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Models for Dispersion in Flow Injection Analysis

by

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B.Sc., G.R.S.C.

A Doctoral Thesis
submitted in partial fulfilment of the requirements
for the award of Doctor of Philosophy
of the Loughborough University of Technology
December 1987

Supervisor: J. F. Tyson, B.Sc., D.I.C., Ph.D.,
C.Chem. F.R.S.C.

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DEDICATION

To my Father
ACKNOWLEDGEMENTS

I would like to take this opportunity to thank my supervisor, Julian Tyson, for his help and encouragement throughout this work. I would also like to thank my industrial supervisor, Mike Sharpe, for his interest and support, and for providing an independent check of the mathematical derivations given in this thesis.

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The work described in this thesis has been the subject of several publications and presentations, which are listed below.

**PUBLICATIONS**


**PRESENTATIONS**


ADDITIONAL PUBLICATIONS AND PRESENTATIONS

The work described in this thesis has also been mentioned in the papers and presentations listed below.


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ABSTRACT

STONE, D. C., Models for Dispersion in Flow Injection Analysis.
Keywords: flow injection, dispersion, models, time injection, diffusion-convection, tanks-in-series, tanks-in-parallel, well-stirred tank.

An extensive study has been made of the factors affecting sample dispersion in flow injection analysis. This has included the effect of different manifold parameters on peak height and shape, and the use of various flow models to describe the physical processes involved.

The factors investigated included sample flow-rate, tube length and diameter, sample volume, and the diffusion coefficient of the solute. The role of the detector has also been investigated, and was found to exert a strong influence on the observed dispersion behaviour of a flow injection manifold. In the light of this, a low volume flow cell was constructed which minimised the contribution of the detector to dispersion. Different modes of sample injection ("time" and "slug" injection) have been studied, and an automatic valve constructed in connection with the work.

Both new and existing flow models have been evaluated in terms of their ease-of-use and ability to predict peak shape. Conditions for which the different models were valid were derived both experimentally and from consideration of the assumptions of the models. The applications of such models to the characterisation and design of flow manifolds has also been considered, and this approach compared to the purely theoretical and empirical approaches that have been described in the literature. The models examined in this thesis are the convection, diffusion, tanks-in-series, well-stirred tank, two-tanks-in-parallel and two-tanks-in-series models.

Finally, the practical implications of this work for the design of flow injection manifolds and the derivation of so-called "rules for dispersion" have been discussed, and suggestions made for further areas of research.
Chapter One

Introduction
1.1 HISTORICAL BACKGROUND

1.1.1 Continuous-flow techniques

Flow injection analysis (FIA) is one of a number of continuous-flow techniques that have been developed over the last thirty years or so. These include chromatographic techniques such as gas-liquid chromatography (GLC) and high performance (or high pressure) liquid chromatography (HPLC), as well as segmented-flow analysis (SFA) and field-flow fractionation (FFF). In general, a continuous-flow technique may be defined as one involving the introduction of a sample into a continuously flowing carrier stream, which then passes through some form of reactor prior to downstream detection and the readout of quantitative and/or qualitative analytical information. Within this broad definition, continuous-flow techniques may be subdivided in a number of different ways. For example, distinctions can be made between those techniques that involve the separation of the sample into its individual components and those that do not, and between those using segmented and unsegmented flow. This is illustrated below (Fig. 1.1).

Clearly, such a scheme is neither definitive nor exhaustive. However, it does illustrate how different continuous-flow techniques can be related to one another, and provides a framework within which to discuss the different approaches that have been used in continuous-flow analysis.

1.1.2 The Development of FIA

The term "flow-injection analysis" was first used by Ruzicka and Hansen [1] in 1975 to describe the use of sample injection into an unsegmented flowing stream for rapid continuous-flow analysis. They demonstrated the feasibility of this technique for the determination of methyl orange, the determination of phosphate by the molybdenum yellow and molybdenum blue methods, and the
Fig. 1.1 Different categories of continuous flow analysis methods
potentiometric determination of ammonia. Simultaneously, and independently, Stewart, Beecher and Hare [2] described the use of the same technique for the rapid determination of trypsin samples. Both groups based their ideas on the work of Skeggs [3], who had first described the use of segmented streams in continuous-flow analysis in 1957.

In SFA, samples are aspirated from individual sample cups by means of a peristaltic pump, and merged with a reagent stream. A wash solution is aspirated between samples to minimise sample carryover. Air bubbles are introduced to preserve sample integrity and promote mixing between the sample and reagent within each sample bolus. These bubbles are removed prior to the downstream-detection of the product formed.

Although SFA is a well-established and widely used technique, it suffers from a number of disadvantages (see later). One of these is that sample carryover is greater for an increased sampling rate, resulting in increased baseline noise and a deterioration in the accuracy and precision of the method. It was this fact that prompted both Ruzicka and Hansen and Stewart et al to investigate the use of unsegmented streams for continuous-flow analysis. This was not a new idea, as both Stewart [4] and Mottola [5] have since pointed out. For example, in 1970 Pungor [6] described the use of sample injection into an unsegmented flowing stream for the continuous-flow voltammetric determination of an electroactive species. A small mixing chamber was situated between the point of injection and the detector cell in order to ensure the reproducible mixing of the sample with the carrier stream. Between 1975 and 1979, Ruzicka and Hansen published their series of ten papers on FIA [1, 7-15] which did much to generate interest in the technique, as well as attracting some initial criticism (see for example the exchange between Margoshes [16] and Ruzicka et al [17] on reagent consumption and pumping pressure). Many workers entered the field during this period, and the apparatus, theory and application of FIA underwent considerable development, as described in an excellent review by Betteridge [18].
In 1979 the first international conference on FIA [19] was held, and marked the beginning of a rapid growth in the flow injection literature. By 1980, FIA was being successfully applied to agricultural, clinical, environmental and pharmaceutical chemistry, whilst the versatility of the technique was becoming increasingly apparent. This was demonstrated in a review by Ruzicka and Hansen [20], which listed nearly 80 papers describing the use of enzymatic reactor columns, gas diffusion cells, gradient techniques, intermittent pumping, merging zones, solvent extraction and stopped flow measurements, with methods of detection ranging from amperometry to spectrophotometry. In a more recent review [21], the same authors cited over 800 references for the period 1975 to 1986, covering the measurement of more than 200 different analytes, the use of 50 different analytical techniques and detection methods, 8 major areas of application, and the measurement of more than 10 different physical and chemical values, such as chemical oxygen demand, diffusion coefficients, pH values, refractive indices and reaction rate constants. Four textbooks have been published to date [22-25], whilst a journal devoted exclusively to FIA is now being published in Japan [26]. All this gives some idea of both the scope and importance of FIA as a modern analytical technique.

1.1.3 A Comparison of FIA, SFA and HPLC

As described earlier, FIA was initially developed by Ruzicka and Hansen and Stewart et al from segmented-flow systems. Stewart et al also employed some of the concepts used in the post-column flow system of the amino acid analyser developed by Spackman, Stein and Moore [27]. It was therefore inevitable that early in the development of FIA, comparisons were drawn with SFA. In the same way, obvious similarities were noted between FIA and HPLC. Therefore it is relevant to compare FIA, SFA and HPLC and examine their similarities and differences.
Flow diagrams for the determination of a single analyte by FIA, SFA and HPLC are shown in Fig. 1.2, together with the corresponding peak shapes observed. This clearly illustrates three of the major differences between FIA and SFA; the absence of air bubbles, the comparative simplicity of the flow-injection manifold and the measurement of a transient, rather than a steady-state signal. Ruzicka and Hansen [1, 22] identified the main disadvantages of air-segmentation as being pulsation of the flow due to the compressibility of air, the need to remove air bubbles prior to detection, and the need to obtain the steady-state signal. Much of the early criticism of FIA seems to have centred around such comparisons of the "advantages" and "disadvantages" of FIA and SFA, and the question of whether or not FIA was a "better" method than SFA (see for example Margoshes [16], Ruzicka and Hansen [17], and Snyder [28]). However, such discussions failed to take into account the fourth major difference between FIA and SFA, namely the different philosophies of the two techniques. In SFA [29], samples are viewed as discrete entities upon which a number of operations are performed in sequence, such as addition of reagent, mixing, heating, etc. Thus the technique is analogous to the manual batch method, with the samples being treated in the same way as a discrete analyser. If FIA is viewed as being SFA without the air bubbles, the same philosophy becomes applied to FIA. But as both Ruzicka [30] and Stewart [31] have pointed out, such an analogy is incorrect. Instead, the underlying principle of FIA is the controlled dispersion of the sample in the carrier or reagent stream, and does not require the steady-state to be obtained. Thus, although FIA was initially developed from SFA, the two techniques represent two different approaches and are better thought of as complementary rather than competing techniques.

At first sight, HPLC and FIA would appear to have much more in common than FIA and SFA. Ranger [32] has compared FIA, HPLC and SFA, listing a number of operating characteristics which provide points of comparison between
Fig 1.2 Flow manifolds for FIA, SFA and HPLC.

A = air; C = column; D = detector; E = eluent; P = pump; R = reagent; S = sample; V = injection valve; W = waste.
the three techniques. He pointed out that both FIA and HPLC employ sample injection rather than sample aspiration, segmented rather than unsegmented flow, and tubes of less than 1 mm rather than 2 mm internal diameter. Indeed, with the increasing use of micro-columns in FIA and post-column derivatisation in HPLC, there is a growing degree of overlap between the two techniques.

However, there is an important difference between the approaches used in the two techniques. In HPLC, the aim is to achieve the separation of a sample into its individual components by exploiting the partitioning of these components between two phases. The ideal peak shape is considered to be Gaussian, the flow manifold being constructed so as to minimise pre- and post-column dispersion effects in order to reduce peak asymmetry and band-broadening. This is in complete contrast to FIA, where the emphasis is on the controlled dispersion of the sample zone, the flow manifold being constructed to produce the required extent of mixing (dispersion) for each specific application.

1.2 THE THEORY OF DISPERSION IN FIA

1.2.1 Fundamental Concepts in FIA

Having reviewed the development of FIA and seen how it relates to other continuous-flow techniques, it is important to consider the development of the underlying principles and theory of FIA.

In their initial proposal for an unsegmented continuous-flow technique, Ruzicka and Hansen [1] suggested that the feasibility of such an approach depended on three things: (a) reproducible sample injection; (b) reproducible flow through the manifold; and (c) reproducible and sufficient mixing of the sample zone with the carrier stream. These three requirements were subsequently embodied in the three principles of FIA which were defined [15] as being (a) sample injection; (b)
reproducible timing; and (c) controlled dispersion of the sample zone.

Flow injection may be defined as the placement of a well-defined sample zone into a flowing stream, in such a way that the movement of the stream is not disturbed. Consider the simple flow injection manifold illustrated in Fig. 1.3.

![Fig 1.3 A simple flow injection manifold.](image)

Following sample injection, the sample zone is propelled by the carrier stream towards the detector. During this transport process, the sample zone becomes increasingly mixed with the carrier stream. This process occurs from the ends of the sample zone in towards the centre. If a suitable reagent is present in the carrier stream, a chemical reaction will also occur as the mixing process progresses. These three processes - transport, mixing and chemical reaction - all contribute to the overall dispersion of the sample zone, which in turn determines the resulting peak shape recorded by the detector. The transport and mixing processes give rise to what will be referred to as "physical dispersion", whilst the contribution of chemical reaction kinetics will be referred to as "chemical dispersion". The term "dispersion" will generally be taken as referring to purely physical dispersion. It was the understanding of the exact nature of these processes that proved to be the
key in the development of the theory of dispersion in FIA. Initially, Ruzicka and Hansen [1] proposed that turbulent flow be used to provide adequate mixing of the sample with the carrier (reagent), whilst limiting the extent of peak broadening. However, it was later established [13, 16] that laminar flow was predominant for the conditions used in FIA. This proved to be an important step in elucidating the mechanism by which the dispersion process occurred.

In part seven of their series of papers on FIA, Ruzicka et al [1] considered that the dispersion process produced a peak with symmetrical rising and falling edges intersecting at a point above the baseline, which constituted the peak height. This description was derived from the work of Thiers et al [33] and Begg [34], who presented results on the origin of the lag phase and peak shape in SFA. This led to the rising edge being described by the equation

\[ c = c_0 [1 - \exp(-Rt/V_s)] \]  \hspace{1cm} (1.1)

where \( V_s \) is the volume of the sample zone, \( R \) the volume of the carrier solution mixed with the sample zone per unit time, \( t \) is time, \( c_0 \) is the initial sample concentration and \( c \) the concentration at time \( t \). No attempt was made by Ruzicka and Hansen to verify this equation for FIA, it simply being stated that the equation was only valid for short line lengths and/or low \( R \) values. In this paper Ruzicka and Hansen also introduced the concept of the dilution factor, \( D \), as a measure of the extent of dispersion occurring in a flow injection manifold. This was defined as the ratio of the sample concentration at the peak maximum to the initial sample concentration. Subsequently [15], this was replaced by the dispersion coefficient, also given the symbol \( D \), which was simply defined as the reciprocal of the dilution factor.

In their ninth paper, Ruzicka et al [14] classified dispersion as being either "small", "medium" or "large". By this time it was well-established that factors such as
reactor geometry, flow-rate and sample volume all had an influence on both the extent of dispersion and the peak shape observed. The authors made use of some of the chemical engineering flow models described by Levenspiel [35, 36] to describe peak shapes encountered in FIA, and to develop their approach to what they termed "continuous-flow titrations". The ideas presented in this paper were more fully developed in the tenth paper in the series [15], in which an attempt was made to provide some practical guidelines for the construction of flow injection manifolds. These were expressed as the "six rules for dispersion". Although it was realised that a comprehensive theory of dispersion in FIA should include the contribution of chemical reaction kinetics, the authors chose to limit their discussion to the simpler case of physical dispersion only.

At this time, the classifications of dispersion were quantified in terms of the dispersion coefficient. Thus limited, medium and large dispersions were defined as being those for which $1 < D < 3$, $3 < D < 10$ and $D > 10$ respectively. Other important concepts introduced at this time were the contributions of laminar flow and molecular diffusion to the dispersion process, and the description of the overall dispersion due to the injection process, the reactor and the detector. The concept of the "half volume", $V_{1/2}$, was also introduced. This was defined as being the sample volume required to obtain a dilution of one half ($D = 2$). Finally, an expression was derived for the overall dispersion coefficient of a flow manifold consisting of a single line of uniform bore. This will be discussed in detail in Chapter 3.

1.2.2 The Development of Dispersion Theory

At the first international conference on FIA [19], several workers introduced important theoretical concepts which added to the work of Ruzicka and Hansen. Significantly, these contributions were derived from liquid chromatographic theory, rather than the chemical
engineering theory used by Ruzicka and Hansen. Tijssen (37) and Van den Berg et al (38) compared the relative performances of coiled tubular, open tubular and packed-bed reactors for FIA. These authors used the equations of Taylor (39,40) and Aris (41) to describe dispersion, which they discussed in terms of band broadening. Dispersion was quantified in terms of the liquid chromatographic concepts of plate height, mean residence time and peak variance. Reijn et al (42) and Poppe (43) employed the same concepts in their discussions of dispersion in flow injection manifolds. Reijn et al employed the work of Taylor (39) and the tanks-in-series model (see for example reference 35) to describe the contributions of the injection and detection processes to the overall dispersion of a flow injection manifold. They also defined two different types of injection process, which they called "time" and "slug" injection (these will be described more fully in Chapter 2). Poppe critically examined the role of the detector in continuous-flow systems, with particular reference to column liquid chromatography (LC) and FIA. Dispersion was again described in terms of band-broadening, the contributions of connecting tubing, dynamic response and finite measuring volume being discussed. Both Reijn et al and Poppe quantified dispersion in terms of the peak variance \( \sigma_t^2 \), making use of the additivity of variances to describe the overall dispersion of a flow injection manifold in terms of the contributions to dispersion from sample injection, sample transport and detection, i.e.

\[
(\sigma_t^2)_{\text{total}} = (\sigma_t^2)_{\text{injection}} + (\sigma_t^2)_{\text{transport}} + (\sigma_t^2)_{\text{detection}} \quad (1.2)
\]

In 1981, Vanderslice et al (44) discussed the theory of dispersion in FIA. They defined dispersion in terms of baseline peak width \( \Delta t_b \), and further characterised a single line manifold in terms of the appearance time of the peak \( t_a \). These authors used the numerical methods of Ananthakrishnan et al (45) and Bate et al (46) to solve the diffusion-convection equation describing the
dispersion of a solute in a laminarly-flowing stream [39]. Equations were derived for both $t_a$ and $\Delta t_b$, and guidelines developed for the design of simple, single line manifolds. However, these equations were subject to certain restrictions, which were clearly set out in a later paper [47].

Reijn et al [48] continued their study of different reactors for use in FIA by describing the use of the single bead string reactor (SBSR). This was constructed using a length of 0.6 mm i.d. tubing packed with large (0.4 mm i.d.) inert glass beads. This had the property of achieving a large extent of mixing without producing excessive dispersion. The semi-quantitative computer simulation of Golay and Atwood [49, 50] was used to describe dispersion in open tubes. In a following paper, Reijn et al [51] examined the role of the reactor in determining dispersion in flow injection systems, thus completing their earlier work on the contributions of the injection process and the detector [42]. Both Taylor's equations [39] and the axially dispersed plug flow or diffusion model of Levenspiel and Smith [36] were used to compare coiled tubes, open tubes, packed-bed reactors and SBSR's.

Ruzicka and Hansen [22] published the first monograph on FIA in 1981. This text incorporated all the theoretical concepts described in parts nine and ten of their series of papers on FIA, a discussion of the effect of chemical reaction kinetics on dispersion, summaries of the work of Tijssen [37], Van den Berg et al [38], Reijn et al [42] and Poppe [43], and an additional rule to the "six rules for dispersion" [15]. These rules may be summarised in the following way:

(i) Dispersion decreases with increasing sample volume according to the equation

$$D = [1-\exp(-K\gamma_s)]^{-1}$$

where $K = \ln 2/V_{\%}$

(ii) Dispersion increases with the square root of the distance travelled.
(iii) Dispersion decreases with decreasing flow-rate.
(iv) Limited dispersion ($10^{-3}$) is obtained for large sample volumes and short line lengths.
(v) Large dispersion ($10^{-10}$) may be obtained using gradient devices.
(vi) Maximum sensitivity is obtained for low dispersion and large extents of reaction.
(vii) Maximum sampling frequency is obtained for low axial dispersion (peak width) and small sample volumes.

Meschi and Johnson [52, 53] applied the work of Taylor [39, 40] to the prediction of the amperometric and coulometric response of tubular electrodes for FIA. They found excellent agreement between theoretical calculations and experimental results for flow-rates in the region of $0.5 \text{ ml min}^{-1}$.

Pardue and Fields [54, 55] described a kinetic model for single- and dual-channel flow injection systems containing a gradient chamber for so-called continuous-flow titrations. They argued that since mixing during the flow process imparted a kinetic character to the measurement, all flow injection determinations could be regarded as kinetic measurements, regardless of whether or not any chemical kinetic process was involved.

Painton and Mottola [56] described their initial study of the contribution of chemical reaction kinetics to dispersion in FIA. They pointed out that the value of the dispersion coefficient yielded no direct information on peak shape when both physical and chemical dispersion effects were involved. To aid their discussion, they defined physical and chemical dispersion coefficients such that

$$D_{\text{chem}} = D_{\text{phys}} + \text{chem} - D_{\text{phys}} \quad (1.3)$$

i.e., $D_{\text{phys}}$ is the dispersion coefficient measured for physical dispersion only, and $D_{\text{phys}} + \text{chem}$ is that measured for both physical and chemical dispersion. Initial results for the reaction of dichromate with L-ascorbic acid showed that values of $D_{\text{chem}}$ as high as 240
could be obtained, indicating the large extents of
dispersion possible in the presence of moderate or slow
chemical reactions.

Ramsing, Ruzicka and Hansen [57] applied the tanks-in-series (TIS) and diffusion models to the design of
manifolds for continuous-flow titrations without gradient chambers. Following their earlier work [15, 22], the
injection process was modelled by the TIS model for the
case of a single tank, and flow through the manifold was
described using the diffusion model [36]. (The equations
derived and conclusions reached in this paper will be
discussed in detail in chapter 3).

Tyson [58, 59] described the well-stirred tank (WST)
model for flow injection-atomic absorption spectrometry
(FIAAS). This model was used to develop a standards
addition method for FIA. Subsequently [60-62], the same
model was used to develop a reagent addition method and a
calibration procedure based on an exponential dilution
flask.

Reijn et al [63] described a possible approach to the
optimisation of single-line manifolds based upon that
employed in HPLC. Equations were derived for the maximum
sampling frequency obtainable within certain design
parameters and constraints, such as reactor type and size,
pressure drop, etc. Open tubular, coiled tubular, packed
bed and single bead string reactors were considered using
liquid chromatographic theory.

Betteridge et al [64] applied the work of Taylor to
the design of an automated viscometer based on the flow
injection principle. They constructed the flow manifold
to satisfy the conditions for which Taylor's equations
were valid, and derived expressions for viscosity and
diffusion coefficient based on the measurement of peak
width.

Hansen et al [65] discussed selectivity in FIA. They
defined the selectivity coefficient, $k_{AB}$, in the same way
as that defined in potentiometry, i.e.
\[ C'_A = C_A + k_{AB}C_B \]  

(1.4)

where \( C'_A \) is the apparent concentration of A, \( C_A \) the actual concentration of A, and \( C_B \) the concentration of an interfering species.

