform concentration. Pump P₂ was an ismatec 840, pump P₁, was an LKB microperpex controlled manually. Samples of 0, 5, 10, 15, 20 mg l⁻¹ chromium in 250 mg l⁻¹ iron were used to test the procedure. The standard used was 73.2 mg l⁻¹ chromium: Both the standard and the diluent contained 883
Figure 4.36

Standard additions FIA manifold

mg l⁻¹ iron.

Pump P₂ was found to deliver 8.83 ml min⁻¹. The manifold was connected to a Baird Atomic A3400 spectrometer burning an air acetylene stoichiometric flame. Results were recorded on a chart recorder.

Procedure

Samples were injected into standards of increasing chromium concentration obtained by increasing the speed of pump P₁. Both the absorbance levels of the standards and the peak heights for the solutions injected into the standards were recorded.
Results

The recorded results are presented as standard addition curves (fig. 4.37) all lines were fitted by eye with a ruler. From equations (4.19-4.22) derived in appendix A1.7, the concentrations of the injected samples were calculated (table 4.11).

Standard Curve Slope \( (k_1) = 0.0186 \)

Sample Solution Curves.

<table>
<thead>
<tr>
<th>Concentration (mg/l)</th>
<th>Chromium (mg/l)</th>
<th>Dispersion Intercept with standard concentration curve</th>
<th>Concentration (mg/l)</th>
<th>Concentration (mg/l)</th>
<th>Concentration (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.0126</td>
<td>3.100</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>5.00</td>
<td>0.0120</td>
<td>2.818</td>
<td>7.10</td>
<td>7.10</td>
<td>7.12</td>
</tr>
<tr>
<td>10.00</td>
<td>0.0118</td>
<td>2.725</td>
<td>13.23</td>
<td>13.23</td>
<td>13.23</td>
</tr>
<tr>
<td>15.00</td>
<td>0.0115</td>
<td>2.620</td>
<td>18.30</td>
<td>18.30</td>
<td>18.30</td>
</tr>
<tr>
<td>20.00</td>
<td>0.0111</td>
<td>2.480</td>
<td>22.70</td>
<td>22.70</td>
<td>22.70</td>
</tr>
</tbody>
</table>

Table 4.11

Results of standard additions calibration

\[ D_{SAM} = \frac{k_1}{(k_1 - k_2)} \]

4.19

\( D_{SAM} \) is the dispersion of the sample, \( k_1 \) the slope of the...
Fig. 4.37

Standard additions calibrations

Mixed standards into which solutions below are injected

Absorbance

Concentration of added standard (mg l⁻¹)

-20 -15 -10 -5 0 5 10 15 20

-20 -15 -10 -5 0 5 10 15

20 mg l⁻¹
15 mg l⁻¹
10 mg l⁻¹
0 mg l⁻¹

0.2
0.3
0.4
0.5
normal standard curve and $k_2$ the slope of the FIA standard additions curve.

At the intercept of the FIA standard additions curve with the concentration axis

$$C_{\text{SAM}}^m = C_{\text{STD}}^m (D_{\text{SAM}}^m - 1)$$  \hspace{1cm} (4.20)

At the intercept of the FIA standard additions curve with the absorbance axis

$$C_{\text{SAM}}^m = A_P^m D_{\text{SAM}}^m / k_1$$  \hspace{1cm} (4.21)

At the intercept of the FIA standard additions curve with the normal standard curve

$$C_{\text{SAM}}^m = C_{\text{STD}}^m$$  \hspace{1cm} (4.22)

In these equations $C_{\text{SAM}}^m$ is the original concentration of the injected sample, $C_{\text{STD}}^m$ the concentration of standard measured at the intercept and $A_P^m$ the absorbance at the intercept.

The results for the concentrations calculated from the different intercepts show good agreement with each other but are all higher than expected. This could be due to several factors: The flowrate of the peristaltic pumps must be known accurately for mixing accurate standard concentrations, these may have drifted or the original...
pump calibration may have been inaccurate. This is the most likely cause of errors. If the interference has not been completely corrected for, the slopes of the standard additions curves will be greater than expected. This would increase the value of $D_{SAM}$ and increase the sample concentration calculated from the intercepts with the axis and cause the calculated concentration from the intercept with the normal calibration to be high. This error could be corrected by inclusion of more iron in the standard and diluent streams. According to Tyson and Idris (1972), the iron to chromium ratio should be kept above 30 for constant depression of the chromium absorbance. In these experiments the minimum ratio was 29.35, when the highest chromium concentration sample was injected into the highest chromium concentration sample. This is very close to the borderline ratio. There is also a decrease in the slopes of the standard additions curves with increasing analyte concentration in the sample. This may be due to the normal curvature observed with increasing concentration for atomic absorption calibration curves, or the incomplete suppression of chromium in the samples.

**Conclusions**

This method of standard additions shows some potential but generally interference compensation by the standard additions method is difficult to achieve unless there is some prior knowledge about interferent and analyte concentration of the sample. The mixed standard must contain all the known interferents in sufficient
quantities to give constant suppression of the analyte signal. This is difficult to achieve and if sufficient information is known, a matrix matching technique would be preferable.

In this method, several samples can be injected into the standards if their matrices are similar. In the case of reverse FIA standard additions, the carrier must be changed for each analysis, as it is the sample.

Several other experiments were carried out with various iron concentrations in both samples and mixed standards. Increasing the iron concentration did not show the expected degree of improvement in accuracy. The inaccuracy therefore, is probably due to other sources of interference e.g. acid concentration etc. Removing the iron from the standards altogether did decrease the accuracy. Further development of this technique is required for this procedure to be useful as an analytical tool.
5.1 Introduction

Pretreatment is used to produce samples and standards suitable for aspiration by the spectrometer. It can involve dissolution of the sample, removal of certain concomitants, addition of reagents and preconcentration or dilution. These procedures are conventionally carried out using glassware but could be carried out on-line.

Getting the sample into solution is often the most lengthy process. The sample is often oxidised and dissolved using concentrated strong acids and heat. This is to ensure that all the element of interest is in solution. A decreased quantity of analyte will occur if incomplete dissolution of the sample occurs. Even so, the relative proportions of the materials dissolved from the sample may be constant throughout dissolution. If the concentration of the element of interest were measured with reference to another element in the sample, analysis with incomplete dissolution may be valid. This could be performed online.

Before it is decided if further pretreatment is necessary, tests must be performed to discover if any of the matrix constituents interfere as described in section 3.3.3. This can be achieved off- or on-line by mixing the potential interferent with a pure sample of the element of
interest.

Once the sample is in solution the different constituents can be converted to appropriate forms to facilitate the removal of interferents. On-line addition of reagents for this can be achieved by merging the sample stream with the reagent. If the two streams are miscible, this will dilute the sample, reducing sensitivity.

Once the conversion has taken place, separation of the species is possible by separation of one of the immiscible streams containing the concomitant or analyte (solvent extraction), exchange of the concomitant ion with another ion (ion exchange) or precipitation. All of these processes could be achieved on-line.

Preconcentration can be performed online using solvent extraction and/or ion-exchange: The sample is collected by the reagent from a larger volume of sample than the volume of eluent used to restore the reagent.

5.2 Preliminary experiments

5.2.1 Dissolution of steel

Several different acid mixtures and dissolution procedures were described as being suitable for dissolution of steel samples [152-154]. The effectiveness of these procedures is described in section 3.3.3.

5.2.2 Studies of Ion exchange materials

Ion exchange materials come in two types: Anion and cation exchange media. Their exchange properties depend on the active group on the medium and the pH at which they
are used. They are divided into four groups:

1. Weakly basic Anion exchangers.
   These exchange anions over a limited pH range:
   Typically 1-7, 1-9.

2. Strongly basic Anion exchangers.
   These exchange anions over a wide pH range:
   Typically 1-12, 1-14.

3. Weakly acidic Cation exchangers.
   These exchange cations over a limited pH range:
   Typically 5-14 or 6-9.

4. Strongly acidic Cation exchangers.
   These exchange cations over a wide pH range:
   Typically 1-14.

Both solid and liquid exchangers are available.

**Speciation using liquid ion-exchange materials**

A brief investigation into the possibility of using liquid ion-exchangers in continuous flow analysis was carried out. These media have been used for separation [162].

These liquids are weakly basic, water insoluble amines of molecular weight 350 to 400 and are usually diluted for use with white spirit, xylene etc. They are immiscible with water but when in high concentrations in
the white spirit, can form emulsions with water [163].

**Apparatus**

The manifold, fig 5.1, was constructed of PTFE tubing (0.78 I.D.). The pumps were two Ismatec 840's pumping each channel at 0.3 ml min⁻¹. Two types of phase separator were used. One constructed by Tecator as a prototype for their commercial FIA system and a small chamber containing glass beads figs. 5.2 and 5.3 respectively. The eluent was approximately 0.2% potassium thiocyanate in dilute ammonia solution.

The ion-exchange resin was 50% Amberlite LA-2 (BDH) in white spirit. This amine is less soluble than LA-1 (the available alternative) in dilute mineral acids. The test solution was a mixture of approximately 50 mg l⁻¹ each of chromium III and dichromate solutions.

**Procedure**

The chromium III dichromate mixture was aspirated via pump P₁. This stream was merged with the ion exchange solution. The resulting segmented stream, flowing at 0.6 ml min⁻¹ was allowed to flow down 1 metre of tubing during which the dichromate was taken into the organic layer. The two phases were then separated and the organic layer was merged with eluent. After flowing down another metre of tubing, the aqueous and organic layers were again separated and the regenerated organic layer returned to the exchange liquid reservoir. The two aqueous solutions emerging from the phase separators were collected in two
Fig. 5.1

Liquid ion-exchange species separation manifold

P₁ and P₂ - pumps

PS₁ - phase separator (fig. 4.38)

PS₂ - phase separator (fig. 4.39)

R - reservoir of liquid ion-exchange medium

A + B - points where separated species emerge
Fig. 5.2
Tecator prototype phase separator

Fig. 5.3
Packed chamber phase separator
beakers and the colours examined visually.

Results

Although the experiment was not conducted under optimised or rigorous conditions, each aqueous solution produced did not show any colouration from the other. After about ten minutes, emulsion collected in the phase separators causing inefficient separation of the organic and aqueous solutions and contamination of the ion exchange solution. Both phase separators were efficient at this flowrate.

Conclusion

The experiment showed the possibility of using liquid ion-exchangers in continuous flow analysis systems. If the concentration of ion-exchanger was reduced in the white spirit, emulsions would be less likely to form. If only purified cations were required the second elution and separation step would not need to be carried out in the manifold. The exchanger solution could be drawn from a layer floating on top of a concentrated eluent solution. As the spent ion-exchanger was returned to the reservoir, it could be introduced into the bottom of the eluent. Droplets would then be regenerated as they rose to the bulk ion-exchange solution. If the eluent was in sufficient excess, the eluent would not need changing during an analysis.
Separation of species using solid ion-exchangers

Several experiments were performed to investigate the applicability of solid ion exchangers to continuous flow analysis. These exchangers are usually in a powder or reticular bead form and can be packed into columns. Several other workers [117, 143-147] have described their use for flow injection atomic absorption analysis.

Construction of columns

Glass tubing (3 mm I.D. and 5 mm O.D.) was glued with araldite to the unthreaded end of the connectors (Anachem M UNF Connectors). A circle of porous PVC was glued between the glass and the connector (fig. 5.4). The column was then packed with damp ion-exchange resin in the macroreticular bead form. If dry resin was used, the connectors were forced off when the resin was moistened. If the resin was very wet, bonding the araldite was difficult. The macroreticular form allowed the pumping of carrier under low backpressure.

Once the column was packed, a second connector and piece of porous PVC was fitted.

Removal of phosphate inference upon calcium

To test the effectiveness of the columns, the method of removal of phosphate interference upon calcium, as described by Kamson and Townshend [117] was tried with a column.
A 70 mm column containing Amberlite IRA400, a strongly basic anion exchange resin, was constructed as described above. This was incorporated across the valve in the manifold (fig. 5.5). This allowed the column to be switched in and out of line.

A 10 mg l$^{-1}$ solution of pure calcium was compared with a similar solution to that used by Townshend. This was 10 mg l$^{-1}$ calcium in 200 mg l$^{-1}$ orthophosphoric acid.

The manifold was connected to a Baird Atomic A3400 spectrometer and results were recorded using a chart recorder.
Fig. 5.5

Interference removal manifold which allows comparison of injections with and without a column

Procedure

Kamson and Townshend operated their system at a flowrate of 3 ml min$^{-1}$. To check the effect of flowrate on the operation of the column used in the studies described here, the effect of the column was tested at various flowrates.

Sample solutions were injected three timed for each set of conditions. The column was regenerated at the end of the experiment with hydrochloric acid.

Results

The results are presented in table 5.1. The ratio of peak heights shows the extent of interference removal, the optimum value being 1.
Table 5.1

Results of phosphate removal at different flowrates on the absorbance of calcium

Although peak heights are lower for flowrates less than 3.0 ml min\(^{-1}\), at, or above this flowrate, the column efficiency is reduced. At 7.83 ml min\(^{-1}\), apparently no phosphate is removed. At 3.00 ml min\(^{-1}\) most, but not all of the phosphate is removed. Below 3.00 ml min\(^{-1}\) all phosphate interference is removed. It is interesting to note that reducing the flowrate to the nebuliser decreases the depression, even without the column. This may be due to more heat being available in the flame as the amount of solvent which must be evaporated will be reduced. This heat is then available for breaking down the calcium/phosphate complex.
Conclusion

The flowrate used by Kamson and Townshend for their system is not suitable for the column and manifold used here, even if the column is larger. This may be due to differences in this manifold causing less dispersion of the sample and therefore shorter residence times in the column for the same flowrates, or the use of different instruments.

On-line removal of phosphate interference in calcium is possible using small columns at flowrates lower than those used for normal sample aspiration.

5.3 Online Pretreatment Methods

5.3.1 Solid Dissolution

The steel dissolution procedures described in section 3.3.3 can take a considerable time to perform. The analyst has to handle concentrated strong acids at elevated temperatures which puts the analyst at risk. Loss of analyte during the dissolution procedure can occur through evaporation and spillages. If the dissolution process can be incorporated into an on-line system, these difficulties can be avoided.

Three designs of dissolution vessels were tried. Packing a column with steel and inserting it into a flow injection manifold into which acid was injected did not produce reproducible peaks due to the formation of gas bubbles in the column. Filling the column with steel was also difficult.

The design and use of the other two chamber type
dissolution vessels is described.

**Chamber 1**

**Apparatus**

The chamber fig 5.6 was an adaptation of a 'Perspex'

![Diagram of Chamber 1](image)

**Fig. 5.6**

Solid dissolution chamber 1

mixing chamber available in the laboratory and used in the peak width experiments (section 4.4.6) and liquid ion exchange experiments (section 5.2.2). An extra inlet was drilled to allow a third tube to be connected. The whole chamber was mounted on the top of a vortex mixer. This was connected into the manifold (fig. 5.7) across an injection valve.
Provision of a second valve with normal sample loop, enabled injection of other solutions. The valve which incorporated the dissolution chamber and the peristaltic pump were controlled by an Apple IIe microcomputer via the interface described in Appendix A3.1.

Reduced pressure was applied from a water operated filter pump to the top of the chamber. Any gas evolved during dissolution was drawn through the teflon membrane which was supported by glass beads. Whatman glass filter
paper GF/D was supported on glass beads before the outlet to filter out the solid steel sample.

The acid mixture used for dissolution was concentrated hydrochloric acid (SG 1.1, Fisons Primar), carrier was water flowing at 6.5 ml min$^{-1}$.

The steel sample used was British Chemical Standard number 256/1. The major components apart from iron were: W 19.9%, Cr 5.3%, V 1.59% and Co 5.7%.

The spectrometer was optimised for chromium analysis with a stoichiometric air acetylene flame.

Results were recorded on a chart recorder.

**Program**

A program (appendix A2.6) was written for the microcomputer which performed the following operations.

With the carrier flow bypassing the dissolution chamber, acid was pumped into the dissolution chamber for a set time at a set rate. The flow of acid was then stopped for a set time and dissolution allowed to proceed. The dissolved contents of the chamber were then injected for a set time, after which the valve was returned to the fill position and the process repeated.

The time delays were achieved by using a delay loop between each instruction. The approximate delay times were as follows.

<table>
<thead>
<tr>
<th>Operation</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fill</td>
<td>20 s</td>
</tr>
<tr>
<td>Dissolution</td>
<td>35 s</td>
</tr>
<tr>
<td>Inject</td>
<td>20 s</td>
</tr>
</tbody>
</table>
**Procedure**

The chamber was filled with approximately 1.5 g of granular steel and the chamber was then connected to the manifold. Reduced pressure was applied to the teflon 'plumbers tape' membrane. Any gas evolved during dissolution is able to diffuse through this. The vortex mixer was then turned on.

The carrier pump was then turned on and the program started. Sixteen dissolutions and injections were carried out, without changing the sample. The acid was pumped into the chamber at approximately 3 ml min\(^{-1}\).

**Results and discussion**

The first four peaks produced, decreased in height from absorbance 0.43. After eight injections the program was stopped for a few seconds, then restarted. The first peak after this break was, again, larger than the rest. Discounting the first four peaks and this intermediate one the other 11 peaks produced a mean absorbance of 0.233 with a relative standard deviation of 1.890%.

A sketch of the peak shape is given in fig. 58. The tail of the peak has been truncated by the valve returning before the whole contents of the chamber had been injected. The peaks would have had long tails had this not occurred, as the volume of the dissolution chamber was about 1 ml. The volume increases, as the amount of steel decreases.

The high peaks obtained at the beginning of the experiment are due to the presence of small steel
particles and the oxide layer on the steel. These dissolve quickly, raising the concentration of analyte in the chamber. Once these have all dissolved, the peaks produced are uniform in height. The high peak which occurred after a break in the program, is due to increased residence time of acid in the chamber which produced increased dissolution.

Considering the surface area of the sample and the amount present decreases as dissolution proceeds, the peaks are highly reproducible with a relative standard deviation value comparable to that normally found for flow injection flame atomic absorption spectrometry.
Conclusion

This on-line dissolution method shows promise. Further increases in sensitivity may be possible by using mixed acids and some interference compensation method to reduce the interference of iron on chromium.

Chamber 2

Apparatus

A second chamber was constructed based on the first but of glass filled PTFE (fig. 5.9)

Fig. 5.9

Solid dissolution chamber 2
A glass frit was used to filter the iron, the chamber was packed with glass beads below the frit to reduce the volume.

The chamber was mounted in a flask shaker and connected into the manifold (fig. 5.10).

---

**Fig. 5.10**

Solid dissolution manifold – chamber 2
A, B, C are 0.3% 1,10-phenanthroline buffered in 2 molar sodium acetate, 10% hydroxylamine hydrochloride to reduce any FeII to FeIII and water respectively.

This manifold performs the analysis of iron as described by Vogel [164] as used by undergraduates at Loughborough [165] but in a flow injection mode [166].

The acid mixture used for dissolution was 2 parts hydrochloric acid \((D = 1.19)\), 2 parts perchloric acid \((D = 1.67)\) and one part nitric acid \((D = 1.4)\). Cantle [167] recommends this mixture for steel dissolution but with 1 part water added. This was not necessary as the dissolution chamber initially contains water which disperses within the acid when the chamber is filled.

Five mixed standards were made up by dilution of a single stock iron and chromium mixture, to cover the range 64 to 800 mg l\(^{-1}\) iron and 4.6 to 58 mg l\(^{-1}\) chromium.

The formation of the iron-phenanthroline complex was monitored at 508 nm with an LKB Ultraspec UV/vis spectrometer. The steel sample was 1.5 g (approx.) of BCS 256/1, as used in the previous experiment.

The atomic absorption spectrometer was a Pye Unicam SP9 burning a nitrous oxide acetylene flame, optimised for chromium determination. The use of a nitrous oxide flame will reduce interference of iron in the chromium analysis.

Results were recorded using a twin channel chart recorder connected to both spectrometers.

Procedure

The program described above was used.
Initially the outlet of the dissolution chamber was monitored directly to check the performance of the dissolution chamber. The chamber was then refilled and connected to the manifold. The standards were then injected via the injection valve and the absorbance of the iron complex monitored using the UV/vis spectrometer. The contents of the dissolution chamber were then injected following acid dissolution.

Results and discussion

Dissolution chamber performance

Four injections of the contents of the dissolution chamber gave a mean absorbance of 1.16 and relative standard deviation of 7.61%. Although the response is greater than the response from use of chamber 1, the precision is considerably poorer.

Injection of standards

The results for the injection of the standards are presented in table 5.2.

The injection of 800 mg l$^{-1}$ produced a double peak due to reagent depletion and loss of buffering capacity. The reagent concentration available for reaction is constant and normally in excess. At high concentrations all the reagent is used, producing flat topped peaks. The pH capacity can be exhausted causing the complex to either, not form, or break down. This is the case for the injection of 800 mg l$^{-1}$ iron.

The response of the atomic absorption spectrometer
Concentration mean absorbance %RSD of Fe (mg l\(^{-1}\)) (4 injections)

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>0.226</td>
<td>1.02</td>
</tr>
<tr>
<td>32</td>
<td>0.455</td>
<td>2.08</td>
</tr>
<tr>
<td>48</td>
<td>0.631</td>
<td>2.94</td>
</tr>
<tr>
<td>64</td>
<td>0.767</td>
<td>0.065</td>
</tr>
<tr>
<td>800</td>
<td>see below</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.2
Results of solid dissolution

was not recorded during these experiments. Visually the chromium signal was small.

Solid dissolution

Although the response for iron was within the calibration range producing an iron concentration of about 50 mg l\(^{-1}\), the peaks for chromium were very small, even when scale expansion was employed. They appeared three seconds after the iron peaks. The reduction in concentration from the outlet of the dissolution chamber when connected into the complete manifold indicated by the reduced chromium peak height, is due to merging the stream with two reagent streams.

Conclusion

Although the dissolution chamber performed well, there were problems with the analysis of the effluent. Vogel [164] indicates that perchlorate (used in these methods) can interfere with the analysis of iron using 1,10-phenanthroline. The method of iron analysis also produced too great a dilution of the effluent from the
chamber for chromium analysis.

Even if an analysis of the iron and chromium content of the chamber effluent was possible, the percentage chromium in the steel could not be calculated: Other constituents of the steel must be taken into account. Only a ratio of chromium to iron content could be calculated. Alloys of just these two metals could be analysed in this way, but will not dissolve except under very vigorous conditions.

5.3.2 Interference Investigation Manifold

This manifold was designed for use for investigation into interference. This system is based on flow injection analysis where the element on which interference acts (the analyte) is used as the reagent and monitored by the atomic absorption spectrometer. Potential interferents are introduced into the system and the response noted. Originally interferents were injected into a single line manifold but the peaks produced not only resulted from interference, but also from dilution of the analyte. This problem was overcome by using a two line manifold.

Apparatus

The manifold (fig. 5.11) was constructed. Initially the analyte and carrier streams were driven by gravity from Marriot bottles. When a pump became available, the flow-rates were controlled by this. An eight port valve V was used with two matched sample loops of 82.2 μl. When a single sample loop is used, the increase in tube length
Interference investigation manifold

Fig. 5.11

when the sample loop is switched into line, changes the
flow resistance of the line, and thus the mixing ratio at
the confluence point. This changes the dilution of the
calcium stream and therefore the baseline level. The
flowrates delivered by gravity were adjusted by moving the
Marriot bottles vertically. The flowrates were adjusted
to match the normal aspiration rate of the spectrometer.
Each line produced a flowrate of $1 \text{ ml min}^{-1}$. The test
element used was $20 \text{ mg l}^{-1}$ calcium, stream A. This was
diluted by the water carrier, stream B.

The manifold was connected to a Hilger Atomspek
spectrometer. Interferents injected included $1000 \text{ mg l}^{-1}$
phosphate as ammonium sodium hydrogen orthophosphate (NH$_4$NaHPO$_4$·4H$_2$O), 1000 mg l$^{-1}$ aluminium, 99% ethanol, methyl isobutyl ketone (MIBK), 1000 mg l$^{-1}$ potassium, 10% lanthanum. A 100 mg l$^{-1}$ calcium solution was also injected to observe the concentration profile for the injections. Results were recorded on a chart recorder.

**Procedure**

Initially, the calcium stream, stream A, was replaced with a water stream and the interferents injected to ensure that no response occurred without calcium present.

The calcium stream was then replaced and each interferent injected at least four times. Finally the phosphate solution was diluted to 100, 50, 20 mg l$^{-1}$ phosphate and reinjected.

**Results and discussion**

Merging the injected interferents with water did not produce any response.

Traces obtained upon injection of interferents into the system with calcium are shown in fig. 5.12. Aluminium causes complete depression of the calcium signal, even at low concentrations. Phosphate on the other hand, shows increasing depression up to a certain concentration. Further increase in phosphate concentration from this level, does not produce increased depression. This is typical of phosphate interference [84]. Ethanol produces a marked signal enhancement. This is due to changes in the surface tension and vapour pressure and therefore in
Fig. 5.12

Trace of injection of interferents into the interference manifold
the nebulised drop size and in flame fuel composition. The enhancement shown by MIBK is due, again, to nebulisation and flame effects but the noise on the peak is due to the stream entering the nebuliser being segmented: MIBK is a water immiscible solvent. Potassium and lanthanum produce enhancement by ionisation suppression but in both cases, either depression of the calcium signal or no enhancement occurs at high concentrations. This could be due to an interference similar to that of iron on chromium i.e. a matrix of interferent forms around the analyte which must be evaporated before the analyte. The interference could also be due to nebulisation effects. The very broad lanthanum peak is due to the high lanthanum concentration originally injected.

During these experiments it was noted that the tail of the phosphate peak produced enhancement. When the diluted phosphate solution was injected, a series of peaks with increasing enhancement with decreasing concentration was produced (fig. 5.13).

At low concentrations the effect of the ammonium ion as a releasing agent and of sodium as an ionisation suppressant counteract the effect of phosphate. These ions are present in the \( \text{NH}_4\text{NaHPO}_4\cdot\text{4H}_2\text{O} \) used.
This manifold enables fast determination of interference upon an analyte. The extent of interference can be observed and if a maximum level of interference is achievable, this will be seen as a flat bottomed peak.

It was intended that the phosphate peak width at the plateau would be used to quantify the concentration of phosphate injected. This would only be possible if no signal enhancing species were present and if the phosphate concentration reached the plateau producing level.

Conclusions

Enhancement and reduction of calcium signal by $\text{NH}_4\text{NaPO}_4\cdot4\text{H}_2\text{O}$
5.3.3 pH Controlled Separation of Species

The different pH ranges over which ion exchange resins operate, can be exploited to decrease the number of steps involved in species separation. In the preliminary experiments, section 5.2.2, Amberlite IRA 400 was used for phosphate removal. Amberlite IRA 400 will remove phosphate ions over the pH range 1-12. Once the phosphate has been bound onto the column, it must be eluted, usually with hydrochloric acid. When included in a flow injection manifold, this means that concentrated acid must be injected after the samples. If the eluent is included in the carrier stream, the elution will take place immediately after the sample slug has passed through the column. If a suitable resin is chosen, the elution of the column can be speeded up by using samples and carrier of different pH.

A diagrammatic representation of the concentration profiles expected as a dispersed sample slug passes through a column, where the carrier and sample are of different pH is shown in fig. 5.14.

Two peaks are predicted for the adsorbed species. The first results from the pH being unfavourable and eluent concentration too high, for adsorption. Once conditions become favourable the species is adsorbed. As the pH and eluent concentration revert back to the carrier conditions, the adsorbed species is eluted producing a second peak.

The unadsorbed species peak is not delayed and follows the injected sample concentration profile.
Fig. 5.14  Predicted peak profiles

- - - Injected sample concentration (unadsorbed)
- - - pH
- - - - Carrier concentration
- - - - - Injected sample concentration (adsorbed)

Separation of species is possible, if the peaks from the adsorbed and unadsorbed species do not overlap. The first peak for the adsorbed species is not suitable as unadsorbed species will always be present at the peak.
Separation of the other peak should be possible if the carrier and sample conditions are chosen carefully. If no diffusion of the sample slug occurred, rectangular concentration profiles would result.

In this case, complete separation is achieved and two 'peaks' obtained. In practice therefore, diffusion of the sample slug must be kept to a minimum.

The carrier/eluent must be chosen so that elution occurs at the desired pH and occurs fast enough so that no bound material remains on the column when the next sample slug passes through: If the bound species was being monitored, the peaks would increase with each injection as sample carry over occurred. The eluent chosen will depend therefore on how strongly the bound species is held on the resin. Some species may be eluted by the OH⁻ ions from an alkali carrier, others may require an alkali carrier containing other anions. Similarly for cation exchange resins.

Three systems based on these ideas were examined.