Van der Linden [66] used a variation of the TIS model to discuss gas diffusion through a membrane in on-line dialysis units for FIA. The dialysis unit was modelled by a series of tanks having a volume \( \Delta V \) on either side of a membrane. The degree of transfer of material through the membrane in each tank was assumed to be proportional to the concentration difference across the membrane.

In 1984, several authors approached the problem of chemical kinetics in FIA. Vanderslice et al [67] described a method for the determination of first and pseudo-first order rate constants based on the comparison of peak shapes obtained both with and without chemical reaction. This method assumed a negligible difference between the diffusion coefficients for the analyte in streams with and without the reagent.

Reijn et al [68] continued their earlier work by discussing kinetics in SBSR's. Physical dispersion was described using the TIS model, and the reaction kinetics assumed to be first or pseudo-first order. Guidelines were derived for the design of SBSR's, and it was shown that under certain conditions, the dispersion of the sample zone was unaffected by the presence of a chemical reaction.

Painton and Mottola [69] published an in-depth study of chemical dispersion in FIA. The diffusion-convection equation was modified to incorporate the effect of a first or pseudo-first order reaction, and solved using the numerical method of Ananthakrishnan et al [45]. Results were compared with those obtained using Taylor's equations [39], and with experimental results for the reaction between L-ascorbic acid and potassium dichromate [56]. Correction factors were used in the calculations to obtain better agreement between experimental results and theoretical calculations. These were chosen to compensate...
for the effects of reactor geometries, and the contribution of the flow cell in particular. It was found that the rate constant appeared to change with time, indicating the complex nature of the kinetics within the sample zone.

Another important development was the work of Ruzicka and Hansen [70] on the miniaturisation of flow manifolds for FIA. Three "scaling factors" were used to aid the design of such manifolds; the dispersion coefficient (D), the residence time (t_r), and the "dispersion factor", defined as V_m/V_r, where V_m is the volume of the flow manifold between the points of injection and detection. This last scaling factor was necessary because of the strong dependence of the dispersion coefficient on sample volume, and better reflects the contribution of manifold geometry to physical dispersion.

Tyson [71] described a peak-width calibration method for FIAAS based on the WST model. Nord and Karlberg [72] discussed dispersion in flow injection liquid-liquid extraction systems, and described the role of film formation in the dispersion mechanism that occurs in such systems (see also the work of Cantwell and Sweileh [73] on phase segmentation in solvent extraction flow injection systems). Betteridge et al [74] described a random walk simulation method for modelling chemical and physical dispersion in FIA. This method used a similar approach to that of Golay and Atwood [49, 50].

In 1985, Bernhardsson et al [75] published their study on dispersion in on-line dialysis units for FIA. They derived numerical solutions for solute transfer across a dialyser membrane for two cases: plug flow in both donor and acceptor streams, and laminar flow in both streams. They compared the results, and those obtained using the model of Van der Linden [66], with experimental data. The models developed by Bernhardsson et al enabled the effect of changing channel dimensions in the dialysis unit to be predicted with reasonable accuracy.

Harrow and Janata [76] discussed the effect of heterogeneous samples on dispersion in FIA, with a
particular emphasis on whole blood samples. They identified two sources of error associated with the determination of heterogeneous samples using homogeneous standards. One is the "tumbling" action of particulate matter in a laminarly flowing stream (see also the work of Riley et al (77)); the second arises from the dilution of the sample zone. For a heterogeneous sample, the sample volume is not the same as the volume of the aqueous (or liquid) phase. Thus if a sample contains 50% particulate matter by volume, and no analyte is exchanged between the solid and liquid phases, a two-fold increase in sample volume results in a three-fold increase in the volume of liquid phase. Harrow and Janata demonstrated that, if the hydrodynamic behaviour of a heterogeneous sample is assumed to be the same as that of a homogeneous sample, then injection of the heterogeneous sample into a manifold yielding $D = 2$ results in an effective dispersion coefficient, $D_{\text{eff}} = 3$. These authors examined the effect of viscosity and cell content on dispersion and peak asymmetry for whole blood samples. They quantified peak asymmetry as the ratio of the widths of the rising and falling portions of the peak at 10% of the peak height, following the definition employed by Foley and Dorsey (78) for describing chromatographic peaks.

1.2.3 Recent Developments in Dispersion Theory

Several recent papers have described important developments in dispersion theory. Appleton and Tyson (79) have studied the kinetics of instrument response in FIAAS. Because of the contribution of instrument response to dispersion in FIAAS, these authors defined the response dispersion coefficient, $D_r$, as being the ratio of the absorbances corresponding to the initial sample concentration and the concentration at the peak maximum, i.e.,

$$D_r = \frac{A_o}{A_p}$$  \hspace{1cm} (1.5)
The WST model was used as the basis of two new models; the two-tanks-in-parallel (TTP) and extended single-tank (EST) models. These were applied to the prediction of instrument response for FIAAS, and a comparison made between predicted and experimental results. Stone and Tyson [80] applied the WST model to the prediction of the effect of the finite volume of the flow cell used for spectrophotometric flow injection determinations.

Vanderslice et al [81] continued their earlier work [44] by describing the shapes of sample zones in a single-line manifold as a function of time for different tube dimensions, flow-rates and molecular diffusion coefficients. Their results clearly show the way in which the shape of the sample zone, and the concentration gradients within that zone are affected by the dispersion processes mechanisms of diffusion and convection.

Tyson [82] discussed peak width methods in FIA, particularly those employing gradient devices for so-called continuous-flow titrations. The concept of reagent dispersion was employed in the derivation of accurate equations for peak width based on the WST model for both single-line and merging-stream manifolds. The reagent dispersion coefficient, $D^R$, was simply defined as the ratio of initial reagent concentration to that at the sample peak maximum (reagent peak minimum). A peak width method was also described based on the formation of "doublet peaks" by reagent depletion.

Gisin et al [83] examined the precision of gradient techniques in FIA. The hydrodynamically limited precision of concentration gradients produced by gradient chambers and straight tubes was examined theoretically using several simple flow models. For gradient chambers, two models were described, resulting from the convolution of an exponential residence time distribution with a plug injection function and an exponential-washout function. For straight tubes, the TIS model was convoluted with a plug injection function and a "delta" injection function (injection of unit concentration in zero time). The resulting concentration gradients were represented
mathematically as the "distribution of the reciprocal dispersion coefficients in terms of reduced time and reduced injection volume", i.e. normalised concentration as a function of reduced time and reduced sample volume.

Pardue and Jager [84] continued the work of Pardue and Fields [54, 55] on the kinetic treatment of flow injection systems incorporating gradient chambers using a linearly-responding electrochemical detector. (The earlier work had used a non-linearly responding photometric detector). Different methods of presenting the calibration data using such systems were discussed.

Wada et al [85] described the effect of chemical reaction on the sample zone using a numerical method to solve the diffusion-convection equation with chemical reaction. Theoretical peak shapes were compared with experimental data, and sample zone shapes and concentration distributions calculated for different physical and chemical parameters. This work complements that of Vanderslice et al [81].

In a special paper containing contributions from over 30 different authors in the field of FIA [86], Van der Linden briefly discussed the implications of "scaling down" a flow injection manifold as suggested by Ruzicka and Hansen [70]. Scaling factors were calculated for the effect of reducing channel radius on such parameters as residence time, pressure drop, peak dispersion and sampling frequency.

Kristensen et al [87] have described a novel method for investigating dispersion in practical flow systems. A microvoltammetric electrode was used as a probe to monitor an electroactive tracer material (dopamine) across the diameter of a tube at the outlet of a flow injection manifold. It was shown that such probes provided useful tools for examining sample dispersion within a manifold for conditions that would be difficult to describe theoretically.

Olsson et al [88] derived the response function of a diffusion-limited amperometric enzyme electrode used as a detector in a simple flow injection manifold. Sample
dispersion within the manifold was approximated by a modified Gaussian function. The response function was obtained by convoluting this with expressions for diffusion into and chemical reaction in the enzyme membrane of the detector.

Finally, Leclerc et al [89] have discussed axial dispersion in coiled tubular reactors. However, although their findings are of interest in considering the effect of coiling on dispersion (see section 2.9), the conditions employed were outside those typically used in FIA.

1.2.4 Summary

In section 1.2.1, flow injection was defined as the placement of a well-defined sample zone into a flowing stream in such a way that the movement of the stream is not disturbed (Fig. 1.4,A). Under conditions of laminar flow, the sample zone will become distorted by the parabolic velocity profile established across the tube diameter (Fig. 1.4,B). Under the action of molecular diffusion, distortion of the sample zone will also occur as molecules near the sample/carrier interface diffuse away from regions of high sample concentration. Because of the distortion produced by laminar flow (the convection process), this results in diffusion in both the axial and radial directions (Fig. 1.4,C). The combined effects of both the diffusion and convection processes establish concentration gradients throughout the sample zone (Fig. 1.4,D), giving rise to the characteristic peak shape observed by a downstream detector. The diffusion and convection processes will be referred to as the primary dispersion mechanism.

Secondary dispersion mechanisms can be induced by tightly coiling the manifold tubing (secondary flow), or by introducing gradient chambers, packed bed or single bead string reactors. Chemical reaction may also be considered as a dispersion mechanism (chemical dispersion).

The parameters used to describe sample dispersion are
Fig. 1.4 Dispersion of the sample zone by the diffusion-convection mechanism. The arrow indicates the direction of flow.

summarised below (Table 1.1 and Fig 1.5), together with definitions and equations where appropriate. The subjects discussed within this chapter are listed in Table 1.2, together with the reference numbers of the relevant papers. This list also includes those papers cited elsewhere in this thesis.
Fig. 1.5 The definition of different parameters relating to peak shape
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Equation/Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample concentration</td>
<td>$C_o$</td>
<td>(see Fig. 1.5)</td>
</tr>
<tr>
<td>Peak concentration</td>
<td>$C_p$</td>
<td>(see Fig. 1.5)</td>
</tr>
<tr>
<td>Dispersion coefficient</td>
<td>$D$</td>
<td>$D = C_o / C_p$</td>
</tr>
<tr>
<td>Effective Dispersion coefficient</td>
<td>$D_{eff}$</td>
<td>Effective value of $D$ for a heterogeneous sample</td>
</tr>
<tr>
<td>Response dispersion coefficient</td>
<td>$D_r$</td>
<td>$D_r = H_o / H_p$</td>
</tr>
<tr>
<td>Chemical dispersion coefficient</td>
<td>$D_{chem}$</td>
<td>$D_{chem} = D - D_{phys}$</td>
</tr>
<tr>
<td>Physical dispersion coefficient</td>
<td>$D_{phys}$</td>
<td>Value of $D$ obtained in the absence of a chemical reaction</td>
</tr>
<tr>
<td>Asymmetry factor</td>
<td>$- $</td>
<td>$A/B \times 100$ (see Fig. 1.5)</td>
</tr>
<tr>
<td>Dispersion factor</td>
<td>$- $</td>
<td>$V_t / V_R$, where $V_R$ is the volume of the manifold</td>
</tr>
<tr>
<td>Appearance time</td>
<td>$t_a$</td>
<td>(see Fig. 1.5)</td>
</tr>
<tr>
<td>Baseline width</td>
<td>$\Delta t_b$</td>
<td>(see Fig. 1.5)</td>
</tr>
<tr>
<td>Time to peak maximum</td>
<td>$t_p$</td>
<td>(see Fig. 1.5)</td>
</tr>
<tr>
<td>Half volume</td>
<td>$V_{1/2}$</td>
<td>Sample volume required to give $D = 2$</td>
</tr>
<tr>
<td>Peak variance</td>
<td>$\sigma_t^2$</td>
<td></td>
</tr>
<tr>
<td>Instrument response</td>
<td>$H$</td>
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Table 1.1 Parameters relating to dispersion in FIA
<table>
<thead>
<tr>
<th>Subject</th>
<th>References</th>
</tr>
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<td><strong>Comparison of Techniques</strong></td>
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<td><strong>Dispersion Theory</strong></td>
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<td><strong>General Development</strong></td>
<td>1, 2, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 20, 21, 22, 30, 31, 51, 70</td>
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<td><strong>Gradient Techniques</strong></td>
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<td><strong>Heterogeneous Samples</strong></td>
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<td><strong>Kinetics</strong></td>
<td></td>
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<td><strong>Manifold Components:</strong></td>
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<td><strong>Coiling</strong></td>
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<td><strong>Detectors</strong></td>
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<td><strong>Dialysis Units</strong></td>
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<td><strong>Injection Process</strong></td>
<td>15, 42, 57</td>
</tr>
<tr>
<td><strong>Mixing Tanks</strong></td>
<td>6, 14, 15, 54, 55, 58, 59, 60, 61, 62, 84, 111, 115, 116, 118</td>
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<td><strong>Packed-bed Reactors</strong></td>
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<td><strong>SBSR's</strong></td>
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<td><strong>Solvent Extraction</strong></td>
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<td><strong>Miniaturisation</strong></td>
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<td><strong>Models:</strong></td>
<td></td>
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<tr>
<td><strong>Convective flow</strong></td>
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<tr>
<td><strong>Diffusion</strong></td>
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<td><strong>EMG</strong></td>
<td>105, 113, 114</td>
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<td><strong>EST</strong></td>
<td>79, 108</td>
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<tr>
<td><strong>Kinetic</strong></td>
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<tr>
<td><strong>Numerical</strong></td>
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<tr>
<td><strong>Simulation</strong></td>
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<td><strong>TIS</strong></td>
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<tr>
<td><strong>TTP</strong></td>
<td>79, 108</td>
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1.3 MODELS FOR DISPERSION IN FIA

1.3.1 Quantifying Sample Dispersion

In the previous section, the development of dispersion theory was reviewed, and the primary dispersion mechanism of diffusion-convection described qualitatively. The different parameters that have been used to quantify dispersion in terms of peak height and shape were also described. It is desirable to be able to relate these empirically derived parameters to theoretically derived expressions for sample dispersion for several reasons. If the effect of changing various manifold parameters (such as flow-rate, tube length, etc.) on the sample dispersion could be predicted, then it would be possible to design manifolds for specific applications without resorting to a time-consuming trial-and-error experimental approach. Also, the ability to accurately define the concentration gradients established within a manifold would offer significant advantages in the development of calibration strategies and gradient techniques. The problem then is to be able to quantify sample dispersion as measured by such parameters as peak height, peak width and peak shape, in terms of the physical parameters under the control of the analyst - tube length and diameter, flow-rate, and so
The obvious starting point in developing methods for achieving this is the work of Taylor [39,40], who described the dispersion of a solute flowing down a tube under conditions of laminar flow by the diffusion-convection equation. This may be expressed in the form

\[
\frac{\partial \Delta C}{\partial t} = D_\text{m} \left( \frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial r^2} + \frac{1}{r} \frac{\partial C}{\partial r} \right) - u_0 \left( 1 - \frac{r^2}{a^2} \right) \frac{\partial C}{\partial x}
\]

where \( C \) is the concentration of solute at the point \((x,r,t)\), \( D_\text{m} \) is the diffusion coefficient, \( x \) is the distance along the tube axis, \( r \) is the radial distance from the tube centre, \( t \) is time, \( a \) is the tube radius, and \( u_0 \) is the linear flow velocity at the tube centre. However, equation 1.6 cannot be solved directly, whilst the conditions for which Taylor derived approximate solutions lie outside those normally encountered in FIA. This has led to the use of numerical techniques, flow models, and empirical methods for quantifying sample dispersion in FIA.

1.3.2 The Modelling Rationale

An important concept underlying both the numerical and modelling approaches to quantifying dispersion in FIA is that of the ideal (or theoretical) manifold. This arises from the commonly made assumptions that (a) conditions of laminar flow exist, (b) the flow is undisturbed by the injection process, (c) flow occurs down a long, straight tube of circular cross-section and (d) the solute concentration is measured in a plane at right angles to the direction of flow. The problem with making such assumptions is that they are not generally applicable to real manifolds. For example, although laminar flow is the primary flow pattern that exists under flow injection conditions, coiling, connections, valves and tight bends will all introduce a variety of secondary flow patterns,
some of which cannot be accurately predicted. Also, the
behaviour of real detectors does not often match the ideal
and, as will be seen later (Chapter 2), can have a
significant effect on the observed dispersion behaviour of
a manifold. Finally, manifolds for practical analyses are
often more complicated than the simple, single-line case
envisaged above. They may, for example, contain one or
more confluence points, phase segmentors and separators,
dialysis units, and so on.

Whilst some of these problems (such as coiling of the
manifold tubing) can be taken into account using numerical
techniques to solve the relevant differential equations
[41, 45, 46], others (such as confluencing) are not
amenable to a rigorous theoretical treatment. On the
other hand, an entirely empirical approach would be time­
consuming, and would have little or no predictive power
beyond the experimental conditions and components used.
The alternative to both these approaches is the use of
suitable flow models.

The modelling approach by definition is semi­
empirical, the starting point for the development of many
flow models being a set of experimental observations (this
will be discussed in detail in Chapter 3). However, this
does not rule out a theoretical approach, since it is
equally possible to develop models based on approximations
to known theoretical expressions (the work of Taylor [39]
is an example of this). Whichever route is adopted,
certain requirements must be met. These are (a)
sufficient experimental observations to adequately state
the problem, and (b) suitable criteria by which to assess
different flow models. Such criteria can readily be
identified as (a) that the model should apply to a wide
range of conditions, (b) that it should take into account
all the relevant flow injection variables, and (c) that it
should be capable of predicting peak height, peak width
and peak shape.
1.4 AIMS AND OBJECTIVES

The aims of the work described in this thesis can be summarised as being (a) to evaluate existing models for sample dispersion in FIA, and (b) to develop and assess new models for dispersion. In order to achieve this, an extensive investigation of the factors affecting physical dispersion has been undertaken, the results of which are presented in Chapter 2. The factors investigated were the contribution of the detector and the effects of coiling, flow-rate, tube length and internal diameter, the injection process and sample volume, the solute used and confluencing. The results obtained were then used to assess existing models for dispersion, and to develop new models (Chapters 3 and 4).

During the course of this work, several results were obtained which have important implications for practical FIA, including the role of the detector and the use of "time" injection. These are discussed in the relevant sections. The findings of this work, and the conclusions drawn from them, are summarised in Chapter 5.

A full bibliography and list of symbols may be found in Appendices A and B respectively.
Chapter Two

Physical Dispersion in FIA
2.1 GENERAL INTRODUCTION

2.1.1 Apparatus and Chemicals

For convenience, the apparatus used is listed below, and will be referred to using the appropriate symbol throughout. Flow injection manifolds were constructed using PTFE tubing of various internal diameters (Anachem, R. S. Components). Connections were made using low pressure LC fittings (Anachem). Omnifit fittings (Anachem) were used in the study of the effect of coiling. Solvent resistant pump tubing (Sterilin Instruments) was used with the peristaltic pump (P2). Plastipak™ syringes were used to fill the valve sample loops, and with pump P1.

Chemicals used were as follows:

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>KMnO₄</td>
<td>AR grade (BDH and Fisons)</td>
</tr>
<tr>
<td>CoCl₂·6H₂O</td>
<td>AR grade (BDH)</td>
</tr>
<tr>
<td>Tartrazine</td>
<td>(Pointing Ltd.)</td>
</tr>
<tr>
<td>Ponceau S</td>
<td>(Fisons Ltd.)</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>(Sigma)</td>
</tr>
</tbody>
</table>

All solutions were made up in distilled or deionised water, using standard volumetric glassware. Calibration standards were prepared by serial dilution of a concentrated stock solution. Unless otherwise indicated, "slug" injection was used throughout (see section 2.7).
Computers:
C1 Dedicated microprocessor, built in-house by Pye-Unicam Ltd.
C2 Apple IIe microcomputer, with interface card built in-house at L.U.T.
C3 SP9 Computer (Pye-Unicam).
C4 PU9090 Data Graphics System (Pye-Unicam).

Detectors:
D1 SP8-500 UV/visible spectrophotometer (Pye-Unicam)
D2 SP8-100 UV/visible spectrophotometer (Pye-Unicam)
D3 SP6-250, visible region spectrophotometer (Pye-Unicam).
D4 SP9 AA spectrometer (Pye-Unicam)
D5 PU 8610 UV/visible spectrophotometer (Pye-Unicam).

Flow cells:
F1 35 μl, 5mm pathlength, prototype model.
F2 8 μl, 10mm pathlength (Hellma).
F3 sub-μl cell, built in-house at L.U.T.
F4 25 μl, absorbance/fluorescence cell (Hellma).
F5 60 μl, 10mm pathlength (Pye-Unicam).

Pumps:
P1 Sage 341A syringe pump (Orion).
P2 Minipuls2 8-channel peristaltic pump (Gilson)

Chart Recorders:
R1 Tarkan 600 (W + W).
R2 PM8251 chart recorder (Philips).
R3 PM8252A two pen recorder (Philips).
R4 TE200 chart recorder (Tekman).

Valves:
V1 5020 injection valve (Rheodyne)
V2 8-port slider valve (Altex)
V3 5020 injection valve configured as a stream switching valve (Rheodyne).
V4 Motorised 5020 injection valve (see section 2.7).
2.1.2 General Procedures

All flow-rates were determined experimentally. This was done either by collecting the effluent from the manifold for a timed period and weighing, or by timing the aspiration of a measured volume of sample solution.

Sample loop volumes were calibrated by collecting the effluent from the manifold for ten replicate injections in a graduated flask (10 or 25 ml), making up to volume and measuring the steady-state absorbance. The loop volume was then calculated from the initial sample concentration and the amount of sample delivered, which was obtained by interpolation from a calibration graph. The loop volume was calculated as the mean value of three replicate measurements.

The response time of the detector system (detector plus chart recorder) was measured where appropriate by rapidly blocking the light beam of the detector, and measuring the time taken to reach the steady-state from the chart recorder.

Data processing was performed on the University's mainframe computer (Honeywell DPS8/4) using programs written in FORTRAN 77. These are described in Appendix D.

2.1.3 Practical Considerations

During the course of some preliminary experimental measurements of dispersion using simple flow injection manifolds, several practical problems were encountered which have a bearing on all the work described in this chapter. These are discussed below, and will be referred to later.

The Presence of Air Bubbles

The presence of air bubbles is one of the problems most commonly encountered in FIA, and results in two main effects. Firstly, they cause changes in the dispersion of the sample zone by disrupting the flow pattern through the
manifold. Secondly, when present in a flow cell, they can cause "spikes" and fluctuations in the baseline. This happens because the air bubbles shorten the effective pathlength of the cell, and introduce additional refraction losses, both of which affect the observed absorbance value. Small air bubbles cause problems when they become lodged in the flow cell, as they expand and contract with changes in pressure within the manifold. Thus any interruption of the flow, such as that caused by switching an injection valve, or any change in pumping pressure, will result in unpredictable changes in the observed baseline. The major sources of air bubbles are leakage through connections and valves, degassing of solutions, and the changing of loading syringes and carrier streams. In this work, degassing of the sample and carrier solutions in the manifold was prevented by the presence of a back-pressure coil on the cell outlet. In order to avoid the entry of air bubbles whilst changing the carrier stream for the measurement of steady-state values, a stream-switching valve was introduced between the pump and the injection valve (Fig. 2.1). This also

![Diagram of a stream switching valve]

Fig. 2.1 Use of a stream switching valve.

C = carrier stream; D = detector; IV = injection valve; P = pump; SV = stream switching valve; W = waste; S = sample.

allowed the flow cell to be checked for the presence of air bubbles, since the pressure in the manifold could be
changed rapidly using a syringe through the stream-switching valve. The presence of an air bubble would then be indicated by corresponding changes in the baseline or steady-state signal. Valves and connections were regularly checked for leakage, and adjusted when necessary.

**Pulsation of the Flow**

Pulsation of the flow is a problem inevitably encountered when using a peristaltic pump. This is a direct result of the action of the rollers on the pump tubing. Pulsation can be reduced to some extent by good design of the pressure plate (platten), the use of a large number of rollers on the pump head, and careful adjustment of the pressure exerted by the platten on the pump tubing. This last point is illustrated in Fig. 2.2, which shows the effect of adjusting the platten pressure on the flow-rate, as measured by a pressure-sensitive device situated downstream of a peristaltic pump. As the platten pressure is increased, so the flow-rate increases up to a maximum value. Beyond this point, increasing platten pressure results only in an increased rate of wear of the pump tubing. Fig. 2.2 also shows that the pulsation of the flow arises from two sources. The first of these is the action of each individual roller; the second is due to the eccentricity of the pump head. With pump P2, this was minimised by using a channel close to the bearing plate of the pump head. The platten pressure was adjusted for each size of pump tubing used, and new pump tubes were "worn in" before use. A depulsing column was placed between the pump and the injection valve, which consisted of a glass 'T'-piece connected to a piece of wide bore pump tubing stoppered at one end and kept full of air.

Pulsation was also experienced with the syringe pump (P1). This appeared to be due to the plunger sticking in the syringe barrel, resulting in an uneven motion. This problem occurred for high delivery rates and manifolds generating high back pressures. The problem was overcome...
Fig. 2.2 Effect of platten pressure on flow rate.
Signal (mV) is proportional to flow-rate. Arrows indicate points at which platten pressure was increased by allowing the syringes to become worn in before use.

The Effect of Breaking and Making Connections

With the apparatus used, good reproducibility was obtained for replicate injections, with values of 1% RSD or less being typical for ten measurements of peak height. However, poorer precision was obtained when the value of D was measured under identical conditions over a period of
several days, or if the measurements involved the breaking and making of connections within the manifold. For example, for a 110 cm length of 0.58 mm i.d. tubing, a sample volume of 113 μl, and a flow-rate of 5.0 ml min⁻¹, the mean of ten individual determinations of the value of D was found to be 1.946 with 95% confidence interval ±0.011. When these measurements were performed over a period of several days with the breaking and making of connections between each determination, the value of D obtained was 1.905 with 95% confidence interval ±0.089. These results were obtained using flanged joints. Similar results were obtained using flangeless connectors (see section 2.3.3).

The variation in the value of D found for identical conditions is probably due to slight variations in tube alignment through the connectors, resulting in different mixing and dead volumes wherever connections are made. Simple butt joints were also tried to see if better precision could be obtained, but the results were inconclusive.

Sample Material

Initial experiments were performed using potassium permanganate as the sample material. However, when the low volume flow cell was used (see section 2.2.3), a high concentration was required which caused deposition of manganese dioxide within the valve, flow cell and manifold tubing. Therefore, alternative sample materials were investigated. The criteria used to assess these were that they should be water-soluble, stable in solution, strongly absorbing in the visible region, and give a linear calibration curve up to 1A.U. when used with the low volume flow cell. Compounds considered included potassium dichromate, bromothymol blue, copper sulphate, safranine O (CI 50240), green S (CI 44090), tartrazine (CI 19140) and ponceau S (CI 27195). Tartrazine was chosen as it satisfied all the above criteria, and shows excellent long-term stability in solution [90].
2.2 EFFECT OF THE FLOW CELL

2.2.1 Preliminary Experiments

During preliminary studies of the effect of various manifold parameters on physical dispersion, results were obtained for the variation of the dispersion coefficient with tube length, flow-rate and sample volume for a simple single-line manifold using both 8 μl and 35 μl flow cells (Fig. 2.3).

Fig. 2.3 Flow cells used during preliminary experiments.
A. \( V_{\text{CELL}} = 8 \mu l \)  B. \( V_{\text{CELL}} = 35 \mu l \)  F indicates the direction of flow; L indicates the light path.

The results showed that the flow cell could dramatically affect both peak height and peak shape. For example, Fig. 2.4 shows the variation of the dispersion coefficient with flow-rate and sample volume obtained for both cells. For the 8 μl cell, the value of D was independent of flow-
rate above 2 ml min\(^{-1}\). However with the 35 µl cell, the value of D increased with increasing flow-rate until a maximum was reached at about 5.5 ml min\(^{-1}\), and then decreased again. These results were obtained for a line length of 30.1 cm and a tube i.d. of 0.58 mm. Comparison of the results obtained for the 8 µl and 35 µl cells for identical manifold conditions revealed that the larger cell always gave the higher dispersion coefficient. Washout times were also significantly longer for the 35 µl cell than for the 8 µl cell, as illustrated by Fig. 2.5.

These results show that the flow cell can make a significant contribution to the observed dispersion behaviour of the sample zone in a flow injection manifold. Comparison of the construction of the two cells (Fig. 2.3) revealed some important differences between the flow paths through the cells. It therefore seemed likely that both the mixing pattern within the cell and the optical volume of the cell were responsible for the observed results. For this reason, it was decided to investigate the role of the flow cell more closely.
2.2.2 Effect of a Finite Measuring Volume

Reijn et al [42] have discussed the contribution of the method of detection to the measured peak variance in FIA systems. They compared the cup-mixing and mean value types of detector. A cup-mixing detector, such as a fraction collector or mass flow detector, records a weighted mean concentration. A mean value detector records the average concentration in a plane perpendicular to the flow. This type of detector is approximated by a conventional spectrophotometric flow cell, such as those commonly used in FIA and HPLC. However, such detectors actually measure the concentration within a finite volume, and are usually arranged so that the detector "looks" along the flow path, rather than across it. Reijn et al did not take a finite measuring volume into consideration in their work.

Poppe [43] has briefly discussed the effect of the finite volume and the dynamic behaviour of the detector electronics on the observed response for continuous-flow detectors. This paper highlighted the fact that it is difficult to predict the response function of a real flow cell because the exact flow pattern through the cell, and the effective measuring volume, are often unknown.

The effect of a finite measuring volume in the absence of any additional mixing due to flow was examined using the well-stirred tank (WST) model (see Appendix C). If a volume \( V_s \) of a sample of unit concentration is allowed to enter a well-stirred mixing tank of volume \( V_m \) at a flow-rate \( Q \), it can be shown that the resulting concentration-time (C-t) curve at the tank outlet is described by the equations

\[
C = 1 - \exp\left(-\frac{Qt}{V_m}\right), \quad 0 < t < t_p \tag{2.1}
\]

\[
C = C_p \cdot \exp\left[-\frac{Q(t - t_p)}{V_m}\right], \quad t > t_p \tag{2.2}
\]

where \( t_p \) is the time to the peak maximum, and \( C_p \) is the
peak concentration. If a detector having a measuring volume $V_d$ is situated immediately at the tank outlet, then the concentration of solute recorded by the detector at any point in time $t$ will be the mean value of the concentration profile existing within the detector at that point. Assuming plug flow to exist throughout the detector volume, then the residence time of any element of fluid entering the detector, $t_d$, will be equal to $V_d/Q$. Therefore, the observed concentration, $C_{obs}$, at time $t$ will be the arithmetic mean of the $C-t$ curve over the interval $(t-t_d)$ to $t$, that is

$$C_{obs} = \frac{1}{t_d} \int_{t-t_d}^{t} C(t) dt \quad (2.3)$$
Because the function \( C(t) \) is discontinuous, equation 2.3 cannot be solved directly. Therefore, a six-strip Simpson's Rule procedure was used to calculate response curves for a range of detector volumes. (The computer program used to perform these calculations is described in appendix D).

The results obtained are shown in Fig. 2.6. Curve A represents the \( C-t \) curve at the tank outlet, and curves B to F show the effect of increasing detector volume on the observed peak shape. The results obtained show that as the detector volume increases, there is an increasing distortion of the observed response curve, characterised by an increase in the observed time to the peak maximum, a decrease in peak height, and an increase in peak width.

The shape of these curves suggested that the detector volume acted so as to introduce a time constant into the detector response function. This being the case, then the effect of the detector volume should be most pronounced for rapidly changing signals. This was investigated by making use of the property of the WST model that

\[
C_p = 1 - \exp\left(-\frac{V}{V_s}\right)
\]  
(2.4)

That is, if the sample and tank volumes are kept equal, a series of \( C - t \) profiles can be generated having the same peak height, but different rates of change in concentration about the peak maximum. Calculations were performed for a range of sample and tank volumes, the differences between the "actual" and "observed" values of \( t_p, C_p \) and \( D \) being calculated. The effect of increasing the sample volume for constant tank volume was also investigated. The \( C - t \) profiles used in these calculations are shown in Fig. 2.7, and the results obtained are summarised in Table 2.1.
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Table 2.1 The Effect of a Finite Measuring Volume on \(t_p\), \(C\), and \(D\), calculated using \(Q = 2.00\) ml min\(^{-1}\) and \(L = 0\) cm.

(a) The difference between the "actual" and "observed" values.

(b) \(\varepsilon_t\) is the maximum error associated with the calculated value of \(t_p\) observed.
Fig. 2.6  C-t profiles for the WST model.  

$Q = 2.0 \ \text{ml min}^{-1}, \ \ V_d = (A) \ 0, \ (B) \ 10, \ (C) \ 25, \ (D) \ 40, \ (E) \ 60 \ \text{and} \ (F) \ 80 \ \mu\text{l.}$

As the sample volume is increased for a constant tank volume, then the value of $C_p$ approaches the steady-state value, resulting in a lower rate of change in concentration up to the peak maximum, and a greater rate of change in concentration immediately after the peak maximum (see Fig. 2.7, curves A and B). This implies that the differences between the actual and observed values of $t_p$ should decrease with increasing sample volume. 

Comparison of the results obtained for a tank volume of
Fig. 2.7 C-t profiles for the WST model.

Q = 2.0 ml min⁻¹. 
(A) $V_s = V_m = 100 \mu l$.
(B) $V_s = 200 \mu l$, $V_m = 100 \mu l$.
(C) $V_s = V_m = 200 \mu l$.
(D) $V_s = V_m = 500 \mu l$.

100 \mu l$, and sample volumes of 100 and 200 \mu l show that this is indeed the case. Comparison of the results obtained for different sample volumes and a constant value of $V_s/V_m$ confirms that the errors in the observed values of $t_p$, $C_p$ and D introduced by a finite detector volume are greatest for the most rapidly changing C-t profile ($V_s = V_m = 100 \mu l$).
To summarise these results, in practical dispersion measurements the finite volume of the detector flow cell will distort the observed peak shapes, resulting in errors in the measured values of $t_p, C_p$, and $D$, even if no extra mixing occurs within the flow cell. These errors will be greatest for large detector volumes and/or rapidly changing $C-t$ profiles.

2.2.3 Construction of a Low Volume Flow Cell

From the results outlined in sections 2.2.1 and 2.2.2, it was clear that if the dispersion properties of the flow injection manifold were to be successfully investigated, the contribution of the detector would have to be minimised. Therefore it was decided to construct a flow cell having as low a volume as possible, with a flow path through the cell matching that of the manifold tubing. The final design is shown in Fig. 2.8.

Fig. 2.8 Construction of a low-volume flow cell.
L = light path; F = flow path; shading indicates optical volume.

The cell was made from a piece of drawn glass tubing having an internal diameter of 0.6 mm, mounted in a black perspex block. In order to minimise both the optical
volume of the cell and the effect of light "channelling" through the tube walls, the light guide was drilled to the same internal diameter as the tube. The light guide was partially drilled out to a diameter of 5 mm on either side of the glass tube to improve the light throughput of the cell. The completed flow cell was placed in a modified Pye Unicam cell mounting, which allowed some adjustment of the cell position to be made. Connections were made to the cell by simple butt joints, using small pieces of pump tubing as sleeving. The optical volume of the cell was estimated to be 0.2 µl.

Because of the small cell aperture, a low light throughput and a high background absorbance were obtained. To overcome these problems, compounds were chosen having an absorption maximum within the optimum wavelength range of both source emission and detector response profiles. Large bandwidths were also used, these being 8nm and 10nm for detectors D3 and D2 respectively. Both instruments had an absorbance backoff facility, which enabled a zero baseline to be established. With the dual beam instrument, it was found necessary to attenuate the reference beam in order to zero the baseline and protect the photomultiplier tube. This was achieved using a simple pin-hole mask. With air in the cell, a transmittance of only 2% was obtained (100% set with air in the beam). Baseline noise levels were low, being of the order of 0.005A (peak-to-peak) for D2, and <0.002A (peak-to-peak) for D3. It was found that care had to be taken to keep both the cell and the sample compartment windows clean, as even small quantities of dust caused a significant stray-light effect. The orientation of the cell with respect to the light beam was found to be quite critical, as small changes of alignment (rotation about the vertical and horizontal axes, or change in cell height) reduced the light throughput, causing either a shift in the baseline or a complete loss of signal. No problems were experienced with refractive index effects. This was attributed to (a) the use of both aqueous carrier and sample solutions, and (b) the small difference in
refractive index between the carrier and sample solutions. For example, when the refractive index of the most concentrated solution of tartrazine used (0.25 g l$^{-1}$ in distilled water, $\lambda_{\text{max}}$ = 426 nm) was measured, it was found to be indistinguishable from that of pure water. (This measurement was made on an Abbé 60 refractometer using a sodium lamp at 589.3 nm).

Within the constraints outlined above the cell performed satisfactorily, although such a cell would not be suitable for general analytical applications with conventional spectrophotometers. Similar flow cells have been described for use in open capillary LC (see for example Yang [91], Ishii et al [92], and Tsuda et al [93]). It is therefore suggested that such cells might well find application in FIA with spectrophotometers having optics specifically designed for small aperture cells.

2.2.4 Experimental Comparison of Different Flow Cells

Having constructed a low volume flow cell, the performance of this cell was compared with that of several conventional flow cells all having different optical volumes. The experimental manifold used is shown in Fig. 2.9. Peak shapes were recorded for cells F2 - F5 in turn for flow-rates of 0.84, 1.99 and 6.03 ml min$^{-1}$. Steady-state absorbance values were also measured, so that the

![Flow manifold for the comparison of different flow cells](image)

Fig. 2.9 Flow manifold for the comparison of different flow cells
peak heights could be normalised for direct comparison, values being read directly from the chart recorder output. Solutions containing either 0.02 or 0.25 g 1\(^{-1}\) of tartrazine were used, depending on the cell pathlength.

The results obtained are shown in Fig. 2.10, plotted as relative peak height against time. For each flow-rate, the curves for the 0.2, 8.0 and 25.0 \(\mu\)l cells lie close together. The slight differences in peak height and shape between these curves may indicate real differences between the cells, but could equally be attributed to the variation produced by the breaking and making of connections required to change cells (see section 2.1.3). Although these three cells appear to be very different in size, the 0.2 and 25 \(\mu\)l cells represent similar measuring conditions because in both cells detection occurs across the flow (see Fig. 2.11). Also, only a small part of the window of the 25 \(\mu\)l cell coincided with the light path of the spectrophotometer, thus reducing the effective optical volume. The biggest difference in peak shape was observed between these cells and the 60 \(\mu\)l cell. Comparison of the results shown in Fig. 2.10 reveals that peak height is significantly reduced for the 60 \(\mu\)l cell, whilst peak width is correspondingly greater. The peak also appears later in time, even though the connecting tubes were carefully cut to keep the distance between the injection valve and the optical volume constant. This is in agreement with the theoretical calculations described in section 2.2.2 for the effect of a finite measuring volume. However, it should be noted that mixing within the flow cell also accounts for a large part of the observed effect. Such mixing will occur by both diffusion-convection and localised turbulence generated by sharp bends, edges and changes in bore [94]. This is supported by the different variations in dispersion coefficient with flow-rate found for different cells (section 2.2.1). It is also likely that dead volumes will exist, particularly in the larger cells, which will give rise to increased washout times and greater peak asymmetry.

The dynamic response of the detector and recorder
Fig. 2.10 (a) & (b) Peak shapes obtained using different flow cells at flow-rates of (a) 6.03 and (b) 1.99 ml min$^{-1}$. $V_{\text{CELL}} = (A) 0.2$, (B) 8.0, (C) 25.0 and (D) 60.0 μl
Fig. 2.10 (c) Peak shape obtained using different flow cells at a flow rate of 0.84 ml min$^{-1}$. $V_{\text{CELL}} = \langle A \rangle 0.2, \langle B \rangle 8.0, \langle C \rangle 25.0$ and $\langle D \rangle 60.0 \mu l$.

Electronics will affect the observed peak shape for rapidly changing profiles (short tubes and high flow-rates). Larkins and Westcott [95] have presented results for the effect of different detector response times on observed peak shape in HPLC. These show a similar effect to that of the finite measuring volume described above. The response time of the detector system used in this experiment was found to be less than 0.5s for full scale deflection. Comparing this with the rise times obtained with the different flow cells shows that the detector system does not make a significant contribution to the observed peak shape under the conditions used.

Refractive index effects will also affect observed peak shape. These occur when regions of different density exist within the flow cell. Such regions can arise from poor mixing of the sample and carrier, or from changes in the temperature of the carrier stream. As will be
discussed later (see section 2.9), poor mixing will result when the sample and carrier have differing physical properties (density, viscosity, etc.). However, as mentioned in section 2.2.3, the sample solution (tartrazine) has a refractive index indistinguishable from that of water. Therefore it seems unlikely that the results obtained show any significant contribution from refractive index effects.

2.2.5 Summary and Conclusions

It has been shown that both the size and construction of a flow cell can influence the observed dispersion behaviour of a flow injection manifold. This is due to (a) additional mixing generated by the flow path through the cell, and (b) the effect of measuring the solute

Fig. 2.11 Construction of (A) 25.0 and (B) 60.0 μl flow cells. L = light path; F = flow path; shading indicates optical volume. For the other cells see Figs. 2.3 and 2.8.
concentration within a finite volume. Refractive index effects are also known to exert an influence on the observed peak shape.

The results obtained suggest that for general flow injection applications, flow cells having pathlengths of 10 mm and volumes of 8 and 18 μl (the most commonly used sizes) are entirely satisfactory. However, for applications requiring the contribution of the detector to be minimised, low volume flow cells (8 μl or less) should be used. This is particularly important if the dispersion processes occurring within the manifold are to be investigated.

2.3 EFFECT OF COILING

2.3.1 Introduction

The effect of coiling the manifold tubing on dispersion in FIA has been discussed both theoretically and experimentally by Tijssen [37], Van den Berg et al [38], Reijn et al [51, 63] and Leclerc et al [89]. In helically coiled tubes, centrifugal forces induce secondary flow patterns in the radial direction. These flow patterns generally act so as to improve mixing whilst reducing peak asymmetry. The extent and exact nature of these flow patterns depends upon a number of factors [37]. These include the flow-rate, coil length, and aspect ratio (λ, the ratio of coil to tube diameters).

It is important to consider the effect of coiling on dispersion for several reasons. It is common practice to use coiled tubing in order to keep flow injection manifolds compact and tidy, whilst tightly coiled tubes have been used to promote mixing between sample and reagent and to limit peak broadening [96]. Also, it is difficult to construct manifolds without introducing some curvature of the tubing at connections to valves and flow cells. Because of this, the effect of coiling was investigated in order to assess whether or not there was a significant contribution to the observed dispersion.
coefficient and peak shape from centrifugally-induced flow under the conditions used in this work.

2.3.2 Experimental

The flow manifold used is shown in Figure 2.12.

![Flow manifold diagram](image)

Fig. 2.12 Flow manifold for the investigation of the effect of coiling

Both flanged and flangeless (Omnifit) joints were used in order to compare the reproducibility obtained on breaking and making connections. Tubing of 0.5 mm i.d. was used throughout. The coil formers had diameters of 12.5 and 6.0 mm, giving aspect ratios of 28 and 15 respectively.