**Cation exchange, Ca²⁺ and PO₄³⁻**

Kamson and Townshend [117] separated calcium and phosphate using a strongly basic anion exchange resin. Calcium could be held on a cation exchange resin and eluted with dilute acid, using pH controlled separation of species.

**Apparatus**

A 60 mm column was constructed as in section 5.2.2
containing Zerolit 226. This is a weakly acidic cation exchange resin. Below a pH of about 3.5, the resin exists entirely in the hydrogen form. This resin is available as Amberlite IRC-50 from BDH Chemicals Ltd. This was connected into the manifold (fig. 5.15) on the outlet of the valve.

![Diagram](image)

**Fig. 5.15**

pH controlled separation of species manifold

The pH of a water carrier was decreased to approximately 2.5 by the dropwise addition of concentrated hydrochloric acid.

Samples of 10 mg l⁻¹ calcium and 10 mg l⁻¹ calcium plus 200 mg l⁻¹ phosphate as phosphoric acid were buffered at pH 9.8 in ammonium acetate buffer. This was made with 55.5 ml acetic acid (99%) and 112.5 ml ammonia solution (25%). pH adjustment was achieved by the dropwise addition of ammonia solution. Both the pH of the samples...
and carrier were measured with narrow range pH paper.

The carrier flowrate was $3.0 \text{ ml min}^{-1}$. Injected volume of sample $82.3 \mu l$. The manifold was connected to a Baird Atomic A3400 spectrometer. Results were recorded on a chart recorder.

**Procedure**

Each sample solution was injected four times and the peaks recorded.

**Results and discussion**

The first peak resulting from a single injection, fig. 5.16, will be depressed by phosphate (see fig. 5.14). The second peak may be depressed by phosphate.

When the ratio of the second peak for the interfered solution to uninterfered solution is calculated (table 5.3) the value is considerably less than 1. This

<table>
<thead>
<tr>
<th>Sample</th>
<th>mean peak absorbance</th>
<th>ratio of peak absorbances</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mg l$^{-1}$ Ca$^{2+}$</td>
<td>0.044</td>
<td></td>
</tr>
<tr>
<td>10 mg l$^{-1}$ Ca$^{2+}$</td>
<td>0.693</td>
<td></td>
</tr>
<tr>
<td>200 mg l$^{-1}$ PO$_4^{3-}$</td>
<td>0.0305</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.3

Results of pH controlled cationic ion exchange of phosphate and calcium mixtures
The two peaks obtained resulting from the injection of calcium into the manifold Fig. 5.15 shows that the tail of the sample slug containing phosphate, has overlapped with the elution of calcium causing interference.

**Conclusion**

Separation of phosphate from calcium was not obtained under these conditions.

Anion Exchange $\text{Ca}^{2+}$ and $\text{PO}_4^{3-}$

In this system, the phosphate is retained on the column. Better separation should be possible than the
cation exchange calcium/phosphate system as the calcium peak will occur at the point where minimum or no elution of phosphate occurs (fig. 5.14). The trough between the phosphate peaks will be larger; The bigger the sample volume injected, the longer the time the conditions for binding exist.

**Apparatus**

Two 60 mm columns constructed as described in section 5.2.2, containing different resins were incorporated into the manifold (fig. 5.15). 82.3 µl and 256 µl were injected into a system with IRA 400 resin (used by Kamson and Townshend [117]). This resin is a strongly basic anion exchanger and will bind anions over the pH range 1-12. At pH's less than 10, the resin is capable of binding virtually all anions [168]. 256 µl was injected into a system with IRA 93 resin. This resin is a weakly basic anion exchange resin which will bind ions over the pH range 1-7, otherwise it is in the free base form [168].

Samples of 10 mg l⁻¹ calcium and 10 mg l⁻¹ calcium plus 200 mg l⁻¹ phosphate were made up with hydrochloric acid (5 ml concentrated HCl (1.1) per 100 ml). The pH of these samples was 1.5-2.0. The carrier was 2.5% ammonium hydroxide (0.88). The approximate pH was 10-11. These pH's were measured using narrow range pH paper.

The manifold was connected to a Baird Atomic spectrometer, results being recorded on a chart recorder.
Procedure

82.3 μl of each sample was injected four times each, into the system incorporating either the IRA 400 resin or the IRA 93 resin.

Results and discussion

The results are given in table 5.4.

<table>
<thead>
<tr>
<th>Resin.</th>
<th>Sample Volume</th>
<th>mean peak absorbance</th>
<th>Peak ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μl</td>
<td>Ca²⁺</td>
<td>Ca²⁺ + PO₄⁻</td>
</tr>
<tr>
<td>IRA 400</td>
<td>82.3</td>
<td>0.110</td>
<td>0.103</td>
</tr>
<tr>
<td></td>
<td>256</td>
<td>0.170</td>
<td>0.169</td>
</tr>
<tr>
<td>IRA 93</td>
<td>256</td>
<td>0.160</td>
<td>0.158</td>
</tr>
</tbody>
</table>

Table 5.4

Results of pH controlled anionic ion exchange of phosphate and calcium mixtures

Because the flow demand of the nebuliser was greater than the flow delivered by the pump, small bubbles were able to form within the column after long periods of operation. This produced a wide variation in peak heights for the injections using IRA 93.

Larger injection volumes produce better separation as shown by the interfered to uninterfered peak height ratios. Because the IRA 400 resin exchanges over a wide pH range, elution of phosphate will be slower than when the IRA 93 resin is used.
Conclusion

Removal of phosphate interference is possible using pH controlled separation of species with anion exchange. Phosphate elution cannot be seen using the spectrometer and the phosphate may be held on the column during subsequent injections.

5.3.4 pH Controlled Speciation

Because the elution of phosphate from columns cannot be seen using an atomic absorption spectrometer whilst analysing for calcium, the speciation of chromium was studied. The separation of chromium III and chromium VI as dichromate should be possible with the system used for phosphate/calcium separation. Cresser and Hargitt [169] used a manual ion-exchange procedure to separate these species.

Apparatus

The manifold used for the phosphate/calcium separation fig. 5.15 was used with an injection volume of 256 µl, and a flowrate of 3 ml min⁻¹. The carrier was an ammonia solution at approximately pH 11. Three sample solutions were made up: 20 mg l⁻¹ of chromium III, 20 mg l⁻¹ chromium VI and a mixture of 20 mg l⁻¹ chromium VI and 20 mg l⁻¹ chromium III. 5 ml of HCl specific gravity 1.1, was incorporated into each solution to produce a pH of approximately 1.5. 1000 mg l⁻¹ stock chromium III supplied by BDH and 1000 mg l⁻¹ chromium VI as potassium dichromate (2.829 g l⁻¹) were used with
suitable dilution.

The manifold was connected to a Baird Atomic A3400 spectrometer burning an air/acetylene flame. A slightly fuel rich flame was used to give moderate sensitivity and reasonable linearity of the response. Results were recorded on a chart recorder.

Procedure

The mixture was injected first, followed by the chromium III and chromium VI solutions respectively. The chromium III and the mixture were then reinjected. Each solution was injected at least five times.

Results

Sketches of the peaks produced are shown in figures 5.17 to 5.19. These traces show that the chromium VI species is only partly eluted from the column by the carrier. A proportion of the chromium VI is retained from each injection, reducing the height of the second peak. Some of this retained species is eluted during subsequent injections. Hence the second peaks' height for the mixture fig. 5.17, decreases for subsequent injections of chromium III and the peak heights increase for injection of chromium VI fig. 5.19.

According to the BDH handbook [168] the affinity for anions for most resins is in the following order.

\[
SO_4^{2-} > CrO_4^{2-} > citrate > tartrate > NO_3^- > AsO_4^{3-}
\]
Peaks produced during pH controlled speciation
Shen-Yang and Ke-An [170] describe the theoretical abundance of the species present at various pH values and concentration of chromium VI. The species present over the pH range (and concentration $10^{-4}$-$10^{-3}$ M) used in this experiment are mainly $\text{CrO}_4^{2-}$ at pH~8 to mainly $\text{HCrO}_4^{-}$ with some $\text{Cr}_2\text{O}_7^{2-}$, $\text{H}_2\text{CrO}_4$ and $\text{CrO}_4^{2-}$ (in order of decreasing abundance) at pH~2-3. The affinity for these species (one of which is neutral) will depend on molecular size, valency and concentration. A possible order of affinity inferred from this and the series above is,

$$\text{Cr}_2\text{O}_7^{2-} \rightarrow \text{CrO}_4^{2-} \rightarrow \text{HCrO}_4^{-} \rightarrow \text{H}_2\text{CrO}_4$$

As the pH of the solution around the resin changes, the species will change. This will affect the elution of Cr VI ions, as a significant proportion of the chromate will be strongly held on the column.

**Conclusion**

The existence of several chromate species at different pH's prevents the use of pH controlled speciation as variable peak heights are obtained. This could be improved by using different pH carriers/samples and inclusion of an eluent species with a strong affinity for the resin.

The thiocyanate ion has a high affinity for anion exchange resins [171] and could be used in the
eluent/carrier.

**pH controlled speciation with thiocyanate**

These experiments were designed to investigate the effect of thiocyanate in the carrier. Two carrier pHs were tried and two sample pHs.

**Apparatus**

The manifold (fig. 5.15) with an injection volume of 256 µl and spectrometer conditions used in previous experiments were used in these experiments.

The carrier solutions were made using 1 g l\(^{-1}\) ammonium thiocyanate for the carrier at pH 6, 1 g l\(^{-1}\) ammonium thiocyanate and 5 ml ammonia (0.88) for the carrier at pH 10.

Six sample solutions were made up to 20 mg l\(^{-1}\) Cr III, 20 mg l\(^{-1}\) Cr VI and 20 mg l\(^{-1}\) Cr III + 20 mg l\(^{-1}\) Cr VI using 5% and 10% hydrochloric acid (1.1), to give solution pHs of approximately 1.5 and <1.5. The flowrate of the carrier solutions was reduced from 3.0 ml min\(^{-1}\) in previous experiments to 2.0 ml min\(^{-1}\), to increase the peak width. Those illustrated in figs. 5.17-5.19 were narrow making evaluation of the separation difficult.

**Procedure**

Samples at pH 1.5 were injected into both carriers at pH 10 and 6 and the peaks recorded. Samples at pH<1.5 were injected into carrier at pH 10. Each injection was performed at least three times.
Results

Repeat injections of Cr III into the ammonium thiocyanate carrier at pH 6 produced reproducible peaks but when Cr VI solutions were injected, the peak heights decayed showing that increasing amounts of Cr VI were bound onto the resin.

Peaks produced upon injection of Cr VI sample solution at pH<1.5 and pH 1.5 into ammonium thiocyanate carrier at pH 10 are shown in figures 5.20 and 5.21 respectively.

Absorbance 0.1

Fig. 5.20

Injected sample pH<1.5

The peak shape in fig. 5.21 resembles that predicted for the eluted species in the introduction to this section (fig. 5.14). The trace in fig. 5.20 has an extra peak. This is probably due to formation of a third species other than Cr III and Cr$_2$O$_7^{2-}$ at this pH e.g. HCrO$_4^-$ which is eluted more slowly from the column.
Fig. 5.21
Injected sample pH 1.5

The peaks produced upon injection of samples at pH 1.5 into the ammonium thiocyanate carrier at pH 10 are shown in fig. 5.22. These peaks are overlaid to show that the trough between the two peaks for the Cr VI trace occurs directly under the peak for Cr III. The peak heights for each injection of solutions at pH 1.5 into carrier pH 10 are given in table 5.5.

Peak height (absorbance)

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<th></th>
<th>Cr III</th>
<th>Cr VI</th>
<th>Mixture</th>
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<tr>
<td></td>
<td>Cr III</td>
<td>Cr VI</td>
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</tr>
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<td>0.298</td>
<td>0.092</td>
<td>0.306</td>
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<td>0.299</td>
<td>0.099</td>
<td>0.304</td>
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<tr>
<td></td>
<td>0.295</td>
<td>0.094</td>
<td>0.304</td>
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<tr>
<td>mean</td>
<td>0.297</td>
<td>0.095</td>
<td>0.305</td>
</tr>
</tbody>
</table>

Table 5.5
Peak heights for chromium speciation experiments
The mean peak heights for the Cr III species in the mixture was 2.7% higher than the peak height for the Cr III species when injected independently. This is due to
there being a small amount of Cr VI present, as indicated by the trough between the two Cr VI peaks not being at baseline (fig. 5.22). If the ratios of Cr III to Cr VI in the mixture were varied, this trough level could give even higher errors: If the Cr VI was in excess of the Cr III, this trough height would cause the Cr III peak from the mixture, to be very much larger than that obtained when injected independently.

Addition of thiocyanate to the carrier enables complete elution of the column: There is no evidence of carry-over or excessive tailing in any of the traces of the peaks.

Conclusion

Speciation of chromium VI from chromium III was achieved but peak separation of chromium III from chromium VI was not completely obtained. Longer columns and lower pH samples may improve this separation. Use of computer software could enable the peak heights to be calculated even if considerable overlap was observed.
CHAPTER 6
Flame Atomic Absorption Spectrometry User Survey

The different approaches to overcoming interferences have been described in section 3.3.3. The analyses conducted in that section were based on methods described in the literature, but the methods described in the literature may not be those used routinely for analysis by practising analytical atomic spectroscopists in 'the real world'. A large part of the time spent performing an analysis will be spent preparing the sample and standards for presentation to the instrument. To discover what methods are used by practising spectroscopists for flame atomic absorption spectrometry, a questionnaire was devised (fig. 6.1 shows one which has been completed) and distributed to the members of the Atomic Spectroscopy Group (A.S.G.) of the Analytical Division of the Royal Society of Chemistry. Initially the survey was sent to members of the committee of the ASG as a pilot survey and suggestions were incorporated in the final version. The questionnaire is divided into a number of sections to enable information to be supplied about (1) the analyte element, sample type and dissolution procedure, (2) treatment of sample to overcome any interference effects, (3) treatment of standards, (4) instrument operating parameters, (5) method of data acquisition, (6) curve fitting to calibration and (7) any other relevant information.
Flame Atomic Absorption Spectrometry Calibration Questionnaire

Please select up to four methods which you consider 'typical' of the various calibration strategies used in your laboratory for analysis. We require methods that employ FLAME AAS only, excluding hydride generation, cold vapour, furnaces. Please include information on one element per analysis A to D, even if the procedure used is for multielement analysis.

In the appropriate column please respond with
(1) Y for yes
(2) N for no
(3) NK for not known
(4) the appropriate number
(5) your own abbreviations if not covered by the above and explain here

-----------------------------------------------------------------------------------------------

-----------------------------------------------------------------------------------------------

-----------------------------------------------------------------------------------------------

In the appropriate spaces, please fill in the information required or respond NA for not applicable or NK for not known.

---

Fig. 6.1

The questionnaire
1. Analytes, Samples and Dissolution

A. Determination of Calcium in Apples
Matrix composition known \( Y \) \( Y/N \) (to a limited extent).
Approximate concentration of analyte known \( Y \) \( Y/N \)
Brief description of dissolution procedure (if appropriate)
DIGESTED BY KELDANIEL METHOD, i.e. 36% H\(_2\)SO\(_4\) + 5% K\(_2\)SO\(_4\) + Cu/CuCl
Catalyst. Ammonium taken for final determination.

B. Determination of Magnesium in Soil
Matrix composition known \( Y \) \( Y/N \) (to limited extent)
Approximate concentration of analyte known \( Y \) \( Y/N \)
Brief description of dissolution procedure (if appropriate)
EXTRACTED WITH M ammonium nitrate, filtered and read

C. Determination of Copper in Plants
Matrix composition known \( Y \) \( Y/N \) (to limited extent)
Approximate concentration of analyte known \( Y \) \( Y/N \)
Brief description of dissolution procedure (if appropriate)
DIGESTED WITH NITRIC/PERCHLORIC ACID MIXTURE TO DRYNESS AND THEN TAKEN UP IN 0.4 M HYDROCHLORIC ACID.

D. Determination of Zinc in Plants
Matrix composition known \( Y \) \( Y/N \) (to limited extent)
Approximate concentration of analyte known \( Y \) \( Y/N \)
Brief description of dissolution procedure (if appropriate)
AS COPPER IN PLANTS.

Fig. 6.1 contd.
2. **Sample Preparation**

Dissolution and/or dilution with

(a) no further pretreatment
(b) addition of releasing agents (eg La)
(c) addition of protecting agents (eg EDTA)
(d) addition of ionization suppressants (eg K)
(e) addition of aliquots of standards (standard additions method)

(f) number of additions

(f) other procedures (eg solvent extraction, chelating column preconcentration)

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<tr>
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<th>D</th>
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<td>✓</td>
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<td>(e)</td>
<td>✓</td>
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</table>

3. **Standard Preparation**

Serial dilution of a stock solution to give range of standards covering desired calibration range, with

(a) no further additions
(b) addition of releasing agents
(c) addition of suppressing agents
(d) addition of ionization suppressants
(e) addition of sample matrix components
(f) other procedures

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<td>(f)</td>
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</table>

(g) Own stock or bought solution (0 or 3)

(h) Blank
   (i) matrix matched
   (ii) distilled water
   (iii) other

<table>
<thead>
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<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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<tbody>
<tr>
<td>(g)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>(h)</td>
<td>✓</td>
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As far as acid concentration, sample preparation.
4. **Instrument Operation**

(a) **air - C₂H₂**

(b) **N₂O - C₂H₂**

(c) **Instrument settings**
   (c1) pre-set
   (c2) optimization for max. sensitivity
   (c3) optimization for max. signal to noise
   (c4) optimization for minimum interference
   (c5) other (eg reduced sensitivity)

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</table>

(d) **Sample presentation**
   (d1) continuous nebulization
   (d2) microsampling (aliquot sampling, discrete nebulization)
   (d3) flow injection
   (d4) other

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<td>✓</td>
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</tr>
</tbody>
</table>

Fig. 6.1 contd.
5. Absorbance Values

(a) reading analogue meter
   (a1) how many significant figures?

(b) reading digital meter
   (b1) how many digits?

(c) analogue integration
   (c1) how many significant figures?
   (c2) integration time (seconds)

(d) digital integration
   (d1) how many digits?
   (d2) integration time (seconds)
   (d3) how many measurements per second?

(e) chart recorder
   (e1) how long at steady state (seconds)?

(f) values obtained from instrument's integral computer

(g) values obtained from own interfaced computer

(h) how many absorbance readings per sample or standard

<table>
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<tr>
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<td>1</td>
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</tr>
</tbody>
</table>

Fig. 6.1 contd.
6. Calibration

6.1 Curve Fitting

(a) How many calibration points excluding blank

(b) manual graphical plot
   (b1) ruler
   (b2) flexicurve

(c) instrument's integral computer
   (c1) equation A
       B
       C
       D

(d) own computer interfaced with instrument
   (d1) equation

(e) own computer not interfaced with instrument
   (e1) equation

(f) algorithm
   (f1) linear least squares
   (f2) non-linear least squares
   (f3) weighted least squares
   (f4) exact, equation solved
   (f5) other

(g) stored calibration data
   (g1) unchanged
   (g2) with reslope on one standard
   (g3) data entered via key board
   (g4) other

Fig. 6.1 contd.
6.2 Assessment of Uncertainty due to Calibration

(a) Calculation of 95% (or other) confidence interval

(b) other

A ....................................... .
B ....................................... .
C ....................................... .
D ....................................... .

6.3 Reasons for Choosing a Particular Calibration Method

(a) speed

(b) accuracy

(c) precision

(d) other

7. Comments

7.1 Other Procedures

Do you use a sample/standard preparation and calibration procedure for flame atomic absorption spectrometry that has not been covered by the above questions? If so please give details:-
7.2 Problems

Have you any comments to make on the shortcomings (or otherwise) of your calibration procedures?

7.3 Any other comments

8. Further Help

Would you be prepared to provide further assistance to this research project by, say, providing specimen calibration data?

If so:- Name: ..................................................
Address: ..................................................
 ..................................................
 ..................................................
 ..................................................

Many thanks for your co-operation.

Fig. 6.1 contd.
Results and Discussion

931 questionnaires were sent out and 105 have been returned correctly filled in, representing 392 routine analyses. Several blank questionnaires were returned as the members have either retired or no longer perform flame atomic absorption analyses, others were handed to colleagues who were more qualified to fill them in. Some were returned with ambiguous answers, most of these were discarded, a few were included if the ambiguous answers could be correlated with another answer. Some respondents filled in a number of elements for each dissolution of a sample i.e. use multielement analysis. In these cases only the first element in the given list was counted, as single element analyses were requested.

The results for the types of analyses, obtained from the questionnaires are presented in table 6.1. This table enables the correlation of sample type, analyte element, method of interference compensation (if any) and the flame used for the analysis with each other. The categories of sample type are those used in 'Annual Reports on Analytical Atomic Spectroscopy' (ARAAS) [172]. Some samples were difficult to place within these categories: a space for complete descriptions of the samples was not included in the reply. Some samples therefore, could be included in several categories; an example is 'plating solution' which may be included in the effluent, chemical or even metal categories.

The method of interference compensation section was divided into the following methods of sample and standard
<table>
<thead>
<tr>
<th>ELEMENT</th>
<th>CHEMICALS</th>
<th>METALS</th>
<th>REFRACTORIES</th>
<th>MINERALS</th>
<th>AIR &amp; PARTICulates</th>
<th>WATERS/ EFFLUENTS</th>
<th>SOIL PLANTS</th>
<th>FOOD &amp; BODY TISSUES</th>
<th>BEVERAGES &amp; FLUIDS</th>
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**Questionnaire Results**

Table 6.1...
Questionnaire Results

Table 6.1

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Table 6.1: Questionnaire Results
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**Table 6.1**

Questionnaire Results

**A** = air-acetylene flame  
**N.O.** = nitrogen oxide-acetylene flame  
**R** = removal from matrix  
**M** = matrix matching of standards & sample  
**I** = method of analysis  
**S** = addition of reagent to overcome interference  
**N** = standard additions method  
**E** = no interference assumed, no sample or standard treatment

* = Na + air burn blankness
treatment. a) removal from the matrix, usually by solvent extraction, b) matrix matching which involved the addition of sample matrix and dissolution components to the standards, c) addition of interference suppression reagents to either the sample, standard or both. This includes ionisation suppressants, protecting agents and releasing agents, d) standard additions, e) no treatment of standards or samples. If an interference suppression reagent was added to samples and standard, this was not counted as matrix matching but as interference compensation, but if the reagent was added and a standard additions procedure performed, the analysis was included in the standard additions category. There will be some bias in the results towards the less common analyses even if the information on routine analyses was requested. If a laboratory performs say 300 analyses of one type and 3000 of another each year, both will occupy one position in a questionnaire and receive equal weighting in table 6.1. Each entry in table 6.1 therefore represents the number of times an analysis is described and not the number of times it is performed.

Of the analyses described, two thirds were analysed with some compensation for interferences, the most popular methods were matrix matching and interference suppression, each fulfilling the requirements for about one quarter the analyses described. Of the remainder, five sixths were by standard additions and a few by matrix removal methods. It is clear that standard additions is not a popular routine technique. Most respondents stated that if the
method of standard additions was used, it was used for samples of unknown composition or in method development. The use of a nitrous oxide flame is not very popular except for aluminium; an element which is difficult to atomise, and calcium; which is prone to interference. In these cases, addition of an ionisation suppressant was common. Other elements including beryllium, molybdenum, phosphorous, silicon, tin, strontium, titanium and scandium are analysed using the nitrous oxide flame but the number of returns to date does not allow any conclusion as to how often this flame is used for these elements.

Most of the samples described are waters/effluents, chemicals or metals. This may be a reflection of the type of sample analysed or it could be a reflection of the type of membership of the ASG of the Analytical Division of the RSC! It is interesting to note that the most popular elements for which analyses are described are the current environmentally important ones i.e. lead, copper, calcium and zinc. Lead and copper are pollutants from exhaust fumes and algicides respectively, calcium is responsible for water hardness and zinc is a pollutant from many industrial effluents.

The results of the survey when studied with respect to curve fitting techniques, are given in figures 6.2 and 6.3. The introduction of curve fitting software used on many modern atomic absorption spectrometers is reflected in the frequent use of the computer for 'normal' calibration. The majority of standard additions
Fig. 6.2

Number of calibration points used in normal calibrations. The upper blocks represent the frequency of use of computer curve fitting. The lower blocks are manual curve fitting methods. Calibrations on the other hand are carried out using manual techniques, but even here, the computer is making an impact. Many of the respondents did not know what type of curve fitting was being performed or the equation being fitted. Some respondents described their own curve
Fig. 6.3

Number of calibration points (number of additions) for standard additions calibrations. The upper blocks represent the frequency of computer curve fitting as opposed to manual methods represented by the lower blocks fitting algorithms. One such algorithm fitted an equation raising absorbance to a fractional power and e to a multiple of absorbance, equation 6.1.

\[ C = aA + bA^{2.5} + c(e^{14A} - 1) \]  \hspace{1cm} 6.1

Another used a rational function, equation 6.2.

\[ A = \frac{a + bC}{1 + cC} \]  \hspace{1cm} 6.2

In both these equations a, b and c are parameters, the
values of which are found by the method of least squares. These equations were included in the comparison of curve fitting algorithms (section 3.3.2) and performed reasonably well, but were not as good as some of the manufacturers own algorithms.

The other algorithms used by respondents were manufacturers own or ones using linear and quadratic functions of absorbance or concentration. Some included weighting to produce a good fit to standards that are close to the blank concentration.

The number of standards used, excluding the blank, reflects the method of analysis and the compromise between speed and accuracy. Standard additions calibrations cannot be performed reliably with one or two additions, as an extrapolation step is involved. When more standards are used, more time is taken preparing the solutions for analysis, especially if a matrix matching technique is used where standards are taken through the same dissolution procedure as the samples. This is why the most popular numbers of calibration points are 3, 4 and 5; a compromise between speed and accuracy. One respondent claimed to use twenty-five calibration points and plotted these by hand, but the reason that was stated for using such a procedure was speed!

The manual methods of calibration described included the inevitable ruler and flexicurve and also a bent ruler. This technique could be useful as a bent ruler if bent by compressing each end, is curved in the middle with two linear portions at each end. The linear portion at one
end would follow the linear section of a calibration curve at the origin. The linear portion at the other end would follow the asymptotic region of the calibration curve.

The total number of analyses represented in fig. 6.2 and 6.3 does not match the total number of analyses described in table 6.1. This is due to some replies containing ambiguous answers or some questions being left unanswered.

The majority of respondents filled in the section requesting information on the reason for choosing a particular calibration method with multiple answers e.g. they ticked both speed and accuracy. These replies were supposed to be mutually exclusive: A method assuming no interference using only two calibration points for the analysis of an element may be fast but is probably less accurate than a matrix matching technique employing five standards. It is suspected that respondents answered the question "Why use flame atomic absorption spectrometry?" rather than "Why use this calibration technique for flame atomic absorption spectrometry?".

Conclusion

The number of responses has not produced a large sample and drawing conclusions from the survey is limited at this stage. The target sample (members of the ASG) may not be representative of the entire population of Flame atomic absorption spectroscopists and the sub-sample of questionnaires returned may also be unrepresentative.

Some shortcomings in the design of the questionnaire have
become apparent. Future questionnaires should include a space to categorise the sample according to ARAAS [172] and emphasise that the questions refer to the calibration method and not to the technique of FAAS.

It is hoped that this survey will continue so that a larger, representative sample can be obtained from which more valid conclusions can be drawn.

Even where the analysis of a particular element is common, no particular method of analysis is most dominant. Use of computers for curve fitting has not superseded the manual methods, though this impression may be due to the bias towards less routine analysis and hence small laboratories which may not have modern instruments incorporating a microcomputer.
Conclusions and Suggestions for Further Work

In the introduction, the breadth of the subject of calibration was discussed. The study described in this thesis therefore, covers a wide range of topics. The literature survey, chapter 2, indicated that a number of calibration strategies are possible.

The lack of an accurate mathematical model for the causes of bending of the normal calibration curves has led manufacturers to develop a number of curve fitting algorithms, each manufacturer adopting a different algorithm for historical reasons, to avoid violating another manufacturers patent, or in the belief that their algorithm is superior. Because of commercial reasons, no comparison of all the various algorithms had been published by any manufacturer. The comparison carried out in this study, (section 3.3.2), indicated that the simple parabolic curve fitting procedures could produce errors of as much as ±28% in the concentration values, due simply to the poor fit of such curves to atomic absorption calibration data. The more complex algorithms used on more advanced instruments, although different to each other, produce similar errors at approximately the ±3% level. Some algorithms produce a better fit to certain curve shapes but, overall, the rational function (equation 7.1) used by Varian as a family of curves, produces the best fit with a maximum error in concentration found in
this study to be ±2.53%.

\[ A = \frac{a + bA + cA^2}{C} \] 

This level of error is crucial to the accurate analysis of a sample and all the algorithms produced results exhibiting significant errors. The prosperity of a company may rely on accurate analysis for quality control and more importantly the safety of the public may depend on such an analysis.