The dispersion coefficient was measured for five replicate injections of a 0.25 g l\(^{-1}\) solution of tartrazine as a function of tube length, flow-rate, coil diameter and the number of loops \(N\) in the coil. Peak shapes were also recorded at fast chart speeds. The error introduced by the breaking and making of connections was estimated for both types of connector by measuring the value of \(D\) for five sets of ten replicate injections using 69.8 cm of 0.5 mm i.d. tubing and a flow-rate of 1.0 ml min\(^{-1}\). The mean value of \(D\), standard deviation, and 95% confidence interval about the mean were then calculated for both types of connector.

2.3.3 Results and Discussion

The results obtained are shown in Tables 2.2 and 2.3.
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<td>0</td>
<td>2.34</td>
</tr>
<tr>
<td>50.9</td>
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<td>15</td>
<td>5</td>
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</tr>
<tr>
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<td>15</td>
<td>10</td>
<td>2.11</td>
</tr>
<tr>
<td>50.9</td>
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<tr>
<td>50.9</td>
<td>4.36</td>
<td>15</td>
<td>15</td>
<td>2.29</td>
</tr>
</tbody>
</table>

Table 2.3 Results obtained using flangeless joints.
For flanged connectors, the mean value of D obtained from five separate determinations was 0.274 with 95% confidence interval ±0.117 (±5.15% of the mean). For flangeless connectors, a value of 2.202 ±0.035 (±1.57% of the mean) was obtained. Thus the variation in the value of D obtained for flanged connections can be attributed to the uncertainty involved in the breaking and making of connections necessary to achieve coiling of the tube. Very little difference in peak shape was observed between straight and coiled tubes for the conditions used with these connectors (Fig. 2.13).

The peak shapes obtained under different conditions for the flangeless connectors are shown in Fig. 2.14. No significant variation in the value of D obtained for straight and coiled tubes could be found for a flow-rate of 1.0 ml min⁻¹, although some slight differences in peak shape were observed. Significant differences in the value of D obtained for straight and coiled tubes were found for a flow-rate of 4.3 ml min⁻¹. This was reflected by differences in peak shape. The values of D obtained for coiled tubes were lower than those obtained for straight tubes. This can be explained by considering the efficiency of mixing of the sample slug with the carrier stream. For a coiled tube, the secondary flow patterns will improve mixing within the sample zone, resulting in a more symmetrical concentration profile than that obtained for a straight tube of the same length and internal diameter. Thus material at the centre of the sample zone should be mixed to a greater extent in coiled rather than straight tubing, resulting in a lower value of D.

- 54 -
Fig. 2.13 Effect of coiling on peak shape for flanged joints, $L = 69.8$ cm and $Q = 1.0$ ml min$^{-1}$.

(A) Straight tubing; (B) 8 loops, $\lambda = 28$;
(C) 15 loops, $\lambda = 15$.

Fig. 2.14 As Fig. 2.13 for flangeless joints. $L = 138.9$ cm; $Q = 1.0$ ml min$^{-1}$ (C, D) and 4.3 ml min$^{-1}$ (A, B); straight tubes (A, C) and coiled tubes (B, D).
2.3.4 Summary and Conclusions

With the apparatus used, secondary flow patterns produced by coiling the manifold tubing do not make a significant contribution to either the peak shape or the value of $D$, provided that the aspect ratio is large (greater than, say, 20) and/or the flow-rate is kept low. In the work described in this chapter, long lengths of tubing were loosely coiled to a minimum diameter of 4 cm ($\lambda = 80$ for 0.5 mm i.d. tubing) for convenience. The results described in this section indicate that this will not introduce any significant differences from the results that would have been obtained using perfectly straight tubes.

2.4 EFFECT OF FLOW-RATE

2.4.1 Experimental

The dispersion coefficient was measured as a function of flow-rate for various lengths of 0.58 mm i.d. tubing using the manifold shown in Fig. 2.15.

![Fig. 2.15 Manifold for the investigation of the effect of flow-rate.](image)

The experimental conditions used are summarised in Table 2.4.
Table 2.4 Values of L and \( V_s \) used.

Ten replicate injections of a solution containing 0.25 g \( \text{L}^{-1} \) of tartrazine were made for each determination of the value of \( D \). Each set of injections was followed by a measurement of the steady-state absorbance. The mean, standard deviation and 95% confidence interval about the mean were calculated for each determination of the dispersion coefficient. The flow-rate range examined (0.1 to 10 ml \( \text{min}^{-1} \)) was chosen to cover the range of flow-rates commonly used in FIA with both flame AAS and solution spectrophotometric detection. Peak shapes were recorded using a fast chart speed, and the data stored on a computer for subsequent analysis.

2.4.2 Results and Discussion

The results obtained for the variation of the dispersion coefficient with flow-rate are shown in Figs. 2.16 and 2.17. With the apparatus used, relative standard deviations for ten replicate injections were typically less than 1% but, as mentioned previously (section 2.1.3), poorer precision was found for the value of \( D \) obtained under identical conditions on a day-to-day basis. This made it difficult to accurately define the way in which the value of \( D \) varied with flow-rate for different tube lengths without much additional and time-consuming work. However, comparison of the confidence intervals for individual points for any given tube length revealed that the observed changes in the value of \( D \) with flow-rate represented a real effect.

For short tube lengths, the value of \( D \) was found to
Fig. 2.16 D vs. Q for a sample volume of 113 μl and L = 
(Δ) 30.0, (+) 70.0, (□) 110, (○) 210 and (X) 360 cm.
(More than one set of results is shown for each set of conditions).
Fig. 2.17 As Fig. 2.16.  

〈○〉 $V_s = 287.0 \, \mu l$, $L = 70.0 \, cm$;  
〈□〉 $V_s = 67.3 \, \mu l$, $L = 30.0 \, cm$;  
〈X〉 $V_s = 67.3 \, \mu l$,  
$L = 100 \, cm$.

(More than one set of results is shown for each set of conditions).
decrease with increasing flow-rate, becoming constant for flow-rates greater than about 5 ml min\(^{-1}\). As the tube length was increased, the dispersion coefficient vs. flow-rate curves began to exhibit a maximum at low flow-rates (<1 ml min\(^{-1}\)), the value of D again becoming constant above a flow-rate of about 5 ml min\(^{-1}\). For long tube lengths, two maxima were observed, one at about 1.5 ml min\(^{-1}\), the other at about 6.5 ml min\(^{-1}\). Dispersion coefficient vs. flow-rate curves obtained for different sample volumes with the same tube length showed the same overall shape, the values of D being higher for the lower sample volumes as expected (see for example curve A of Fig. 2.16 and curve A of Fig. 2.17). Curve B of Fig. 2.17 highlights the problem of the poor day-to-day reproducibility of the value of D. This problem is compounded by the fact that for high values of D (low peak heights), the relative error associated with each individual measurement is much greater than for low values of D. Poorer precision for replicate injections was obtained for this set of conditions, values of 1 - 2% RSD being obtained for flow-rates greater than about 3 ml min\(^{-1}\). Better precision (<1% RSD), and better agreement between successive sets of measurements was obtained for flow-rates below 3 ml min\(^{-1}\). This was attributed to a lesser degree of pulsation arising from the pump head at low pump speeds.

The observed variation in the dispersion coefficient with flow-rate for the different tube lengths reflects a variation in the relative contributions of the diffusion and convection mechanisms to the overall dispersion of the sample zone. It is well-known that the convection process (distortion due to laminar flow) will dominate for short tube lengths and high flow-rates, whilst the diffusion process will tend to dominate for long tubes and low flow-rates [35]. Using Taylor's equations for dispersion by convection alone [39], it can be shown that for the introduction of a volume \(V_s\) of sample of unit concentration into a length of tubing of volume \(V_t\) at a flow-rate \(Q\), then the resulting concentration-time
distribution at the tube outlet will be given by

\[ t_{a} < t < t'_{p}, \quad C = 1 - \frac{V_{r}}{2Qt} \]  \hspace{1cm} (2.5)

\[ t = t'_{p}, \quad C = \frac{V_{p}}{(V_{s} + V_{p})} \]  \hspace{1cm} (2.6)

\[ t > t'_{p}, \quad C = \frac{V_{s}}{2Qt} \]  \hspace{1cm} (2.7)

where the appearance time, \( t_{a} \), is given by \( \frac{V_{r}}{2Q} \), and \( t'_{p} \) is given by \( \frac{(V_{r} + V_{s})}{2Q} \) (see Appendix C).

Equation 2.7 predicts that, for dispersion by convection alone, the value of \( D_{p} = \frac{1}{C_{p}} \) for a sample of unit concentration) will be independent of flow-rate. Thus the results obtained for short tube lengths may be interpreted as representing the situation where dispersion occurs predominantly by convection, with an increasing contribution from molecular diffusion for decreasing flow-rate. The dispersion mechanism operating at high flow-rates for short tubes is unlikely to be that of convection alone, however. There are two reasons for this: (a) the convection model predicts infinite tailing of the peaks, which is not observed in practice, and (b) the values of \( D \) obtained experimentally are consistently lower than those calculated from equation 2.7 (see Table 2.5). This is because there will always be some contribution from molecular diffusion, whilst secondary dispersion mechanisms such as localised turbulence at connections, etc., will also have some effect.

As the residence time of the sample zone in the manifold is increased (longer tube length, lower flow-rate), there will be an increasing contribution from molecular diffusion to the dispersion of the sample zone. This is reflected by the increasing complexity of the dispersion coefficient vs. flow-rate curves for increasing tube length, although the results obtained are not amenable to a full interpretation. The observed peak
Table 2.5 Values of the Dispersion Coefficient Calculated from Equ. 2.7.

<table>
<thead>
<tr>
<th>$V_s/\mu l$</th>
<th>$L/cm$</th>
<th>$V_f/\mu l$</th>
<th>$D$</th>
</tr>
</thead>
<tbody>
<tr>
<td>113</td>
<td>30.1</td>
<td>79.5</td>
<td>1.704</td>
</tr>
<tr>
<td>113</td>
<td>70.0</td>
<td>185</td>
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<tr>
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<td>291</td>
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<td>113</td>
<td>210</td>
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<td>113</td>
<td>360</td>
<td>951</td>
<td>9.417</td>
</tr>
<tr>
<td>287</td>
<td>70.0</td>
<td>185</td>
<td>1.644</td>
</tr>
<tr>
<td>67.3</td>
<td>30.1</td>
<td>79.5</td>
<td>2.181</td>
</tr>
<tr>
<td>67.3</td>
<td>100</td>
<td>286</td>
<td>4.945</td>
</tr>
</tbody>
</table>

Notes: (a) Assuming $d = 0.58$ mm; (b) value of $D$ obtained from Equ. 2.7.

shapes will be discussed fully in Chapter 3.

Ruzicka and Hansen [15] have reported that the dispersion coefficient increases with flow-rate. Since this is not in full agreement with the results presented here, it is important to consider why this is so. Examination of the experimental results of Ruzicka and Hansen (Fig. 16 of reference 9) shows agreement with their "rule" that dispersion increases with flow-rate. However, these authors only presented results for flow-rates of 0.25, 0.75 and 1.5 ml min$^{-1}$, using an 18 $\mu l$ flow cell. The results presented here were obtained over a wider flow-rate range, using a much smaller cell ($\approx 0.2$ $\mu l$). It has been shown (see section 2.2.1) that the volume of the flow cell can have a critical effect on the observed variation of the dispersion coefficient with flow-rate. For example, when measurements were made using an 8 $\mu l$ cell, the dispersion coefficient was found to increase with flow-rate over the range 1 to 6 ml min$^{-1}$ for tube lengths of more than 90 cm, and to be independent of flow-rate over the same range for tube lengths of less than 90 cm. Therefore, the differences between the results of Ruzicka and Hansen and those presented here may be explained by the sizes of flow cell used and the range of flow-rates examined.
2.4.3 Summary and Conclusions

It has been shown that the dispersion coefficient varies with flow-rate in a complex manner, and is strongly dependent on tube length. Although the primary dispersion process is that of diffusion-convection, it is probable that secondary mechanisms are also involved in determining the dispersion behaviour of the manifold in this work.

The results obtained for the variation of the dispersion coefficient with flow-rate are not in agreement with those of Ruzicka and Hansen. This has been attributed to the different experimental conditions used. It is believed that the results presented here more accurately reflect the dispersion processes occurring within the manifold itself, the contribution from the flow cell being small by comparison.

2.5 EFFECT OF TUBE LENGTH

2.5.1 Experimental

The dispersion coefficient was measured as a function of tube length using the manifold shown in Fig. 2.18.

Fig. 2.18 Manifold for the investigation of the effect of tube length.

The conditions used are summarised in Table 2.6. Each measurement of the dispersion coefficient was based on the mean peak height for ten replicate injections of a solution containing 0.25 g 1⁻¹ of tartrazine. The steady-state absorbance was measured after each set of...
injections, and the mean, standard deviation and 95% confidence interval about the mean calculated in the usual manner. Peak shapes were recorded using a fast chart speed. In a separate experiment, the uncertainty in the value of D obtained was evaluated by performing the measurements in triplicate, with the breaking and making of connections between each measurement. In this case, each determination of the value of D was based on the mean peak height obtained for five replicate injections. The mean value of D and 95% confidence interval about the mean were calculated for each tube length (see also section 2.10).

\[ Q \text{/ml min}^{-1} \quad 1.00 \quad 2.01 \quad 2.22 \quad 5.00 \]

\[ V_s \text{/ul} \quad 113 \quad 113 \quad 67.3 \quad 113 \]

Table 2.6 Values of Q and \( V_s \) used.

2.5.2 Results and Discussion

The results obtained for the variation of the mean dispersion coefficient with tube length are shown in Fig. 2.19, the error bars indicating the 95% confidence interval for each value of D. The results obtained based on single measurements of D are shown in Fig. 2.20, and all follow the same basic trend, with dispersion increasing with increasing tube length. This is to be expected since both residence time and dispersion volume are increased for increasing tube length under otherwise identical conditions. The error bars in Fig. 2.19 reflect the variation in the value of D introduced by the breaking and making of connections, as discussed earlier (see section 2.1.3). Such errors are also associated with the data in Fig. 2.20, which should be borne in mind when interpreting the results.

For each set of conditions used, the variation of D
Fig. 2.19 D vs. L for $Q = 2.11 \text{ ml min}^{-1}$, $V_s = 67.3 \mu\text{l}$.

Error bars represent 95% confidence intervals for $n = 3$. 
Fig. 2.20 $D$ vs. $L$ for $Q = \frac{1}{2}$ (O) 1.00, (Δ) 2.01, (+) 2.22 and (□) 5.00 ml min. $V_s = 113 \mu l \ (O, \Delta, \square)$ and 67.3 $\mu l \ (+)$. 
with tube length was found to follow an 'S'-shaped curve, with the fastest rate of change occurring for tube lengths between about 50 and 100 cm. Similar results were obtained for a sample volume of 67.3 \( \mu l \) and a flow-rate of 5.71 ml min\(^{-1} \) using potassium permanganate as the sample solution (see section 2.10, Fig. 2.56). These results also showed good linearity for tube lengths below 70 cm. The results obtained for a sample volume of 67.3 \( \mu l \) and a flow-rate of 2.22 ml min\(^{-1} \) (Fig. 2.20) show a similar trend. Linear regression analysis on this data for tube lengths from 20.0 to 70.4 cm gave a correlation coefficient of 0.993 with slope 0.0149 cm\(^{-1} \) and intercept 1.088. From equation 2.7 (section 2.4.2), it can be shown that

\[
C_p = V_s / [V_s + (\pi d^2 L / 4)]
\]  

(2.9)

which on rearranging gives

\[
D = 1 + \pi d^2 L / 4V_s
\]

(2.10)

where \( d \) is tube internal diameter and \( L \) is tube length. This predicts that for dispersion by convection alone, a plot of dispersion coefficient against tube length will be linear with slope \( \pi d^2 / 4V_s \) and an intercept of unity. Comparing this with the values obtained for the linear portion of the data for \( Q \approx 2.22 \) ml min\(^{-1} \) suggests that the dispersion mechanism operating over the range of tube lengths 20.0 to 70.4 cm is predominantly that of convection. However, although the intercept is indeed close to unity, when values of \( d \) and \( V_s \) were substituted into the term for the slope of the graph, a value of 0.0393 cm\(^{-1} \) was obtained, which is some 2.5 times greater than the experimentally derived value. Similar calculations were performed for the results shown in Fig. 2.19. In this case, linear regression analysis on the data for tube lengths of 19.3 to 99.8 cm gave a slope of
0.0220 cm\(^{-1}\) with intercept 1.018 and correlation coefficient 0.988. These results suggest that the convection mechanism is predominant for short tubes under certain conditions, but that other dispersion mechanisms cannot be neglected (see also the discussion on the effect of flow-rate, section 2.4.2). The peak shapes obtained will be described and discussed fully in Chapter 3.

Ruzicka and Hansen [15] have reported that dispersion increases with the square root of the distance travelled, or with the square root of the residence time (equivalent to the time taken for the appearance of the peak maximum). Experimental results were presented for the variation of peak height and \(D_f\) (the dispersion coefficient "of the flow arrangement") against \(t^p\), it being assumed that

\[
t_p = \pi d^2 L / 4Q
\]

(Equ. 16 of reference 15). These results showed a linear relationship between \(C_p\) and \(L\). However, close examination of Fig. 15 of reference 15 reveals some deviation from the rule that \(D = KL^{1/2}\), where \(K\) is a constant (Equ. 22 of reference 15). It should also be noted that this rule fails to satisfy the requirement that \(D = 1\) when \(L = 0\). For this reason, Appleton and Tyson [79] have proposed the relationship

\[
(D - 1)^2 = KL
\]

where \(K\) is a constant. In order to test this relationship, the data shown in Figs. 2.19 and 2.20 was plotted as \((D - 1)^2\) against \(L\). The results are shown in Fig. 2.21. The best agreement with equation 2.12 was found for the data for a flow-rate of 5.00 ml min\(^{-1}\) and a sample volume of 113 \(\mu\)l. Linear regression analysis on the data gave a slope of 0.0119 cm\(^{-1}\), with intercept -0.612 and correlation coefficient 0.999. The remaining data showed either limited or no agreement with equation 2.12. These results should be compared with those
Fig. 2.21 $(D-1)^2$ vs. $L$ for the data shown in Figs. 2.19 and 2.20. $Q = (O) 1.00, (+) 2.01, (\Delta) 2.11, (X) 2.22$ and $(\Box) 5.00$ ml min$^{-1}$. $V_s = 113$ $\mu$l $(O, +, \Box)$ and 67.3 $\mu$l $(\Delta, X)$. 
obtained using a flame AA spectrometer as the detector (section 2.10).

2.5.3 Summary and Conclusions

These results confirm that dispersion increases with increasing tube length, as predicted by simple theoretical equations. However, neither the "rule for dispersion" of Ruzicka and Hansen \(D = KL^k\), nor the relationship proposed by Appleton and Tyson (equation 2.12) are entirely satisfactory for describing the variation of the dispersion coefficient with tube length obtained under the conditions used in these experiments. Direct comparison with the experimental results of Ruzicka and Hansen is not possible because, as discussed earlier (section 2.4.2), the experiments were performed under different conditions.

2.6 EFFECT OF TUBE INTERNAL DIAMETER

2.6.1 Experimental

The dispersion coefficient was measured at different flow-rates over the range 0.5 to 6.0 ml min\(^{-1}\) for tube internal diameters of 0.3, 0.5, 0.8 and 1.0 mm. These diameters were chosen because they could all be supplied to a uniform specification by one manufacturer. The tube length was varied with the internal diameter in order to keep the tube volume constant. The tube volumes calculated from the length and internal diameter were 106, 215 and 410 \(\mu\)l. The manifold used is shown in Fig. 2.22. Ten replicate injections of 75.9 \(\mu\)l of a 0.25 g l\(^{-1}\) solution of tartrazine were made for each determination of \(D\), the mean, standard deviation and relative standard deviation being calculated in each case. Peak shapes were also recorded using a fast chart speed. The internal diameter of the sample loop was not matched to that of the manifold in order to maintain a constant volume.

The experiment was repeated using a constant tube length of 25.2 cm for internal diameters of 0.3, 0.5, 0.8
Fig. 2.22 Manifold for the investigation of the effect of tube i.d.

and 1.0 mm, a flow-rate of 0.5 ml min$^{-1}$ and a sample volume of 67.3 µl (a new valve had to be used for this experiment). Values of the dispersion coefficient were measured and peak shapes recorded as before.

2.6.2 Results and Discussion

The results obtained for the variation of the dispersion coefficient with flow-rate for different tube diameters and constant tube volume are shown in Fig. 2.23. It was found that very few results could be obtained for tubing of 0.3 mm i.d. with the apparatus used, because of the high back pressure generated by such tubing. As mentioned earlier, some caution must be exercised in interpreting the results because of the uncertainty in the value of $D$ introduced by the breaking and making of connections. The uncertainty associated with each value of $D$ was estimated from the confidence intervals calculated in earlier experiments (see section 2.1.3). This indicated that the observed results represented a real effect. The results obtained for tube diameters of 0.5, 0.8 and 1.0 mm show that the effect of changing the tube diameter on the value of $D$ is dependent on flow-rate. In general, the value of $D$ was found to increase with increasing tube diameter, although for some conditions no clear trend emerged.
Fig. 2.23 D vs. Q for different tube diameters and constant reactor volumes of (A) 106, (B) 215 and (C) 410 µl. d = (△) 0.3, (X) 0.5, (□) 0.8 and (○) 1.0 mm.
The peak shapes obtained for the variation of tube diameter with constant tube length are shown in Fig. 2.24. The value of $D$ was found to increase with increasing tube diameter. This was expected, since if the diameter is increased for a fixed tube length, then the effective dispersing volume is also increased, resulting in a greater degree of mixing (increased value of $D$), and increased appearance and mean residence times.

Considerable variation in peak shape was observed for the different tube diameters used. Representative examples of the different types of peak shape are shown in Fig. 2.25. These will be referred to as (a) skewed Gaussian, (b) triangular, (c) humped, (d) tailed, and (e) exponential. Skewed Gaussian peaks were obtained for 0.5 mm i.d. tubing for all the flow-rates and tube volumes examined. Humped and exponential peaks were obtained for both 0.8 and
Fig. 2.25 Typical peak shapes in FIA. \( d = (a) \ 0.5, \ (b,c) \ 0.8 \) and \( (d,e) \ 1.0 \) mm. \( Q = (a) \ 2.88, \ (b) \ 2.91, \ (c) \ 0.72, \ (d) \ 2.90 \) and \( (e) \ 0.55 \) ml min\(^{-1}\). \( V_r = (a,b,d) \ 410, \ (c) \ 106 \) and \( (e) \ 215 \) \( \mu l \).

1.00 mm i.d. tubing. Triangular peaks were observed only for 0.8 mm i.d. tubing, whilst tailed peaks were observed only for 1.0 mm i.d. tubing. These five categories can be used to describe all the peak shapes obtained in the experiments described in this chapter.

The observed differences in peak shape may be explained from theoretical considerations in terms of the diffusion-convection mechanism. However, a more empirical approach can be adopted using the principles employed in chemical engineering [97]. These two approaches will be discussed in detail in chapters 3 and 5.

Ruzicka and Hansen [15] found that "within the range of tube lengths and diameters used...........\( D_f \) is
independent of the tube diameter for the same residence time", whereas the results obtained in this work show a marked variation of the dispersion coefficient with tube diameter for a constant residence time (Fig. 2.23). However, the same considerations apply here as for the results for the effect of flow-rate (section 2.4.2) and tube length (section 2.5.2); since different experimental conditions were used, the results are not directly comparable. Ruzicka and Hansen did not discuss the effect of tube diameter on peak shape.

2.6.3 Summary and Conclusions

It has been shown that the way in which the dispersion varies with tube internal diameter depends on the flow-rate, and whether the tube length or the tube volume is kept constant. This variation is reflected by changes in the peak shape obtained for different manifold parameters. These will be discussed more fully in chapter 3. The results obtained suggest that for practical FIA, 0.5 mm i.d. tubing is preferable to wider bore tubing, as this limits peak broadening.

2.7 EFFECT OF THE INJECTION PROCESS

2.7.1 Construction of an Automatic Valve

So far, all the experimental results described have been obtained using "slug" injection, as defined by Reijn et al [42] (see chapter 1). However, in studying the effect of the injection process, the mode of injection must be taken into account as well as the actual sample volume. Therefore both "slug" and "time" injection were investigated. In order to achieve the latter, it was necessary to be able to accurately and precisely control both the switching rate and the delay time between switching and returning the injection valve, since both these factors affect the reproducibility of the sample volume obtained. It was therefore decided to build a
motorised injection valve, which would be capable of being operated in either "time" or "slug" injection modes, and could also be operated via a computer. The arrangement adopted is shown in Fig. 2.26. A Rheodyne 5020 injection valve was coupled to a Philips 31104 stepper motor via a 75:1 reduction gear drive (McLennan Servo Supplies) and a universal coupling (Huco). The reduction gear drive was necessary in order to achieve reasonable switching rates, since the injection valve required considerable torque to operate it—typically about 200 mN m. A later version of the valve used a Philips 31004 motor, which is identical to the original model but of slightly lower power.

Initially, the valve was controlled by a combined power supply and microprocessor unit provided by Pye Unicam. This could be programmed to give any desired switching sequence, rate and delay time to a resolution of 1 ms. Using this system, the valve could be switched between positions in 0.5 s, with good precision for replicate injections (typically <1% RSD).

Later, a special interface was designed and constructed to allow the valve to be controlled by an Apple IIe microcomputer. This is described in Appendix E.
2.7.2 Time Injection

Experimental

The flow manifold used for the initial experiments is shown in Fig. 2.27. The valve was fitted with a 600 µl loop, and connected to the flow cell using 50 cm of 0.58 mm i.d. tubing. By varying the delay time between switching and returning the valve, different sample volumes were obtained. The sample volume was calculated from the volumetric flow-rate and the delay time, it being assumed that the valve had a negligible residual volume. A sample solution containing 0.02 g l⁻¹ of tartrazine was used for these experiments. Peak shapes were recorded for sample volumes over the range 10 to 600 µl, using a flow-rate of 1.23 ml min⁻¹ and a fast chart speed. The dispersion coefficient was measured for sample volumes over the range 3.2 to 315 µl using a flow-rate of 1.89 ml min⁻¹. The range of sample volumes was chosen so that no diluted sample entered the manifold from the sample loop. Ten replicate injections were made for each measurement, and the mean, standard deviation and 95% confidence interval about the mean were calculated.

Results and Discussion

The peak shapes obtained for different delay times,
hence different sample volumes, are shown in Fig. 2.28. Under the conditions used in this experiment, the steady-state was achieved for sample volumes of greater than 287 μl. For sample volumes of 10.0 to 410 μl, the fall curve was found to be the reverse of the rise curve to the steady-state (Fig. 2.28, curves A to F). This was not the case when the full sample volume was injected into the manifold (Fig. 2.28, curve H). This highlights the difference between slug and time modes of injection. Used in the conventional mode (slug injection), the sample loop is first filled with solution (Fig. 2.29, A). When the valve is switched to the "inject" position, the contents of the loop are propelled into the manifold by the carrier stream (Fig. 2.29, B). This means that, whilst the front of the sample zone undergoes dispersion in the manifold, the rear of the sample zone undergoes dispersion in both the manifold and the sample loop. However, if the valve is switched back to the "fill" position before the dispersed tail of the sample zone enters the manifold, then the rear of the sample zone will become dispersed to the same extent as the front. This corresponds to time injection, and explains the relationship between the fall and rise curves noted above. Curve G of Fig. 2.28 shows what happens when the valve is switched back to the "fill" position after the dispersed tail of the sample zone has started entering the manifold: the fall curve initially follows that obtained for slug injection, and then changes to that obtained for time injection once the valve has been switched. It is expected that the differences between the two modes of injection will be most significant when the sample volume is large compared to the total volume of the manifold (low dispersion systems), since for small sample volumes (high dispersion systems), any dispersion occurring in the sample loop will be negligible in comparison to that occurring in the rest of the manifold.

Values of the dispersion coefficient were calculated from the peaks shown in Fig. 2.28, and plotted with the results obtained for a flow-rate of 1.89 ml min⁻¹ as l/D

- 78 -
Fig. 2.28 Peak shapes for time injection. $Q = 1.23$ ml min$^{-1}$. $V_s = (A)$ 10, (B) 41, (C) 82, (D) 205, (E) 287, (F) 410, (G) 533 and (H) 600 $\mu$l.
Fig. 2.29 Operation of the Injection Valve. (A) The "fill" position. (B) The "inject" position.

against $V_s$, the sample volume (Fig. 2.30). The value of $1/D$ became constant at very low sample volumes, with poor reproducibility being obtained for replicate injections. This is because the residual volume of the valve due to the switching channels and entry ports becomes increasingly significant for low sample volumes.

Ruzicka and Hansen [15, 57] have reported the relationship

$$1/D = 1 - \exp(-K \cdot V_s)$$  \hspace{1cm} (2.13)

where $K = 0.693/V_{\text{w}}$, and $V_{\text{w}}$ is defined as the sample volume required to give a dilution of 1/2 ($D = 2$). (Equation 2.13 should be compared to the corresponding equation for the well-stirred tank model, Appendix C). On rearranging, equation 2.13 gives

$$-\ln(1 - 1/D) = K \cdot V_s$$  \hspace{1cm} (2.14)

Therefore, if equation 2.13 is valid, a plot of $-\ln(1 - 1/D)$ against $V_s$ should be linear with a slope equal to
Fig. 2.30 $1/D$ vs. $V_s$ for flow-rates of (D) 1.23 and (O) 1.89 ml min$^{-1}$.
0.693/V. The results obtained are shown in Fig. 2.31. For a flow-rate of 1.89 ml min\(^{-1}\), good agreement was found between the experimental data and equation 2.13 except for low sample volumes. Linear regression analysis on the data gave a correlation coefficient of 0.9996 with slope 0.0186, giving \(V_{12} = 37 \mu l\). The value of \(V_{12}\) obtained by interpolation from Fig. 2.30 was 40 \(\mu l\). Poorer agreement was found for the data obtained at 1.23 ml min\(^{-1}\). Linear regression analysis of the data for sample volumes up to 200 \(\mu l\) gave a correlation of 0.9994 with slope 0.0158 and \(V_{12} = 44 \mu l\). The value obtained by interpolation was 40 \(\mu l\). The breakdown of equation 2.13 for values of \(D\) approaching unity (peak heights close to the steady-state) can be explained by consideration of the exponential term involving \(K\) and \(V_s\). This predicts the steady-state \((D = 1)\) will only be obtained for an infinite sample volume, whereas the steady-state was actually achieved for sample volumes greater than 200 and 315 \(\mu l\) for flow-rates of 1.23 and 1.89 ml min\(^{-1}\) respectively. Therefore, equation 2.13 will always fail for sample volumes giving peak heights close or equal to the steady-state value.

2.7.3 Slug Injection

Experimental

Following the work on time injection, the use of slug injection was investigated for a range of experimental conditions. The manifold used is shown in Fig. 2.32. In order to achieve low sample volumes, the flying leads from the valve were replaced with the shortest possible lengths of 0.58 mm i.d. tubing. Different sample volumes were achieved by inserting different lengths of the same tubing into the sample loop. The loop volumes were determined using the procedure outlined in section 2.1.2. Values of the dispersion coefficient were measured for replicate injections of a 0.25 g l\(^{-1}\) solution of tartrazine for flow-rates of 1.1 and 5.2 ml min\(^{-1}\), and tube lengths of 19.3 and 169.3 cm (0.58 mm i.d.). Since changing the
Fig. 2.31 $-\ln(1-1/D)$ vs. $V_s$ for flow-rates of (□) 1.23 and (○) 1.89 ml min$^{-1}$. 
Fig. 2.32 Manifold for the investigation of slug injection

sample loop involved breaking connections, each
determination of the value of D was made in triplicate,
and the mean, standard deviation and 95% confidence
interval about the mean calculated, a connection being
broken and remade between each measurement. Peak shapes
were also recorded using a fast chart speed. In order to
compare these results with those obtained by time
injection, the automated valve was used with the Apple IIe
microcomputer, measurements being made in the same way.

Results and Discussion

The results obtained are given below in Table 2.7,
and shown graphically in Figs. 2.33 and 2.34. Examination
of the plots of $-\ln(1 - 1/D)$ against t shows that there is
good agreement between the experimental data and equation
2.13 for a tube length of 169.3 cm, but poorer agreement
for the results obtained for a tube length of 19.3 cm.
The values of $-\ln(1 - 1/D)$ for $V_s = 242.5 \mu l$ seem to show
some systematic error, although it is not clear why this
should be so. The results obtained from linear regression
analysis on the first five points of the data for a tube
length of 169.3 cm are given below (Table 2.8). The
values of $V_s$ derived from the analysis agree favourably
with those obtained by interpolation from Fig. 2.33. The
results obtained for a tube length of 19.3 cm did not show
such good agreement, the plots of $-\ln(1 - 1/D)$ against $V_s$
Fig. 2.33 $1/D$ vs. $V_S$ for slug injection. $Q = (X, □) 1.11$ \text{ml min}^{-1}$ and $(Δ, O) 5.16$ ml min$^{-1}$. $L = (Δ, X) 19.3$ and $(O, □) 169.3$ cm.
Fig. 2.34. $-\ln(1 - 1/D)$ vs. $V_s$ for slug injection. Symbols are the same as for Fig. 2.33.
(a) $L = 19.3 \text{ cm}, \ Q = 1.11 \text{ ml min}^{-1}$.

<table>
<thead>
<tr>
<th>$V_s/\mu l$</th>
<th>D</th>
<th>±95%CI</th>
<th>1/D</th>
<th>$-\ln(1 - 1/D)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.1</td>
<td>2.666</td>
<td>0.499</td>
<td>0.375</td>
<td>0.4715</td>
</tr>
<tr>
<td>53.6</td>
<td>1.799</td>
<td>0.176</td>
<td>0.556</td>
<td>0.8116</td>
</tr>
<tr>
<td>77.3</td>
<td>1.370</td>
<td>0.034</td>
<td>0.730</td>
<td>1.3091</td>
</tr>
<tr>
<td>112.1</td>
<td>1.241</td>
<td>0.043</td>
<td>0.806</td>
<td>1.6389</td>
</tr>
<tr>
<td>164.7</td>
<td>1.152</td>
<td>0.015</td>
<td>0.868</td>
<td>2.0254</td>
</tr>
<tr>
<td>242.5</td>
<td>1.047</td>
<td>0.017</td>
<td>0.955</td>
<td>3.1035</td>
</tr>
<tr>
<td>274.2</td>
<td>1.045</td>
<td>0.008</td>
<td>0.957</td>
<td>3.1451</td>
</tr>
</tbody>
</table>

(b) $L = 169.3 \text{ cm}, \ Q = 1.11 \text{ ml min}^{-1}$.

<table>
<thead>
<tr>
<th>$V_s/\mu l$</th>
<th>D</th>
<th>±95%CI</th>
<th>1/D</th>
<th>$-\ln(1 - 1/D)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.1</td>
<td>9.325</td>
<td>0.397</td>
<td>0.107</td>
<td>0.1134</td>
</tr>
<tr>
<td>53.6</td>
<td>4.427</td>
<td>0.130</td>
<td>0.226</td>
<td>0.2560</td>
</tr>
<tr>
<td>77.3</td>
<td>3.548</td>
<td>0.090</td>
<td>0.282</td>
<td>0.3311</td>
</tr>
<tr>
<td>112.1</td>
<td>2.587</td>
<td>0.042</td>
<td>0.387</td>
<td>0.4887</td>
</tr>
<tr>
<td>164.7</td>
<td>1.906</td>
<td>0.024</td>
<td>0.525</td>
<td>0.7437</td>
</tr>
<tr>
<td>242.5</td>
<td>1.322</td>
<td>0.019</td>
<td>0.756</td>
<td>1.4123</td>
</tr>
<tr>
<td>274.2</td>
<td>1.336</td>
<td>0.008</td>
<td>0.749</td>
<td>1.3803</td>
</tr>
</tbody>
</table>

Table 2.7 Results Obtained for Slug Injection.
(c) \( L = 19.3 \text{cm}, \ Q = 5.16 \text{ ml min}^{-1}. \)

<table>
<thead>
<tr>
<th>( V_\varepsilon /\mu l )</th>
<th>( D )</th>
<th>±95%CI</th>
<th>( 1/D )</th>
<th>(-\ln (1 - 1/D))</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.1</td>
<td>1.965</td>
<td>0.071</td>
<td>0.508</td>
<td>0.7095</td>
</tr>
<tr>
<td>53.6</td>
<td>1.347</td>
<td>0.057</td>
<td>0.742</td>
<td>1.3563</td>
</tr>
<tr>
<td>77.3</td>
<td>1.258</td>
<td>0.067</td>
<td>0.795</td>
<td>1.5843</td>
</tr>
<tr>
<td>112.1</td>
<td>1.161</td>
<td>0.013</td>
<td>0.861</td>
<td>1.9756</td>
</tr>
<tr>
<td>164.7</td>
<td>1.103</td>
<td>0.016</td>
<td>0.907</td>
<td>2.3711</td>
</tr>
<tr>
<td>242.5</td>
<td>1.038</td>
<td>0.012</td>
<td>0.963</td>
<td>3.0705</td>
</tr>
<tr>
<td>274.2</td>
<td>1.038</td>
<td>0.009</td>
<td>0.963</td>
<td>3.0705</td>
</tr>
</tbody>
</table>

(d) \( L = 169.3 \text{cm}, \ Q = 5.16 \text{ ml min}^{-1}. \)

<table>
<thead>
<tr>
<th>( V_\varepsilon /\mu l )</th>
<th>( D )</th>
<th>±95%CI</th>
<th>( 1/D )</th>
<th>(-\ln (1 - 1/D))</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.1</td>
<td>9.120</td>
<td>0.470</td>
<td>0.110</td>
<td>0.1161</td>
</tr>
<tr>
<td>53.6</td>
<td>4.339</td>
<td>0.264</td>
<td>0.230</td>
<td>0.2620</td>
</tr>
<tr>
<td>77.3</td>
<td>3.423</td>
<td>0.166</td>
<td>0.292</td>
<td>0.3455</td>
</tr>
<tr>
<td>112.1</td>
<td>2.327</td>
<td>0.220</td>
<td>0.430</td>
<td>0.5617</td>
</tr>
<tr>
<td>164.7</td>
<td>1.874</td>
<td>0.083</td>
<td>0.534</td>
<td>0.7628</td>
</tr>
<tr>
<td>242.5</td>
<td>1.307</td>
<td>0.125</td>
<td>0.765</td>
<td>1.4486</td>
</tr>
<tr>
<td>274.2</td>
<td>1.309</td>
<td>0.011</td>
<td>0.764</td>
<td>1.4437</td>
</tr>
</tbody>
</table>

Table 2.7 cont.
Table 2.8 Comparison of values of $V_e$.

<table>
<thead>
<tr>
<th>$L$/cm</th>
<th>$Q$/ml min$^{-1}$</th>
<th>slope/$\mu$l$^{-1}$</th>
<th>corr</th>
<th>$V_e$/ml</th>
<th>$V_e$/$\mu$l$^{(a)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>169.3</td>
<td>1.11</td>
<td>0.00457</td>
<td>0.9980</td>
<td>152</td>
<td>155</td>
</tr>
<tr>
<td>169.3</td>
<td>5.16</td>
<td>0.00482</td>
<td>0.9957</td>
<td>144</td>
<td>150</td>
</tr>
<tr>
<td>19.3</td>
<td>1.11</td>
<td>0.0178</td>
<td>0.9944</td>
<td>38.9</td>
<td>46.5</td>
</tr>
<tr>
<td>19.3</td>
<td>5.16</td>
<td>0.0275$^{(b)}$</td>
<td>-</td>
<td>25.2</td>
<td>29.5</td>
</tr>
</tbody>
</table>

Notes: (a) obtained by interpolation from Fig. 2.33; (b) calculated from the first two points, Fig. 2.34.

showing a definite change in slope at a sample volume of about 60 $\mu$l. The values of $V_e$ obtained from the initial slopes were similar to the values obtained by interpolation from Fig. 2.33 (Table 2.8). Values of $V_e$ obtained from the latter part of the graphs were 74.7 and 79.1 $\mu$l for flow-rates of 1.11 and 5.16 ml min$^{-1}$ respectively. Thus although a linear relationship between $-\ln(1 - 1/D)$ and $V_e$ exists for sample volumes above ~60 $\mu$l, the slope is not equal to 0.693/$V_e$. Comparison of the sample volumes used with the manifold volumes (50 and 450 $\mu$l for tube lengths of 19.3 and 169.3 cm respectively) suggests that equation 2.13 is only valid for slug injection when the sample volume is less than the manifold volume. Above this point, the value of $K$ in the exponential term changes.

The results obtained for time injection are shown in Figs. 2.35 and 2.36 for comparison. The plots of $-\ln(1 - 1/D)$ against $V_e$ were curved, showing poor agreement with equation 2.13. For a tube length of 169.3 cm, the value of $V_e$ obtained from the slope of the best straight line through the first eight points of Fig. 2.36 was 130 $\mu$l, compared to a value of 128 $\mu$l by interpolation from Fig. 2.35. No agreement was found when the same calculations were performed on the data for $L = 19.3$ cm.
Fig. 2.35 $1/D$ vs. $V_s$ for time injection and a flow-rate of 1.17 ml min$^{-1}$. $L = (\square) 19.3$ and $(O) 169.3$ cm.
Fig. 2.36 $-\ln(1 - 1/D)$ vs. $V_s$ for time injection. Symbols are the same as for Fig. 2.35.
The peak shapes obtained for time and slug injection are shown in Fig. 2.37 and 2.38. For a tube length of 19.3 cm, there are significant differences in peak shape between time and slug injection for all the sample volumes used ($V_s = 30.1 \text{ to } 274.2 \mu l$, $D = 2.67 \text{ to } 1.05$ for slug injection). Very similar peak shapes were obtained for a tube length of 169.3 cm for sample volumes up to about 100 $\mu l$ ($V_s = 30.1 \text{ to } 112.1 \mu l$, $D = 9.33 \text{ to } 2.59$ for slug injection). Differences between slug and time injection became increasingly significant for sample volumes above 100 $\mu l$. This is in agreement with the prediction made earlier (see page 78).

2.7.4 Summary and Conclusions

The use of both slug and time modes of injection has been demonstrated. With the apparatus used, significant differences between peak shapes obtained using slug and time injection were found for conditions giving a value of $D$ greater than about 2.6. Time injection would seem to offer a convenient means of increasing sample throughput whilst maintaining sensitivity by eliminating the contribution of the injection valve to peak broadening.