The results of the survey, chapter 6, show that the majority of calibrations are generated using at least three standards and the usual number of standards that is used is five. The results of the comparison of curve fitting algorithms implies that the results of routine analysis in laboratories therefore suffer from an error of at least ±3% due simply to curve fitting.

Use of any calibration function is limited to a concentration range from the detection limit to the concentration of the most concentrated standard. This forces the analyst to adopt a trial and error procedure to discover within what concentration range the sample lies and if any dilution or preconcentration is required. Once a suitable range of standards has been prepared and the sample dissolved and/or diluted to be within the calibration range, an analyst may still be uncertain as to whether the sample absorbance has been affected by an interferent. The survey shows that many different routine
methods are employed to try and compensate for interferences.

All these factors mean that a great deal of time is taken in the analysis of samples by atomic absorption spectrometry. Even when a result is obtained, there is some uncertainty as to the accuracy of the result.

In order to try and improve these deficiencies in calibration a number of alternative strategies for sample treatment and calibration were studied.

If the 'mix and match' technique (section 4.3.3) of standard dilution and sample dilution and analysis is used, the need for curve fitting is removed and samples which are off the range of the standards available can be automatically diluted to a limited extent to bring them on range. The system developed so far is slow, taking approximately 1 minute to 'mix and match' the absorbance of a sample. If more appropriate hardware could be developed, these operations could be speeded up.

Peristaltic pumps, although convenient to use, do not produce pulse-free, accurate flow. High pressure liquid chromatography pumps are too expensive to be a practical alternative. If such hardware were available, sample introduction by flow injection could be used, greatly reducing the volume of sample required and speeding up the 'mix and match' processes. The 'intelligent instrument' concept on which this system was based, where the instrument responds to an analytical reading, may be useful for future work.

The study of peak width calibrations (section 4.4.6)
was made in order to evaluate such a procedure for producing an extended calibration range. Use of a logarithmic function is the principal behind such a calibration, but by their very nature, measurement of peak widths and conversion to concentrations through such a function, gives imprecise results. The single well stirred tank model predicts that peak width calibrations will be linear if the function 7.2 is used.

\[ t' = \langle V/u \rangle \ln(\langle C_m/C' \rangle - 1) + K \]  \hspace{1cm} 7.2

where \( t' \) is the peak width, \( V \) is the volume of the tank, \( u \) the volumetric flow rate, \( C' \) the concentration at which the peak width is measured, \( C_m \) the concentration injected and \( K \) a constant. In this study S-shaped peak width calibration curves were produced, indicating that this model is an oversimplification of what really occurs within a manifold which includes a nebuliser. If a more appropriate model was devised, linear peak width calibration curves would be expected, increasing the accuracy of such techniques and enabling injections of a single standard to be used to calibrate the instrument for a wide range of concentrations. The use of a characteristic peak by Gine et al [138] in the zone sampling technique indicates that problems of calculating the concentration of dispersed solutions at certain times along a flow injection profile using a model, have been encountered by other workers.

Timed injection into a flow injection manifold
(section 4.4.5) holds promise as an online dilution technique as it would enable rapid calibration from a single standard and dilution of samples. In this study equipment was used which did not allow accurate timing or sufficient resolution of the injection time to be obtained. Once accurate timing of the injection becomes possible, calculation of standard concentration from the valve timing would be possible. In its present form, the system could be used to obtain arbitrary dilutions which must be characterised against a calibration curve.

Gradient calibration techniques were not studied. The two pump manifold (as used for the mix and match method) could be used under computer control to produce gradients whose shape and range could be specified by the operator. Use of this manifold to produce linear gradients would enable the conventional type of calibration to be prepared quickly and automatically from a single standard.

Exponential dilution tanks can provide gradients for calibration but their use entails non-linear concentration time functions.

Removal of interferences and pH controlled separation of the species (sections 5.3.3 and 5.3.4) shows the value of on-line reactors for general flow-injection analysis. The concept of analyte or interferent capture on ion-exchange resins could be extended to include the capture of reagent material prior to injection of sample. This material would then be released by the passage of the sample zone through the reactor, enabling reaction to take place. In this way the presence of an interferent which
has an affinity for the column may become an advantage.

It was noticed that the conventional nebuliser, although appropriate for conventional steady state absorbance measurement, may be inappropriate to flow injection peak measurement. The experiments using peak width measurement indicated that the spray chamber distorts peak shapes from those profiles generated within a manifold. If an online reactor is used, at low flowrates bubbles may appear in the reactor due to the reduced pressure generated by the nebuliser, degassing the carrier solution. Air compensation [173] could be used to overcome this and can be an advantage where interference is a problem as this may be reduced.

The nebuliser chamber with its associated ball and/or baffles was designed to produce a uniform mist of analyte droplets. When such a system is used, much of the analyte goes to waste. When the analyte is being measured conventionally, this loss of analyte is partially counteracted by the continuous replacement of analyte in the nebuliser chamber to produce the steady state. When flow injection sample introduction is used, only a small amount of sample is injected. Much of this goes to waste and is not replaced. Not only does the analyte go to waste, but the analyte droplets which do form a mist are diluted by the mist already in the chamber and that formed from the carrier after the analyte slug has completely entered the chamber. The analyte loss and mist dilution increases the dispersion of a flow-injection flame atomic absorption system and distorts the peak shape. Although
the nebuliser and nebuliser chamber may be appropriate for conventional, steady state measurements, they are inappropriate for flow injection sample introduction techniques. If the analyte could be introduced into the flame by another means, the sensitivity of flow-injection flame atomic absorption spectrometry could be increased. One possible method of introduction is feeding a carbon fibre ribbon into the flame as a continuous loop. The solution from the outlet of a manifold could be pumped onto the ribbon and hence carried into the flame.

Development of online sample dissolution methods (section 5.3.1) suffered from many problems. This topic, related to calibration by the need to quantify the amount of analyte dissolved during each dissolution cycle, was only partially examined, although some considerable time was spent on its study. The advantages of such a system to the analyst, were development possible, would be immense, hence this topic warrants further study.

Use of a liquid ion-exchange medium was only briefly studied (section 5.2.2) but these media may offer advantages over their solid counterparts because they can be moved about within a manifold enabling them to be removed from the reaction part of the manifold enabling regeneration to be performed continuously.

The user survey (chapter 6) highlighted the fact that flow injection analysis with its advantages of fast sample throughput, ease of automation etc. is seldom used where routine analyses are performed. In the industrial environment, development of new analytical methods is time
consuming and consequently, especially when techniques that work have already been developed, are not readily undertaken. This problem is further compounded by the lack of a well developed flow injection instrument. Those that are available are generally crude or expensive or both. Much of the work carried out in the field of flow-injection analysis is performed using individual components designed for other uses and made into a flow injection system by the user. Until the instrument quality and sophistication as applied to high pressure liquid chromatography is available, the technique will not be adopted on a large scale.

This study provides some of the information required to enable the choice of calibration strategy to be made for calibration of instruments which have a non-linear response and to be performed in a simple and accurate manner. Although the majority of the aims of the study were fulfilled, new areas for future study have been discovered. This is the inevitable consequence of the progress of a broad research project.
Appendix 1: Mathematical derivations

Common terms used:

A - absorbance
C - concentration
D - dispersion
u - flowrate
V - volume
t - time
t' - peak width
SD - standard deviation
RSD - relative standard deviation

Sub- and superscripts

m - steady state value
p - peak value
STD - the value resulting from the standard
SAM - the value resulting from the sample
i - the incremental value
c - calculated values
k - known values

Other terms are described within the text.
A1.1 Derivation of equations for the standard deviations of SSPD and RMSPD [85]

\[
\text{SSPD} = \sum_{i=1}^{N} \left[ \left( \frac{C_i^i - C_k^i}{C_k^i} \times 100 \right)^2 \right] \tag{A1.1.1}
\]

\[
\text{RMSPD} = \sqrt{\frac{\text{SSPD}}{N}} \tag{A1.1.2}
\]

Let each term in the summation be designated \( T_i \)

\[
\therefore \text{SSPD} = 10^4 (T_1 + \ldots \ldots + T_7) \tag{A1.1.3}
\]

where

\[
T_i = \left( \frac{C_i^i - C_k^i}{C_k^i} \right)^2
\]

assume a RSD of 1% in all \( C_c \) values

\[
i.e. \ \text{SD}(C_c) = 0.01C_c \tag{A1.1.4}
\]

Rules of propagation of random errors [86]

(a) if \( y = q + q_a a + q_b b + \ldots \ldots + q_n n \)

\[
\text{SD}(y) = \sqrt{\left( q_a \text{SD}(a) \right)^2 + \left( q_b \text{SD}(b) \right)^2 + \ldots \left( q_n \text{SD}(n) \right)^2}
\]
(b) if \( y = qa \)
\[
SD(y) = kSD(a)
\]

(c) if \( y = a^2 \)
\[
SD(y) = 12aSD(a)
\]

(d) if \( y = qa^{\frac{1}{2}} \)
\[
SD(y) = 1(q/2a^{\frac{1}{2}})SD(a)
\]

from (a) \( SD \) of \( C^i_c - C^i_k = 0.01C^i_c \)

from (b) \( SD \) of \( (C^i_c - C^i_k)/C^i_k = 0.01C^i_c/C^i_k \)

from (c) \( SD \) of \( [(C^i_c - C^i_k)/C^i_k]^2 \)
\[
= \left| \frac{2(C^i_c - C^i_k) \times 0.01 C^i_c}{C^i_k} \right| \left| \frac{C^i_c}{C^i_k} \right|
\]
\[
= \left| \frac{0.02 C^i_c (C^i_c - C^i_k)}{(C^i_k)^2} \right|
\]

Therefore from (a) \( SD(SPD) \) from equation A1.1.3
\[
= 10^4 \left\{ \sum_{i=1}^{N} \left[ \frac{0.02 C^i_c (C^i_c - C^i_k)}{(C^i_k)^2} \right] \right\}^{\frac{1}{2}}
\]
and from (d) with \( q = \frac{1}{N} \), \( \text{SD(RMSPD)} \)

\[
= \left( \frac{1}{N} \right) \frac{1}{2} \text{SD(SSPD)} \frac{1}{2} \text{SSPD}
\]
A1.2 Concentration of a solution at a merging point.

Considering the manifold, figure A1.2.1

\begin{align*}
C_3 &= \frac{F_1 C_1 + F_2 C_2}{F_1 + F_2} \\
\text{Fig. A1.2.1}
\end{align*}

$C_1$ is the concentration of one solution flowing to the merging point at a flowrate $F_1$. $C_2$ is the concentration of the other solution flowing at a flowrate of $F_2$. $C_3$ is the concentration of the resultant solution which flows at a flowrate of $F_1 + F_2$.

In unit time $F_1$ volumes of $C_1$ merge with $F_2$ volumes of $C_2$.

$\therefore$ concentration $C_3 = \frac{F_1 C_1 + F_2 C_2}{F_1 + F_2}$

A1.2.1
A1.3 Concentration of a solution produced in a 'two pump manifold'

Considering the manifold, figure A1.3.1

![Diagram showing concentration of solutions](image)

Fig. A1.3.1

$C_1$ is the concentration of one solution merged with another of concentration $C_2$ which is flowing to the merging point at $F_2$. The flowrate of $C_1$ entering the system at a flowrate of $F_1$ is adjusted by removing some of the flow, to produce a flowrate of $F_1 - F_2$. The flowrate of the resultant solution, concentration $C_3$, therefore leaves the system at a flowrate of $F_1$.

If this system is compared with that in Appendix A1.2, the flowrate of the resultant solution is only dependent on the flowrate of one solution rather than on both solutions merged.

Rewriting equation A1.2.1 so that the flowrates at the merging point of $C_1$ is $F_1 - F_2$ and $C_2$ is $F_2$, as described above, produces equation A1.3.1
\[ C_3 = \frac{(F_1 - F_2)C_1 + F_2C_2}{F_1} \quad \text{A1.3.1} \]

when \( C_1 = 0 \)

\[ C_3 = \frac{F_2C_2}{F_1} \]

when \( C_2 = 0 \)

\[ C_3 = \frac{(F_1 - F_2)C_1}{F_1} \quad \text{A1.3.2} \]
A1.4 Calculation of flowrate produced from a pump controlled by the computer interface appendix A3.1

Initially, 10,000 pulses are sent to the pump and the volume, \(VOL\), of solution pumped measured. The flowrate produced by the pump when the interface operates in the free running mode, depends on the frequency of pulses produced, equation A3.1.1, and the volume pumped during each pulse. The equation A1.4.1 is derived directly from equation A3.1.1.

\[
\text{Flowrate} = \frac{\text{VOL} \times 1 \times 10^6 \times 60}{10000 (2n + 3.5)^2 / 16}
\]

where \(n\) is the two byte number \(LB + (256 \times HB)\), and \(A\) is a multiple of 16.
A1.5 The number of absorbance values read during operation of the two pump system

500 computer generated pulses produce one revolution of the pump head. The pump head has 10 rollers, therefore the number of computer generated pulses required to produce one pulse in the mixing (section 4.3.2) is 50. If 10 000 computer pulses pumping VOL volumes of liquid, the pulsed mixing pulse rate

\[
= \frac{\text{Flowrate} \times 10 \, 000}{\text{VOL} \times 50} \quad \text{mixing pulses per minute}
\]

where Flowrate is expressed in volumes per minute

Therefore the duration of each mixing pulse

\[
= \frac{\text{VOL} \times 50 \times 50}{\text{Flowrate} \times 10 \, 000} \quad \text{seconds}
\]

If the A/D converter can convert at 50 data values per second, the number of data points required to be read over two mixing pulses

\[
= 50 \times 2 \times \frac{\text{VOL} \times 50 \times 60}{\text{Flowrate} \times 10 \, 000}
\]

\[
= \frac{\text{VOL} \times 30}{\text{Flowrate}}
\]

As the flowrate increases this value decreases, so the actual number of readings read \( R \) is increased by a constant number (200), equation A1.5.1
Flowrate is calculated by equation A1.6.1, and the value VOL is entered during the setting up of the system.
A1.6 Simplification of the peak with calibration equation

\[ t' = \frac{V \ln \left( \frac{C_m}{C'} - 1 \right)}{u} - \frac{V \ln(D-1)}{u} \quad \text{A1.6.1} \]

if \( C' \ll C_m \)

then \( \frac{C_m}{C'} \approx 1 \) and therefore \( \frac{C_m - 1}{C'} \approx \frac{C_m}{C'} \)

therefore

\[ t' = \frac{V \ln C_m}{u} - \frac{V \ln(D-1)}{u} - \frac{V \ln C'}{u} \quad \text{A1.6.2} \]

\[ t' = \frac{V \ln C_m}{u} - \frac{V \ln[C'(D-1)]}{u} \quad \text{A1.6.3} \]

If the concentration is measured at a fixed level, \( C' \) becomes constant, and if the manifold is kept constant (i.e. \( V, u \) and \( D \) are constant) then the term \( \frac{V \ln[C'(D-1)]}{u} \), equation A1.6.3 can be considered as a constant, if the width is measured at a set concentration \( C' \), therefore \( t' \) becomes proportional to \( \ln C_m \).
A1.7 Derivation of Flow Injection Standard Additions

Calibration equations

When samples are injected into a standard stream containing analyte,

\[ C_p = C_{SAM}^p + C_{STD}^p \quad \text{A1.7.1} \]

but \( C_{SAM}^p = \frac{C_{SAM}}{D_{SAM}} \) and \( C_{STD}^p = \frac{C_{STD}}{D_{STD}} \)

\[ C_p = \frac{C_{SAM}}{D_{SAM}} + \frac{C_{STD}}{D_{STD}} \]

but \( D_{STD} = \frac{D_{SAM}}{D_{SAM} - 1} \)

\[ C_p = \frac{C_{SAM}}{D_{SAM}} + \frac{C_{STD}}{D_{STD}} \left( \frac{D_{SAM}}{D_{SAM}} - 1 \right) \quad \text{A1.7.3} \]

When a linear calibration is obtained, as is used for standard additions calibrations, absorbance is proportional to concentration. This gives

\[ A_m^{STD} = k_1 C_m^{STD} \quad \text{A1.7.4} \]

where \( k_1 \) is the slope of a normal calibration graph. In the case of a standard additions calibration, it is
assumed that the standards contain sufficient concentration of interferent to give a constant degree of depression with increasing analyte concentration.

Combining equations A1.7.3 and A1.7.4 gives

\[ A_p = \frac{k \cdot C_{\text{SAM}}}{D \cdot \text{SAM}} + \frac{k \cdot C_{\text{STD}}}{D \cdot \text{SAM}} (D \cdot \text{SAM} - 1) \]

By plotting the peak absorbances obtained when a sample solution is injected into various concentrations of standard, against the concentrations of the standards, a line with slope

\[ k_2 = k_1 \left( \frac{D \cdot \text{SAM} - 1}{D \cdot \text{SAM}} \right) \]

is obtained. The line will intercept the absorbance axis when

\[ A_p = \frac{k \cdot C_{\text{SAM}}}{D \cdot \text{SAM}} \]

and the concentration axis when

\[ C_{\text{SAM}} = -C_{\text{STD}} \left( \frac{D \cdot \text{SAM} - 1}{D \cdot \text{SAM}} \right) \]

If the absorbances of the standards into which injection takes place are plotted against the concentration of these
standards, the slope $k_1$ of this line (a normal calibration) can be measured. Equation A1.7.6 will then yield the sample dispersion $D_{\text{SAM}}$ by substitution of the two slopes of both calibration lines $k_1$ and $k_2$. Equations A1.7.7 and A1.7.8 will then yield the sample concentration.

A third point can be used to calculate the sample concentration. If the two calibration lines intersect then

$$A_{m}^{\text{STD}} = A_{p}$$  \hspace{1cm} \text{A1.7.9}$$

and

$$k_1 C_{m}^{\text{STD}} = k_1 C_{m}^{\text{SAM}} + \frac{k_1 C_{m}^{\text{STD}} (D_{\text{SAM}} - 1)}{D_{\text{SAM}}}$$

$$C_{m}^{\text{SAM}} = C_{m}^{\text{STD}}$$ \hspace{1cm} \text{A1.7.10}$$

Use of the intercept with the absorbance axis, equation A1.7.7, allows concentration to be calculated without extrapolation or interpolation, as the peak absorbance when a sample is injected into the blank can be easily measured. Use of the intercept with the concentration axis, equation A1.7.8, involves extrapolation, similar to a normal standard additions calibration. Both these methods rely on the calculation of sample dispersion from the slopes of the calibration curves, and in one case, the slope of the calibration is used again. Use of the intersection of the two calibration curves involves
interpolation only.
Appendix 2: Computer programs

A2.1 Curve fitting programs
A2.1.1 Baird Quadratic

10 PRINT"C"
20 CLR
30 PRINT"***********************************************POLYNOMIA"
40 INPUT"LABEL ";D$
50 PRINT/P D$
60 INPUT"MAX. NO. OF TERMS REQUIRED [<6] =";TM:IF TM>5 GOTO 60
70 INPUT"NUMBER OF STANDARDS+BLANK [>1] =";N1:IF N1=1 GOTO 7 0
80 INPUT"HIGHEST CONCN. STANDARD =";ZM
90 ZB$=STR$(ZM)
100 MODE GR:REM PLOT ROUTINE****
110 MOVE 20,0
120 AXIS 0,-42,10:RMOVE 10,0:GPRINT [0,31,ZB$
130 MOVE 20,-420
140 AXIS 1,42,10
150 MOVE 20,-420
160 ZB$="+ZH$
170 HSET:MOVE 20,-20:GPRINT [0,0],ZB$="+ZH$
180 DEF FNO(6)=C1 (1)+C1 (2)*X+C1 (3)*X^2+C1 (4)*X^3+C1 (5)*X^4
190 A$="####### ##F#F### ##F### ##F###
200 C$="###F### ##F##### ###F##
210 M1=35
220 DIM Z(TM),A(TM,TM),C1(5),Y(35),U(35,TM),ZP(5,5)
230 DIM W(TM,1),B(TM,TM),I2(TM,3),X(35),Y1(35)
240 DIM Y2(35),R3(35),E2(TM)
250 PRINT"
260 REM
270 IF N2=0 THEN GOSUB 390
280 IF N2=TM GOTO 2060
290 IF N2=N2+1
300 L3=(N1-1)*2+1
310 IF N4=1 THEN 460
320 REM SORT THE DATA
330 GOSUB 470:REM SET UP MATRIX
340 GOSUB 930:REM SQUARE UP THE MATRIX
350 GOSUB 1080:REM GAUSS SOLN.
360 GOSUB 560:REM PRINT RESULTS
370 GOSUB 1450:REM PLOT DATA
380 GOTO 240:REM NEXT
390 REM GET DATA
400 PRINT " " INPUT CONCN. & ABS. VALUES "
410 PRINT"INCLUDE BLANK eg. 0,0":PRINT
420 FOR I=1 TO N1
430 INPUT Y1(I),X(I)
440 CIRCLE Y1(I)*420/ZM,X(I)*420/ZI,2,0,360,90
450 NEXT I
460 RETURN:REM FROM DATA INPUT
470 REM SET UP DATA MATRIX
480 FOR I=1 TO N1
490 U(I,1)=1
500 FOR J=2 TO N2
510 U(I,J)=U(I,J-1)*X(I)
520 NEXT J

-299-
530 Y(I)=Y1(I)
540 NEXT I
550 RETURN:REM FROM SETTING UP DATA MATRIX
560 REM CALC. RESIDS. & PRT RESULTS
570 S7=0
580 S8=0
590 T6=0
600 FOR I=1 TO N1
610 Y2=0
620 FOR J=1 TO N2
630 Y2=Y2+C1(J)*U(I,J)
640 NEXT J
650 R3(I)=Y2-Y(I)
660 Y2(I)=Y2
670 T6=T6+R3(I)*R3(I)
680 S7=S7+Y(I)
690 S8=S8+Y(I)*Y(I)
700 NEXT I
710 IF N2>0 THEN C3=SQR(1-T6/(S8-S7*S7/N1))
720 IF N1=N2 THEN E5=SQR(T6)
730 IF N1<N2 THEN E5=SQR(T6/(N2-N1))
740 IF N1>N2 THEN E5=SQR(T6/(N1-N2))
750 FOR J=1 TO N2
760 E2(J)=E5*SQR(C88(B(J,J)))
770 NEXT J
780 PRINT" ABSO CONCN CONCN*C RESID" 
790 FOR I=1 TO N1
800 PRINT USING A$;I;X(I),Y(I),Y2(I),R3(I)
810 NEXT I
820 PRINT"
830 PRINT"Coefficients Errors"
840 PRINT"
850 FOR I=1 TO N2
860 PRINT USING C$;C1CII,E2CII
870 NEXT I
880 PRINT"
890 PRINT"CORR COEF=1";C3-1
900 ZP(N2,0)=C3
910 RETURN:REM FROM RESULTS PRINT
920 REM U&Y CONVERTED TO A&Z
930 FOR K=1 TO N2
940 FOR L=1 TO K
950 A(K,L)=0
960 FOR I=1 TO N1
970 A(K,L)=A(K,L)+U(I,L)*U(I,K)
980 IF K<L THEN A(L,K)=A(K,L)
990 NEXT I
1000 NEXT L
1010 NEXT K
1020 Z(K)=0
1030 FOR I=1 TO N1
1040 Z(K)=Z(K)+Y(I)*U(I,K)
1050 NEXT I
1060 NEXT K
1070 RETURN:REM FROM SQ
1080 REM GAUSS INV. & SOLN.
1090 E1=0
1100 IS=1
1110 N3=1
1120 FOR I=1 TO N2

-300-
1130 FOR J=1 TO N2
1140 B(I,J)=A(I,J)
1150 NEXT J
1160 W(I,1)=Z(I)
1170 I2(I,3)=0
1180 NEXT I
1190 D3=1
1200 FOR I=1 TO N2
1210 B1=0
1220 FOR J=1 TO N2
1230 IF I2(J,3)=1 THEN 1320
1240 FOR K=1 TO N2
1250 IF I2(K,3)>1 THEN 1920
1260 IF I2(K,3)=1 THEN 1310
1270 IF B1>=ABS(B(J,K)) THEN 1310
1280 I3=J
1290 I4=K
1300 B1=ABS(B(J,K))
1310 NEXT K
1320 NEXT J
1330 I2(I4,3)=I2(I4,3)+1
1340 I2(I,1)=I3
1350 I2(I,2)=I4
1360 IF I3=I4 THEN 1490
1370 D3=-D3
1380 FOR L=1 TO N2
1390 H1=B(I3,L)
1400 B(I3,L)=B(I4,L)
1410 B(I4,L)=H1
1420 NEXT L
1430 IF N3<1 THEN 1490
1440 FOR L=1 TO N3
1450 H1=W(I3,L)
1460 W(I3,L)=W(I4,L)
1470 W(I4,L)=H1
1480 NEXT L
1490 P1=B(I4,I4)
1500 D3=D3*P1
1510 B(I4,I4)=1
1520 FOR L=1 TO N2
1530 B(I4,L)=B(I4,L)/P1
1540 NEXT L
1550 IF N3<1 THEN 1590
1560 FOR L=1 TO N3
1570 W(I4,L)=W(I4,L)/P1
1580 NEXT L
1590 FOR L1 =1 TO N2
1600 IF L1=I4 THEN 1700
1610 T=B(L1,I4)
1620 B(L1,I4)=0
1630 FOR L=1 TO N2
1640 B(L1,L)=B(L1,L)-B(I4,L)*T
1650 NEXT L
1660 IF N3<1 THEN 1700
1670 FOR L=1 TO N3
1680 W(L1,L)=W(L1,L)-W(I4,L)*T
1690 NEXT L
1700 NEXT L1
1710 NEXT I
1720 FOR I=1 TO N2