The validity of equation 2.13 has been examined for both slug and time injection. Preliminary results for time injection obtained using an 8 $\mu l$ flow cell showed good agreement with equation 2.13, although later results obtained using a smaller cell ($\approx 0.2 \mu l$) did not show such good agreement. This may be due to the effect of the cell volume on dispersion, but may also be due to the valve used (see section 4.1.4). Equation 2.13 was found to be valid for slug injection only for short tube lengths and low sample volumes. Equation 2.13 will always break down for conditions giving values of $D$ close to unity.
Fig. 2.37 Peak shapes for slug injection and a flow-rate of 1.11 ml min\(^{-1}\). \(L = (a) 19.3\) and \(b) 169.3\) cm. \(V_s = (A) 30.1, (B) 53.6, (C) 77.3, (D) 112.1, (E) 164.7\) and \(F) 274.2\) µl.
Fig. 2.38 Peak shapes for time injection and a flow-rate of 1.17 ml min$^{-1}$. (a) $L = 19.3$ cm; $V_s =$ (A) 17.3, (B) 48.7, (C) 74.9, (D) 101.0, (E) 155.9 and (F) 293.4 μl. (b) $L = 169.3$ cm; $V_s =$ (A) 20.8, (B) 47.2, (C) 73.6, (D) 128.2, (E) 184.8 and (F) 347.8 μl.
2.8 EFFECT OF THE SOLUTE

2.8.1 Experimental

The value of the dispersion coefficient was measured as a function of flow-rate for five different solutes using the manifold shown in Fig. 2.39. The 8 µl cell (F2) was used in order to keep the cell volume small whilst having a long enough pathlength to allow a range of solutes to be examined. The relevant data for the solutes used is summarised in Table 2.9. Ten replicate

![Manifold for the investigation of the effect of the solute](image)

Table 2.9 Available data on the solutes used.

<table>
<thead>
<tr>
<th>Solute</th>
<th>$M_r/\text{g mol}^{-1}$</th>
<th>$D_m/10^{-5} \text{ cm}^2 \text{ s}^{-1}$</th>
<th>$\lambda_{\text{max}}/\text{nm}$</th>
<th>C/g l$^{-1}$</th>
<th>$A_{\text{max}}$ (a)</th>
<th>R.I. (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KMnO$_4$</td>
<td>158.04</td>
<td>0.435–1.5 (c)</td>
<td>526</td>
<td>0.053</td>
<td>0.819</td>
<td>1.3330</td>
</tr>
<tr>
<td>CoCl$_2$.6H$_2$O</td>
<td>237.93</td>
<td>–</td>
<td>513</td>
<td>35.3</td>
<td>0.739</td>
<td>1.3372</td>
</tr>
<tr>
<td>Tartrazine</td>
<td>543.37</td>
<td>–</td>
<td>426</td>
<td>0.020</td>
<td>0.906</td>
<td>1.3330</td>
</tr>
<tr>
<td>Ponceau S</td>
<td>760.58</td>
<td>–</td>
<td>532</td>
<td>0.020</td>
<td>0.960</td>
<td>1.3330</td>
</tr>
<tr>
<td>Vitamin B$_{12}$</td>
<td>1355.4</td>
<td>–</td>
<td>550</td>
<td>0.135</td>
<td>0.781</td>
<td>–</td>
</tr>
</tbody>
</table>

Notes: (a) measured in a 10 mm pathlength cell at $\lambda_{\text{max}}$ with water as a baseline; (b) measured on an Abbé 60 refractometer using a sodium lamp. R.I. of water was 1.3330; (c) for 0–0.06M KMnO$_4$ in water from Ref. 39.
injections were made for each sample at various flow-rates in the range 0.3 to 10.0 ml min$^{-1}$. Each set of injections was followed by a measurement of the steady-state absorbance. The mean, standard deviation and 95% confidence interval about the mean were calculated for each measurement. These measurements were made by varying the flow-rate for each solute in turn. The same measurements were repeated for the five solutes using fixed flow-rates of 2.91, 2.03 and 1.14 ml min$^{-1}$. Peak shapes were recorded for the five solutes at flow-rates of 0.8, 1.1, 1.8, 2.4 and 4.4 ml min$^{-1}$, using a fast chart speed.

2.8.2 Results and Discussion

The results obtained for the variation of D with flow-rate for the five different solutes are shown in Fig. 2.40. Generally, the dispersion coefficient versus flow-rate curves for the same solute recorded on different days were in good agreement, some differences being observed at low flow-rates. For all the solutes, the value of D tended to become constant above a flow-rate of about 6 or 7 ml min$^{-1}$. The greatest differences between the solutes were observed at low flow-rates. Although it was not possible to accurately define the way in which D varied with flow-rate for each solute (see section 2.1.3), comparison of the confidence intervals for individual points for any given solute revealed that the observed variation of D with flow-rate represented a real effect. This was confirmed by comparison with the results obtained for tartrazine presented in section 2.4.

The results obtained for the value of D measured for different solutes at fixed flow-rates are summarised in Table 2.10. Examination of these results reveals that potassium permanganate consistently yields lower values of D for the conditions used, whilst the greatest number of significant differences between the solutes is found at the lowest flow-rate (1.14 ml min$^{-1}$).

Differences in dispersion between the solutes were
<table>
<thead>
<tr>
<th>Solute</th>
<th>Q/ml min⁻¹</th>
<th>D</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>2.91</td>
<td>1.882</td>
<td>1.847-1.918</td>
</tr>
<tr>
<td>C</td>
<td>2.91</td>
<td>2.049</td>
<td>2.041-2.057</td>
</tr>
<tr>
<td>T</td>
<td>2.91</td>
<td>2.056</td>
<td>2.042-2.069</td>
</tr>
<tr>
<td>P</td>
<td>2.91</td>
<td>2.055</td>
<td>2.049-2.061</td>
</tr>
<tr>
<td>V</td>
<td>2.91</td>
<td>2.067</td>
<td>2.047-2.087</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Solute</th>
<th>Q/ml min⁻¹</th>
<th>D</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>2.03</td>
<td>1.857</td>
<td>1.846-1.858</td>
</tr>
<tr>
<td>C</td>
<td>2.03</td>
<td>2.005</td>
<td>2.027-2.051</td>
</tr>
<tr>
<td>T</td>
<td>2.03</td>
<td>2.044</td>
<td>2.008-2.048</td>
</tr>
<tr>
<td>P</td>
<td>2.03</td>
<td>2.028</td>
<td>2.026-2.058</td>
</tr>
<tr>
<td>V</td>
<td>2.03</td>
<td>2.042</td>
<td>2.026-2.058</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Solute</th>
<th>Q/ml min⁻¹</th>
<th>D</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>1.14</td>
<td>1.946</td>
<td>1.936-1.956</td>
</tr>
<tr>
<td>C</td>
<td>1.14</td>
<td>1.950</td>
<td>1.937-1.964</td>
</tr>
<tr>
<td>T</td>
<td>1.14</td>
<td>2.423</td>
<td>2.406-2.441</td>
</tr>
<tr>
<td>P</td>
<td>1.14</td>
<td>2.273</td>
<td>2.266-2.280</td>
</tr>
<tr>
<td>V</td>
<td>1.14</td>
<td>2.292</td>
<td>2.262-2.323</td>
</tr>
</tbody>
</table>

**Table 2.10** Values of D obtained for different solutes at fixed flow rates.

Notes:  
(a) calculated for n=5;  (b) peak heights were identical to within the measurement error. K = KMnO₄; C = CoCl₂·6H₂O; T = tartrazine; P = ponceau S; V = vitamin B₁₂.
Fig. 2.40 D vs. Q for (A) tartrazine, (B) KMnO<sub>4</sub>, (C) ponceau S, (D) CoCl<sub>2</sub>·6H<sub>2</sub>O and (E) vitamin B<sub>12</sub>
also apparent from the peak shapes (see Fig. 2.41). The peak shapes obtained for potassium permanganate and cobalt (II) chloride were very similar, and generally showed the greatest peak height. They most closely resembled the skewed Gaussian shape described earlier (see section 2.6.2). The peak shapes for tartrazine and ponceau S were very similar, showing a greater extent of tailing and peak asymmetry than those for the two inorganic solutes. The highest extent of tailing and asymmetry was shown by the peak shapes for vitamin B$_{12}$. These most closely resembled the triangular and exponential of peak shapes defined earlier.

These observations are explained by the different molecular (or ionic) sizes, charges and hydrodynamic radii (radii of the solvated species) of the five solutes, which give rise to different diffusion coefficients.

Fig. 2.41 Peak shapes obtained for a flow-rate of 1.14 ml min$^{-1}$. (A) tartrazine, (B) KMnO$_4$, (C) ponceau S, (D) CoCl$_2$$\cdot$6H$_2$O and (E) vitamin B$_{12}$. 

- 99 -
Unfortunately, values of the diffusion coefficient are not available for all five compounds used in this work (see Table 2.9). However, the molecular masses of the different solutes can be used as a guide to their relative diffusion coefficients. Thus permanganate ion and hexaaquacobalt (II) ion are expected to have the highest diffusion coefficients of the five, giving rise to a significant contribution to dispersion from the diffusion mechanism. Vitamin B_{12} is expected to have the lowest diffusion coefficient, and so show the greatest contribution from convection to the dispersion of the sample zone. As will be seen later (see chapter 3), the observed peak shapes do indeed correspond to those expected for different contributions from the diffusion and convection mechanisms. The differences in peak shape between the solutes could be due in part to differences in refractive index between the sample solutions and the water carrier stream. However as mentioned earlier (see section 2.2.3), such differences are unlikely to be significant since (a) aqueous solutions were used throughout, and (b) the solutes used were present only in low concentrations, giving rise to negligible changes in density over that of pure water.

In a subsequent experiment, the dispersion coefficients and peak shapes obtained for magnesium, calcium and strontium were measured using flow injection-atomic absorption spectroscopy (FIAAS) to see if any significant differences in dispersion behaviour could be observed between them. The results of this experiment were, however, inconclusive.

2.8.3 Summary and Conclusions

These results confirm that the physical nature of the solute is important in determining the observed dispersion behaviour of a flow injection manifold. Although this is apparent from the diffusion-convection equation (see p. 26), the contribution of molecular diffusion to dispersion has been exploited only by a few authors (see for example
Betteridge et al. [64] and Gerhardt and Adams [98]). One implication of the results is that any solute used as a tracer for optimising a flow injection manifold should have similar physical properties (i.e. diffusion coefficient) to the analyte being determined. This will be particularly important for applications involving large molecules.

2.9 EFFECT OF A CONFLUENCE POINT

2.9.1 Introduction

Although many manifolds for practical FIA applications incorporate one or more stream confluence points (see for example references 7, 9, 99, 100), none of the major studies of dispersion theory gives consideration to the effect of such devices on the observed dispersion behaviour of a manifold. Very little has been published in the flow injection literature on the relative performances of different designs of confluence point, either. Frei and Lawrence [101] have discussed the mixing properties of different types of confluence points for post-column derivatisation flow systems in LC. They showed that a 30°/30° T-piece (Fig. 2.42, A) was a more efficient mixer than a 90°/90° T-piece (Fig. 2.42, B).

Fig. 2.42 Different designs of T-piece. See text for details
However, Silfwerbrand-Lindh et al [96] found no difference in performance between acute and right angle T-pieces in their flow injection system. Experience obtained within the research group at Loughborough University indicates that the mixing properties of any confluence point are heavily dependent on both the design and manufacture of such devices. For example, Marsden [102] has found that a Y-piece having a small mixing volume at the actual point of confluence is a more efficient mixer than a T-piece having a uniform internal diameter throughout.

Fernández et al [103] have applied hydrodynamic considerations to a simple flow injection network consisting of a split and confluence point (Fig. 2.43). They showed that for a network consisting of tubing of uniform diameter, the fraction of the sample volume passing through each side of the network, and the fractional flow-rates through each side, depend only on the relative lengths of the two sides. These authors did not examine dispersion at the splitting and confluence points.

Fig. 2.43 Manifold with a split and confluence point.
C = carrier; D = detector; IV = injection valve; \( L_1, L_2 \) = different tube lengths; P = pump; S = sample; W = waste.

Tyson [82] has employed a model incorporating a confluence point after a well-stirred mixing tank. In
this model, all dispersion is assumed to take place within
the tank, the effect of the confluence point being the
dilution of the effluent stream by a carrier stream.

In the light of the above, it was clear that the role
of the confluence point needed closer examination, in
order to evaluate different methods for incorporating
confluence points into flow models. A preliminary
investigation was carried out, the results of which are
presented in this section.

2.9.2 Experimental

The effect of introducing a confluence point into the
manifold was investigated using a 90°/90° T-piece (Altec,
05-40-5101), having a uniform internal diameter of 0.58
mm. The T-piece was used in the three configurations
shown in Fig. 2.44. The T-piece was kept in a horizontal
plane throughout these experiments.

In order to evaluate the mixing properties of the T­
piece, the manifold was operated in configuration I
without the injection valve. A solution containing
0.25 g l\(^{-1}\) of tartrazine was merged with distilled water,
and the resulting absorbance monitored continuously using
the chart recorder. Equal flow-rates were used for both
streams, the net flow-rate being 4.6 ml min\(^{-1}\).
Measurements were performed with 12, 72 and 112 cm lengths
of 0.58 mm i.d. tubing between the T-piece and the flow
cell. The experiment was repeated using configurations II
and III. Visual inspection of the mixing process within
the T-piece was carried out with the aid of a magnifying
glass.

The effect of the confluence point on dispersion and
peak shape was investigated for all three configurations
using a 112 cm length of 0.58 mm i.d. tubing between the
T-piece and the flow cell. This was chosen to give
adequate mixing of the two streams prior to detection (see
later). For each configuration, ten replicate injections
of 67.3 \(\mu l\) of the tartrazine solution were performed for
net flow-rates of 1.6, 3.4 and 4.4 ml min\(^{-1}\), equal
Fig. 2.44 Manifolds for the investigation of confluencing flow-rates being used for both streams. Peak shapes were also recorded using a fast chart speed. Steady-state absorbance values were obtained by stopping the flow through the diluent channel (Q1) and pumping tartrazine solution through the injection channel (Q2). For every flow-rate, the above procedure was repeated with no flow through the carrier channel and (i) the original flow-rate, (ii) the net flow-rate through the injection channel.
2.9.3 Results and Discussion

The results obtained for the mixing of water and tartrazine streams using configuration I are shown in Fig. 2.45. A very noisy signal was obtained for a mixing length of 12 cm, indicating a low mixing efficiency. Absorbance and peak-to-peak noise values for all the mixing lengths and configurations used are summarised in Table 2.11. These results show that the T-piece used is not a good mixer, the majority of mixing occurring in the post-confluence tubing. Visual examination of the point of confluence revealed that the two streams maintained their integrity through the actual confluence point, even when the two streams met head-on. These results are in agreement with those of Silfwerbrand-Lindh et al [96]. Close examination of the recorder traces obtained suggests that there are two noise components involved. One is believed to be intermittent mixing due to the pulsation of the flow in both streams introduced by the rollers of the peristaltic pump (see section 2.1.3). This would account for the higher frequency noise component. The second source of noise is likely to be caused by uneven wearing of the pump tubes, resulting in changes in the flow ratio between the two streams. This would account for the lower frequency noise component, and explain the different
Table 2.11 The effect of different post-confluence mixing lengths.

<table>
<thead>
<tr>
<th>Configuration</th>
<th>Mixing Length/cm</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>12</td>
<td>0.262±0.028</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>0.262±0.006</td>
</tr>
<tr>
<td></td>
<td>112</td>
<td>0.264±0.002</td>
</tr>
<tr>
<td>II</td>
<td>12</td>
<td>0.274±0.009</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>0.276±0.004</td>
</tr>
<tr>
<td></td>
<td>112</td>
<td>0.280±0.002</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>0.232±0.035</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>0.282±0.004</td>
</tr>
<tr>
<td></td>
<td>112</td>
<td>0.282±0.002</td>
</tr>
</tbody>
</table>

Table 2.11 The effect of different post-confluence mixing lengths.

The absorbance values obtained for the different conditions used (see Table 2.11).

The results obtained for the dispersion measurements are summarised in Table 2.12. These results show that the effect of the confluence point is not to simply achieve the dilution of the sample zone by a factor dependent on the ratio of the flow-rates entering the confluence point. Qualitatively, the peak shapes obtained with merging streams were similar to those obtained with only a single stream. Values of the dispersion coefficient obtained with merging streams were consistently higher than those obtained with a single stream at the same net flow-rate, reflecting the dilution effect of the second stream. The results obtained do not suggest any significant difference in dispersion behaviour between the three configurations used.
<table>
<thead>
<tr>
<th>Configuration</th>
<th>Q1/ml min⁻¹</th>
<th>Q2/ml min⁻¹</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2.20</td>
<td>2.16</td>
<td>4.536</td>
</tr>
<tr>
<td></td>
<td>2.24</td>
<td>-</td>
<td>3.459</td>
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<td></td>
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<td>-</td>
<td>3.305</td>
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<td>I</td>
<td>1.76</td>
<td>1.73</td>
<td>3.995</td>
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<td></td>
<td>1.80</td>
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<td></td>
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<td>-</td>
<td>2.952</td>
</tr>
<tr>
<td>I</td>
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<td>0.83</td>
<td>4.289</td>
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<td></td>
<td>0.88</td>
<td>-</td>
<td>3.572</td>
</tr>
<tr>
<td></td>
<td>1.69</td>
<td>-</td>
<td>3.213</td>
</tr>
<tr>
<td>II</td>
<td>2.13</td>
<td>2.09</td>
<td>4.159</td>
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<tr>
<td></td>
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<td></td>
<td>4.26</td>
<td>-</td>
<td>3.090</td>
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<tr>
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<td></td>
<td>1.73</td>
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<td></td>
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<td>3.121</td>
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<td></td>
<td>1.64</td>
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<td>3.843</td>
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<tr>
<td>III</td>
<td>2.11</td>
<td>2.24</td>
<td>4.259</td>
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<td>-</td>
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<td></td>
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<td>4.35</td>
<td>3.438</td>
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<td>-</td>
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</tr>
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<td></td>
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<td>3.40</td>
<td>3.001</td>
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</tr>
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<td>-</td>
<td>0.84</td>
<td>3.629</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.52</td>
<td>3.347</td>
</tr>
</tbody>
</table>

Table 2.12 Results obtained for different configurations and flow rates.
2.9.4 Conclusions and Summary

The effect of a simple T-piece on dispersion in a flow injection manifold has been investigated. The results obtained show that such devices do not provide adequate mixing of the two merging streams - a situation made worse when the two streams have widely differing physical properties [102]. Ideally, a confluence point should have the properties of a zero dead volume combined with the instantaneous mixing of all elements of fluid reaching the actual point of confluence. In practice, it is difficult to achieve efficient mixing at a confluence point without incorporating some form of mixing volume. The implications of this for flow models will be discussed later (see chapter 5). The results presented in this section do not provide sufficient information to allow any more detailed discussion of the effect of confluence points on dispersion in FIA.

2.10 EFFECT OF A FLAME AA SPECTROMETER

2.10.1 Introduction

In section 2.2, the contribution of the flow cell to the overall dispersion behaviour of a flow injection manifold was discussed. Other types of detector will show a similar effect - flame atomic absorption spectrometers, electrochemical cells and inductively-coupled plasmas, for example. However, as mentioned in chapter one, theoretical studies of dispersion in FIA often assume that the detector has a negligible effect on the observed dispersion of the sample zone. It is therefore important to give some consideration to detector response in FIA.

Various workers have investigated the response functions of a range of different detectors. For example, Meschi and Johnson [52, 53] derived equations for the response of amperometric and coulometric tubular electrodes in FIA using the analysis of Taylor [39] to model dispersion in the manifold. Pratt and Johnson [104]
have derived response equations for a vibrating wire electrode for FIA. More recently, Olsson et al [88] have derived response equations for amperometric enzyme electrodes, assuming a gaussian function for the dispersion generated by the manifold. Harris [105] has derived response equations for a laser-based, low volume detector.

Appleton and Tyson [79] used various models to describe instrument response in flow injection-atomic absorption spectroscopy (FIAAS). These authors identified three main reasons why the AA spectrometer behaves as a non-ideal flow injection detector. These are (i) the analytical signal is related to the concentration of sample entering the flame, rather than that entering the nebuliser; (ii) the nebulisation and transport processes take time, so that instrument response is not instantaneous; and (iii) calibration curves in AAS have a limited linear range. Under these circumstances, the contribution of the detector to the overall dispersion of a flow injection system cannot be neglected. Appleton and Tyson used simple flow models based on a single well-stirred tank to represent the instrument response for a step change in concentration and the effect of flow-rate on the nebuliser performance.

In this section, the observed dispersion behaviour of a single line manifold are compared for a flame AA spectrometer, and a low volume spectrophotometric detector. The response of the nebuliser is also investigated, and the results compared with those of Appleton and Tyson.

2.10.2 Experimental

Preliminary experiments were performed using a solution containing 1.00 μg ml⁻¹ of magnesium, which was prepared by serial dilution from a stock solution containing 1.00 mg ml⁻¹ (BDH Chemicals, Spectrosol® solution). Later experiments were performed using manganese solutions containing up to 100 μg ml⁻¹ (diluted
from 1.00 mg ml\(^{-1}\) stock solution, BDH Chemicals) and 500 µg ml\(^{-1}\) (1.44 g l\(^{-1}\) KMnO\(_4\), Fisons, AR grade), made up in deionised water. When the flame AA spectrometer was used with a pump, the pump was adjusted to give a sample flow-rate close to the natural aspiration rate of the nebuliser. Where appropriate, all absorbance values were converted to concentration values using a freshly constructed calibration curve.