-301-
1730 L=N2-I+1
1740 IF I2(L,1)=I2(L,2) THEN 1920
1750 T=I2(L,1)
1760 I4=I2(L,2)
1770 FOR K=1 TO N2
1780 H1=E(K,1)
1790 B(K,13)=B(K,14)
1800 B(K,14)=H1
1810 NEXT K
1820 NEXT I
1830 FOR K=1 TO N2
1840 IF I2(K,3)<1 THEN 1920
1850 NEXT K
1860 E1=0
1870 FOR I=1 TO N2
1880 C1(I)=W(I,1)
1890 NEXT I
1900 IF IS=1 THEN 1940
1910 PRINT""
1920 E1=1
1930 PRINT"ERROR*MATRIX SINGULAR"
1940 RETURN:REM FROM G. SUBR
1950 REM PLOT ROUTINE************
1960 IF S=3 THEN S=-1
1970 S=S+1:GF=0 :PCOLOR S
1980 PHOME
1990 FOR I=0 TO ZI STEP .02*ZI
2000 IF FNQ(I)*420/ZM>420 THEN MOVE 420,420+I/ZI:GOTO 2030
2010 IF FNQ(I)*420/ZM<0 THEN MOVE 0,420+I/ZI:GOTO 2030
2020 LINE FNQ(I)*420/ZM ,I*420/ZI
2030 NEXT I
2040 PHOME
2050 RETURN
2060 MOVE -20,-50:MODE TN
2070 PRINT/P "PARAMETERS, INCLUDING CONSTANT"
2080 PRINT/P "="
2090 PRINT/P "CONS x x^2 x^3 x^4 CONSTANT"
2100 PRINT/P "-----------------------------------""1
2110 DS=3: IF N2<3 LET DS=N2
2120 FOR T=1 TO DS:PCOLOR T:PRINT/P T:NEXT T
2130 IF N2=4 THEN PCOLOR 0:PRINT/P 4
2140 IF N2=5 THEN PCOLOR 1:PRINT/P 5
2150 SKIP -N2:MODE TS
2160 ZW$="####,##### "
2170 FOR I=1 TO N2
2180 PRINT/P " " USING ZW$:ZP(I,1),ZP(I,2),ZP(I,3),ZP(I,4)
2190 PRINT/P " WHICH FUNCTION DO YOU REQUIRE? 1>";N2:INPUT ZJ
2200 PRINT/P " DO YOU WANT RESULTS PRINTED?":DD$:IF LEFT$(DD$, 1)="Y" THEN PLOT
2210 PRINT/P "ESCAPE 'ABS TRAP' TYPE ANY LETTER"
2220 INPUT "DO YOU WANT RESULTS PRINTED? ":DD$:IF LEFT$(DD$, 1)="Y" THEN PLOT
2230 DEF FNW(ZS)=ZP(ZJ,1)+ZP(ZJ,2)*ZS+ZP(ZJ,3)*ZS^2+ZP(ZJ,4)
2240 PRINT/P " EQUATION ":ZJ
2250 PRINT/P " NOW GIVE ME ABSORBANCE VALUES AND I'LL RETURN CONCENTRATIONS"
2260 COLOR ,.,,ZJ
2270 ON ERROR GOTO 2300
2280 INPUT "ABS=": ZS
2290 PRINT "CONC=": FNW(ZS): GOTO 2280
2300 PLOTOFF: PCOLOR 0: COLOR, 7, 1: END
A2.1.2 Baird Rational
10 PRINT "C"
20 CLR
30 PRINT "****************************************************************** QUOTIENT"
   "LEAST* SQUARES CALIBRATION ******************************************************************"
   "****************************************************************** PRINT PRINT"
40 INPUT "LABL=":D$
50 PRINT/P D$
55 PRINT/P "ABS/CONC=P+Q(ABS^2)+....."
60 INPUT "MAX. NO. OF TERMS REQUIRED [6] =":TM:IF TM>5 GOTO 60
70 INPUT "NUMBER OF STANDARDS+BLANK [>1] =":N1:IF N1=1 GOTO 70
80 INPUT "HIGHEST CONCN. STANDARD =":ZM
85 INPUT "HIGHEST ABSORBANCE =":ZI
90 ZB$=STR$(ZM)
95 ZH$=STR$(ZI)
100 MODE GR:REM PLOT ROUTINE**
110 MOVE 20,0
115 ZG$=" "
120 AXIS 0,-42,10:MOVE -10,0:GPRINT [0,3],ZB$
130 MOVE 20,-420
140 AXIS 1,42,10
150 MOVE 20,-420
160 ZX$=" "
170 HSET:MOVE 20,-20:GPRINT [0,0],ZX$:PHOME:PCOLOR 3
180 DEF FN0(G)=G*(C1(1)+C1(2)+G+C1(3)*G^2+C1(4)*G^3+C1(5)*G^4)
190 A$=" ### "
200 C$=" ### "
210 M1=35
220 DIM Z(TM),A(TM,TM),C1(5),Y(35),U(35,TM),ZP(5,5)
230 DIM W(TM,1),B(TM,TM),I2(TM,3),X(35),Y1(35)
240 DIM Y2(35),R3(35),E2(TM)
250 PRINT"
260 REM
270 IF N2=0 THEN GOSUB 390
280 IF N2=TM GOTO 2060
290 N2=N2+1
300 LI=(N1-1)*2+1
310 IF N4=1 THEN 460
320 REM SORT THE DATA
330 GOSUB 470:REM SET UP MATRIX
340 GOSUB 930:REM SQUARE UP THE MATRIX
350 GOSUB 1090:REM GAUSS SOLN.
360 GOSUB 560:REM PRINT RESULTS
370 GOSUB 1950:REM PLOT DATA
380 GOTO 240:REM NEXT
390 REM GET DATA
400 PRINT " INPUT CONCN. & ABS. VALUES "
410 PRINT "INCLUDE BLANK eg. 0,0":PRINT
420 FOR I=1 TO N1
430 INPUT Y1(I),X(I)
440 CIRCLE Y1(I)*420/ZM,X(I)*420/ZI,2,0,360,90
450 NEXT I
460 RETURN:REM FROM DATA INPUT
470 REM SET UP DATA MATRIX
480 FOR I=1 TO N1
490 U(I,1)=1
500 FOR J=2 TO N2

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510 U(I,J)=U(I,J-1)*X(I)
520 NEXT J
530 Y(I)=X(I)/Y1(I)
540 NEXT I
550 RETURN:REM FROM SETTING UP DATA MATRIX
560 REM CALC. RESIDS. & PRRT. RESULTS
570 S7=0
590 S8=0
600 T6=0
610 FOR I=1 TO N1
620 Y2=0
630 Y2=Y2+C1(J)*U(I,J)
640 NEXT J
650 R3(I)=Y2-Y(I)
660 Y2=Y2
670 T6=T6+R3(I)*R3(I)
680 S7=S7+Y(I)
690 S8=S8+Y(I)*Y(I)
700 NEXT I
710 IF N2>0 THEN C3=SQR(1-T6/(S8-S7*S7/N1))
720 IF N1=N2 THEN C5=SQR(T6)
730 IF N1<N2 THEN C5=SQR(T6/(N2-N1))
740 IF N1>N2 THEN C5=SQR(T6/(N1-N2))
750 FOR J=1 TO N2
760 E2(J)=C5*SQR(ABS(B(J,J)))
770 NEXT J
780 PRINT" ABSO CONCN CONCN*C RESID"
790 FOR I=1 TO N1
800 PRINT USING "A$;I;X(I),Y(I),Y2(I),R3(I)
810 NEXT I
820 PRINT""
830 PRINT"Coefficients Errors"
840 PRINT"
850 FOR I=1 TO N2
860 PRINT USING C$;C1(I),E2(I)
870 ZP(N2,I)=C1(I)
880 NEXT I
890 PRINT"
900 PRINT"CORR COEF=1+": C3=1
910 ZP(N2,0)=C3
920 RETURN:REM FROM RESULTS PRINT
930 REM U&Y CONVERTED TO A&Z
940 FOR K=1 TO N2
950 FOR L=1 TO K
960 A(K,L)=0
970 FOR I=1 TO N1
980 A(K,L)=A(K,L)+U(I,L)*U(I,K)
990 IF K>L THEN A(L,K)=A(K,L)
1000 NEXT I
1010 NEXT L
1020 Z(K)=0
1030 FOR I=1 TO N1
1040 Z(K)=Z(K)+Y(I)*U(I,K)
1050 NEXT I
1060 NEXT K
1070 RETURN:REM FROM SQ
1080 REM GAUSS INV. & SOLN.
1090 E1=0
1100 I5=1

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1110 N3=1
1120 FOR I=1 TO N2
1130 FOR J=1 TO N2
1140 B(I,J)=A(I,J)
1150 NEXT J
1160 W(1,1)=Z(1)
1170 I2(I,3)=0
1180 NEXT I
1190 D3=1
1200 FOR I=1 TO N2
1210 B1=0
1220 FOR J=1 TO N2
1230 IF I2(J,3)=1 THEN 1320
1240 FOR K=1 TO N2
1250 IF I2(K,3)>1 THEN 1920
1260 IF I2(K,3)=1 THEN 1310
1270 IF B1>=ABS(B(J,K)) THEN 1310
1280 I3=J
1290 I4=K
1300 B1=ABS(B(J,K))
1310 NEXT K
1320 NEXT J
1330 I2(I4,3)=I2(I4,3)+1
1340 I2(I,1)=I3
1350 I2(I,2)=I4
1360 IF I3=I4 THEN 1490
1370 D3=-D3
1380 FOR L=1 TO N2
1390 H1=B(I3,L)
1400 B(I3,L)=B(I4,L)
1410 B(I4,L)=H1
1420 NEXT L
1430 IF N3<1 THEN 1490
1440 FOR L=1 TO N3
1450 H1=W(I3,L)
1460 W(I3,L)=W(I4,L)
1470 W(I4,L)=H1
1480 NEXT L
1490 P1=B(I4,I4)
1500 D3=D3*P1
1510 B(I4,I4)=1
1520 FOR L=1 TO N2
1530 B(I4,L)=B(I4,L)/P1
1540 NEXT L
1550 IF N3<1 THEN 1590
1560 FOR L=1 TO N3
1570 W(I4,L)=W(I4,L)/P1
1580 NEXT L
1590 FOR L1 =1 TO N2
1600 IF L1=I4 THEN 1700
1610 T=B(L1,I4)
1620 B(L1,I4)=0
1630 FOR L=1 TO N2
1640 B(L1,L)=B(L1,L)-B(I4,L)*T
1650 NEXT L
1660 IF N3<1 THEN 1700
1670 FOR L=1 TO N3
1680 W(L1,L)=W(L1,L)-W(I4,L)*T
1690 NEXT L
1700 NEXT L1
1710 NEXT I
1720 FOR I=1 TO N2
1730 L=N2-I+1
1740 IF I2(L,1)=I2(L,2) THEN 1320
1750 I3=I2(L,1)
1760 I4=I2(L,2)
1770 FOR K=1 TO N2
1780 H(I)=B(K,I3)
1790 B(K,I3)=B(K,I4)
1800 B(K,I4)=H(I)
1810 NEXT K
1820 NEXT I
1830 FOR K=1 TO N2
1840 IF I2(K,3)<1 THEN 1920
1850 NEXT K
1860 E1=0
1870 FOR I=1 TO N2
1880 C1(I)=W(I,1)
1890 NEXT I
1900 IF I3=1 THEN 1940
1910 PRINT"
1920 E1=1
1930 PRINT"ERROR*MATRIX SINGULAR"
1940 RETURN:REM FROM G. SUBR
1950 REM PLOT ROUTINE***********
1960 IF S=3 THEN S=-1
1970 S=S+1:GF=0:PCOLOR S
1980 PHOME
1990 FOR I=0 TO ZI STEP .02*ZI
2000 IF FN0(I)*420/ZM>420 THEN MOVE 420,420*I/ZI:GOTO 2030
2010 IF FN0(I)*420/ZM<0 THEN MOVE -420,420*I/ZI:GOTO 2030
2020 LINE FN0(I)*420/ZM,1*420/ZI
2030 NEXT I
2040 PHOME
2050 RETURN
2060 MOVE -20,-50:HSET:MODE TN
2070 PRINT/P "PARAMETERS:INCLUDING CONSTANT"
2080 PRINT/P "=====================================
" 2090 PRINT/P "CONS x x^2 x^3 x^4 CDDCOE"
2100 PRINT/P "---------------------------------------" 2110 DS=3: IF N2=<3 LET DS=N2
2120 FOR T=1 TO DS:PCOLOR T:PRINT/P: T:NEXT T
2130 IF N2=4 THEN PCOLOR 0:PRINT/P 4
2140 IF N2=5 THEN PCOLOR 1:PRINT/P 5
2150 SKIP -N2:MODE TS
2160 ZWS="##lt# ##" "
2170 FOR I=1 TO N2
2180 PRINT/P " " ; USING ZWS;ZP(I,1),ZP(I,2),ZP(I,3),ZP(I,4),ZP(I,5),ZP(I,0):NEXT I
2190 MODE TN
2200 PRINT[7,3] "WHICH FUNCTION DO YOU REQUIRE? 1>";N2:INPUT ZJ
2210 PRINT"TO ESCAPE "ABS TRAP" TYPE ANY LETTER"
2220 INPUT "DO YOU WANT RESULTS PRINTED? 1>";DD$:IF LEFT$(DD$, 1)="Y" THEN PLT0N
2230 DEF FNW(ZS)=ZS/(ZP(ZJ,1)+ZP(ZJ,2)*ZS+ZP(ZJ,3)*ZS^2+ZP(ZJ,4)*ZS^3+ZP(ZJ,5)*ZS^4)
2240 PRINT "EQUATION " ;ZJ
2250 PRINT "NOW GIVE ME ABOIlRANCE VALUES AND I'LL RETURN C
ONCENTRATIONS"
2260 COLOR ,,7,2
2270 ON ERROR GOTO 2300
2280 INPUT"ABS=";ZS
2290 PRINT"CONC=";FNW(ZS):GOTO 2280
2300 PLOTcff:PCOLOR 0:COLOR,,7,1:END
**A2.1.3 IL Cubic 1**

10 PRINT "C"
20 CLR
30 PRINT "*********************************************************************************** INS LAB LEAST-SQUARES CALIBRATION ***********************************************************************************:*PRINT:PRINT
40 INPUT "LABEL "; D#
50 PRINT /F D#
55 PRINT /F " CONCNC=O(ABS)+R(ABS^2)+...
60 INPUT " MAX. NO. OF TERMS REQUIRED [=3?] "; TM: IF TM<>5 GoTo 60
70 INPUT " NUMBER OF STANDARDS+BLANK [=11] "; N1: IF N1<>1 GoTo 70
80 INPUT " HIGHEST CONCNC. STANDARD ="; ZM
85 INPUT " HIGHEST ABSORBANCE ="; ZI
90 ZB$=STR$(ZM)
95 ZH$=STR$(ZI)
100 MODE GR:REM PLOT ROUTINE****
110 MOVe 20,0
115 ZG$=" 
120 AXIS 0,-42,10:RMovE -10,0:GPRINT [0,31,Zh$]
130 MOVe 20,-420
140 AXIS 1,42,10
150 MOVe 20,-420
160 ZG$=" CONCENTRATION 0">=ZG$+"ppm"
170 HSET:MOVe 20,-20:GPRINT [0,0],ZH$:PHome:FCOLoR 3
180 DEF FNOIGI=C1111•G+C1121•G-2+C1131•G-3+C1141•G-4+C1151•G-5
190 A$=" 
200 C$=" 
210 TM=35
220 DIM Z(TM),A(TM,TM),C1(5),Y(35),U(35,TM),ZP(5,5)
230 DIM W(TM,1),B(TM,TM),I2(TM,3),X(35),Y1(35)
240 DIM Y2(35),R3(35),E2(TM)
250 PRINT"
260 REM
270 IF N2=0 THEN GOSUB 390
280 IF N2=TM GOTO 2060
290 N2=N2+1
300 L3=(N1-1)*2+1
310 IF N4<>1 THEN 460
320 REM SORT THE DATA
330 GOSUB 470:REM SET UP MATRIX
340 GOSUB 930:REM SQUARE UP THE MATRIX
350 GOSUB 1080:REM GAUSS SOLN.
360 GOSUB 560:REM PRINT RESULTS
370 GOSUB 1950:REM PLOT DATA
380 GOTO 240:REM NEXT
390 REM GET DATA
400 PRINT " INPUT CONCNC. & ABS. VALUES 
410 PRINT " INCLUDE BLANK eg. O,0":PRINT
420 FOR I=1 TO N1
430 INPUT Y1(I),X(I)
440 CIRCLE Y1(I)*420/ZM,X(I)*420/ZI,2,0,360,90
450 NEXT I
460 RETURN:REM FROM DATA INPUT
470 REM SET UP DATA MATRIX
480 FOR I=1 TO N1
490 Y1(I,1)=X(I)
500 FOR J=2 TO N2

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510 U(I,J)=U(I,J-1)*X(I)
520 NEXT J
530 Y(I)=Y1(I)
540 NEXT I
550 RETURN: REM FROM SETTING UP DATA MATRIX
560 REM CALC. RESIDS. & PRT RESULTS
570 S7=0
580 SB=0
590 T6=0
600 FOR I=1 TO N1
610 Y2=0
620 FOR J=1 TO N2
630 Y2=Y2+C1(J)*U(I,J)
640 NEXT J
650 R3(I)=Y2-Y(I)
660 Y2(I)=Y2
670 T6=T6+R3(I)*R3(I)
680 S7=S7+Y(I)
690 SB=SB+Y(I)*Y(I)
700 NEXT I
710 IF N2=0 THEN C3=SQR(1-T6/(S8-S7*S7/N1))
720 IF N1=N2 THEN E5=SQR(T6)
730 IF N1<N2 THEN E5=SQR(T6/(N2-N1))
740 IF N1>N2 THEN E5=SQR(T6/(N1-N2))
750 FOR J=1 TO N2
760 E2(J)=E5*SQR(ABS(B(J,J)))
770 NEXT J
780 PRINT" ABSO CONCN CONCN*C RESID"
790 FOR I=1 TO N1
800 PRINT USING A$;I;X(I),Y(I),Y2(I),R3(I)
810 NEXT I
820 PRINT"
830 PRINT"Coefficients Errors"
840 PRINT"
850 FOR I=1 TO N2
860 PRINT USING C$;C1(I),E2(I)
870 ZP(N2,I)=C1(I)
880 NEXT I
890 PRINT"
900 PRINT"CORR COEF=1+"; C3-1
910 ZP(N2,0)=C3
920 RETURN: REM FROM RESULTS PRINT
930 REM U&Y CONVERTED TO A&Z
940 FOR K=1 TO N2
950 FOR L=1 TO K
960 A(K,L)=0
970 FOR I=1 TO N1
980 A(K,L)=A(K,L)+U(I,L)*U(I,K)
990 IF K>L THEN A(L,K)=A(K,L)
1000 NEXT I
1010 NEXT L
1020 Z(K)=0
1030 FOR I=1 TO N1
1040 Z(K)=Z(K)+Y(I)*U(I,K)
1050 NEXT I
1060 NEXT K
1070 RETURN: REM FROM SQ
1080 REM GAUSS INV. & SOLN.
1090 E1=0
1100 I5=1
1110 N3=1
1120 FOR I=1 TO N2
1130 FOR J=1 TO N2
1140 B(I,J)=A(I,J)
1150 NEXT J
1160 W(I,1)=Z(I)
1170 I2(I,3)=0
1180 NEXT I
1190 D3=1
1200 FOR I=1 TO N2
1210 B1=0
1220 FOR J=1 TO N2
1230 IF I2(J,3)=1 THEN 1320
1240 FOR K=1 TO N2
1250 IF I2(K,3)>1 THEN 1920
1260 IF I2(K,3)=1 THEN 1310
1270 IF B1=ABS(B(J,K)) THEN 1310
1280 I3=J
1290 I4=K
1300 B1=ABS(B(J,K))
1310 NEXT K
1320 NEXT J
1330 I2(I4,3)=I2(I4,3)+1
1340 I2(I,1)=I3
1350 I2(I,2)=I4
1360 IF I3=I4 THEN 1490
1370 D3=-D3
1380 FOR L=1 TO N2
1390 H1=B(I3,L)
1400 B(I3,L)=B(I4,L)
1410 B(I4,L)=H1
1420 NEXT L
1430 IF N3<1 THEN 1490
1440 FOR L=1 TO N3
1450 H1=W(I3,L)
1460 W(I3,L)=W(I4,L)
1470 W(I4,L)=H1
1480 NEXT L
1490 P1=B(I4,I4)
1500 D3=D3*P1
1510 B(I4,I4)=1
1520 FOR L=1 TO N2
1530 B(I4,L)=B(I4,L)/P1
1540 NEXT L
1550 IF N3<1 THEN 1590
1560 FOR L=1 TO N3
1570 W(I4,L)=W(I4,L)/P1
1580 NEXT L
1590 FOR L1=1 TO N2
1600 IF L1=I4 THEN 1700
1610 T=B(L1,I4)
1620 B(L1,I4)=0
1630 FOR L=1 TO N2
1640 B(L1,L)=B(L1,L)-B(I4,L)*T
1650 NEXT L
1660 IF N3<1 THEN 1700
1670 FOR L=1 TO N3
1680 W(L1,L)=W(L1,L)-W(I4,L)*T
1690 NEXT L
1700 NEXT L
1710 NEXT I
1720 FOR I=1 TO N2
1730 L=N2-I+1
1740 IF I2(L,1)=I2(L,2) THEN 1820
1750 IS=I2(L,1)
1760 I4=I2(L,2)
1770 FOR K=1 TO N2
1780 H1=B(K,IS)
1790 B(K,I4)=B(K,I4)
1800 B(K,I4)=H1
1810 NEXT K
1820 NEXT I
1830 FOR K=1 TO N2
1840 IF I2(K,3)>1 THEN 1920
1850 NEXT K
1860 E1=0
1870 FOR I=1 TO N2
1880 CI(I)=W(I,1)
1890 NEXT I
1900 IF IS=1 THEN 1940
1910 PRINT""
1920 E1=1
1930 PRINT"ERROR-MATRIX SINGULAR"
1940 RETURN REM FROM 6. SUBR
1950 REM PLOT ROUTINE***********
1960 IF S=3 THEN S=-1
1970 S=S+1: GF=0: PCOLOR S
1980 PHOME
1990 FOR I=0 TO Zi STEP .02*ZI
2000 IF FNO(I)*420/ZM>420 THEN MOVE 420, 420*: GOTO 2030
2010 IF FNO(I)*420/ZM<0 THEN MOVE 0,420*1/ZM: GOTO 2030
2020 LINE FNO(I)*420/ZM, I*420/ZM
2030 NEXT I
2040 PHOME
2050 RETURN
2060 MOVE -20, -50: HSET: MODE TN
2070 PRINT/P "PARAMETERS"
2080 PRINT/P "==================================" 
2090 PRINT/P "x x^2 x^3 x^4 x^5 CODE" 
2100 PRINT/P "=================================="
2110 DS=3: IF N2<3 LET DS=N2
2120 FOR T=1 TO DS:PCOLOR T:PRINT/P T:NEXT T
2130 IF N2>=4 THEN PCOLOR 0:PRINT/P 4
2140 IF N2=5 THEN PCOLOR 1:PRINT/P 5
2150 SKIP -N2: MODE TS
2160 ZW$="####.##~--~--~", 
2170 FOR I=1 TO N2
2180 PRINT/P "\ USING ZW$=ZP(I,1),ZP(I,2),ZP(I,3),ZP(I,4),ZP(I,5),ZP(I,6)" 
2190 PRINT/P "\ WHICH FUNCTION DO YOU REQUIRE? 1="; N2:INPUT ZJ
2200 PRINT"TO ESCAPE 'ABS TRAP' TYPE ANY LETTER"
2210 PRINT"DO YOU WANT RESULTS PRINTED? "; DD$: IF LEFT$(DD$, 1)="Y" THEN PLTTON
2220 IF FFN(WZ)=ZP(ZJ,1)*ZS+ZP(ZJ,2)*ZS^2+ZP(ZJ,3)*ZS^3+ZP(ZJ,4)*ZS^4+ZP(ZJ,5)*ZS^5 THEN 2230 DEF FNW(ZS)=ZP(ZJ,1)*ZS+ZP(ZJ,2)*ZS^2+ZP(ZJ,3)*ZS^3+ZP(ZJ,4)*ZS^4+ZP(ZJ,5)*ZS^5
2240 PRINT" EQUATION " ZJ
2250 PRINT"NOW GIVE ME ABORBEANCE VALUES AND I'LL RETURN C ONCENTRATIONS"
2260 COLOR ,,7,2
2270 ON ERROR GOTO 2300
2280 INPUT "ABS=":ZS
2290 PRINT "CONC=":ENW(ZS):GOTO 2280
2300 PLOTOFF:PCOLOR 0:COLOR ,,7,1:END
A2.1.4 IL Cubic 2

10 PRINT"C"
20 CLR
30 PRINT"******************************************************************************NEW VERSION OF IL A=f(A) CALIBRATION******************************************************************************"PRINT:PRINT
40 INPUT"LABEL ":D$
50 PRINT"F INPUT";D$
60 TM=3
70 INPUT"NUMBER OF STANDARDS=";N1
80 INPUT"HIGHEST CONC. STANDARD =";ZM
90 INPUT"HIGHEST ABSORBANCE =";ZI
100 ZB$=STR$(ZM)
110 ZH$=STR$(ZI)
120 MODE GR:REM PLOT ROUTINE****
130 MOVE 20,0
140 ZG$=" ABSORBANCE 0>"+ZH$
150 AXIS 0,-42,10:RMOVE -10,0:GPRINT 0,33,ZB$
160 MOVE 20,-420
170 AXIS 1,42,10
180 MOVE 20,-420
190 ZX$=" CONCENTRATION 0>"+ZB$+"ppm"
200 HSET:MOVE 20,-200:GPRINT 0,01,ZX$:PHOME:PCOLOR 3
210 DEF FND(G)=C1(1)*G+C1(2)*G^2+C1(3)*G^3
220 A$=" " REM GET DATA
230 C$=" " REM GET DATA
240 M1=35
250 DIM Z(TM),A(TM,TM),C1(5),Y(35),U(35,TM),ZP(5,5)
260 DIM W(TM,1),B(TM,TM),I2(TM,3),X(35),Y1(35)
270 DIM Y2(35),R3(35),E2(TM)
280 PRINT"
290 REM
300 IF N2=0 THEN GOSUB 420
310 IF N2=TM GOTO 2090
320 N2=N2+1
330 L3=(N1-1)*2+1
340 IF N4=1 THEN 490
350 REM SORT THE DATA
360 GOSUB 500:REM SET UP MATRIX
370 GOSUB 960:REM SQUARE UP THE MATRIX
380 GOSUB 1110:REM GAUSS SOLN.
390 GOSUB 590:REM PRINT RESULTS
400 GOSUB 1980:REM PLOT DATA
410 GOTO 270:REM NEXT
420 REM GET DATA
430 PRINT " INPUT CONC. & ABS. VALUES ";PRINT
440 PRINT"INCLUDE BLANK eg. 0,0";PRINT
450 FOR I=1 TO N1
460 INPUT Y1(I),X(I)
470 CIRCLE Y1(I)*420/ZM,X(I)*420/ZI,2,0,360,90
480 NEXT I
490 RETURN:REM FROM DATA INPUT
500 REM SET UP DATA MATRIX
510 FOR I=1 TO N1
520 U(I,1)=Y1(I)
530 FOR J=2 TO N2
540 U(I,J)=U(I,J-1)*Y1(I)
550 NEXT J
560 Y(I)=X(I)
570 NEXT I

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580 RETURN: REM FROM SETTING UP DATA MATRIX
590 REM CALC. RESIDS. & PRT RESULTS
600 S7=0
610 SB=0
620 T6=0
630 FOR I=1 TO N1
640 Y2=0
650 FOR J=1 TO N2
660 Y2=Y2+C1(J)*U(I,J)
670 NEXT J
680 R3(I)=Y2-Y(I)
690 Y2(I)=Y2
700 T6=T6+R3(I)*R3(I)
710 S7=S7+Y(I)
720 SB=SB+Y(I)*Y(I)
730 NEXT I
740 IF N2>0 THEN C3=SQR((1-T6/(SB-S7*S7/N1))
750 IF N1=N2 THEN E5=SQR(T6)
760 IF N1>N2 THEN E5=SQR(T6/(N2-N1))
770 IF N1>N2 THEN E5=SQR(T6/(N1-N2))
780 FOR J=1 TO N2
790 E2(J)=E5*SQR(ABS(B(J,J)))
800 NEXT J
810 PRINT" ABDI CONCN CONCN*C RESID"
820 FOR I=1 TO N1
830 PRINT USING A$;I;X(I),Y(I),Y2(I),R3(I)
840 NEXT I
850 PRINT"
860 PRINT"Coefficients Errors"
870 PRINT"
880 FOR I=1 TO N2
890 PRINT USING C$;C1(I),E2(I)
900 ZIP(N2,I)=C1(I)
910 NEXT I
920 PRINT"
930 PRINT"CORR COEF=1+"; C3-1
940 ZIP(N2,0)=C3
950 RETURN: REM FROM RESULTS PRINT
960 REM U&Y CONVERTED TO A&Z
970 FOR K=1 TO N2
980 FOR L=1 TO K
990 A(K,L)=0
1000 FOR I=1 TO N1
1010 A(K,L)=A(K,L)+U(I,L)*U(I,K)
1020 IF K<>L THEN A(L,K)=A(K,L)
1030 NEXT I
1040 NEXT L
1050 Z(K)=0
1060 FOR I=1 TO N1
1070 Z(K)=Z(K)+Y(I)*U(I,K)
1080 NEXT I
1090 NEXT K
1100 RETURN: REM FROM SQ
1110 REM GAUSS INV. & SOLN.
1120 E1=0
1130 IS=1
1140 N3=1
1150 FOR I=1 TO N2
1160 FOR J=1 TO N2
1170 B(I,J)=A(I,J)
1180 NEXT J
1190 W(I,1)=Z(I)
1200 IZ(I,3)=0
1210 NEXT I
1220 D3=1
1230 FOR I=1 TO N2
1240 B1=0
1250 FOR J=1 TO N2
1260 IF IZ(J,3)=1 THEN 1350
1270 FOR K=1 TO N2
1280 IF IZ(K,3)>1 THEN 1950
1290 IF IZ(K,3)=1 THEN 1340
1300 IF B1>=ABS(B(J,K)) THEN 1340
1310 I3=J
1320 I4=K
1330 B1=ABS(B(J,K))
1340 NEXT K
1350 NEXT J
1360 IZ(I4,3)=IZ(I4,3)+1
1370 IZ(I,1)=I3
1380 IZ(I,2)=I4
1390 IF I3=I4 THEN 1520
1400 D3=-D3
1410 FOR L=1 TO N2
1420 H1=B(I3,L)
1430 B(I3,L)=B(I4,L)
1440 B(I4,L)=H1
1450 NEXT L
1460 IF N3<1 THEN 1520
1470 FOR L=1 TO N3
1480 H1=W(I3,L)
1490 W(I3,L)=W(I4,L)
1500 W(I4,L)=H1
1510 NEXT L
1520 P1=B(I4,I4)
1530 D3=D3*P1
1540 B(I4,I4)=1
1550 FOR L=1 TO N2
1560 B(I4,L)=B(I4,L)/P1
1570 NEXT L
1580 IF N3<1 THEN 1620
1590 FOR L=1 TO N3
1600 W(I4,L)=W(I4,L)/P1
1610 NEXT L
1620 FOR L1=1 TO N2
1630 IF L1=I4 THEN 1730
1640 T=B(L1,I4)
1650 B(L1,I4)=0
1660 FOR L=1 TO N2
1670 B(L1,L)=B(L1,L)-B(I4,L)*T
1680 NEXT L
1690 IF N3<1 THEN 1730
1700 FOR L=1 TO N3
1710 W(L1,L)=W(L1,L)-W(I4,L)*T
1720 NEXT L
1730 NEXT L1
1740 NEXT I
1750 FOR I=1 TO N2
1760 L=N2-I+1
1770 IF IZ(L,1)=IZ(L,2) THEN 1850
1780 I3=I2(L,1)
1790 I4=I2(L,2)
1800 FOR K=1 TO N2
1810 H1=B(K,13)
1820 B(K,13)=B(K,14)
1830 B(K,14)=H1
1840 NEXT K
1850 NEXT I
1860 FOR K=1 TO N2
1870 IF I2(K,3)<1 THEN 1950
1880 NEXT K
1890 E1=0
1900 FOR I=1 TO N2
1910 C1(I)=W(I,1,1)
1920 NEXT I
1930 IF I5=1 THEN 1970
1940 PRINT""
1950 E1=1
1960 PRINT"ERROR\MATRIX SINGULAR"
1970 RETURN:REM FROM G. SUBR
1980 REM PLOT ROUTINE**********
1990 IF S=3 THEN S=-1
2000 S=S+1:GF=0 :PCOLOR S
2010 PHOME
2020 FOR I=0 TO ZM STEP .02*ZM
2030 IF FNO(I)*420/ZI>420 THEN MOVE 420,420*1/ZM :GOTO 2060
2040 IF FNO(I)*420/ZI<0 THEN MOVE 0,420+1/ZM:GOTO 2060
2050 LINEI*420/ZM,FNO(I)*420/ZI
2060 NEXT I
2070 PHOME
2080 RETURN
2090 MOVE -20,-50:HSET:MODE TN
2100 PRINT/P "PARAMETERS, INCLUDING CONSTANT"
2110 PRINT/P "--------------------------------------" CDOE
2120 PRINT/P "--------------------------------------"
2130 PRINT/P "--------------------------------------"
2140 DS=3: IF N2=3 LET DS=N2
2150 FOR T=1 TO DS:PCOLOR T:PRINT/P T:NEXT T
2160 IF N2=>4 THEN PCOLOR 0:PRINT/P 4
2170 IF N2=5 THEN PCOLOR 1:PRINT/P 5
2180 SKIP-N2:MODE TS
2190 ZW$=" #\#\#\#\#\#\#\#\#\#\#\#\#\#" 
2200 FOR I=1 TO N2
2210 PRINT/P " USING ZW$;ZP(I,1),ZP(I,2),ZP(I,3),ZP(I,0)
2220 NEXT I
2230 MODE TN
2240 PRINT[7,3] " WHICH FUNCTION DO YOU REQUIRE? 1">":N2:INPUT ZJ
2250 PRINT"TO ESCAPE 'ABS TRAP' TYPE ANY LETTER"
2260 PRINT"DO YOU WANT RESULTS PRINTED? ";DD$:IF LEFT$(DD$, 1)="Y" THEN PLOTN
2270 PRINT "EQUATION ";ZJ
2280 PRINT NOW GIVE ME ABSORBANCE VALUES AND I'LL RETURN CONCENTRATIONS"
2290 COLOR,,7,2
2300 ON ERROR GOTO 2400
2310 INPUT"ABS";ZS
2320 T1=.0000001:X=3
2330 GOSUB 2340
2340 GOTO 2390
2340 X1=X
2350 GOSUB 2385
2360 D6=F/F1:X=X1-D6
2370 IF (ABS(D6) >= ABS(T1*X)) THEN 2340
2380 RETURN
2385 F=ZP(ZJ,1)*X+ZP(ZJ,2)*X^2+ZP(ZJ,3)*X^3-ZS
2386 F1=ZP(ZJ,1)+ZP(ZJ,2)*X+ZP(ZJ,3)*X^2
2387 RETURN
2390 PRINT "CONC=":X:GOTO 2300
2400 PLOT OFF:PCOLOR 0:COLOR,.7,1:END
A2.1.5 PE 2/3 Coefficient