**Nebuliser Response**

The effect of the flow-rate on the nebuliser was investigated using the manifold shown in Fig. 2.46. The natural aspiration rate of the nebuliser was measured by the timed aspiration of 10 ml of deionised water. A slightly fuel-lean air-acetylene flame was used, and the instrument optimised for maximum sensitivity for the relevant element using conventional aspiration. A solution containing 1.00 µg ml\(^{-1}\) of magnesium was pumped into the nebuliser at flow-rates covering the range 1 to 12 ml min\(^{-1}\). Deionised water was used as the blank. The absorbance obtained for each flow-rate was measured at 285.2 nm. Ten readings were taken for each flow-rate using a 0.1 s integration time, and the mean and relative standard deviation calculated using the SP9 computer. In order to examine the effect of flow-rate on peak height
for FIAAS, valve V3 was replaced by an injection valve (V1) fitted with a 113 µl sample loop. Ten replicate injections were performed at each flow-rate, and the mean and relative standard deviation calculated.

The response of the nebuliser to a step change in concentration was investigated using the same manifold (Fig. 2.46) and sample solution. A calibration curve was constructed over the range 0.10 to 1.00 µg ml⁻¹ of magnesium using standards prepared by serial dilution. The stream switching valve was connected to the nebuliser using a 15 cm length of 0.58 mm i.d. PTFE tubing. A step change in concentration was achieved by switching the valve between the water and sample streams. The resulting growth curves were recorded using a fast chart speed. The effect of flow-rate was also investigated. A different SP9 had to be used for this part of the experiment, the nebuliser being connected to the valve using a 10 cm length of 0.58 mm i.d. tubing. The same instrumental conditions were used, and both instruments optimised for magnesium (see earlier). The flow-rates examined covered the range 1 to 6 ml min⁻¹, a fresh calibration curve being constructed for each flow-rate used. Because the signal becomes noisier at low flow-rates, three growth curves were recorded for each flow-rate and averaged manually by using tracing paper to superimpose them.

**Comparison of Dispersion Behaviour**

The dispersion coefficient was measured for different lengths of 0.58 mm i.d. tubing over the range 19.3 to 350 cm using both solution spectrophotometric and flame AAS detection. Potassium permanganate was used as the sample because it can be detected directly using both techniques. A high concentration of permanganate ion was necessary in order to maintain similar conditions between the two sets of measurements. Optimisation of the AA detector was carried out using solutions containing up to 100 µg ml⁻¹ of manganese. Calibration curves were prepared for wavelengths of 279.5, 280.1 and 403.1 nm, in order to
assess the most suitable wavelength for monitoring high levels of potassium permanganate. A wavelength of 403.1 nm was subsequently chosen. A flow-rate of 5.8 ml min\(^{-1}\) was used throughout, which corresponded to the natural aspiration rate of the nebuliser under the conditions used. For solution spectrophotometric detection, the absorbance of permanganate ion was monitored at 526 nm. Since the flow cell had a very low volume (<0.2 μl), the observed dispersion very closely approximated that due to the manifold alone, allowing the additional dispersion introduced by the nebuliser/spray chamber of the AA spectrometer to be evaluated.

Values of the dispersion coefficient were calculated from the mean peak heights of five replicate injections of 67.3 μl of sample, the peak heights being first converted into concentration values using a calibration graph. The sample volume was chosen to ensure that a dispersion coefficient of greater than unity was obtained for all tube lengths. Because of the uncertainty in the value of D introduced by the breaking and making of connections (see section 2.1.3), each determination was carried out in triplicate, a connection being broken and remade each time, and the mean, standard deviation and 95% confidence interval about the mean were calculated. Peak shapes and growth curves were recorded using both detectors for different tube lengths.

2.10.3 Results and Discussion

Nebuliser Response

The results obtained for the effect of flow-rate on the steady-state absorbance are shown in Fig. 2.47. As the flow-rate is increased, the absorbance also increases, passing through a maximum value at or near the natural aspiration rate of the nebuliser for the conditions used. This was found to be 5.38 ml min\(^{-1}\). Similar results have been obtained by Tyson and co-workers using a variety of different instruments [58, 79, 106]. The relationship
The effect of flow-rate on peak height for FIAAS is shown in Fig. 2.48. As the flow-rate is increased, the peak height (here expressed in units of absorbance) rapidly increases to a maximum and then decreases again. This is different to the behaviour expected for the same manifold with an ideal detector (c.f. section 2.5), for which peak height is expected to increase towards a
maximum value and then remain constant with increasing flow-rate. This difference can be explained by considering the dynamic response of the AA spectrometer to changes in concentration.

When a step change in concentration was introduced to the nebuliser of the AA spectrometer, the resulting growth curves were found to resemble exponential growth curves (Fig. 2.49). For a sample flow-rate of 6.42 ml min$^{-1}$, some 4 seconds were taken for the steady-state to be reached, yet the maximum of a flow injection peak recorded under identical conditions was reached in less than 1 second. This means that the detector cannot accurately follow the concentration profile generated by a flow injection manifold. This is because sample entering the nebuliser/spray chamber of an AA spectrometer is not instantaneously transported into the flame, whilst it takes time for the amount of sample in the spray chamber to build up to its equilibrium value. The net effect of
Fig. 2.49 Typical growth and decay curves for AAS. Time is shown increasing from right to left.

this is similar to that of the volume of an optical flow cell as described earlier (section 2.2): the observed peak height will be reduced from that expected for an ideal detector, this effect being greater for rapidly changing concentration profiles than for slowly changing ones. This explains the shape of the peak height vs. flow-rate graph obtained. As the flow-rate is increased, although peak height increases as expected, the observed peak height becomes depressed to an increasing extent by the response time of the detector. This is not a complete
explanation of the observed variation of peak height with flow-rate, since such factors as the dependence of nebulisation efficiency on flow-rate have not been taken into account. However, as mentioned earlier, it is difficult to derive an exact theoretical description of the processes occurring within the nebuliser/spray chamber assembly, and therefore difficult to account fully for the effect of these processes.

It was noted above that the growth curves obtained resembled exponential rise curves. It was this fact that led Tyson [58] to develop the well-stirred tank (WST) model for FIAAS. In this model, the response of an AA spectrometer to a step change in concentration is represented by a single well-stirred mixing tank of volume \( V_m \). For a step change in concentration from \( C = 0 \) to \( C = C_o \), the resulting rise curve to the steady-state is given by the equation

\[
C = C_o \left[ 1 - \exp\left( -\frac{Qt}{V_m} \right) \right] \quad (2.15)
\]

Similarly for the fall curve \( (C = C_o \) to \( C = 0) \),

\[
C = C_o \exp\left( -\frac{Qt}{V_m} \right) \quad (2.16)
\]

Although this model has been successfully applied to FIAAS [58 - 61], it was found that the model did not properly describe growth curves obtained experimentally [79, 107]. Since the results of Appleton and Tyson were obtained using different instruments (Shandon Southern A3400 and Perkin Elmer 290B) to the one used here (Pye Unicam SP9), the growth curves obtained were tested for agreement with the WST model. Equations 2.15 and 2.16 can be re-written as

\[
-ln(1 - \frac{C}{C_o}) = \frac{Qt}{V_m} \quad (2.17)
\]

\[
-ln(\frac{C}{C_o}) = \frac{Qt}{V_m} \quad (2.18)
\]

Therefore, a plot of either \(-\ln(1 - \frac{C}{C_o})\) or \(-\ln(\frac{C}{C_o})\)
against $t$ should be linear with a slope equal to $Q/V_m$. The experimental data was analysed using a specially written computer program (see Appendix D), and the results obtained are summarised below (Table 2.13, Fig. 2.50).

**Fig. 2.50** Fit of the WST model to the growth curve for AAS. See text for details. Line indicates predicted curve for $V_m = 88.7 \mu l$; circles show actual data points.

Results for the growth curves show good agreement between the model and the experimental data. However, when the decay curves were analysed, the plots of $-\ln(C/C_0)$ against $t$ showed two linear portions having different slopes.
Table 2.13 Results for the WST Model.

Notes:  
(a) calculated from slope of linear portion  
(b) Pearson correlation coefficient for linear portion  
(c) alternative linear portion.

indicating a breakdown of the model. This may be explained by the configuration of the valve used, which meant that an additional length of tubing was effectively introduced into the manifold when the valve was switched to obtain the decay curve. Since some dispersion of the sample/carrier boundary will occur in even a short length of tubing this meant that the concentration gradient entering the nebuliser was less like a step change than for the growth curves. The growth curves obtained for different flow-rates were analysed in the same way. The plots of $-\ln(1 - C/C_0)$ against $t$ also showed two linear portions. This suggests that the WST model may only describe the response of the AA spectrometer to step changes in concentration adequately under certain limiting conditions, and that a more general model is required.

Appleton and Tyson [79] have described the two tanks-in-parallel (TTP) model for instrument response in FIAAS (Fig. 2.51). For a step change in concentration from $C = 0$ to $C = C_0$, this gives rise to the response function
Fig. 2.51 TTP model for AAS. C = carrier or sample stream; D = detector; $f_1$, $f_2$ = flow fractions to each tank; $Q$ = net volumetric flow-rate; $V_1', V_2 = tank volumes; W = waste. AA'$ and BB' are assumed to be negligible.

$$C = C_0 \left[ 1 - f_1 \exp \left( \frac{-f_1 Qt}{V_1} \right) - f_2 \exp \left( \frac{-f_2 Qt}{V_2} \right) \right] \quad (2.19)$$

(see appendix C for a full derivation).

The method described by Appleton [108] was used to test the experimental data for agreement with equation 2.19. If it is assumed that the initial and final gradients of the plots of $-\ln(1 - C/C_0)$ against $t$ are due to tanks one and two respectively, then it follows from equation 2.17 that

$$G_1 = \frac{Q_1}{V_1} \quad \therefore \quad V_1 = f_1 \cdot \frac{Q}{G_1} \quad (2.20)$$

$$G_2 = \frac{Q_2}{V_2} \quad \therefore \quad V_2 = f_2 \cdot \frac{Q}{G_2} \quad (2.21)$$

Initial and final gradients were calculated for all the rise curves from the plots of $-\ln(1 - C/C_0)$ against $t$, and
used to calculate values of $V_1$ and $V_2$ for different values of $f_1$. The best fit of the model data to the experimental growth curves was determined by visual inspection. The results obtained are summarised below (Fig. 2.52 and Table 2.14). A good fit was obtained for all the curves except

\[ \begin{align*}
C/C_0 & \quad 0.00 \quad 0.20 \quad 0.40 \quad 0.60 \quad 0.80 \quad 1.00 \\
0.00 \quad 2.00 \quad 4.00 \quad 6.00 \quad 8.00 \quad t/s
\end{align*} \]

Fig. 2.52 Fit of the TTP model to the growth curve for AAS. Line shows predicted curve for $V_1 = 57.31 \mu l$, $V_2 = 26.84 \mu l$, $f_1 = 0.80$.

that for a flow-rate of 1.23 ml min$^{-1}$. Appleton and Tyson also reported good agreement between the TTP model and their experimental data, which suggests that the TTP model is more widely applicable than the WST model.

The final model described by Appleton and Tyson is the extended single tank (EST) model (see chapter 4). This was developed to try and model the effect of flow-rate on absorbance for FIAAS, and was found to give good agreement with the experimental data. However, the equations derived for the model are complex, and can only
Table 2.14 Fit of the TTP model to experimental growth curves. "Best fit" values determined visually.

---

be simplified if the concentration ranges involved are assumed to be within the linear calibration range for the element and instrument used. Therefore this model was not investigated in this study.

When the growth curves obtained for different flow-rates were plotted as normalised concentration ($C/C_0$ as determined from absorbance-time data via a calibration curve) against time, it was found that all the curves were identical within experimental error apart from that obtained at the lowest flow-rate (Fig. 2.53). Since the growth curves were recorded three times each, and the same result obtained, it is unlikely that this difference was caused by an abrupt change in conditions during the course of the experiment. If changing the sample flow-rate only affected the rate at which sample entered the flame, then the absorbance-time ($A$-$t$) curves would be different at different flow-rates, but the concentration-time ($C$-$t$) curves would be the same. But if the performance of the nebuliser/spray chamber varied with the flow-rate, the concentration-time curves would be different.

In fact the sample flow-rate does affect nebuliser
Fig. 2.53 Effect of flow-rate on AAS growth curves. \( Q = (\Delta) 5.76, (+) 4.20, (X) 3.16, (\square) 2.18 \) and (O) 1.23 ml min\(^{-1}\).

performance [109]: a decrease in flow-rate results in an increased proportion of small droplets in the aerosol, thus increasing the amount of sample usefully entering the flame. This could explain the observed difference in the C-t curves. If the rate of transport of small droplets into the flame is determined only by the fuel and oxidant flow-rates, then the initial portion of the C-t growth curve will be due to this rapid process, and will show the same rate of growth for any flow-rate, although different growth rates will be obtained for the A-t curves. However, those processes involving large droplets - which are less well understood - may be slower and have a more significant effect at low sample flow-rates, giving rise
to the later, less rapid rate of growth observed in Fig. 2.53 for a flow-rate of 1.23 ml min\(^{-1}\). This could also explain the poorer fit of the TTP model to the growth curve obtained at this flow-rate.

**Dispersion Behaviour**

The rise curves obtained for both detectors were analysed as above for agreement with the WST and TTP models. The results are given below (Tables 2.15 and

<table>
<thead>
<tr>
<th>Detector</th>
<th>L/cm</th>
<th>(V_m/\mu l)(^{(a)})</th>
<th>(r)(^{(b)})</th>
<th>Fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAS</td>
<td>10.0</td>
<td>115.89</td>
<td>0.9994</td>
<td>YY</td>
</tr>
<tr>
<td>AAS</td>
<td>20.1</td>
<td>105.66</td>
<td>0.9992</td>
<td>Y</td>
</tr>
<tr>
<td>AAS</td>
<td>30.1</td>
<td>116.99</td>
<td>0.9995</td>
<td>Y</td>
</tr>
<tr>
<td>AAS</td>
<td>40.0</td>
<td>115.48</td>
<td>0.9993</td>
<td>YY</td>
</tr>
<tr>
<td>AAS</td>
<td>50.4</td>
<td>155.20</td>
<td>0.9991</td>
<td>?</td>
</tr>
<tr>
<td>AAS</td>
<td>80.5</td>
<td>210.46</td>
<td>0.9982</td>
<td>X</td>
</tr>
<tr>
<td>AAS</td>
<td>110.0</td>
<td>183.41</td>
<td>0.9989</td>
<td>X</td>
</tr>
<tr>
<td>AAS</td>
<td>160.0</td>
<td>210.80</td>
<td>0.9965</td>
<td>?</td>
</tr>
<tr>
<td>UV</td>
<td>20.1</td>
<td>48.61</td>
<td>0.9994</td>
<td>X</td>
</tr>
<tr>
<td>UV</td>
<td>30.1</td>
<td>40.22</td>
<td>0.9989</td>
<td>Y</td>
</tr>
<tr>
<td>UV</td>
<td>40.0</td>
<td>43.16</td>
<td>0.9999</td>
<td>Y</td>
</tr>
<tr>
<td>UV</td>
<td>50.4</td>
<td>54.02</td>
<td>0.9993</td>
<td>?</td>
</tr>
<tr>
<td>UV</td>
<td>80.5</td>
<td>-</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>UV</td>
<td>110.0</td>
<td>-</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>UV</td>
<td>150.0</td>
<td>-</td>
<td>-</td>
<td>X</td>
</tr>
</tbody>
</table>

**Table 2.15** Fit of the WST model to rise curves obtained for AAS and UV detection. YY = very good fit; Y = good fit; ? = partial fit; X = no/poor fit.

**Notes:**
(a) calculated from the slope of the linear portion
(b) Pearson correlation coefficient for the linear portion.
Table 2.16 Fit of the TTP model, as for Table 2.15
Notes: (a) equivalent to the WST model, $V_m = 115.47\mu l$

2.16, Figs 2.54 and 2.55). The growth curves obtained using AAS detection show good agreement with the WST model for tube lengths up to 40.0 cm, and with the TTP model for tube lengths up to 50.4 cm. For the growth curves obtained using solution spectrophotometric detection, the WST model fitted the data well up to 40.0 cm, similar results being obtained for the TTP model. A tube length of 10.0 cm could not be used because a minimum length of 15.0 cm was required to make a connection between the injection valve and the flow cell. The results obtained for a tube length of 20.1 cm were not included because the data showed several discontinuities, indicating that it was unreliable.

The values of $V_m$ obtained from the AA data for the
Fig. 2.54 Fit of the WST (A,C) and TTP (B,D) models to FIAAS growth curves. (A) $L = 30.1$ cm, $V_m = 116.99$ μl. (B) $L = 30.1$ cm, $V_1 = 58.49$ μl, $V_2 = 58.13$ μl, $f_1 = 0.500$. (C) $L = 80.5$ cm, $V_m = 110.82$ μl. (D) $L = 80.5$ cm, $V_1 = 84.18$ μl, $V_2 = 66.29$ μl, $f_1 = 0.400$. 
Fig. 2.55 Fit of the WST (A,C) and TTP (B,D) models to FIA growth curves. Tube lengths as Fig. 2.54. (A) $V_m = 35.48\ \mu l$. (B) $V_1 = 17.74\ \mu l, V_2 = 20.11\ \mu l, f_1 = 0.500$. (C) $V_m = 63.58\ \mu l$. (D) $V_1 = 39.21, V_2 = 38.15\ \mu l, f_1 = 0.400$. 
WST model were significantly greater than those obtained from the UV/visible data. This reflects the contribution of the nebuliser/spray chamber of the AA spectrometer to the observed dispersion of the manifold. The value of $V_m$ obtained from the AA data for tube lengths up to 40.0 cm is fairly constant, suggesting that the processes occurring within the nebuliser/spray chamber are the predominant factor in determining the observed shape of the growth curves for short tube lengths. Both models break down for increasing tube length for both methods of detection. That is, as the extent to which the sample/carrier boundary becomes dispersed within the manifold increases, it becomes harder to represent the combination of this with the "dispersion" generated by the AA spectrometer.

The results obtained for the variation in the observed dispersion coefficient with tube length are shown in Fig. 2.56. These results again show the effect of the AA spectrometer on the observed sample dispersion. Comparing the results obtained using the two methods of

![Graph showing D vs. L for FIAAS (X) and FIA (O).](image-url)

**Fig. 2.56** $D$ vs. $L$ for FIAAS (X) and FIA (O).
detection shows that the nebuliser/spray chamber makes a large contribution to the observed sample dispersion for short tube lengths, this effect becoming less significant as the tube length is increased up to 250 cm.

Both sets of results show a linear portion up to a tube length of about 100 cm. For the AA data, linear regression analysis on the first three points gave a correlation coefficient of 0.9970 with slope 0.0103 and intercept 2.60. For the solution spectrophotometric data, analysis of the first five points gave a correlation coefficient of 0.9986 with slope 0.0138 and intercept 1.07. These results are in agreement with those described in section 2.5. The results obtained were tested for agreement with equation 2.12 (section 2.5),

\[(D - 1)^2 = KL\]

by plotting \((D - 1)^2\) against L (Fig. 2.57). For the AA data, linear regression analysis for tube lengths of 19.3 to 249.8 cm gave a correlation coefficient of 0.9967 with slope 0.0453 and intercept 2.337. For the solution spectrophotometric data, two linear portions were found. These results show that for FIAAS, equation 2.12 holds for tube lengths up to 250 cm for the conditions used, but does not accurately describe the relationship between tube length and dispersion coefficient for the flow manifold alone. Appleton and Tyson also found limited agreement between their experimental data and equation 2.12 for FIAAS.

2.10.4 Summary and Conclusions

It has been demonstrated that the nebuliser/spray chamber of a flame AA spectrometer has a significant effect on the observed sample dispersion of a flow injection manifold. This can be seen in both growth curves to the steady-state and the peak heights obtained for FIAAS, as compared to those obtained from the manifold alone.
Fig. 2.57 \((D - 1)^2\) vs. \(L\) for FIAAS (X) and FIA (O)

When the sample flow-rate entering the nebuliser of an AA spectrometer is close to the natural aspiration rate of the nebuliser, then the concentration-time growth curves may be described using either the WST or TTP models for short lengths of connecting tubing. The TTP model was also found to be valid for lower sample flow-rates with the instrument used, but both models broke down for tube lengths above about 40 cm. For FIAAS, the relationship between the dispersion coefficient and tube length may be usefully described by the equation \((D - 1)^2 = KL\).

The implications of this work for modelling dispersion in flow injection manifolds will be discussed in Chapters 4 and 5.
Chapter Three

Flow Models in FIA
3.1 THE DESCRIPTION OF PEAK SHAPE

3.1.1 Introduction

In section 2.6, the peak shapes recorded experimentally were divided into five categories: skewed Gaussian, triangular, humped, exponential and tailed. It was mentioned there that these peak shapes could be explained from theoretical considerations, or using a modelling approach based on that employed in chemical engineering. Both of these approaches will now be described, and their advantages and disadvantages discussed.

3.1.2 The Theoretical Description of Peak Shape

The origin of peak shape in FIA was described in general terms in section 1.2.4 as the result of the primary dispersion mechanism of diffusion-convection. It was also mentioned that the differential equation describing this mechanism (equation 1.6, p 26) could not be solved directly for all conditions. However it is possible to use numerical techniques to solve this equation, a method which has been used by various workers to study sample dispersion in flowing streams. The results of these workers may be used to explain the different categories of peak shape described in chapter 2. Golay and Atwood [49, 50] and Vanderslice et al [44, 47, 81] predicted the appearance of a range of peak shapes under conditions of laminar flow through straight tubes, and confirmed their predictions experimentally. Reijn et al [51] also observed such peak shapes. The calculations of Golay and Atwood and Vanderslice et al show that the range of peak shapes observed for different experimental conditions (tube length, flow-rate, etc.) is due to different relative contributions to the dispersion mechanism from the diffusion and convection (laminar flow) processes. The method of injection can also make a contribution to the observed peak shape, although this was
not taken into account by these authors, who all employed conditions of slug injection in their work.