10 PRINT"C"
20 CLR
30 PRINT"********************************************************************** PER ELM
LEAST-SQUARES CALIBRATION *******************************************************************";
40 PRINT;PRINT
40 IF N1=1 GOTO 70
50 PRINT"LABEL":; D$
50 PRINT/F D$
55 PRINT/F "CONC=(K1(ABS)+K3(ABS^2))/(K2(ABS)-1)"
60 TM=3
70 IF N1=1 GOTO 1060
80 IF N2=0 THEN GOSUB 390
80 IF N2=TM GOTO 260
90 N2=N2+1
100 L3=(N1-1)*2+1
110 IF N4=1 THEN 460
120 REM SORT THE DATA
130 GOSUB 470:REM SET UP MATRIX
140 GOSUB 930:REM SQUARE UP THE MATRIX
150 GOSUB 1080:REM GAUSS SOLN.
160 GOSUB 560:REM PRINT RESULTS
170 GOSUB 1950:REM PLOT DATA
180 GOTO 240:REM NEXT
190 REM GET DATA
200 PRINT"INPUT CONCN. & ABS. VALUES "
210 PRINT"INCLUDE BLANK eg. 0,0" :PRINT
220 FOR I=1 TO N1
230 INPUT Y1(I),X1
240 CIRCLE Y1(I)*420/2M,X1*420/2I,2,0,360,90
250 NEXT I
260 RETURN:REM FROM DATA INPUT
270 GOSUB 70 5:REM SET UP DATA MATRIX
280 PRINT FOR I=1 TO N1
290 U(I,1)=-X(I)
300 U(I,2)=X(I)*Y1(I)
310 U(I,3)=X(I)^2
320 Y1(I)=Y1(I)
330 PRINT"***************~~-~-*******~-~-***~·*****~-*~·
340 PER ELM L
350 E:4GT-~·SOU{-·~ES
360 CAL I
370 Eil:::.:{.4T I
380 DN·il!·*··;&;··io!··;(··K·>f·-~--;::--_(·?l"· >t--~-J- -i!:--~·-lli··j(·-~--~iii·*·!~!-·il=: *-*·~-*-!( ~­
540 NEXT I
550 RETURN:REM FROM SETTING UP DATA MATRIX
560 REM CALC. RESIDS. & PRT RESULTS
570 S7=0
580 S8=0
590 T6=0
600 FOR I=1 TO N1
610 Y2=0
620 FOR J=1 TO N2
630 Y2=Y2+C1(J)*U(I,J)
640 NEXT J
650 R3(I)=Y2-Y(I)
660 Y2(I)=Y2
670 T6=T6+R3(I)*R3(I)
680 S7=S7+Y(I)
690 S8=S8+Y(I)*Y(I)
700 NEXT I
710 IF N2>0 THEN C3=SQRT(1-T6/(S8-S7*S7/N1))
720 IF N1=N2 THEN E5=SQRT(T6)
730 IF N1<N2 THEN E5=SQRT(T6/(N2-N1))
740 IF N1>N2 THEN E5=SQRT(T6/N1-N2))
750 FOR J=1 TO N2
760 E2(J)=E5*ABS(B(J,J))
770 NEXT J
780 PRINT"ABS CONCN CONCN*C RESID"
790 FOR I=1 TO N1
800 PRINT USING A$;I;X(I),Y(I),Y2(I),R3(I)
810 NEXT I
820 PRINT"
830 PRINT"Coefficients Errors"
840 PRINT"
850 FOR I=1 TO N2
860 PRINT USING C$;C1(I),E2(I)
870 ZP(N2,I)=C1(I)
880 NEXT I
890 PRINT"
900 PRINT"CORR COEF=1+"; C3-1
910 ZP(N2,0)=C3
920 RETURN:REM FROM RESULTS PRINT
930 REM U&Y CONVERTED TO A&Z
940 FOR K=1 TO N2
950 FOR L=1 TO K
960 A(K,L)=0
970 FOR I=1 TO N1
980 A(K,L)=A(K,L)+U(I,L)*U(I,K)
990 IF K>L THEN A(L,K)=A(K,L)
1000 NEXT I
1010 NEXT L
1020 Z(K)=0
1030 FOR I=1 TO N1
1040 Z(K)=Z(K)+Y(I)*U(I,K)
1050 NEXT I
1060 NEXT K
1070 RETURN:REM FROM SQ
1080 REM GAUSS INV. & SOLN.
1090 E1=0
1100 IS=1
1110 N3=1
1120 FOR I=1 TO N2
1130 FOR J=1 TO N2
-320-
1140 B(I,J)=A(I,J)
1150 NEXT J
1160 W(I,1)=Z(I)
1170 I2(I,3)=0
1180 NEXT I
1190 D3=1
1200 FOR I=1 TO N2
1210 B1=0
1220 FOR J=1 TO N2
1230 IF I2(J,3)=1 THEN 1320
1240 FOR K=1 TO N2
1250 IF I2(K,3)>1 THEN 1920
1260 IF I2(K,3)=1 THEN 1310
1270 IF B1=ABS(B(J,K)) THEN 1310
1280 I3=J
1290 I4=K
1300 B1=ABS(B(J,K))
1310 NEXT K
1320 NEXT J
1330 I2(I4,3)=I2(I4,3)+1
1340 I2(I,1)=I3
1350 I2(I,2)=I4
1360 IF I3=I4 THEN 1490
1370 D3=-D3
1380 FOR L=1 TO N2
1390 H1=B(I3,L)
1400 B(I3,L)=B(I4,L)
1410 B(I4,L)=H1
1420 NEXT L
1430 IF N3<1 THEN 1490
1440 FOR L=1 TO N3
1450 H1=W(I3,L)
1460 W(I3,L)=W(I4,L)
1470 W(I4,L)=H1
1480 NEXT L
1490 P1=B(I4,I4)
1500 D3=D3*P1
1510 B(I4,I4)=1
1520 FOR L=1 TO N2
1530 B(I4,L)=B(I4,L)/P1
1540 NEXT L
1550 IF N3<1 THEN 1590
1560 FOR L=1 TO N3
1570 W(I4,L)=W(I4,L)/P1
1580 NEXT L
1590 FOR L=1 TO N2
1600 IF L1=I4 THEN 1700
1610 T=B(L1,I4)
1620 B(L1,I4)=0
1630 FOR L=1 TO N2
1640 B(L1,L)=B(L1,L)-B(I4,L)*T
1650 NEXT L
1660 IF N3<1 THEN 1700
1670 FOR L=1 TO N3
1680 W(L1,L)=W(L1,L)-W(I4,L)*T
1690 NEXT L
1700 NEXT L1
1710 NEXT I
1720 FOR I=1 TO N2
1730 L=N2-I+1
1740 IF I2(L,1)=I2(L,2) THEN 1820
1750 I3=I2(L,1)
1760 I4=I2(L,2)
1770 FOR K=1 TO N2
1780 H1=B(K,I3)
1790 B(K,I3)=B(K,I4)
1800 B(K,I4)=H1
1810 NEXT K
1820 NEXT I
1830 FOR K=1 TO N2
1840 IF I2(K,3)>1 THEN 1920
1850 NEXT K
1860 E1=0
1870 FOR I=1 TO N2
1880 C1(I)=W(I,1)
1890 NEXT I
1900 IF I5=1 THEN 1940
1910 PRINT"
1920 E1=1
1930 PRINT"ERROR*MATRIX SINGULAR"
1940 RETURN:REM FROM 6. SUBR
1950 REM PLOT ROUTINE**********
1960 IF S=3 THEN S=-1
1970 S=S+1:GF=O:PCOLOR S
1980 PHOME
1990 FOR I=0 TO ZI STEP 0.02*ZI
2000 IF FNO(1)*420/ZM>420 THEN MOVE 420,420*1/ZI:GOTO 2030
2010 IF FNO(1)*420/ZM<0 THEN MOVE 0,420*1/ZI:GOTO 2030
2020 LINE FNO(1)*420/ZM ,1*420/ZI
2030 NEXT I
2040 PHOME
2050 RETURN
2060 MOVE -20,-50:HSET:MODE TN
2070 PRINT/P "PARAMETERS"
2080 PRINT/P "------------------------------------------·
2090 PRINT/P "K1 K2 K3 COCOE"
2100 PRINT/P "------------------------------------------·
2110 DS=3: IF N2<3 LET DS=N2
2120 FOR T=1 TO DS:PCOLOR T:PRINT/P T:NEXT T
2130 IF N2>3 THEN PCOLOR 0:PRINT/P 4
2140 IF N2=5 THEN PCOLOR 1:PRINT/P 5
2150 SKIP -N2:MODE TS
2160 ZW$="#####.##### "
2170 FOR I=1 TO N2
2180 PRINT/P " :USING ZW$;ZP(I,1),ZP(I,2),ZP(I,3),ZP(I,4),ZP(I,5),ZP(I,6):NEXT I
2190 MODE TN
2200 PRINT7,J1 "WHICH FUNCTION DO YOU REQUIRE? 1>");N2:INPUT ZJ
2210 PRINT"TO ESCAPE "ABS TRAP" TYPE ANY LETTER"
2215 PRINT6,21 " DO NOT EXCEED CALIBRATION RANGE!!! "
2220 INPUT "DO YOU WANT RESULTS PRINTED? ".DD$:IF LEFT$(DD$, 1)="Y" THEN PLOTCH
2230 DEF FNW(ZS)= (ZP(ZJ,1)*ZS+ZP(ZJ,3)*ZS^2)/(ZP(ZJ,2)*ZS-1)
2240 PRINT" EQUATION ":ZJ
2250 PRINT"NOW GIVE ME ABSORBANCE VALUES AND I’LL RETURN CONCENTRATIONS"
2260 COLOR ,,7,2
2270 ON ERROR GOTO 2300
2280 INPUT"ABS=";ZS
2290 PRINT"CONC=":FNW(ZS):GOTO 2280
2300 PLOTOFF:FCOLOR 0:COLOR,7,1:END
A2.1.6 Varian Rational

10 PRINT "******************************************************************"
20 PRINT " VARSIM RATIONAL  SET CALIBRATION" 
30 PRINT "******************************************************************"
40 INPUT " NUMBER OF STANDARDS = " ; N4
50 INPUT " MAX CONCN. = " ; CM
60 INPUT " MAX ABS. = " ; AM
70 INPUT " LABEL " ; XW$ 
80 PRINT / P XW$ 
90 DIM PX(N4,3), X(N4), Y(N4+1)
100 Y(N4+1)=9.9E+30 :
110 PRINT " INPUT CONCN. AND ABS. VALUES "
120 FOR I=1 TO N4
130 INPUT X(I), Y(I)
140 NEXT I
150 IF N4=1 GOTO 1430
160 IF N4>3 THEN N1=N4: GOTO 180
170 N1=N4
180 PRINT / P " CURVES FOUND STARTING AT ORIGIN "
190 PRINT / P " A/D = r + 6A + tA^2 "
200 REM SIM SOLN. BY GAUSS ELIM 26.11.84
210 REM PAGE 66 BASIC PRODS BOOK
220 A$="##.###AAAA"
230 B$="##.#####AAAA"
240 C$="##.#####AAAA"
250 D$="##.#####AAAA"
260 M1=8
270 FOR FF=0 TO N4-3
280 DIM Z(8), A(8,8), C1(8), W(8), B(8,8)
290 GOSUB 530: REM INPUT SUBR.
300 GOSUB 650: REM GAUSS ELIM.
310 REM
320 IF N1<5 THEN 400
330 PRINT " MATRIX CONSTANTS "
340 FOR I=1 TO N1
350 FOR J=1 TO N2
360 PRINT USING A$; A(I,J); 
370 NEXT J
380 PRINT USING B$; Z(I)
390 NEXT I
400 PRINT
410 IF E1=1 THEN 500
420 PRINT " SOLN."
430 PRINT
440 PRINT / P " ";
450 FOR I=1 TO N2
460 PRINT USING C$; PX(FF,I); 
470 PRINT / P USING D$; PX(FF,I); 
480 NEXT I
490 PRINT
500 NEXT FF; GOTO 1150
510 REM
520 REM
530 REM
540 PRINT: REM INPUT DATA
550 IF N1<2 THEN 1130
560 N2=N1
570 FOR I=1 TO N1
580 A(I,1)=X(FF+I)
590 FOR J=2 TO N2
600 A(I,J)=A(I,J-1)*Y(FF+I)
610 REM
620 NEXT J
630 Z(I)=Y(FF+I)
640 NEXT I
650 RETURN:REM FROM INPUT ROUTINE
660 REM SIM SOLN BY GAUSS
670 FOR I=1 TO N2
680 FOR J=1 TO N2
690 B(I,J)=A(I,J)
700 NEXT J
710 W(I)=Z(I)
720 NEXT I
730 E1=0
740 FOR I=1 TO N2-1
750 B1=ABS(B(I,I))
760 L=I
770 I1=I+1
780 FOR J=I1 TO N2
790 IF(ABS(B(J,I))<B1)THEN 820
800 B1=ABS(B(J,I))
810 L=J
820 NEXT J
830 IF L=I THEN 930
840 FOR J=1 TO N2
850 H1=B(L,J)
860 B(L,J)=B(I,J)
870 B(I,J)=H1
880 NEXT J
890 H1=W(L)
900 W(L)=W(I)
910 W(I)=H1
920 FOR J=I1 TO N2
930 T=B(J,I)/B(I,I)
940 FOR K=I1 TO N2
950 B(J,K)=B(J,K)-T*B(I,K)
960 NEXT K
970 NEXT J
980 W(J)=W(J)-T*W(I)
990 NEXT J
1000 NEXT I
1010 IF B(N2,N2)=0 THEN B(N2,N2)=1
1020 PX(FF,I)=W(N2)/B(N2,N2)
1030 REM BACK SUBST.
1040 FOR I=N2-1 TO 1 STEP -1
1050 S6=0
1060 FOR J=I+1 TO N2
1070 S6=S6+B(I,J)*PX(FF,J)
1080 NEXT J
1090 PX(FF,I)=(W(I)-S6)/B(I,I)
1100 NEXT I
1110 RETURN:REM NORM
1120 E1=1
1130 PRINT"ERROR\ MATRIX SINGULAR"
1140 RETURN:FROM GAUSS ROUT
1150 PRINT/\ MODE GR
1160 MOVE 20,0
1170 AXIS 0,-42,10
1180 AXIS 1,42,10
1190 MOVE 20,-420

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HSET
MA$="ABSORBANCE O>"+STR$(AM)
MC$="CONCENTRATION O>"+STR$(CM)
MOVE 20,-20:GPRINT [0,01,MC$
MOVE -5,20:GPRINT [0,31,MA$
FOR I=1 TO N4:CIRCLE X(I)*420/CM,Y(I)*420/AM,2,0,360,90:NEXT I:PHOME
FOR FF=0 TO N4-3:REM***********
IF VV=3 THEN VV=-1
VV=VV+1:PCOLOR VV
IF N4<3 GG=0:GOTO 1320
MOVE X(FF+1)*420/CM,Y(FF+1)*420/AM
GG=Y(FF+1):IF GG=1 THEN GG=0:PHOME
FOR I=GF TO Y(FF+N1)+.1 STEP (Y(FF+N1)-Y(FF+1))/10
ON ERROR GOTO 1370
ZZ=I/CPXC11,1+PX(I-1,2)*I+PX(I-1,3)*I^2)
IF ZZ*420/CM>420 THEN GOTO 1380
LINE ZZ*420/CM,I*420/AM
NEXT I
NEXT FF
MODE TN:PCOLOR 0:PRINT/P:PRINT/P
GOSUB 1530
CLS:END
PRINT"EQUATION IS SIMPLE C=A.r TYPE""EQUATION IS SIMPLE C=A.r TYPE"
RT=X(1):TY=Y(1)
B=RT/TY
ON ERROR GOTO 1520
PRINT"INPUT ABS & I'LL RETURN CONCN."
INPUT EE
PRINT/P "ABS=";EE,"CONC=";EE*B
GOTO 1490
1510 GOTO 1490
1520 END
PRINT"INPUT ABS.VALUES "
ON ERROR GOTO 1530
INPUT EE
FOR I=1 TO N4
IF EE<=Y(I+2) THEN FG=EE/(PX(I-1,1)+PX(I-1,2)*EE+PX(I-1,3)*EE^2):PRINT/P"A=";EE,"C=";FG:GOTO 1550
NEXT I
GOTO 1550
A2.1.7 PU Quadratics

5 PRINT "C"
10 PRINT "*********************************************************************"
20 PRINT "SIMULTANEOUS PARABOLAS SET CALIBRATION"
30 PRINT "*********************************************************************"
40 INPUT "NUMBER OF STANDARDS= " ; N
50 INPUT "MAX CONCN. " ; CM
60 INPUT "MAX ABS. " ; AM
70 INPUT "LABEL " ; XW$
80 PRINT/P XW$
90 N4=2*N
100 DIM PX(N4-2,3), X(N4), Y(N4)
110 PRINT "INPUT CONCN. AND ABS. VALUES"
120 FOR I=2 TO N4 STEP 2
130 INPUT X(I), Y(I)
140 REM
150 NEXT I
151 REM
160 FOR I=3 TO N4 STEP 2
170 MA=(Y(I-1)+Y(I+1))/2
180 HJ=(Y(I-1)-Y(I-3))/(X(I-1)-X(I-3))
190 HK=(Y(I+1)-Y(I-1))/(X(I+1)-X(I-1))
220 MS=(HJ+HK)/2 : IF SGN(HJ)=-1 THEN MS=-MS
230 X(I)=(MA-Y(I-1))/MS+X(I-1) : Y(I)=MA
240 PRINT X(I), Y(I)
241 EI=I
250 NEXT I
251 REM
260 N1=3
270 PRINT/P "CURVES FOUND STARTING AT ORIGIN"
280 PRINT/P " C= r + sA + tA^2"
290 REM SIM SOLN. BY GAUSS ELIM 28.11.84
300 REM PAGE 66 BASIC PROGS BOOK
310 A$="#.#.#..... "
320 B$="#.#.#....."
330 C$="#.#.#....."
340 D$="#.#.#....."
350 M1=8
360 FOR FF=1 TO N4-3 STEP 2:REM*********
370 DIM Z(8), A(8,8), C1(8), W(8), B(8,8)
380 GOSUB 620:REM INPUT SUBR.
390 GOSUB 750:REM GAUSS ELIM.
400 REM
410 IF N1>5 THEN 490
420 PRINT " MATRIX CONSTANTS"
430 FOR I=1 TO N1
440 FOR J=1 TO N2
450 PRINT USING A$; A(I,J)
460 NEXT J
470 PRINT USING B$; Z(I)
480 NEXT I
490 PRINT
500 IF EI=1 THEN 590
510 PRINT " SOLN."
520 PRINT
530 PRINT/P " ";
540 FOR I=1 TO N2
550 PRINT USING C$; PX(FF, I)
560 PRINT/P USING D$; PX(FF, I)
570 NEXT I
580 PRINT

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590 NEXT FF: GOTO 1240
600 REM
610 REM
620 REM
630 PRINT: REM INPUT DATA
640 IF N1<2 THEN 1160
650 N2=N1
660 FOR I=1 TO N1
670 A(I,1)=1
680 FOR J=2 TO N2
690 A(I,J)=A(I,J-1)*Y(FF+I)
700 REM
710 NEXT J
720 Z(I)=X(FF+I)
730 NEXT I
740 RETURN: REM FROM INPUT ROUTINE
750 REM SIM SOLN BY GAUSS
760 FOR I=1 TO N2
770 FOR J=1 TO N2
780 B(I,J)=A(I,J)
790 NEXT J
800 W(I)=Z(I)
810 NEXT I
820 E1=0
830 FOR I=1 TO N2-1
840 B1=ABS(B(I,I))
850 L=I
860 I1=I+1
870 FOR J=I1 TO N2
880 IF(ABS(B(J,I))<B1) THEN 910
890 B1=ABS(B(J,I))
900 L=J
910 NEXT J
920 IF B1=0 THEN 1210
930 IF L=I THEN 1020
940 FOR J=1 TO N2
950 H1=B(L,J)
960 B(L,J)=B(I,J)
970 B(I,J)=H1
980 NEXT J
990 H1=W(L)
1000 W(L)=W(I)
1010 W(I)=H1
1020 FOR J=I1 TO N2
1030 T=B(J,I)/B(I,I)
1040 FOR K=I1 TO N2
1050 B(J,K)=B(J,K)-T*B(I,K)
1060 NEXT K
1070 W(J)=W(J)-T*W(I)
1080 NEXT J
1090 NEXT I
1100 IF B(N2,N2)=0 THEN 1210
1110 PX(FF,I)=W(N2)/B(N2,N2)
1120 REM BACK SUBST.
1130 FOR I=N2-1 TO 1 STEP -1
1140 S6=0
1150 FOR J=I+1 TO N2
1160 S6=S6+B(I,J)*PX(FF,J)
1170 NEXT J
1180 PX(FF,I)=(W(I)-S6)/B(I,I)
1190 NEXT I
1200 RETURN:REM NORM
1210 EI=1
1220 PRINT"ERROR=MATRIX SINGULAR"
1230 RETURN:FROM GAUSS ROUT#
1240 PRINT/P: MODE GR
1250 MOVE 20,0
1260 AXIS 0,-42,10
1270 AXIS 1,42,10
1280 MOVE 20,-420
1290 HSET
1300 MA$="" ABSORBANCE 0:"+STR$(AM)
1310 MC$="" CONCENTRATION 0:"+STR$(CM)
1320 MOVE 20,-20:GPRINT [0,0],MC$
1330 MOVE -5,20:GPRINT [0,3],MA$
1340 FOR I=2 TO N4 STEP 2:CIRCLE XCI>*420/CM,Y(1)*420/AM,2,0
1350,90:NEXT I:PHOME
1360 LINE X(2)*420/CM,Y(2)*420/AM
1370 FOR FF=1 TO N4-3 STEP 2
1380 IF VV=3 THEN VV=-1
1390 VV=VV+1:FCOLOR VV
1400 MOVE X(FF+1)*420/CM,Y(FF+1)*420/AM
1410 FOR I=Y(FF+1) TO Y(FF+3)+.1 STEP (Y(FF+3)-Y(FF+1))/10
1420 ZZ=(PX(FF,1)+PX(FF,2)*I+PX(FF,3)*I^2)
1430 IF ZZ*420/CM>420 THEN GOTO 1450
1440 LINE ZZ*420/CM,I*420/AM
1450 PHOME
1460 NEXT FF
1470 MODE TN:PCOLOR 0
1480 CLS:PRINT/P:PRINT/P:PRINT/P
1490 INPUT"DO YOU WANT THE RESULTS PRINTED?";VC$
1500 IF LEFT$(VC$,1)="Y" THEN PLOTON
1510 PRINT"INPUT ABS VALUES FOR CONVERSION"
1525 ON ERROR GOTO 9999
1530 INPUT "ABS ":BY
1540 FOR I=4 TO N4 STEP 2
1550 IF EV<Y(2) THEN NB=EV/Y(2) :PRINT"CONS ";NB:GOTO 1530
1560 IF BV<=Y(1) THEN NB=PX(I-3,1)+PX(I-3,2)*BV+PX(I-3,3)*BV^2:PRINT "CONS ";NB:GOTO1530
1570 NEXT I
9999 PLOTOFF:CLS:END
A2.1.8 Ques 1

Ques 1 was lost by overwriting the program tape. The program was a variation of the preceding programs.
A2.1.9 Ques 2

10 PRINT "C"
20 CLR
30 PRINT "*************** IN WATSON'S CURVE FIT ***************"
40 INPUT "LABEL "; D$
50 PRINT / P D$
60 TM=3
70 INPUT "NUMBER OF STANDARDS="; N1
80 INPUT "HIGHEST CONCNI.: STANDARD ="; ZM
90 INPUT "HIGHEST RESPONSE ="; ZI
100 ZB$=STR$(ZM)
110 ZH$=STR$(ZI)
120 MODE 8: REM PLOT ROUTINE****
130 MOVE 20,0
140 ZG$=" RESPONSE 0."+ZH$
150 AXIS 0,-42,10; RMOVE -10,0; GPRINT [0,31,ZG$
160 MOVE 20,-420
170 AXIS 1,42,10
180 MOVE 20,-420
190 ZX$=" CONCENTRATION 0."+ZB$+" ppm"
200 HSET;MOVE 20,-20; GPRINT [0,0]; ZX$: PHOME; PCOLOR 3
210 DEF FNO(Gl)= (C1(1)+(C1(2)*G))/ (1+(C1(3)*G))
220 A$=" 
230 C$=" 
240 M1=35
250 DIM Z(TM), A(TM, TM), C1(5), Y(35), U(35, TM), ZP(5, 5)
260 DIM W(TM, 1), B(TM, TM), I2(TM, 3), X(35), Y1(35)
270 DIM Y2(35), R3(35), E2(TM)
280 PRINT " 
290 REM
300 IF N2=0 THEN GOSUB 420
310 IF N2=TM GOTO 2090
320 N2=N2+1
330 L3=(N1-I)*2+1
340 IF N4=1 THEN G90
350 REM SORT THE DATA
360 GOSUB 500: REM SET UP MATRIX
370 GOSUB 960: REM SQUARE UP THE MATRIX
380 GOSUB 1110: REM GAUSS SOLN.
390 GOSUB 590: REM PRINT RESULTS
400 GOSUB 1980: REM PLOT DATA
410 GOTO 270: REM NEXT
420 REM GET DATA
430 PRINT " INPUT CONCN. & RESP. VALUES "
440 PRINT "INCLUDE BLANK eg. 0,0": PRINT
450 FOR I=1 TO N1
460 INPUT Y1(I),X(I)
470 CIRCLE Y1(I)*420/ZM, X(I)*420/ZI, 2.0, 360, 90
480 NEXT I
490 RETURN: REM FROM DATA INPUT
500 REM SET UP DATA MATRIX
510 FOR I=1 TO N1
520 U(I,1)=1
530 U(I,2)=Y1(I)
540 U(I,3)=-Y1(I)*X(I)
550 Y(I)=X(I)
570 NEXT I
580 RETURN: REM FROM SETTING UP DATA MATRIX

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REM CALC. RESIDS. & PRT RESULTS
590 S7=0
600 S8=0
610 T6=0
620 FOR I=1 TO N1
630 FOR J=1 TO N2
640 Y2=0
650 FOR J=1 TO N2
660 Y2=Y2+C1(J)*U(I,J)
670 NEXT J
680 R3(I)=Y2-Y(I)
690 Y2(I)=Y2
700 T6=T6+R3(I)*R3(I)
710 S7=S7+Y(I)
720 S8=S8+Y(I)*Y(I)
730 NEXT I
740 IF N2>0 THEN C3=SQRT(1-T6/(S8-(S7*S7/N1)))
750 IF N1=N2 THEN E5=SQRT(T6)
760 IF N1<N2 THEN E5=SQRT(T6/(N2-N1))
770 IF N1>N2 THEN E5=SQRT(T6/(N1-N2))
780 FOR J=1 TO N2
790 E2(J)=E5*SQRT(ABS(B(J,J)))
800 NEXT J
810 PRINT" ABSO CONCN CONCN*C RESID"
820 FOR I=1 TO N1
830 PRINT USING A$;X(I),Y(I),Y2(I),R3(I)
840 NEXT I
850 PRINT" Coefficients Errors"
860 PRINT""
870 PRINT""
880 FOR I=1 TO N2
890 PRINT USING C$;C1(I),E2(I)
900 ZP(N2,I)=C1(I)
910 NEXT I
920 PRINT""
930 PRINT" CORR COEF=1+": C3-1
940 ZP(N2,0)=C3
950 RETURN: REM FROM RESULTS PRINT
960 REM U&Y CONVERTED TO A&Z
970 FOR K=1 TO N2
980 FOR L=1 TO K
990 A(K,L)=0
1000 FOR I=1 TO N1
1010 A(K,L)=A(K,L)+U(I,L)*U(I,K)
1020 IF K>L THEN A(L,K)=A(K,L)
1030 NEXT I
1040 NEXT L
1050 Z(K)=0
1060 FOR I=1 TO N1
1070 Z(K)=Z(K)+Y(I)*U(I,K)
1080 NEXT I
1090 NEXT K
1100 RETURN: REM FROM SQ
1110 REM GAUSS INV. & SOLN.
1120 E1=0
1130 IS=1
1140 N3=1
1150 FOR I=1 TO N2
1160 FOR J=1 TO N2
1170 B(I,J)=A(I,J)
1180 NEXT J
1190 NEXT I
1190 W(I, 1) = Z(I)
1200 IZ(I, 3) = 0
1210 NEXT I
1220 D3 = 1
1230 FOR I = 1 TO N2
1240 B1 = 0
1250 FOR J = 1 TO N2
1260 IF IZ(J, 3) = 1 THEN 1350
1270 FOR K = 1 TO N2
1280 IF IZ(K, 3) > 1 THEN 1950
1290 IF IZ(K, 3) = 1 THEN 1340
1300 IF B1 >= ABS(B(J, K)) THEN 1340
1310 I3 = J
1320 I4 = K
1330 B1 = ABS(B(J, K))
1340 NEXT K
1350 NEXT J
1360 IZ(I4, 3) = IZ(I4, 3) + 1
1370 IZ(I, 1) = I3
1380 IZ(I, 2) = I4
1390 IF I3 = I4 THEN 1520
1400 D3 = -D3
1410 FOR L = 1 TO N2
1420 H1 = B(I3, L)
1430 B(I3, L) = B(I4, L)
1440 B(I4, L) = H1
1450 NEXT L
1460 IF N3 < 1 THEN 1520
1470 FOR L = 1 TO N3
1480 H1 = W(I3, L)
1490 W(I3, L) = W(I4, L)
1500 W(I4, L) = H1
1510 NEXT L
1520 P1 = B(I4, I4)
1530 D3 = D3 * P1
1540 B(I4, I4) = 1
1550 FOR L = 1 TO N2
1560 B(I4, L) = B(I4, L) / P1
1570 NEXT L
1580 IF N3 < 1 THEN 1620
1590 FOR L = 1 TO N3
1600 W(I4, L) = W(I4, L) / P1
1610 NEXT L
1620 FOR L1 = 1 TO N2
1630 IF L1 = I4 THEN 1730
1640 T = B(L1, I4)
1650 B(L1, I4) = 0
1660 FOR L1 = 1 TO N2
1670 B(L1, L) = B(L1, L) - B(I4, L) * T
1680 NEXT L
1690 IF N3 < 1 THEN 1730
1700 FOR L1 = 1 TO N3
1710 W(L1, L) = W(L1, L) - W(I4, L) * T
1720 NEXT L
1730 NEXT L1
1740 NEXT I
1750 FOR I = 1 TO N2
1760 L = N2 - I + 1
1770 IF IZ(L, 1) = IZ(L, 2) THEN 1850
1780 I3 = IZ(L, 1)
1790  I4=I2(L, 2)
1800  FOR  K=1  TO  N2
1810  H1=B(K,13)
1820  B(K,13)=B(K,14)
1830  B(K,14)=H1
1840  NEXT  K
1850  NEXT  I
1860  FOR  K=1  TO  N2
1870  IF  I2(K,3)<1  THEN  1950
1880  NEXT  K
1890  E1=0
1900  FOR  I=1  TO  N2
1910  C1(I)=W(I,1)
1920  NEXT  I
1930  IF  IS=1  THEN  1970
1940  PRINT"
1950  E1=1
1960  PRINT"ERROR*MATRIX  SINGULAR"
1970  RETURN:REM  FROM  E.  SUBR
1980  REM  PLOT  ROUTINE************
1990  IF  S=3  THEN  S=-1
2000  S=S+1:GF=0:PCOLOR  S
2010  PHOME
2020  FOR  I=0  TO  ZM  STEP  .02*ZM
2030  IF  FNQ(I)*420/ZI>420  THEN  MOVE  420,420*I/ZM :GOTO  2060
2040  IF  FNQ(I)*420/ZI<0  THEN  MOVE  0,420*I/ZM:GOTO  2060
2050  LINEI*420/ZM,FNQ(I)*420/ZI
2060  NEXT  I
2070  PHOME
2080  RETURN
2090  MOVE  -20,-50:HSET:MODE  TN
2100  PRINT/P  "PARAMETERS, INCLUDING  CONSTANT"
2110  PRINT/P  "=============================================
2120  PRINT/P  " A  B  C  COCOE"
2130  PRINT/P  "=============================================
2140  DS=3:IF  N2<3  LET  DS=N2
2150  FOR  T=1  TO  DS:PCOLOR  T:PRINT/P  T:NEXT  T
2160  IF  N2>4  THEN  PCOLOR  0:PRINT/P  4
2170  IF  N2>5  THEN  PCOLOR  1:PRINT/P  5
2180  SKIP  -N2:MODE  TS
2190  ZW="####.##AAAA  "
2200  FOR  I=1  TO  N2
2210  PRINT/P  " ;USING  ZW$;ZP(I,1),ZP(I,2),ZP(I,3),ZP(I,0)
2220  NEXT  I
2230  MODE  TN
2240  PRINT7,31  "WHICH  FUNCTION  DO  YOU  REQUIRE?  1>";N2:INPUT
2250  IF  N2<1  THEN  2490
2260  PRINT"TO  ESCAPE 'TRAP'  TYPE  ANY  LETTER"
2270  IF  N2>1  THEN  PRINT"DO  YOU  WANT  RESULTS  PRINTED?  ";DD$:IF  LEFTS(DD$,1)="Y"  THEN  PLOTON:
2280  PRINT:PRINT:PRINT
2290  PRINT"EQUATION  ":ZJ
2300  DEF  FNQ(ZJ)=(ZZ-ZP(ZJ,1))/((ZP(ZJ,2)-(ZP(ZJ,3)*ZJ))
2310  PRINT"NOW  GIVE  ME  ABSORBNCE  VALUES  AND  I'LL  RETURN  CONCENTRATIONS"
2320  FOR  I=1  TO  N2
2330  PRINT"CDNC=";FNQ(ZS):GOTO  2300
2340  NEXT  I
2350  PLOTOFF:PCOLOR  0:COLOR,,7,1:END
**A2.1.10 Linear Interpolations**

```
10 CLS
20 PRINT(0,51)"*************LINEAR INTERPOLATION***********"
30 PRINT
40 PRINT
50 PRINT
60 PRINT"NUMER OF STANDARDS "
70 INPUT" INCLUDING BLANK";NS
80 PRINT"MAXIMUM CONCENTRATION";CM
90 PRINT"MAXIMUM ABSORBANCE";AM
100 INPUT"LABEL";L$
110 CLS
120 PRINT L$
130 DIM A(NS),C(NS),D(2,NS)
140 PRINT"ENTER IN INCREASING ORDER"
150 PRINT"ALL STANDARDS INCLUDING BLANK"
160 FOR I=1 TO NS
170 INPUT"CONS,ABS";C(I),A(I)
180 NEXT I
190 PRINT"INTERPOLATION EQUATIONS"
200 REM D(1,?)=SLOPES
210 REM D(2,?)=INTERCEPTS
220 FOR I=1 TO NS-1
230 D(1,I)=(C(I)-C(I+1))/(A(I)-A(I+1))
240 D(2,I)=D(1,I)*A(I)-C(I)
250 NEXT I
260 PRINT"EQUATIONS"
270 PRINT"mA = n "
280 FOR I=1 TO NS-1
290 PRINT";D(1,I),D(2,I)
300 NEXT I
305 S=NS-1
306 ON ERROR GOTO 400
310 PRINT"INPUT ABSORBANCES FOR RETURN OF CONCENTRATIONS"
320 INPUT"ABS=";V:IF V>A(NS) THEN PRINT"OFF CALIBRATION":GOT 0 320
340 FOR I=2 TO S
350 IF V<=A(I) GOTO 370
360 NEXT I
370 Z=(D(1,I-1)*V)-D(2,I-1)
380 PRINT" CONC=";Z
390 GOTO320
400 END
```
A2.2 Two pump manifold programs
A2.2.1 2-Pump Calibration with Curve fitting

1 AN = STD:FQ = VOL / 5: FOR D = 1 TO 4: GOSUB 1900: NEXT D:
   GOSUB 2000: WSA = V(STD)
8 PRINT
10 DIM Z(4), A(4,4), C1(5), Y(10), U(10,4), W(4,1), B(4,4), IZ(4,3)
   , X(10), Y1(10), Y2(10), R3(10), E2(4), V(2)
11 E = 16:F = 256: SL = - 16175: VL = - 16176:
12 CH = - 16172: CN = - 16166: CF = - 16165
20 BASE = 49312: SAM = .1: STD = 0: HOME: PRINT: PRINT
25 POKE CH, 0
30 PRINT " PUMPEO CALIBRANTS"
31 PRINT " SRB"
32 PRINT: PRINT: PRINT
33 REM CALIBRATE PUMPS
34 POKE BASE + 2, 255
35 PRINT "COLLECT EFFLUENT FROM COMP PUMP": PRINT "INPUT IT VOLUME WHEN THE PUMP STOPS": PRINT
36 PRINT "PRESS ANY KEY TO CONTINUE"
37 GET J$: IF J$ = "" GOTO 37
38 HOME: PRINT "OK"
39 FOR J = 1 TO 10000
40 POKE BASE, 128: POKE BASE, 0
41 NEXT J
42 INPUT "INPUT THE COLLECTED VOLUME (mL) ": VOL
43 PRINT "MEASURE FLOWRATE OF FIXED SPEED PUMP"
44 INPUT "INPUT VOLUME COLLECTED MEASURED (mL, sec) "; ML, SEC
45 FR = ML * 60 / SEC
46 CR = VOL * 1.166521
47 TSA = 0: WSA = 0
49 INPUT "INPUT STANDARD CONCENTRATION": SC; TC = CR * SC / FR
49 POKE BASE + 3, 255: POKE BASE, 0: POKE BASE + 11, 192
50 REM PURGE: IF PU = 1 THEN POKE BASE + 4, 10: POKE BASE + 5, 10: GOSUB 1900: GOSUB 2000: PU = 0
51 AN = STD: FQ = VOL / 5: FOR D = 1 TO 4: GOSUB 1900: NEXT D:
   GOSUB 2000: WSA = V(STD)
52 FOR I = 1 TO 10
53 Y1(I) = TC * I / 10
54 FQ = Y1(I) * FR / SC
55 BITS = 0.5 * ((VOL * 6000) / (2 ^ (DIV / 16) * FQ) - 3.5)
   : HB = INT (BITS / 256): LB = INT (BITS - HB * 256)
56 IF HB > 255 THEN DIV = DIV + 16: GOTO 55
57 POKE BASE, DIV: POKE BASE + 4, LB: POKE BASE + 5, HB
58 GOSUB 1900: GOSUB 2000
59 X(I) = V(STD) - WSA
60 NEXT I
65 POKE BASE + 11, 0
70 REM CURVE FIT TO A/C=a+bA+cA^2
153 N1 = 10: N2 = 3
160 REM SORT DATA
162 GOSUB 1001
163 GOSUB 1100
164 GOSUB 1140
168 GOSUB 1400
170 GOTO 1500
1001 REM SET UP DATA MATRIX
1002 FOR I = 1 TO N1
1004 U(I, 1) = 1

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1006 FOR J = 2 TO N2
1008 U(I,J) = U(I,J - 1) * X(I)
1010 NEXT J
1012 Y(I) = X(I) / Y1(I)
1014 NEXT I
1016 RETURN : REM FROM DATA MATRIX SET UP
1100 REM U&Y CONVERTED TO A&Z
1102 FOR K = 1 TO N2
1104 FOR L = 1 TO K
1106 A(K,L) = 0
1108 FOR I = 1 TO N1
1110 A(K,L) = A(K,L) + U(I,L) * U(I,K)
1112 IF K < L THEN A(L,K) = A(K,L)
1114 NEXT I
1116 NEXT L
1118 Z(K) = 0
1120 FOR I = 1 TO N1
1122 Z(K) = Z(K) + Y(I) * U(I,K)
1124 NEXT I
1126 NEXT K
1128 RETURN : REM FROM MATRIX SQUARING
1140 REM GAUSS INV.& SOLN.
1141 E1 = 0: I5 = 1:N3 = 1
1142 FOR I = 1 TO N2
1143 FOR J = 1 TO N2
1144 B(I,J) = A(I,J)
1145 NEXT J
1146 NEXT I
1150 D3 = 1
1152 FOR I = 1 TO N2
1153 B1 = 0
1154 FOR J = 1 TO N2
1156 IF I2(J,3) = 1 THEN 1166
1157 FOR K = 1 TO N2: IF I2(K,3) > 1 THEN 1239
1158 IF I2(K,3) = 1 THEN 1164
1159 IF B1 > = ABS(B(J,K)) THEN 1164
1160 I3 = J: I4 = K
1161 B1 = ABS(B(J,K))
1164 NEXT K
1166 NEXT J
1168 I2(I4,3) = I2(I4,3) + 1
1170 I2(I,1) = I3
1171 I2(I,2) = I4
1172 IF I3 = I4 THEN 1185
1173 D3 = - D3
1174 FOR L = 1 TO N2
1175 H1 = B(I3,L)
1176 B(I3,L) = B(I4,L)
1177 B(I4,L) = H1
1178 NEXT L
1179 IF N3 < 1 THEN 1185
1180 FOR L = 1 TO N3
1181 H1 = W(I3,L)
1182 W(I3,L) = W(I4,L)
1183 W(I4,L) = H1
1184 NEXT L
1185 P1 = B(I4,I4)
1186 D3 = D3 * P1
FOR L = 1 TO N2.
B(I4,L) = B(I4,L) / P1
NEXT L
IF N3 < 1 THEN 1195
FOR L = 1 TO N3
W(I4,L) = W(I4,L) / P1
NEXT L
FOR L1 = 1 TO N2
IF L1 = I4 THEN 1206
T = B(L1,I4)
B(L1,I4) = 0
FOR L = 1 TO N2
B(L1,L) = B(L1,L) - B(I4,L) * T
NEXT L
IF N3 < 1 THEN 1206
FOR L = 1 TO N3
W(L1,L) = W(L1,L) - W(I4,L) * T
NEXT L
NEXIT L1
NEXT I
IF I2(L1,2) THEN 1228
I3 = I2(L,1)
I4 = I2(L,2)
FOR K = 1 TO N2
H1 = B(K,I3)
B(K,I3) = B(K,I4)
B(K,I4) = H1
NEXT K
NEXT I
IF I2(K,3) < > 1 THEN 1239
NEXT K
E1 = 0
FOR I = 1 TO N2
C1(I) = W(I,1)
NEXT I
IF I5 = 1 THEN 1250
PRINT
E1 = 1
PRINT "ERROR...FIT NOT POSSIBLE WITH THIS DATA.....
...MATRIX SINGULAR"
RETURN : REM FROM GAUSS SUBR.
REM CALC. CALIB LINE AND SHOW ERRORS
S7 = 0;S8 = 0;T6 = 0
FOR I = 1 TO N1
Y2 = 0
FOR J = 1 TO N2
Y2 = Y2 + C1(J) * U(I,J)
NEXT J
R3(I) = Y2 - Y(I)
Y2(I) = Y2
T6 = T6 + R3(I) * R3(I)
S7 = S7 + Y(I)
S8 = S8 + Y(I) * Y(I)
NEXT I
IF N2 > 0 THEN C3 = SQR (1 - T6 / (S8 - S7 * S7 / N1))
1414 IF N1 = N2 THEN ES = SQRT (T6)
1415 IF N1 < N2 THEN ES = SQRT (T6 / (N2 - N1))
1416 IF N1 > N2 THEN ES = SQRT (T6 / (N1 - N2))
1417 FOR J = 1 TO N2
1418 E2(J) = ES * SQRT (ABS (B(J,J)))
1419 NEXT J
1420 PRINT : PRINT
1430 PRINT "Coefficients Errors"
1431 PRINT "-----------------------------"
1432 FOR I = 1 TO N2
1433 PRINT C1(I),E2(I)
1434 NEXT I
1435 PRINT
1436 PRINT "CORRELATION COEF";C3
1437 RETURN : REM FROM PRINT RESULTS
1500 DEF FN K(SA) = SA / (C1(1) + C1(2) * SA + C1(3) * SA ^ 2)
1510 REM SAMPLE TEN TIMES BEFORE RECALIBRATING
1520 FOR SNO = 1 TO 10
1525 POKE BASE + 1,1
1530 PRINT "PRESENT SAMPLE": PRINT : PRINT : PRINT "PRESS A NY KEY WHEN ABSORBANCE IS STEADY"
1540 GET XL$: IF XL$ = "" GOTO 1540
1550 HOME : PRINT "SAMPLING"
1560 AN = SAM:FO = VOL / 5: GOSUB 2000
1583 IF (V(SAM) - WSA) > = X(10) THEN GOSUB 3000
1590 PRINT "CONCENTRATION OF SAMPLE"; (FN K(V(SAM) - WSA))
* DR
1600 DR = 1: NEXT SNO
1650 POKE BASE + 1,2
1700 GOTO 49
1900 REM DELAY LOOP
1910 FOR ZX = 0 TO 5000: NEXT ZX
1920 RETURN
2000 REM READ VALUE
2010 POKE CN,0: POKE CF,0
2020 V(AN) = 0
2030 KER = INT (VOL * 30 / FO) + 200
2040 FOR RD = 1 TO KER
2050 EY = PEEK (SL): YE = PEEK (VL)
2060 POKE CN,0: POKE CF,0
2070 V(AN) = V(AN) + (EY - E) * F + YE
2080 NEXT RD
2090 V(AN) = V(AN) / KER
2100 RETURN
3000 REM **** DILUTION ****
3010 PRINT "SWITCH DILUTION VALVE TO DILUTE"
3020 PRINT "THEN PRESS ANY KEY"
3030 GET Q$: IF Q$ = "" GOTO 3030
3040 POKE BASE + 11,192: POKE BASE + 4,10: POKE BASE + 5,10
3050 POKE BASE + 11,0: POKE BASE + 11,0
3060 GOSUB 1900: GOSUB 1900
3060 FO = CR
3070 GOSUB 2000
3080 DR = FR / (FR - CR)
3090 PRINT "RETURN DILUTION VALVE TO STANDARD"
3100 PRINT "THEN PRESS ANY KEY"
3110 GET Q$: IF Q$ = "" GOTO 3110
3115 POKE BASE + 11,0:PU = 1
3120 RETURN
A2.2.2 2-Pump Calibration - Mix & Match

10 BASE = 49312: SAM = 1: STD = 0
20 HOME : PRINT : PRINT : PRINT : PRINT : PRINT
30 PRINT " MATCHED STANDARD CALIBRATION"
40 PRINT "SRB 2.8.85"
50 PRINT : PRINT : PRINT
60 REM CALIBRATE PUMP FLOWRATE
70 POKE BASE + 2,255
80 PRINT "COLLECT EFFlUENT FROM COMP PUMP": PRINT "INPUT IT" 
90 PRINT "'S VOLUME WHEN PUMP STOPS": PRINT
100 GET J$: IF J$ = "" GOTO 100
110 HOME : PRINT "OK"
120 FOR J = 1 TO 10000
130 POKE BASE,128
140 POKE BASE,0
150 NEXT J
160 INPUT "INPUT COLLECTED VOLUME (ml) ": VOL.
170 PRINT "MEASURE FLOWRATE OF FIXED SPEED PUMP"
180 INPUT "INPUT VOLUME COLLECTED AND TIME TAKEN" 
190 FR = ML * 60 / SEC
200 CR = VOL * 1.166521
210 REM INPUT OPERATING VALUES
220 DIM V(1),CAL(21),CCAL(21)
230 E = 16:F = 256:SL = - 16175:VL = - 16176
240 CH = - 16172:CN = - 16166:CF = - 16165
250 TSA = 0
260 WSA = 0
270 POKE CH,0
280 REM INITIALISE PUMP SEQUENCE
290 POKE BASE + 3,255
300 POKE BASE,0
310 POKE BASE + 11,192
320 INPUT "INPUT STANDARD CONCENTRATION"; SC
330 TC = (CR * SC) / FR
340 PRINT "TOP STANDARD=:";TC
345 ARI = TC / 20
350 REM MIX TOP STANDARD
360 POKE BASE + 4,10
370 POKE BASE + 5,10
380 PRINT "STRD"
390 GOSUB 910: REM DELAY
400 REM CALCULATE TOP STD LEVEL
410 AN = STD
420 FQ = CR
430 GOSUB 950
440 TSA = V(0)
450 POKE BASE + 11,0
460 HOME
465 PRINT "WATER"
467 ITE = 0
470 GOSUB 910
475 AN = STD
480 REM CALCULATE WATER LEVEL
490 FQ = VOL / 5
500 GOSUB 950
510 WSA = V(0)
520 REM MATCHING SEQUENCE
530 POKE BASE + 11,192
540 PRINT "PRESS AN
Y KEY TO CONTINUE"
540 GET XL$: IF XL$ = "" GOTO 227.
560 REM  SWITCH VALVE TO SAMPLE
570 FOR DD = 1 TO 3: GOSUB 910: NEXT DD
610 REM  READ SAMPLE
620 AN = SAM
630 FQ = VOL / 5
640 GOSUB 950
650 REM  PUMP AT SLOWEST SPEED
660 POKE BASE + 11,192
670 POKEBase + 4,255: POKE Base + 5,255
700 REM  ESTIMATE STD CONC (AND HENCE PUMP SPEED)
710 FOR I = 1 TO 20
720 IF CAL(I) > VAM THEN GOTO 725
725 NEXT I
730 V(STD) = CAL(I): TM = CCAL(I)
740 M = (V(STD) - WSA) / TM
750 ITE = 1 + ITE
760 WSA = V(SAM) + .07 * (V(SAM) - WSA)
770 FOR I = 1 TO 20
780 IF CAL(I) > VAM THEN GOTO 775
790 NEXT I
800 VAM = V(SAM) + .07 * (V(SAM) - WSA)
810 REM  READ STD SAMPL.
820 AN = STD: GOSUB 950
830 TM = FQ * SC / FR
840 GOTO 740
850 END
860 REM  READ VALUE SEQUENCE
950 POKE CN,0: POKE CF,0
960 V(AN) = 0
970 KER = INT (VOL * 30 / FQ) + 200
980 FOR I = 1 TO KER
  990 EY = PEEK (SL): YE = PEEK (VL)
1000 POKE CN,0: POKE CF,0
1010 V(AN) = V(AN) + (EY - E) * F + YE
1020 NEXT I
1030 V(AN) = V(AN) / KER
1040 RETURN
1200 PRINT "SWITCH DILUTION VALVE TO DILUTE"
1210 PRINT "THEN PRESS ANY KEY"
1220 GET Q$: IF Q$ = "" GOTO 1220
1230 POKE BASE + 11,192: POKE BASE + 4,10: POKE BASE + 5,10:
  POKE BASE,0
1240 GOSUB 910: GOSUB 910
1250 FQ = CR
1260 GOSUB 940
1270 DR = FR / (FR - CR)
1280 PRINT "RETURN DILUTION VALVE TO STANDARD"
1290 PRINT "THEN PRESS ANY KEY"
1300 GET Q$: IF Q$ = "" GOTO 1300
1310 RETURN
A2.2.3 2-Pump Calibration - Mix & Match with flow rate device

10 BASE = 49312: SAM = 1: STD = 0
20 HOME: PRINT: PRINT: PRINT: PRINT: PRINT
30 PRINT "MATCHED STANDARD CALIBRATION"
40 PRINT "SRG 2.8.85"
50 PRINT: PRINT: PRINT
60 REM CALIBRATE PUMP FLOWRATE
70 POKE BASE + 2, 255
170 INPUT "INPUT FLOWRATE FROM DEVICE": FR
210 REM INPUT OPERATING VALUES
220 DIM V(1), CAL(21), CCAL(21)
230 E = 16: F = 256: SL = -16175: VL = -16176
240 CH = -16172: CN = -16166: CF = -16165
250 TSA = 0
260 WSA = 0
270 POKE CH, 0
280 REM INITIALISE PUMP SEQUENCE
290 POKE BASE + 3, 255
300 POKE BASE, 0
310 POKE BASE + 11, 192
320 INPUT "INPUT STANDARD CONCENTRATION": SC
330 REM MIX TOP STANDARD
340 POKE BASE + 4, 90
370 POKE BASE + 5, 11
380 PRINT "STANDARD"
381 INPUT "INPUT FLOWRATE FROM DEVICE": DF
382 INPUT "INPUT FLOWRATE FROM DEVICE": DF
383 VOL = 0.96925 * (FR - DF)
385 CR = VOL * 1.164521
386 TC = ((CR * SC)) / FR
387 PRINT "TOP STANDARD": TC
388 ARI = TC / 20
390 GOSUB 910: REM DELAY
400 REM CALCULATE TOP STD LEVEL
410 AN = STD
420 FQ = CR
430 GOSUB 950
440 TSA = V(0)
450 POKE BASE + 11, 0
460 HOME
465 PRINT "WATER"
467 ITE = 0
470 GOSUB 910
475 AN = STD
480 REM CALCULATE WATER LEVEL
490 FQ = VOL / 5
500 GOSUB 950
510 WSA = V(0)
520 REM MATCHING SEQUENCE
530 POKE BASE + 11, 192
550 PRINT: PRINT "PRESENT SAMPLE": PRINT: PRINT "PRESS AN Y KEY TO CONTINUE"
560 GET XL#
570 HOME: PRINT "SAMPLING"
580 REM SWITCH VALVE TO SAMPLE
590 POKE BASE + 1, 1
600 FOR DD = 1 TO 3: GOSUB 910: NEXT DD
610 REM READ SAMPLE
620 AN = SAM
630 FQ = VOL / 5
640 GOSUB 950
641 DR = 1
642 IF V(SAM) + 5 > (TSA) THEN GOSUB 1200
643 HOME : PRINT "OK"
644 POKE BASE + 1,2: GOSUB 910: GOSUB 910: POKE BASE + 11,0

645 IF V(SAM) + 5 > (TSA) THEN PRINT "TOO CONCENTRATED": GOTO 465
646 REM START PUMP AT SLOWEST SPEED
647 POKE BASE + 11,192
648 POKE BASE + 4,255: POKE BASE + 5,255
649 VAM = V(SAM) + .07 * (V(SAM) - WSA)
650 REM ESTIMATE STD CONC (AND, HENCE PUMP SPEED)
651 FOR I = 1 TO 20
652 IF CAL(I) > VAM THEN GOTO 725
653 NEXT I
654 NEXT I
655 V(STD) = CAL(I): TM = CCAL(I)
656 IF V(STD) = 0 THEN V(STD) = TSA; TM = TC
657 M = (V(STD) - WSA) / TM: DIV = 0
658 ITE = 1 + ITE
659 CQ = (V(SAM) - WSA) / M: HOME : PRINT "SAMPLE ABS INDEX"
660 "V(SAM) - WSA: PRINT "STANDARD ABS INDEX": V(STD) - WSA"
661 PRINT : PRINT "ESTIMATED SAMPLE CONC =": TM * DR: PRINT
"NEXT ITERATION &": CQ * DR
662 NA = INT (TM / ARI)
663 CAL(NA) = V(STD)
664 CCAL(NA) = TM
665 H = V(STD) - V(SAM): IF ABS (H * 100 / (V(SAM) - WSA)) < = 2 THEN POKE BASE + 11,0: GOSUB 910: GOTO 465
666 IF ITE = 6 THEN PRINT " 5 ITERATIONS......RESAMPLE": POKE BASE + 11,0: GOSUB 910: GOTO 465
667 FOR FQ = CQ * FR / SC
668 BITS = 0.5 * ((VOL * 6000) / (2 ^ (DIV / 16) * FQ) - 3.5)
669 HB = INT (BITS / 256): LB = INT (BITS - HB * 256)
670 IF HB > 255 THEN DIV = DIV + 16: GOTO 790
671 POKE BASE,DIV
672 POKE BASE + 6, LB
673 POKE BASE + 7, HB
674 GOSUB 910
675 REM READ STD
676 AN = STD: GOSUB 950
677 TM = FQ * SC / FR
678 GOTO 740
679 END
680 REM DELAY LOOP
681 FOR I = 1 TO 12000: NEXT I
682 RETURN
683 REM READ VALUE SEQUENCE
684 POKE CN,0: POKE CF,0
685 V(AN) = 0
686 KER = INT (VOL * 30 / FQ) + 200
687 FOR I = 1 TO KER
688 EY = PEEK (SL): YE = PEEK (VL)
689 POKE CN,0: POKE CF,0
690 V(AN) = V(AN) + (EY - E) * F + YE
691 NEXT I
692 NEXT I
693 V(AN) = V(AN) / KER
694 RETURN
695 PRINT "SWITCH DILUTION VALVE TO DILUTE"
1210 PRINT "THEN PRESS ANY KEY"
1220 GET Q$: IF Q$ = "" GOTO 1220
1230 POKE BASE + 11,192: POKE BASE + 4,90: POKE BASE + 5,11
   POKE BASE, 0
1240 GOSUB 910: GOSUB 910
1250 FR = CR
1260 GOSUB 940
1270 DR = FR / (FR - CR)
1280 PRINT "RETURN DILUTION VALVE TO STANDARD"
1290 PRINT "THEN PRESS ANY KEY"
1300 GET Q$: IF Q$ = "" GOTO 1300
1310 RETURN
A2.3 Timed injection program
5 HOME
10 REM ****** TIMED INJECTION MSC ******
15 GOSUB 3000: REM SET FILL SPEED
20 REM ****** SRB 1986 ******
30 REM
40 REM
50 REM ****** MAIN PROGRAM ******
60 GOSUB 5000: REM SET UP
65 REM ****** CHARACTERISATION ******
70 PRINT "PRESENT A STANDARD ON LINEAR RANGE"
80 PRINT "THEN: PRESS ANY KEY \": GET Y$
90 RT = 500: AS = 1
100 FOR J = 1 TO 3
110 GOSUB 6000: REM FILL LOOP
120 GOSUB 11000: REM INJECT
130 GOSUB 10000: REM PEAK SEARCH
140 GOSUB 7000: REM SS VALUE
150 S1 = SV + S1:F1 = F1 + U
160 SX = SX + 8:PX = PX + SP
170 GOSUB 2000: REM PRINT INFO
180 NEXT J
190 S1 = S1 / 3:F1 = F1 / 3:PX = PX / 3:SX = SX / 3
200 RT = INT ((SX - PX) / 3): AS = 0
210 RF = RT * W1
220 FOR J = 1 TO 3
230 GOSUB 6000: REM FILL LOOP
240 GOSUB 11000: REM INJECT
250 GOSUB 10000: REM PEAK SEARCH
260 GOSUB 2000: REM PRINT INFO
265 PRINT ER
270 F2 = F2 + U:P1 = AM + P1
290 NEXT J
300 P1 = P1 / 3:F2 = F2 / 3
310 DS = S1 / P1
320 FR = (F1 + F2) / 2
330 VI = FR * (RT * W1 - ER) / 60
340 VMC = - VI / LOG (1 - 1 / DS)
350 HOME : PRINT "PRESENT THE STANDARD"
360 INPUT "THEN INPUT IT'S CONCENTRATION \":TS
370 RT = INT ((SX - PX) + (ER / W1))
390 GOSUB 6000: REM FILL LOOP
400 GOSUB 11000: REM INJECT
410 GOSUB 10000: REM PEAK SEARCH
420 GOSUB 2000: REM PRINT INFO
440 F3 = U:P2 = AM
470 REM ****** SAMPLING ******
480 PRINT "PRESENT SAMPLE, THEN PRESS ANY KEY\": GET Y$
490 RT = INT ((SX - PX + (ER / W1)):IN = 0:F3 = P2:TS = TS:SA = 0
500 GOSUB 6000: REM FILL LOOP
510 GOSUB 11000: REM INJECT
520 GOSUB 10000: REM PEAK SEARCH
530 GOSUB 2000: REM PRINT INFO
550 IF AM * 1.02 > = P2 AND IN = 0 THEN RT = INT (RT / 2)
      GOTO 500
560 IN = IN + 1
570 SA = SA + AM
580 IF IN < 3 GOTO 500

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SA = SA / 3: RV = RT * W1 - ER; TTS = TS
REM SEEK CONCENTRATION
PRINT "PRESENT STANDARD, THEN PRESS ANY KEY ": GET Y#
M = P3: TTS
CA = SA / M
TQ = VMC * 60 * (LOG (TS / (TS - CO))) / FR
RT = INT ((TD + ER) / W1)
RS = RT * W1 - ER
CQ = (1 - EXP (- FR * RS / (60 * VMC))) * TS
IF CQ > TS THEN PRINT "SAME STD ": GOTO 470
600 GOSUB 6000: REM FILL LOOP
601 GOSUB 11000: REM INJECT 
602 GOSUB 10000: REM PEAK SEARCH
603 GOSUB 2000: REM PRINT INFO
610 IF ABS ((SA - AM) * 100 / AM) < = 2 THEN CQ = CQ / (1 - EXP (- FR * RV / (60 * VMC))): PR#: 1: PRINT : PRINT "SA
MFE CONC ":; CQ: PR#: 0: PRINT : GOTO 470
720 TTS = CQ: P3 = AM
730 GOTO 620
740 REM ************************************************************
750 REM ************************************************************
760 REM ***** SUB ROUTINES *****
780 REM **** DELAY ****
790 FOR DE = 1 TO 10000: NEXT DE
800 RETURN
800 REM ***** PRINT INFO *****
810 HOME : PR#: 1
820 PRINT "INJECT =":; RT * W1 - ER; " FLOWRATE="; U
830 PRINT "STEADY STATE="; SV; " PEAK MAX="; AV(AI)
840 FR#: 0: RETURN
850 REM ***** FILL SPEED *****
860 INPUT "INPUT FILL SPEED, 2-100 "; FS
870 DIV = 0
880 BITS = 0.5 * (665950 / (2 ^ (DIV / 16) * FS) - 3.5)
890 A = INT (BITS / 256): B = INT (BITS - A * 256)
900 IF A > 255 THEN DIV = DIV + 16: GOTO 3030
910 RETURN
930 REM ***** SET UP *****
940 DIM AV(510): SN = 10
950 E = 16: F = 1056; SL = - 16175; VL = - 16175; CH = - 1617
960 CN = - 16166; CF = - 16165
970 SF = 0.2419
980 BASE = 49312
990 POKE CH, 0: POKE BASE + 2, 255: POKE BASE + 3, 255
1000 FD = 1000
1010 ER = 0.2: W1 = 0.04
1020 RETURN
1040 REM ***** FILL & BASELINE *****
1050 POKE BASE + 11, 192: POKE BASE, DIV: POKE BASE + 4, B: PO KE BASE + 5, A
1060 BL = 0; BM = 0; BV = 0
1070 FOR I = 1 TO FD
1080 POKE CN, 0: POKE CF, 0
1090 IF BV > BM THEN BM = BV
1100 BV = ( Peek (SL) - E) * F + Peek (VL)
1110 BL = BL + BV
1120 NEXT I
1130 BL = BL / FD
1140 BM = (BM - BL) * SF / 150
6110 POKE BASE + 11,0
6140 RETURN
7000 REM **** STEADY STATE CALC ****
7010 SV = 0
7020 IF S = SE OR RT < 450 THEN RETURN
7030 FOR I = S TO SE: SV = AV(I) + SV: NEXT I
7040 SV = SV / (SE + 1 - S)
7050 RETURN
9000 REM **** STEADY ST SEARCH ****
9010 SS = AV(I): SI = AV(I - SN)
9020 IF S < 1 AND (SS - SI) / SN < 0.002 AND DH = 1 THEN S = I - SN
9030 IF S > 1 AND (SS - SI) / SN < - 0.004 THEN SE = I - S
9040 EV = 1: RETURN
9040 REM
9050 RETURN
10000 REM **** PEAK SEARCH ****
10010 DH = 0: AM = 0: AI = 0: EF = 0: GG = 0: SF = 0
10020 SV = 0: SS = 0: S = 0: EV = 0
10030 FOR I = 5 TO 500
10040 IF AV(I) > 1.5 * BM AND DH = 0 THEN DH = 1: SP = I - 1
10050 GG = I + SN
10060 ARM = 0
10070 FOR L = - 2 TO 2
10080 ARM = AV(I + L) + ARM
10090 NEXT L
10100 ARM = ARM / 5
10110 IF ARM > AM THEN AM = ARM: AI = I
10120 IF I = GG AND AS = 1 THEN GG = GG + SN: IF DH = .1 THEN GOSUB 9000: REM STEADY STATE SEARCH
10130 IF EV = 1 THEN GOTO 10090
10140 NEXT I
10150 U = 60 * 132.5E-3 / ((W1 * SP) - ER + 0.04)
10160 RETURN
11000 REM **** INJECT & READ ****
11010 POKE BASE + 1,1
11020 FOR I = 0 TO 500
11030 POKE CN,O: POKE CF,O
11040 IF I = RT THEN POKE BASE + 1,2
11050 AD = (PEEK(SL) - E) * F + PEEK(VL)
11060 AV = (AD - BL) * SF
11070 AV(I) = AV / 150
11100 NEXT I
11110 POKE BASE + 1,2
11120 RETURN
A2.4 Peak width programs
A2.4.1 Peak width calibration - full equation

1 PRINT 0
2 HOME: PRINT "SET STREAM SWITCH VALVE TO STD"
3 PRINT " ASPIRATE WATER "
4 DIM V(200),TI*(200),M(4),FW(200,6),T(200,6)
5 DIM TH(12),GI(3)
6 PRINT
10 D$ = CHR$ (4)
11 E = 16;F = 256;SL = -16175;VL = -16176;
12 CH = -16172;CN = -16166;CF = -16165
13 N2 = 3;Z1 = 1
15 POKE CH,0
16 PRINT "SET GAIN TO GIVE 0 ABSORBANCE"
17 PRINT " THEN PRESS ANY KEY"
18 GET A$: IF A$ = "" GOTO 18
20 POKE CN,0: POKE CF,0
22 FOR O = 1 TO 30
24 EY = PEEK (SL):YE = PEEK (VL)
26 POKE CN,0: POKE CF,0
28 XA = (EY - 16) * 256 + YE
30 IF XA > XB THEN XB = XA
32 XC = XC + XA
34 NEXT O
36 DF = XC / 30
38 PRINT "SET GAIN TO 1 ABSORBANCE"
40 PRINT " THEN PRESS ANY KEY"
42 GET A$: IF A$ = "" GOTO 42
44 POKE CN,0: POKE CF,0
46 FOR O = 1 TO 30
48 EY = PEEK (SL):YE = PEEK (VL)
50 POKE CN,0: POKE CF,0
52 XD = XD + (EY - 16) * 256 + YE
54 NEXT O
56 FD = XD / 30
58 Z = FD - DF
59 N = (XB - DF)
60 PRINT
62 PRINT "NOISE (MAX DEV FROM MEAN BASELINE) "":N / Z: PRINT
T
63 PRINT "SET GAIN TO 0"
64 N7 = N2 + 1
150 REM NORMAL CALIBRATION
151 GOSUB 153
152 GOTO 1450
153 PRINT : INPUT "INPUT NUMBER OF STANDARDS ":N1
155 DIM Z(N7),A(N7,N7),C1(5),Y(50),U(50,N7),W(N7,1),E(N7,N7)
157 REM
159 GOSUB 200
160 REM SORT DATA
162 GOSUB 1001
163 GOSUB 1100
164 GOSUB 1140
168 GOSUB 1400
170 RETURN
200 FOR I = 1 TO N1
202 INPUT "ASPIRATE A STANDARD THEN INPUT IT'S CONCENTRATION ":Y1(I)
204 POKE CN,0: POKE CF,0

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205 XA = 0
206 FOR O = 1 TO 30
207 EY = PEEK (SL): YE = PEEK (VL)
210 POKE CN,0: POKE CF,0
212 XA = (EY - 16) * 256 + YE + XA
214 NEXT O
216 XA = XA / 30
218 X(I) = (XA - 128) / O
219 PRINT X(I), Y1(I)
220 NEXT I
220 RETURN
240 REM END OF DATA COLLECTION
1001 REM SET UP DATA MATRIX
1002 FOR I = 1 TO N1
1004 U(I,1) = 1
1006 FOR J = 2 TO N2
1008 U(I,J) = U(I,J - 1) * X(I)
1010 NEXT J
1012 Y(I) = X(I) / Y1(I)
1014 NEXT I
1016 RETURN: REM FROM DATA MATRIX SET UP
1100 REM U&Y CONVERTED TO A&Z
1102 FOR K = 1 TO N2
1104 FOR L = 1 TO K
1106 A(K,L) = 0
1108 FOR I = 1 TO N1
1110 A(K,L) = A(K,L) + U(I,L) * U(I,K)
1112 IF K < L THEN A(L,K) = A(K,L)
1114 NEXT I
1116 NEXT L
1118 Z(K) = 0
1120 FOR I = 1 TO N1
1122 Z(K) = Z(K) + Y(I) * U(I,K)
1124 NEXT I
1126 NEXT K
1128 RETURN: REM FROM MATRIX SQUARING
1140 REM GAUSS INV.& SOLN.
1141 E1 = 0: I5 = 1: N3 = 1
1142 FOR I = 1 TO N2
1143 FOR J = 1 TO N2
1144 B(I,J) = A(I,J)
1145 NEXT J
1146 W(I,1) = Z(I)
1147 I2(I,1) = 0
1148 NEXT I
1150 D3 = 1
1152 FOR I = 1 TO N2
1153 B1 = 0
1154 FOR J = 1 TO N2
1156 IF I2(J,3) = 1 THEN 1166
1157 FOR K = 1 TO N2: IF I2(K,3) > 1 THEN 1239
1158 IF I2(K,3) = 1 THEN 1164
1159 IF B1 > = ABS (B(J,K)) THEN 1164
1160 I3 = J; I4 = K
1161 B1 = ABS (B(J,K))
1164 NEXT K
1166 NEXT J
1168 I2(I,3) = I2(I,3) + 1
1170 I2(I,1) = I3
1171 I2(I,2) = I4

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IF I3 = I4 THEN 1185
D3 = - D3
FOR L = 1 TO N2
H1 = B(I3, L)
B(I3, L) = B(I4, L)
B(I4, L) = H1
NEXT L
IF N3 < 1 THEN 1185
FOR L = 1 TO N3
H1 = W(I3, L)
W(I3, L) = W(I4, L)
W(I4, L) = H1
NEXT L
P1 = B(I4, I4)
D3 = D3 * P1
B(I4, I4) = 1
FOR L = 1 TO N2
B(I4, L) = B(I4, L) / P1
NEXT L
IF N3 < 1 THEN 1185
FOR L = 1 TO N3
W(I4, L) = W(I4, L) / P1
NEXT L
FOR L1 = 1 TO N2
IF L1 = I4 THEN 1206
T = B(L1, I4)
B(L1, I4) = 0
FOR L = 1 TO N2
B(L1, L) = B(L1, L) - B(I4, L) * T
NEXT L
IF N3 < 1 THEN 1206
FOR L = 1 TO N3
W(L1, L) = W(L1, L) - W(I4, L) * T
NEXT L
NEXT L1
NEXT I
FOR I = 1 TO N2
L = N2 - I + 1
IF I2(L, 1) = I2(L, 2) THEN 1228
I3 = I2(L, 1)
I4 = I2(L, 2)
FOR K = 1 TO N2
H1 = B(K, I3)
B(K, I3) = B(K, I4)
B(K, I4) = H1.
NEXT K
NEXT I
FOR K = 1 TO N2
IF I2(K, 3) < 1 THEN 1239
NEXT K
E1 = 0
FOR I = 1 TO N2
C1(I) = W(I, 1)
NEXT I
IF IS = 1 THEN 1250
PRINT
PRINT "ERROR...FIT NOT POSSIBLE WITH THIS DATA.......
.....MATRIX SINGULAR"
RETURN : REM FROM GAUSS SUBR.
1400 REM CALC. CALIB LINE AND SHOW ERRORS
1401 S7 = 0: S8 = 0: T6 = 0
1402 FOR I = 1 TO N1
1403 Y2 = 0
1404 FOR J = 1 TO N2
1405 Y2 = Y2 + C1(J) * U(I,J)
1406 NEXT J
1407 R3(I) = Y2 - Y(I)
1408 Y2(I) = Y2
1409 T6 = T6 + R3(I) * R3(I)
1410 S7 = S7 + Y(I)
1411 S8 = S8 + Y(I) * Y(I)
1412 NEXT I
1413 IF N2 > 0 THEN C3 = SQRT(1 - T6 / (S8 - S7 * S7 / N1))
1414 IF N1 = N2 THEN E5 = SQRT(T6)
1415 IF N1 < N2 THEN E5 = SQRT(T6 / (N2 - N1))
1416 IF N1 > N2 THEN E5 = SQRT(T6 / (N1 - N2))
1417 FOR J = 1 TO N2
1418 E2(J) = E5 * SQRT(ABS(B(J,J)))
1419 NEXT J
1420 PRINT : PRINT
1421 PRINT "Coefficients Errors"
1422 PRINT "--------------------------"
1423 FOR I = 1 TO N2
1424 PRINT C1(I), E2(I)
1425 NEXT I
1426 PRINT
1427 PRINT "CORRELATION COEF=": C3
1428 RETURN : REM FROM PRINT RESULTS
1429 G1(1) = C1(1): G1(2) = C1(2): G1(3) = C1(3)
1430 DEF FN K(SA) = SA / (G1(1) + G1(2) * SA + G1(3) * SA ^ 2)
1431 PRINT "ASPIRATE WATER, SET GAIN TO 0"
1432 IF W1 = 2 GOTO 9100
1433 REM INJECT ONE STD SEVERAL TIMES
1434 FOR NUM = 1 TO 6
1435 INPUT "ENTER INJECTION STD CONCENTRATION \\
1436 FOR CN, CF"
1437 GCJSUB 9100
1438 FOR L = 1 TO 200
1439 POKE CN, 0: POKE CF, 0
1440 PRINT D$: TI$(L)
1441 W1 = PEEK(SL): W2 = PEEK(VL)
1442 PW(L, NUM) = (W1 - 16) * 256 + W2
1443 NEXT L
1444 PRINT D$: "IN#0": PRINT D$: "PR#0"
1445 FOR I = 200 TO 1 STEP - 1
1446 REM CALCULATE WIDTHS OVER WHOLE PEAK
1447 EOP = I: IF PW(I, NUM) > DF + 2 * N GOTO 1730
1448 NEXT I
1449 IF PW(I, NUM) > DF + 2 * N GOTO TO 1732
1450 FOR I = 1 TO EOP: SOP = I: IF PW(I, NUM) > DF + 2 * N GOTO 1732
1451 NEXT I
1452 FOR I = SOP + 1 TO EOP: IF PW(I, NUM) > X(N1) * Z + DF THEN MP = I: GOTO 1735
1453 IF PW(I, NUM) > DW THEN MP = I: DW = PW(I, NUM)
1454 NEXT I

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FOR I = SOP TO MP
FOR J = EDP TO (MP + 1) STEP - 1
T(I,NUM) = VAL (RIGHT$(TI$(J),6)) - VAL (RIGHT$(T I$(I),6))
EOP = J
IF SGN (T(I,NUM)) = -1 THEN T(I,NUM) = 60 + T(I,NUM)
IF PW(J,NUM) > 2 * N + PW(I,NUM) GOTO 1747
NEXT J
REM
PW(I,NUM) = LOG ((WW / ( FN K((PW(I,NUM) - DF) / Z))) - 1)
NEXT I
PJ = FJ + MP - SOP + 1
TH(NUM) = SOP:TH(NUM + 6) = MP
DW = 0
NEXT NUM
PRINT "PREPARE FOR SAMPLE INJECTION"
PRINT " PEAK WIDTH CALIBRATION"
J = 0:N2 = N2 + 1:IS = N1
REM LSF PW
FOR B = 1 TO 6
FOR I = TH(B) TO TH(B + 6)
U(J,1) = 1
NEXT I
NEXT B
NEXT II
FOR II = 2 TO N2
U(J,II) = U(J,II - 1) * FW(I,B)
NEXT II
J = J + 1
NEXT I
NEXT B
N1 = J
GOSUB 163
NUM = 0
REM SAMPLE INJECTION
POKE CN,0: POKE CF,0
GOSUB 9000
FOR L = 1 TO 200
POKE CN,0: POKE CF,0
INPUT "";TI$1LI
W1 = PEEK (EL);W2 = PEEK (VL)
PW(L,NUM) = (W1 - 16) * 256 + W2
NEXT L
PRINT D$;"IN#0";PRINT D$;"FR#0"
FOR I = 200 TO 1 STEP - 1
REM CALCULATE WIDTHS OVER WHOLE PEAK
EOP = I: IF PW(I,NUM) > DF + 2 * N GOTO 2150
NEXT I
FOR I = 1 TO EOP:SOP = I: IF PW(I,NUM) > DF + 2 * N GO TO 2170
NEXT I
FOR I = SOP + 1 TO EDP: IF PW(I,NUM) > X(IS) * Z + DF THEN MP = I: GOTO 2190
IF PW(I,NUM) > CE THEN MP = I:CE = PW(I,NUM)
NEXT I
MP = MP - 1
FOR I = SOP TO MP
FOR J = EDP TO (MP + 1) STEP - 1
EOP = J
-356-
SS = VAL ( RIGHT$ (TI$ (J), 6)) - VAL ( RIGHT$ (TI$ (I), 6))

IF SGN (SS) = -1 THEN SS = 60 + SS
IF PW (J, NUM) > 2 * N + PW (I, NUM) GOTO 2255
NEXT J

IF SGN (CSS) > -1 THEN SS = 60

IF PW < NUM) THEN PW = NUM
NEXT J

CE = FN K((PW (I, NUM) - DF) / Z)
T1 = 0.000001
X1 = X
D6 = F / F1
X = X1 - D6

IF (ABS (D6) > = ABS (T1 * X)) THEN 2265
RETURN

F = C1(1) + C1(2) * X + C1(3) * X^2 + C1(4) * X^3 - SS
F1 = C1(2) + 2 * C1(3) * X + 3 * C1(4) * X^2
RETURN

CS = CS + (CE * (EXP (X) + 1))
NEXT I
CS = CS / (MP - SOP + 1)
PRINT "ESTIMATE BY PW OF SAMPLE CONCENTRATION"

CS = 0: CE = 0
REM CALC CONC OF SAMPLE
REM FROM SEVERAL WIDTHS
REM IF OFF SCALE CALCULATES DILUTION FACTOR (PERHAPS EVEN DILUTE)
PRINT "ESTIMATE BY PW OF SAMPLE CONCENTRATION"

END
REM INJECT SUBROUTINE
PRINT "CHECKING MEMORY"
PRINT "NOW"
PRINT "NORMAL" 
PRINT D$: "NOMON", O, C"
PRINT D$: "IN#4": PRINT D$: "PR#4"
PRINT : RETURN
PRINT "ASPIRATE SAMPLE, PRESS ANY KEY WHEN OUTPUT IS ST EADY"
XS = 0
9110  GET S$:  IF S$ = ""  GOTO 9110
9115  POKE CN,0:  POKE CF,0
9120  FOR O = 1 TO 30
9125  EY = PEEK (SL):  YE = PEEK (VL)
9130  POKE CN,0:  POKE CF,0
9135  XS = (EY - 16) * 256 + YE + XS
9140  NEXT O
9145  SA = XS / 30
9150  SA = (SA - DF) / Z
9154  GH = GH + 1
9155  PRINT "CONCENTRATION OF SAMPLE",GH, FN K(SA)
9200  RETURN
A2.4.2  Peak width calibration - abbreviated equation

10  PRINT 0: AV = 4
20  PRINT " ASPIRATE WATER "
30  DIM V(20), M(4), SC(20), T(20), IS(20), IC(20)
40  D$ = CHR$(4)
50  E = 16: F = 256: SL = - 16175: VL = - 16176:
60  CH = - 16172: CN = - 16166: CF = - 16165
70  BASE = 49312: POKE BASE + 3, 255
80  POKE CH,0
90  PRINT "SET GAIN TO GIVE 0 ABSORBANCE"
100 PRINT " THEN PRESS ANY KEY"
110 GET A$
120 POKE CN,0: POKE CF,0
130 FOR O = 1 TO 30
140 EY = PEEK (SL): YE = PEEK (VL)
150 POKE CN,0: POKE CF,0
160 XA = (EY - 16) * 256 + YE
170 IF XA > XB THEN XB = XA
180 XC = XC + XA
190 NEXT O
200 XF = XC / 30
210 PRINT "SET GAIN TO 1 ABSORBANCE"
220 PRINT " THEN PRESS ANY KEY"
230 GET A$
240 POKE CN,0: POKE CF,0
250 FOR O = 1 TO 30
260 EY = PEEK (SL): YE = PEEK (VL)
270 POKE CN,0: POKE CF,0
280 XD = XD + (EY - 16) * 256 + YE
290 NEXT O
300 FD = XD / 30
310 Z = FD - DF
320 N = (XB - DF)
330 PRINT
340 PRINT "NOISE (MAX DEV FROM MEAN BASELINE) "; N / Z: PRINT
350 PRINT "SET GAIN TO 0"
360 REM PW CALN
370 PRINT: INPUT "INPUT NUMBER OF STANDARDS "; N1
380 REM
390 REM INJECT ONE STD SEVERAL TIMES
400 INPUT "ABSORBANCE LEVEL REQUIRED"; AL: LA = (AL * Z)
410 FOR M = 1 TO N1: GOSUB 420: MT = MT + T(M): MLC = . MLC + S
420 PRINT "INPUT CONCENTRATION OF STANDARD "; M: INPUT SC(M)
430 FOR XX = 1 TO AV: IF M > N1 THEN H = H + 1: PRINT "SAMPLE " ; H
440 FOR B = 1 TO INT (2 * AL): NEXT B: PRINT "FILL LOOP THEN PRESS ANY KEY" : POKE BASE + 1,2: GET A$
450 PRINT D$: " NODNI,0,C": PRINT : PRINT D$: " IN#4"
460 POKE CN,0: POKE CF,0
470 DF = 0
480 FOR B = 1 TO 30
490 POKE CN,0: POKE CF,0
500 W1 = PEEK (SL): W2 = PEEK (VL)
510 DF = DF + (W1 - 16) * 256 + W2
520 NEXT B
530 DF = DF / 30

-359-
AL = LA + DF: POKE BASE + 1,1
FOR L = 1 TO 200
W1 = PEEK (SL): W2 = PEEK (VL)
POKE CN,0: POKE CF,0
PW = (W1 - 16) * 256 + W2
IF PW > AL GOTO 610
NEXT L
INPUT ":"; T1$: PRINT CHR$ (7)
FOR F = 1 TO 2000
W1 = PEEK (SL): W2 = PEEK (VL)
POKE CN,0: POKE CF,0
PW = (W1 - 16) * 256 + W2
IF PW < AL GOTO 680...
NEXT F
INPUT ":"; T2$
PRINT D$"IN#0": PRINT D$"PR#0"
REM CALCULATE WIDTH
T = VAL (RIGHT$ (T2$, 6)) - VAL (RIGHT$ (T1$, 6))
IF SGN (T) = -1 THEN T = 60 + T
T(M) = T + T(M)
NEXT XX
RETURN
IF AV = 4 THEN GOSUB 830
GOSUB 430
CW = FN CL(T(M))
FOR D = 2 TO N1
IF T(M) < T(D) THEN GOTO 786
NEXT D
D = D - 1
CL = 2.71828 ^ (((T(M) - IC(D - 1)) / IS(D - 1))
T(M) = 0
HOME
PRINT "SAMPLE ";H;" CONCENTRATION BY LSF=";CW
PRINT "BY LIN. INTERP. =";CL
GOTO 770
REM FIT ST LINE
FOR I = 1 TO (N1 - 1)
T(I) = T(I) / 4
SR = SR + ((T(I) - MT) * (SC(I) - MLC))
SSQ = SSQ + (SC(I) - MLC) ^ 2
SUQ = SUQ + (T(I) - MT) ^ 2
IS(I) = (T(I + 1) / 4 - T(I)) / (SC(I + 1) - SC(I))
IC(I) = T(I) - (SC(I) * IS(I))
NEXT I
T(I) = T(I) / 4
SR = SR + ((T(I) - MT) * (SC(I) - MLC))
SSQ = SSQ + (SC(I) - MLC) ^ 2
SUQ = SUQ + (T(I) - MT) ^ 2
BS = SR / SSQ: BC = MT - BS * MLC
CC = SR / ((SSQ * SUQ) ^ 0.5)
PRINT "CORRELATION COEF. ="; CC
DEF FN CL(TI) = 2.71828 ^ ((TI - BC) / BS)
RETURN
A2.5 Flow-injection program
1 DIM AV(290):SN = 10
2 HOME: SD = 0: REM SET UP PARAMETERS
4 SF = 0.2419
5 POKE CH, 0
6 ID = 3
7 BASE = 49312
10 POKE BASE + 2, 255: REM PORTS OUTPUT
20 POKE BASE + 3, 255: REM PORTS OUTPUT
30 POKE BASE + 11, 192: REM SET UP TIMER PIN 7
31 INPUT "DO YOU WANT CHARTS PRINTED? Y/N "; A1$: IF A1$ = "Y" THEN A2$ = "Y": GOTO 35
32 INPUT "DO YOU WANT THE RESULTS PRINTED? Y/N "; A2$
33 HOME
35 INPUT "INPUT PUMP SPEED, 0.1-100 "; PS
36 IF A2$ = "Y" THEN PR# 1: PRINT "PUMP SPEED=": PS: PR# 0
40 DIV = 0
45 BITS = 0.5 * ((665550 / (2 ^ (DIV + 16)) * PS) - 3.5)
50 A = INT (BITS / 256): B = INT (BITS - A * 256)
55 IF A > 255 THEN DIV = DIV + 16: GOTO 45
56 POKE BASE, DIV
60 POKE BASE + 4, B
70 POKE BASE + 5, A: REM LOAD TIMER DATA
85 REM
91 PRINT "TO CHANGE PUMP SPEED PRESS 'P'")
92 PRINT "TO INJECT PRESS SPACE BAR"
100 GET A$: IF A$ = "": GOTO 100
105 IF A$ = "P" GOTO 35
110 BL = 0: BM = 0: BV = 0
115 FOR I = 1 TO 60
120 POKE CN, 0: POKE CF, 0
130 IF BV > BM THEN BM = BV
140 BV = (PEEK(SL) - E) * F + PEEK(VL)
150 BL = BL + BV
160 NEXT
170 BL = BL / 60
180 BM = (BM - BL) * SF / 150
190 HGR
200 HPLOT 0, 150 TO 0, 0 TO 279, 0 TO 279, 150 TO 0, 150
210 POKE BASE + 1, 1
220 FOR I = 0 TO 279
230 POKE CN, 0: POKE CF, 0
235 FOR QW = 1 TO 5: NEXT QW
240 AD = (PEEK(SL) - E) * F + PEEK(VL)
250 AV = (AD - BL) * SF
260 HFT = 150 - AV
270 AV(I) = AV / 150
280 IF HFT < 0 THEN HFT = 0
285 IF HFT > 159 THEN HFT = 159
290 HPLOT TO I, HFT
310 NEXT I
320 POKE BASE + 1, 2
325 AM = 0
330 FOR I = 0 TO 279
340 IF AV(I) > AM THEN AM = AV(I)
350 NEXT I
356 PRINT : PRINT : PRINT
360 IF A1$ = "Y" THEN PR# 1: PRINT : CHR$ (27);"O": PRINT.
    CHR$ (7);"B"
365 IF A2$ = "Y" THEN PR# 1
370 PRINT "PEAK MAX =":AM
380 PR# 0
390 GOTO 85
A2.6 Solid dissolution program
10 HOME: ANAL = 0
20 INPUT "INPUT PUMP SPEED 0.1,100": PS
22 PRINT "OK"
30 DIV = 0
40 BITS = 0.5 * (665950 / (2 ^ (DIV / 16) * PS) - 3.5)
50 A = INT (BITS / 256): B = INT (BITS - A * 256)
60 IF A > 255 THEN DIV = DIV + 16: GOTO 40
70 E = 16: F = 256: SL = -16175: VL = -16176: CH = -16172:
   CN = -16166: CF = -16165: SF = 0.2419: BASE = 49312
80 POKE CH, 0
90 POKE BASE + 2, 255: REM: PORTE: OUTPUT
100 POKE BASE + 3, 255: REM: PORTA OUTPUT
110 POKE BASE + 11, 192: REM: SET UP TIMER PIN 7
120 POKE BASE, DIV
130 POKE BASE + 4, B
140 POKE BASE + 5, A: REM: LOAD TIMBR DATA
150 IF ANAL = 0 GOTO 230
160 FR#: 1
170 PRINT "ANALYSIS": ANAL
180 PRINT "ABSORBANCE"; INT (AM * 100)) / 100
190 PRINT: PRINT
200 FR#: 0
210 GOTO 240
220 FOR I = 1 TO 100: NEXT
230 FOR I = 1 TO 100: ANAL = ANAL + 1: BL = 0
240 IF I = 1 TO 100
250 POKE CN, 0: POKE CF, 0
260 IF T = T THEN T = T
270 BV = (PEEK (SL) - E) * F + PEEK (VL)
280 BL = BL + BV
290 NEXT
300 BL = BL / 100
310 POKE BASE + 11, 0
320 FOR I = 1 TO 8000: NEXT
330 HGR
340 HPLOT 0, 150 TO 0, 0 TO 279, 0 TO 279, 150 TO 0, 150
350 AM = 0
360 POKE BASE + 1, 1
370 FOR I = 1 TO 5000: IF I = 1000 THEN POKE BASE + 1, 2
375 IF I = 0 TO 279
380 POKE CN, 0: POKE CF, 0
390 FOR I = 0 TO 279
400 AD = (PEEK (SL) - E) * F + PEEK (VL)
410 AV = (AD - BL) * SF
420 HPT = 150 - AV
430 AV = AV / 150
440 IF AV > AM THEN AM = AV
450 IF HPT < 0 THEN HPLOT TO I, 0: GOTO 465
460 IF HPT > 159 THEN HPLOT TO I, 159: GOTO 465
470 NEXT I
480 POKE BASE + 1, 2
490 GOTO 90
-365-
A2.7 *Segmented tube model*
10 CLS
20 MODE GR:=HOME
30 PRINT"FIA Manifold Model"
40 PRINT"SRR 26/11/86"
50 PRINT
60 PRINT
70 PRINT
80 INPUT" INPUT TUBE LENGTH OF MANIFOLD (mm)"
90 INPUT" INPUT TUBE LENGTH OF SAMPLE LOOP (mm)"
100 INPUT" INPUT TUBE LENGTH DETECTOR (mm)"
110 INPUT" INPUT TUBE INTERNAL DIAMETER (mm)"
120 INPUT" INPUT FLOWRATE (m1/s)"
130 E=2*F/(\pi*(ID/2)^2)/100
140 X=INT(X/E); Y=INT(Y/E); W=INT(W/E)
150 Z=X+Y+W; S=1; C=0
160 DIM TC(Z+1), P(2*Z)
170 INPUT" IS THIS NORMAL (N) OR REVERSED (R) FIA?"
180 IF Q$="R" THEN S=0; C=1
190 FOR I=0 TO W+X
200 FOR I=W+X TO Z
210 TC(I)=C
220 NEXT I
230 FOR I=Z TO Z+1
240 TC(I)=S
250 NEXT I
260 TC(Z+1)=C
270 FOR I=1 TO Z+2
280 FOR Q=1 TO W: P(I)=P(I)+TC(Q): NEXT Q
290 FOR J=1 TO Z
300 TC(J)=(TC(J)/3)+(TC(J+1)*(2/3))
310 NEXT J
320 NEXT I
330 FOR I=0 TO 2*Z
340 P(I)*G; (-I*5)
350 NEXT I
360 NEXT I
Appendix 3: Ancilliary Topics


Introduction

The board provides the Apple user with a digital input/output facility. In effect, eight lines are available for input or output of digital information, four lines are available for output only and a single line can be configured to produce a wide range of frequencies of a serial waveform. A shift register is also available for the output of serial streams. The interface is based around a 6522 Versatile Interface Adaptor (VIA), a detailed description of which is given in reference [174]. Figure A3.1.1 shows a functional block diagram of the interface. The input/output lines available on a single connector are given in table A3.1.1.
Fig. A3.1.1

Block Diagram of Pump/Valve Interface

1 - 6522 Versatile interface adaptor
2 - Multiplexor
3 - device select logic
4 to 9 - Dividers
<table>
<thead>
<tr>
<th>Pin number</th>
<th>line</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CB2</td>
</tr>
<tr>
<td>2</td>
<td>CB1</td>
</tr>
<tr>
<td>3</td>
<td>PB3</td>
</tr>
<tr>
<td>4</td>
<td>PB2</td>
</tr>
<tr>
<td>5</td>
<td>PB1</td>
</tr>
<tr>
<td>6</td>
<td>PB0</td>
</tr>
<tr>
<td>7</td>
<td>PB7</td>
</tr>
</tbody>
</table>

These four lines are the inverted output of the VIA. Each pin can drive ten LS TTL loads.

PB7 - This line is used to output a stream of pulses under control of VIA timer number 1.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>CA1</td>
</tr>
<tr>
<td>9</td>
<td>CA2</td>
</tr>
<tr>
<td>10</td>
<td>PA0</td>
</tr>
<tr>
<td>11</td>
<td>PA1</td>
</tr>
<tr>
<td>12</td>
<td>PA2</td>
</tr>
<tr>
<td>13</td>
<td>PA3</td>
</tr>
<tr>
<td>14</td>
<td>PA4</td>
</tr>
<tr>
<td>15</td>
<td>PA5</td>
</tr>
<tr>
<td>16</td>
<td>PA6</td>
</tr>
<tr>
<td>17</td>
<td>PA7</td>
</tr>
</tbody>
</table>

**Table A3.1.1**

*Operation of the Interface*

The interface appears to the Apple as a series of
memory addresses, given in table A3.1.2 where BASE is the memory location specific to the wiring on the interface board. In this case BASE = 49312.

<table>
<thead>
<tr>
<th>Address</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>BASE</td>
<td>PORT B I/O register (ORB)</td>
</tr>
<tr>
<td>BASE+1</td>
<td>PORT A I/O register (ORA)</td>
</tr>
<tr>
<td>BASE+2</td>
<td>PORT B Data direction register (DDRB)</td>
</tr>
<tr>
<td>BASE+3</td>
<td>PORT A Data direction register (DDRA)</td>
</tr>
<tr>
<td>BASE+4</td>
<td>Timer 1 control</td>
</tr>
<tr>
<td>BASE+5</td>
<td>(described later)</td>
</tr>
<tr>
<td>BASE+6</td>
<td></td>
</tr>
<tr>
<td>BASE+7</td>
<td></td>
</tr>
<tr>
<td>BASE+8</td>
<td>NOT AVAILABLE IN THIS INTERFACE</td>
</tr>
<tr>
<td>BASE+9</td>
<td></td>
</tr>
<tr>
<td>BASE+10</td>
<td>Shift register control</td>
</tr>
<tr>
<td>BASE+11</td>
<td>Auxilliary Control Register (ACR)</td>
</tr>
<tr>
<td>BASE+12</td>
<td>These registers are described</td>
</tr>
<tr>
<td>BASE+13</td>
<td>in reference [174]</td>
</tr>
<tr>
<td>BASE+14</td>
<td></td>
</tr>
<tr>
<td>BASE+15</td>
<td></td>
</tr>
</tbody>
</table>

Table A3.1.2

Operation of the interface consists of three steps.

(I) Selection of an input or output facility on individual pins of the two data transfer ports.
This is achieved by addressing the appropriate data direction register with the correct number e.g. To make port A pins PA0, PA2, PA3 and PA7 output, with the rest to act as input, the instruction in BASIC would read:

POKE BASE+3, 141

where 141 is 10001101 in binary, each bit representing pins PA7 to PA0.

(II) Selection of the operation mode of the VIA. If the VIA is to be used for simple input and output this step is unnecessary, as this simple mode is preselected. If interrupts or handshaking for input/output are required, specific 'words' of data must be read to the auxiliary control register (ACR) [174]. If a stream of pulses is required on pin PB7 the free running mode of the VIA must be selected. This is achieved by using the BASIC instruction

BASE+11, 192

where 192 is 11000000 in binary and selects the VIA free running mode. Shift register operations are selected in a similar manner.

(III) Reading and writing to the appropriate registers of the VIA, with either the data to be transferred, or the data to be used in timing operations, or the data that is to be used in the shift register operations. Reading and writing is achieved using
the BASIC commands PEEK and POKE respectively.

Operation of timer 1 for production of a variable pulse frequency is achieved by loading a number into either addresses BASE+4 and BASE+5 or addresses BASE+6 and BASE+7, to produce a two byte number. Loading data to address BASE+5 causes the contents of the two registers to be automatically loaded into the timers counters. Once the counter has counted down from the number loaded to zero, the output on pin PB7 is inverted and the data in addresses BASE+4 and BASE+5 is unloaded into the counter and the countdown and inversion process repeated. This repetitive inversion of the output on pin PB7 produces square waves, the frequency of which depends on the number loaded into addresses BASE+4 and BASE+5. Entering data into locations BASE+6 and BASE+7 does not cause the countdown process to be interrupted and restarted with the new data (as with location BASE+5) but this data is loaded into the counter when the counter has reached zero.

As stated earlier, the number which is counted down is a two byte number. The minimum value for this is zero. The maximum pulse frequency is obtained when the smallest value is counted down i.e. zero. The minimum pulse frequency is obtained when the largest value is counted down. This is a two byte number represented by 255 and 255 loaded in each location i.e. 65535. Counting is at the system clock rate (2 MHz for the Apple IIe) but the time for countdown and inversion for a positive edge is $n+1.5$ clock cycles and for a negative edge is $n+2$ clock cycles where $n$ is the number to be counted down (174).
This is represented in fig. A3.1.2

The complete square wave cycle from Port B pin 7 of the VIA is therefore $2n+3.5$ counts in duration. The count rate is that of the Apple's internal clock i.e. 1 MHz.

To enable very slow pump speeds to be used, six dividers were provided on the interface each capable of halving the frequency of the square wave produced at Port B pin 7. These dividers were selected using a multiplexer controlled by pins 3 to 6 Port B. The signals obtained from the multiplexer when the appropriate number is written to Port B are given in table A3.1.3.
Decimal Number Written to Port B | Frequency of Signals from multiplexer output (units as those of the signal PB7)
---|---
0 | PB7
16 | PB7/2
32 | PB7/4
48 | PB7/8
64 | PB7/16
80 | PB7/32
96 | PB7/64

Table A3.1.3

Examination of table A3.1.3 shows that the frequency of the multiplexer signals when a number $N$ (a multiple of sixteen) is written to port B is $\frac{PB7}{2^{N/16}}$. The divisor is selected by entering POKE BASE,$N$.

The final signal from the multiplexer therefore, will produce a pulse rate of

$$1 \times 10^6 \times \frac{60}{(2n + 3.5) \times 2^{N/16}} \text{ pulses min}^{-1}$$

Application to valve and pump control

The P.S. Analytical valve used, has control facilities built in which require TTL inputs. These were wired directly to lines 0 and 1 of port A. When binary 10 (decimal 2) was output by the VIA port A, the valve moved to the inject position. When binary 01 (decimal 1) was output by the VIA port A, the valve returned to the fill
position.

Line 7 of port B was connected to the pulse input connection on the LKB 2132 pump. 500 interface generated pulses produce one revolution of the pump head.

Examples of the use of this interface are the programs given in Appendices A2.2, A2.3, A2.4.2, A2.5 and A2.6.
A3.2 Design and operation of a simple device for measurement of flowrate

During the project, the need arose to measure flowrate accurately. As a flame atomic absorption spectrometer consumes the material which it aspirates, the flowrate at the manifold outlet cannot be measured. Measurement of the uptake rate of reagent is impractical, as the change in volume of a large amount of reagent etc. will be imperceptible. O'Grady et al (157) used a pressure transducer (Radio Spares 303-343) to monitor the pressure in the capillary just before the nebuliser, and showed that this could be used to indicate changes in flowrate especially where a blockage of the capillary was involved. The transducer (fig. A3.2.1) compares the pressure at an inlet port with the pressure at a port which is open to the atmosphere. If a constant voltage is applied to the device the output in millivolts will be proportional to the difference in pressure between the two ports. Although use of this device gives an indication of flowrate and changes in flowrate, the output will vary for different manifolds, nebuliser and the method by which the flow is produced: Nebulisers produce flow by reduced pressure, pumps produce flow by increased pressure. Although each system may produce the same flowrate, pressures in these manifolds will differ. Hence the pressure sensor does not indicate flowrate for any system.

An examination of the Poiseuille equation [175] equation A3.2.1 shows that the pressure drop, p, across a
length of tubing $l$ of radius $a$, generated by the flow of a fluid of viscosity $\eta$, is proportional to the volumetric flowrate $Q$. This equation holds if the flow through the tube is laminar (non-turbulent) i.e. where the Reynolds number $R$, (equation A3.2.2) [176] is less than 1000.

$$R = \frac{2Q \rho}{\pi a \eta} \quad \text{A3.2.2}$$

where $\rho$ is the density of water.
The transducer was modified to allow differential pressure to be measured across a fixed length of tubing. The atmospheric reference port was sealed to a length of steel tubing to enable connection to the device illustrated in fig. A3.2.2

1m coil of 0.5 mm I.D.
Teflon tubing

**Diagram**

- **PM** PTFE membrane
- **PT** Pressure transducer
- **S** Squalane
- **GF** Glass filled PTFE Blocks

*Fig. A3.2.2*

The transducer was separated from the fluid in the manifold by PTFE membranes and squalane. This enables the use of the device for measuring the flowrate of most
fluids. The part of the transducer which is normally open to the atmosphere contains the electronics and this is protected by the squalane which is chemically saturated and an electrical insulator. The squalane also acts as a hydrostatic fluid, transmitting the pressure generated at each PTFE membrane to the pressure transducer.

The dimensions of the coil across which the pressure is measured were length: 1 m and internal diameter: 0.5 mm. If the density and viscosity of all the measured fluids are considered to be that of, or close to water the relationship between flowrate and pressure reduces to equation A3.2.3 by substitution into equation A3.2.1 thus:

\[
Q = \pi \left( \frac{0.5 \times 10^{-3}}{2} \right)^4 \frac{p}{1.002 \times 10^{-3} \times 1} \ m^3 \ s^{-1}
\]

\[
= 1.531 \times 10^{-12} \ p \ m^3 \ s^{-1}
\]

Usually, flowrates are expressed as ml min\(^{-1}\)

therefore, changing units gives

\[
Q = 9.186 \times 10^{-5} \ p \ ml \ min^{-1} \quad A3.2.3
\]

where pressure \(p\) is measured in pascals. The flowrate which gives a Reynolds number of 1000 is given by equation A3.2.4 from equation A3.2.2.
\[ Q = \frac{1000 \times 0.25}{10.57} \]

\[ = 23.65 \text{ ml min}^{-1} \]

The predicted linear range of this device should therefore be from 0 to 23.65 ml min\(^{-1}\). The sensitivity of the transducer is given in the specification as 6.67 mV psi\(^{-1}\) when excited by a 10 V supply. This is equivalent to \(9.68 \times 10^{-4}\) mV Pa\(^{-1}\).

The predicted sensitivity, \(S\), of the device in terms of flowrate, therefore is given by combining this pressure sensitivity with equation A3.2.3 to yield

\[ S = \frac{9.68 \times 10^{-4}}{9.186 \times 10^{-5}} \text{ mV ml}^{-1} \text{ min} \]

\[ = 10.54 \text{ mV ml}^{-1} \text{ min} \]

The device was calibrated by connecting it to the outlet of a peristaltic pump and the time taken to deliver specific volumes was measured whilst the output from the device was monitored using a chart recorder. Fig. A3.2.3 is the calibration graph obtained. Although the calibration curve bends slightly away from the flowrate axis, a reasonable straight line calibration can be obtained for most flow injection applications where flowrate is usually less than 8 ml min\(^{-1}\). The slope of the calibration in fig. A3.2.3 is 6.25 mV ml\(^{-1}\) min. This is less than the predicted value of 10.54 mV ml\(^{-1}\) min.
Fig. A3.2.3 Flowrate (ml min\(^{-1}\))
The actual excitation voltage of the transducer was 9.8 V which accounts for some of the discrepancy: How the transducer sensitivity varies with excitation voltage is not known but is probably linear. The pressure response of the particular transducer used has not been measured and this could differ from the specification.

Problems of air bubbles appearing in the squalane and leakage of squalane have occurred.

This device has been widely used and has enabled problems with pumping and injection to be diagnosed, optimisation of pump pressure plate position and monitoring of flowrate. Figures A3.2.4 and A3.2.5 show sketches of typical traces obtained. Fig. A3.2.4 shows the flowrate changes due to the pump rollers and the rotation of the pump head when flow is supplied by a peristaltic pump. Fig. A3.2.5 shows the changes in flow that occur upon repeat injections using a rotary valve when the flow is supplied from a pressure bottle: Initially the flow is stopped to produce a spike as the valve is turned, but returns to a decreased level due to increased backpressure from the sample loop. The flow is stopped again to produce a second spike as the valve is turned back, but then returns to the original level.

This device can also be used to observe the reduction of pressure pulses produced by a peristaltic pump (fig. A3.2.4), by air column pulse dampers, the efficacy of an injection valve bypass (which reduces the "spikes" in fig. A3.2.5) and the disadvantages of using wide bore tubes at a slow pump speed compared with narrow bore tubes at a
high pump speed.

Fig. A3.2.4
Pulses in flow produced by, a, the precession of the pump head and, b, by the pump rollers

Fig. A3.2.5
Changes in flow produced by repeat injections a and b when flow is produced using a pressure bottle
A3.3 Segmented tube model of sample behaviour in a flow injection manifold

If the parts of a manifold are considered to be divided into a number of segments (fig. A3.3.1) the process of sample transport can be modelled by calculating the concentration of material in each segment after a proportion of material has been transferred between neighbouring segments in unit time.

Laminar flow

When flow is applied to the tube, the linear velocity of the fluid increases across the tube from zero at the walls to twice the mean velocity at the tube centre, to give a parabolic profile. This distorts an originally cylindrical plug to a hollow 'bullet' shape (fig. A3.3.2). If the length of each segment is the distance travelled by the centre of the plug in one time unit, 2/3 of the
material in any segment will be transferred to the next segment in a direction towards the detector (fig. A3.3.3).

In fig. A3.3.3 the volume of a segment in a tube is represented by the square ABCD. Where AB is the centre of the tube and CD is the wall. When flow is applied to the tube the contents of the segment moves towards the detector and is distorted to occupy the volume ACDE which is characterised by the parabolas AC and DE. Integration of the parabola \( y = x^2 \) gives that the volume ACD is \( \frac{1}{3} \) the volume ABCD i.e. \( \frac{2}{3} \) the volume of a segment is transferred to the next segment in one unit of time. The mean velocity of the fluid is the volumetric flowrate divided by the cross sectional area. The length of each
segment can therefore be made to depend on the volumetric flowrate and the diameter of the tubing. The number of segments can then be calculated by dividing the length of a section of tubing (i.e. detector, manifold, etc.) by the length of a segment.

Normally, transport of material in a tube is considered to be by convection and diffusion [177]. The two processes, in the model, of sample transfer and
calculation of overall concentration in a segment, can be considered to model the processes of convection and diffusion.

Algorithm and Program

The program Appendix A2.7 is based on the following algorithm.

Initial conditions of tube length and diameter are entered (lines 80-120) and the length of the elements calculated (line 140). The length of each element is twice the mean linear velocity of the fluid. The mean velocity of the fluid is the volumetric flowrate divided by the cross sectional area of the tubing. The number of segments in each manifold element (sample loop; manifold tubing; detector) is then calculated (line 150). The program then puts values of units in the arrays which represent the manifold, where sample is present (lines 180-260). The concentration at each unit time (one operation of loop 300-320) in each segment is calculated and the mean concentration in the detector is calculated (lines 280 and 290). These values are stored and printed on the screen (lines 290 and 310). The transfer and calculation process is repeated for twice the number of manifold segments (lines 270 and 360). During operation of the program, the results can be plotted in a similar manner to a chart trace (line 350), producing visual representations of the results.
Results and discussion

Figures A3.3.4-A3.3.7 show the output of the program for various manifold conditions. The conditions were as follows except where varied:

Figure A3.3.4, Manifold 10, Sample 7, Internal diameter 0.5 and flowrate 5.

Figure A3.3.5, Sample 7, Detector 2, Internal diameter 0.5 and flowrate 5.

Figure A3.3.6, Manifold 10, Detector 5, Internal diameter 0.5 and flowrate 5.

Figure A3.3.7, Manifold 20, Sample 7, Detector 2 and Internal diameter 0.5.

No attempt was made to correlate real units for the above variables.

The types of curve are as expected. For varied detector volumes the trace fig. A3.3.4 shows increasing apparent dispersion as more of the diluted (dispersed) sample is contained within the detector. The trace for the largest detector shows a flat topped peak. This plateau is where all the sample is contained within the detector. As the length of the manifold is increased (fig. A3.3.5) dispersion increases. As injected volume is increased (fig. A3.3.6) the dilution (dispersion) of the centre of the slug is reduced until, eventually, a flat topped peak is obtained. When flowrate is decreased fig. A3.3.7 the peaks are broader, as they take longer to pass through the detector. This model predicts though, that peak height (and therefore dispersion) will decrease if the flowrate is decreased. As the model stands at
Fig. A3.3.4
Varying volume of detector

Fig. A3.3.5
Varying manifold length
Fig. A3.3.6
Varying injected volume

Fig. A3.3.7
Varying flowrate
present, increasing the internal diameter of the tubing changes all the other parameters especially the volume injected and the volume of detector.

The peak shapes appear rather symmetrical. Whilst the model was being developed it was noted that this symmetry is dependent on the amount of material transferred between adjacent segments. Although two thirds of the material in one segment will be transported by the laminar flow to the adjacent, 'downstream', segment, some of this material will diffuse back into the original segment. It is hoped that the model could be adjusted to enable traces, comparable with actual data, to be produced. The intention of this project however was not to produce models for flow injection analysis and hence further development has not taken place. The algorithm could be modified to allow different internal diameter tube sizes to be used in each manifold element. Different devices which are included in manifolds could be modelled e.g. a well stirred mixing chamber could be modelled by considering one segment to have the volume of the mixing chamber but with the same amount of material being transferred in and out of it as the other, smaller segments of the manifold. Timed injection could be modelled by not including a sample loop element but by having undiluted material entering the manifold for the injection period and being replaced by material of zero concentration after injection. Inclusion of reaction parameters and product monitoring could be modelled by multiplying the concentrations of reagent and sample
together and dividing by a scaling factor. This model does not produce an equation of concentration with respect to time and therefore peak widths etc. cannot be calculated directly. It is hoped that further developments of the model would enable it to be used for the prediction of the behaviour of a manifold, before it is constructed.
Appendix 4: References


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