The extreme cases considered by Golay and Atwood, Vanderslice et al and Reijn et al correspond to Taylor's approximate solutions to the diffusion-convection equation (39, 40). These are convective flow (Taylor 'A') and diffusion-controlled dispersion (Taylor 'B'). As will be seen later, the peak shapes obtained for convective flow are of the tailed or exponential type, whilst for diffusion-controlled dispersion they are of the skewed Gaussian type. For intermediate conditions, the rise part of the peak will be less affected by radial diffusion than the tail, since it is resident within the manifold for a shorter period of time. Golay and Atwood and Vanderslice et al have shown that this explains the humped, or under certain conditions double, peaks that can be observed. It follows that the triangular peak shape described earlier can be explained as an intermediate form of the skewed Gaussian and humped peaks.

The conditions for which the different mechanisms operate are generally discussed in terms of dimensionless parameters. These are reduced retention time \( \tau_p \), reduced length \( X_R \), and the Peclet number \( P_e \), which are given by (44)

\[
\tau_p = \frac{t_p D_m}{a^2} \quad (3.1)
\]

\[
X_R = \frac{L D_m}{a^2 v_o} \quad (3.2)
\]

\[
P_e = \frac{a v_o D_m}{L} \quad (3.3)
\]

where \( a \) is the tube radius, \( D_m \) is the diffusion coefficient, \( L \) is the tube length, \( t_p \) is the time to the peak maximum, and \( v_o \) is the linear flow velocity at the tube centre. In order to compare the experimental results obtained here with the findings of Taylor, Golay and Atwood and Vanderslice et al, the peak shapes recorded were classified into the five categories defined earlier, and values of \( \tau_p \), \( X_R \) and \( P_e \) calculated. A value of $1 \times 10^{-5}$
cm² s⁻¹ was assumed for the diffusion coefficient of tartrazine in water, since no value could be found in the literature. The results obtained are shown in Fig. 3.1, the regions within which the different peak shapes occur being indicated by the shaded areas.

The tailed and exponential peaks occur predominantly for low residence times and short tube lengths. Skewed Gaussian peaks occur for high residence times and long tube lengths. This is in agreement with the conditions for which the convection and diffusion processes will dominate the overall dispersion of the sample zone. Humped and triangular peaks are the intermediate form of these two extremes. These different groups will therefore be referred to as convection, diffusion and intermediate peak shapes respectively.

The 'spikes' observed in the data presented in Fig. 3.1 require some explanation. These are the result of varying the sample volume whilst keeping all other manifold parameters constant, and were obtained for both time and slug injection. As the sample volume is increased, $t_p$ (and hence $\tau_p$) also increases. However, this does not affect the value of $X_R$. This highlights one of the problems of using the dimensionless parameters as defined above, namely that none of them takes into account either the sample volume or the method of injection. This will be discussed further in chapter 5.

The boundary conditions for which different dispersion processes operate have been examined by several workers. Taylor [39] gave the condition $\tau_p > 0.07$ for diffusion controlled dispersion. Reijn et al [51] derived the more exact criterion $\tau_p > 0.8$ from the work of several authors. Wen and Fan [97] have calculated the regions of applicability for various solutions to the diffusion-convection equation in terms of $P_e$ and $\tau_p$, as have Vanderslice et al [44]. Wen and Fan give the conditions for pure convection as being $0.001 < \tau_p < 0.01$, $P_e > 100$. Vanderslice et al [81] have predicted the appearance of convection peaks for $\tau_p < 0.01$, diffusion peaks for $\tau_p > 0.1$, 

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Fig. 3.1 Distribution of experimental peak shapes. Horizontal shading = convection, vertical shading = intermediate and diagonal shading = diffusion peak shapes. Outliers for the three categories are shown by O, □ and Δ respectively. and intermediate peaks for 0.01 < τ_p < 0.1. It should be noted that the calculations of all these authors were performed assuming an ideal manifold and slug injection. The data shown in Fig. 3.1 was replotted as P_e against τ_p using the three categories of peak shape defined above (Fig. 3.2). This shows a clear distinction between the diffusion and convection peak shapes, but considerable
Fig. 3.2 Distribution of experimental peak shapes. Key is the same as for Fig. 3.1.

overlap between the convection and intermediate peak shapes.

Comparison of the experimental data with the boundary conditions calculated by Wen and Fan and Vanderslice et al is complicated by the lack of an accurate value for $D_m$. If a value of $1 \times 10^{-5}$ cm$^2$ s$^{-1}$ is assumed, then none of the data points for convection peaks fall within the convection region, whilst only a few points fall within the Taylor 'B' region (diffusion-controlled dispersion). But if a value of $1 \times 10^{-6}$ cm$^2$ s$^{-1}$ is assumed, then the
majority of the data points for convection peaks fall within the convection region, whilst the majority of the diffusion peaks fall within the intermediate region. The overlap between the data for the convection and intermediate peak shapes suggests that the first situation is the more likely, i.e. that the data does not correspond to conditions for which dispersion occurs by pure convection. This is supported by the calculations performed on the experimental data in sections 2.4 and 2.5, where the observed dispersion behaviour for certain conditions was similar to that expected for pure convection, but could not be explained solely on this basis. In other words, the convection peak shapes observed are not those expected for dispersion by convection alone. This was confirmed by comparison of the experimental data with peak shapes generated using the convection model (see section 3.2).

3.1.3 The Modelling Approach to Peak Shape

An alternative approach to the problem of describing peak shape in FIA is to use models based on experimental observations, following the method used in chemical engineering [97]. Whilst not strictly accurate, this approach has the advantage of allowing the non-ideal nature of real flow manifolds to be taken into account. For example, although Vanderslice et al predicted the appearance of double peaks [44], and generally obtained a good correlation between theory and experimental measurements, they were unable to observe such peaks in practice. This was attributed to imperfections in the flow manifold, such as pulsation from the pump. (See also section 1.3.2).

If skewed Gaussian peaks are assumed to arise from pure diffusion-convection for manifolds approximating to the ideal, then the remaining peak shapes may be explained by considering different deviations from ideal behaviour. These are the presence of a bypass ("channelling"), a dead volume, or a mixing volume (Fig. 3.3). Humped peaks may
Fig. 3.3 Non-ideal manifold behaviour and resulting peak shapes. C = carrier; D = detector; DV = dead volume; MV = mixing volume; P = pump; S = sample; V = valve; W = waste.
be explained by channelling. In this case, the flow is divided between two paths having different lengths, and then recombined prior to detection (Fig. 3.3, B). This will result in two overlapping - but different - concentration-time profiles, giving rise to a humped or double peak. Triangular peaks can be explained as being due to a limited degree of channelling. Tailed peaks may be explained by the presence of a dead volume (Fig. 3.3, C). This will cause part of the flow to be delayed, giving rise to a rapid peak with a long tail. Exponential peaks are readily explained by the presence of a mixing volume (Fig. 3.3, D), since it is well-established that a well-stirred mixing tank will give rise to exponential growth and decay curves for step changes in concentration at the inlet.

It is possible to relate these descriptions to the non-ideal behaviour of real manifolds. For example, it is likely that channelling will occur in wide bore tubing where connections are made using narrow bore tubing. In this case, some of the flow will "stream" through the centre of the tubing, whilst the remainder will flow around the walls, turbulence at the connections giving rise to what is effectively a longer flow path (Fig. 3.4).

Fig. 3.4 Flow patterns in wide bore tubing

This provides an explanation for the peak shapes observed using 1.0 mm i.d. tubing that were described in section 2.6. This explanation is in some ways preferable to that based on a pure diffusion-convection mechanism, since the same problems encountered by Vanderslice et al (non-ideal behaviour of the flow manifold) also apply to the experimental results described in this thesis.
Dead volumes will be caused by regions of stagnant water. These are likely to exist at sharp corners or edges, such as those at connections, valves, or in flow cells (Fig. 3.5). Solute will be exchanged between the regions of stagnant and flowing water, resulting in a prolonged washout of the solute from the manifold. Mixing volumes may arise in similar ways to channelling and dead volumes, or through the use of a real mixing tank.

3.1.4 Summary and Conclusions

In comparing the theoretical and modelling approaches to the description of peak shape in FIA, it is important to remember that real flow injection manifolds do not conform to the ideal manifold that is often assumed in such treatments (see section 1.3.2). Thus although the theoretical approach discussed above (section 3.1.2) is, the more accurate, it fails to take into account secondary dispersion mechanisms such as localised turbulence, dead volumes, etc. Neither would it be easy to take into account the effect of confluence points, or the use of different types of packed reactor. A further problem with the theoretical approach is that, as pointed out by Vanderslice et al [44] and Wada et al [85], it is difficult even with single-line manifolds to obtain
experimental conditions close to those assumed in theoretical calculations.

Therefore, the modelling approach seems to offer considerable advantages in that it does not rely on certain assumed conditions being realised, the model simply being modified to take into account non-ideal behaviour. This of necessity introduces an empirical element into the description of peak shape, which may well limit the applicability of results obtained using one set of equipment to the prediction of results obtained using different equipment. It also appears from the experimental results - particularly the peak shapes obtained - that more than one model will be required to describe dispersion in FIA fully, especially if the method of injection is to be taken into account.

3.2 THE CONVECTIVE FLOW MODEL

3.2.1 Description of the Model

The convective flow model is one of the simplest flow models available, and is based on the assumption that dispersion in an ideal manifold occurs only by the distortion produced by laminar flow [39]. Under such conditions, the linear flow velocity profile is given by the Poiseuille equation,

\[ u_r = \frac{p}{4\eta L} \left( a^2 - r^2 \right) \]  

(3.4)

where \( u_r \) is the linear flow velocity at a radial distance \( r \) from the tube centre, \( p \) is the pressure drop across a length \( L \) of straight tubing of uniform radius \( a \), and \( \eta \) is the viscosity of the fluid. If a volume \( V_s \) of sample solution of initial concentration \( C_0 \) is introduced at the point \( x = 0 \) (Fig. 3.6), then the resulting concentration-time (C-t) profile observed in a plane perpendicular to the flow at the point \( x = (L+L_s) \) will be given by
Fig. 3.6 The convective flow model. See text for details. The arrow indicates the direction of flow.

\[ 0 < t < t_a, \quad C/C_0 = 0 \quad (3.5) \]

\[ t_a < t < t_p, \quad C/C_0 = 1 - (V_r/2Qt) \quad (3.6) \]

\[ t = t_p, \quad C_p/C_0 = V_s/(V_s + V_r) \quad (3.7) \]

\[ t > t_p, \quad C/C_0 = V_s/2Qt \quad (3.8) \]

(See appendix C for a full derivation of these equations). This predicts a peak having an infinitely long tail, the dispersion coefficient being given by the expression \((V_s + V_r)/V_s\). An example of the model is shown in Fig. 3.7. This was generated for a flow-rate of 1.00 ml min\(^{-1}\), a sample volume of 100 \(\mu\)l, and a 100 cm length of 0.5 mm i.d. tubing. For these conditions, the following values were obtained: \(t_a = 5.89\) s; \(t_p = 8.89\) s; \(C_p/C_0 = 0.337\); \(D = 2.963\). The concentration fell to 10% of the peak value after a total of 89 s, and to 1% after a total of 889 s, demonstrating the tailing predicted by the model.
Fig 3.7 Peak shape predicted by the convective flow model. See text for details.

3.2.2 Discussion and Conclusions

Although the convection model is one of the simplest flow models, it has found very little application in flow injection analysis. Reijn et al [42] used the model as the basis of theoretical calculations to demonstrate the effect of cup-mixing and mean value modes of detection. Bernhardsson et al [75] assumed convective flow as one case in their study of solute transfer in flow-through dialysers. (The other case was plug flow, where the linear flow velocity is constant across the tube diameter). The use of models in continuous-flow analysis has been reviewed by Painton and Mottola [110]. However, although the convection model was briefly described by these authors, no examples of its application to FIA were cited.

The reason for the limited application of this model
can be seen by considering the conditions for which it is valid. For straight tubes, the flow pattern is characterised in terms of the Reynold's number, \( R_e \), which is given by the equation [37]

\[
R_e = \frac{2\pi \bar{u} \rho}{\eta}
\]  

(3.9)

where \( \bar{u} \) is the mean linear flow velocity, and \( \rho \) is the density of the fluid. There is some variation in the boundary conditions for laminar and turbulent flow cited by different authors, but these may be taken as \( R_e < 1,000 \) for laminar and \( R_e > 2,000 \) for turbulent flow. From equation 3.9, it can be shown that laminar flow exists for the conditions commonly used in FIA (see appendix C.1). However, this is not the case for the boundary conditions for which dispersion by pure convection can be assumed. Wen and Fan [97] and Vanderslice et al give the condition \( \tau_p < 0.01 \). Wen and Fan also give the condition \( \tau_p > 100 \), whilst Ananthakrishnan et al give \( \tau_p > 1000 \).

Substituting for \( \tau_p \) and \( \tau_p \) gives the conditions

\[
\tau_p < 0.01 \cdot a^2/D_m
\]  

(3.10)

\[
u_o > 1000 \cdot D_m / a
\]  

(3.11)

Assuming values of \( a = 0.25 \text{ mm} \) and \( D_m = 1.00 \times 10^{-9} \text{ m}^2 \text{ s}^{-1} \) equation 3.10 yields the conditions \( \tau_p < 0.625 \text{ s}, \nu_o > 4.00 \text{ mm s}^{-1} \) (Q>0.06 ml min^{-1}). For a tube length of 1.00 m, this first condition can only be satisfied for flow-rates in excess of 18 ml min^{-1}. Similarly, for a flow-rate of 1.00 ml min^{-1} a tube length of less than 5 cm is required. Clearly, such conditions are not met in practical FIA, where flow-rates are typically in the range 0.5 - 2.0 ml min^{-1} and tube lengths in the range 0.5 - 5.0 m. These calculations lend support to the conclusion that the exponential and tailed peak shapes described in section 3.1 cannot be explained by pure convective dispersion. When experimentally recorded peak shapes were compared
with those predicted by the convection model for the same conditions, no agreement was found (see Fig. 3.8).

![Graph](image)

Fig. 3.8 Fit of the convective flow model. Peak shapes were recorded using cell F3 with tartrazine as the solute using 0.58 mm i.d. tubing. (A) $Q = 1.10$ ml min$^{-1}$, $L = 19.3$ cm, $V_s = 53.6$ µl, slug injection. (B) $Q = 0.35$ ml min$^{-1}$, $L = 30.0$ cm, $V_s = 113$ µl, slug injection. Dashed curve shows model prediction.

Generally, the model predicted too low a peak height, and too long a washout time.

In summary, although the convection model is mathematically simple, it does not adequately describe
observed peak shape, neither is it applicable to the conditions generally employed in FIA.

3.3 THE DIFFUSION MODEL

3.3.1 Diffusion-limited Dispersion

Diffusion-limited dispersion was described by Taylor [39] as one of two cases for which a solution to the diffusion-convection equation may be obtained. (The other case, that of pure convection, was described in the preceding section). The underlying assumptions of Taylor's analysis are that (a) \( D_m \) is independent of the solute concentration, (b) the concentration distribution is symmetrical about the tube axis, (c) longitudinal diffusion is negligible in comparison with the effect of convective flow, and (d) radial variations in concentration caused by convective flow are reduced to a fraction of their initial value by the effects of radial diffusion. Under these conditions, Taylor found that the solute became dispersed in a manner analogous to diffusion across a plane moving at the mean linear flow velocity \( \bar{U} = \frac{1}{2} u_o \), with an apparent diffusion coefficient

\[
D_a = a^2 u_o^2 / 192. D_m
\]  

(3.12)

The boundary condition for which assumption (d) is valid was derived as being

\[
L/u_o << a^2 / 3.8^2 D_m
\]  

(3.13)

The condition for which the effects of longitudinal diffusion are negligible was subsequently given as [40]

\[
D_m << a^2 u_o^2 / 192. D_m
\]  

(3.14)

Equations 3.13 and 3.14 reduce to give the expression

\[
4L/a >> \bar{U}a/D >> 6.9
\]  

(3.15)
Following the work of Taylor, several authors described methods for extending the applicability of Taylor's work by removing some of the limiting assumptions described above. Of particular interest is the work of Aris [41] and Bate et al [46]. Aris took into account the variation of $D_m$ with concentration and spatial coordinate, and derived a modified form of equation 3.12,

$$D_a = D_m + \left( a^2 u_o^2 / 192 D_m \right)$$  \hspace{1cm} (3.16)

Bate et al used a numerical technique to solve the diffusion-convection equation and derived the condition for which assumption (c) above is valid as being $P_e > 200$. Since $P_e^2 = a^2 u_o^2 / D_m^2$, this yields the condition

$$40,000 < a^2 u_o^2 / D_m^2$$  \hspace{1cm} (3.17)

which should be compared with equation 3.14.

For the conditions outlined above, and putting $z = r/a$, the diffusion-convection equation reduces to

$$\frac{\delta^2 C}{\delta z^2} + \left( \frac{1}{z} \right) \frac{\delta C}{\delta z} = \left( \frac{a^2}{D_m} \right) \frac{\delta C}{\delta t} + \left( \frac{a^2 u_o}{D_m} (1-z^2) \right) \frac{\delta C}{\delta x}$$  \hspace{1cm} (3.18)

If the solute concentration is measured as the mean concentration in a plane at right angles to the direction of flow ($C_m$), Taylor [39] showed that equation 3.18 further reduces to give

$$D_a \frac{\delta^2 C_m}{\delta x_1^2} = \frac{\delta C_m}{\delta t}$$  \hspace{1cm} (3.19)

where $x_1 = x - \frac{1}{2} u_o t$. Taylor considered two cases for which solutions to equation 3.19 can be derived. These were (a) injection of a mass $M$ of solute at a point $x = 0$ at time $t = 0$ ("delta" injection), and (b) introduction of sample of uniform concentration $C_o$ at a constant flow-rate at $x = 0$ starting at time $t = 0$ (see fig. 3.9).
Fig. 3.9 Initial conditions for the diffusion model for (A) delta injection and (B) continuous sample introduction. See text for details.

These cases yield the solutions

\[ C = \frac{M}{2\pi a^2} \left( \frac{1}{\sqrt{\pi D_a t}} \right) \exp \left( -\frac{x_1^2}{4D_a t} \right) \]  

(3.20)

and

\[ \frac{C}{C_o} = \frac{1}{2} \pm \text{erf} \left( \frac{x_1}{2\sqrt{D_a t}} \right) \]  

(3.21)

where \( \text{erf}(\alpha) = 2\pi^{-\frac{1}{2}} \int_0^\alpha e^{-\alpha^2} d\alpha \)

respectively. An example of the peak shape obtained for typical flow injection conditions from equation 3.20 is shown in Fig. 3.10.
Fig. 3.10 Typical peak shapes predicted for the diffusion model. Calculations were performed for \( L = 100 \) cm, \( d = 0.5 \) mm and \( V_s = 100 \) ml, \( Q = 1.00 \) ml \( \text{min}^{-1} \) \( \text{(A-D)} \) and \( 0.50 \) ml \( \text{min}^{-1} \) \( \text{(E)} \). \( D_m = \text{(A, E)} \) 1.0, \( \text{(B)} 2.0, \text{(C)} 3.0 \) and \( \text{(D)} 0.5 \times 10^{-9} \) m\(^2\) s\(^{-1}\).

3.3.2 Comparison with the Diffusion Model

Levenspiel and Smith [36] have described the diffusion (or axially-dispersed plug flow) model to describe dispersion in long pipes. This model starts with the finding of Taylor [39] that, under certain conditions, dispersion in a long straight tube or pipe could be treated as being analogous to one-dimensional molecular diffusion (Fick's second law). This leads to the relationship
\[
\frac{\delta C}{\delta t} = D_a \frac{\delta^2 C}{\delta x^2}
\]  

(This is equivalent to equation 3.19).

The solution to this equation derived by Levenspiel and Smith is identical to that described by Taylor (equation 3.20), although it should be noted that Levenspiel and Smith used dimensionless units for t and \(D_a\) (these are defined below).

Levenspiel and Smith investigated the properties of the model in terms of the "longitudinal dispersion number", \(\frac{D}{\bar{u}L}\) (here given the symbol \(\delta\)). They found that for values of \(\delta < 0.01\), equation 3.20 could be simplified to give a normal error function

\[
\frac{C}{C_0} = \frac{4}{\pi}\delta \exp\left(\frac{-\left(1-\delta^2\right)}{4\delta}\right)
\]

(3.23)

where \(\delta = t\frac{Q}{V_r}\). That is, when \(\delta\) is small, the solute distribution at any instant is normal and does not change appreciably during the time period required for the volume of fluid occupied by the solute to pass the point of detection. For values of \(\delta > 0.01\), this approximation does not hold, and the resulting peak shapes show an increased skewness.

Levenspiel and Smith also investigated the calculation of the time to peak maximum. They derived the expression

\[
\theta_{\text{max}} = \frac{t\frac{Q}{V_r}}{(\delta^2 + 1)^{1/2} - \delta}
\]

(3.24)

This was compared to the assumption that \(\theta_{\text{max}} = 1\). For very small values of \(\delta\), equation 3.24 does indeed give \(\theta_{\text{max}} = 1\). However, for large values of \(\delta\), equation 3.24 can be expressed as \(\theta_{\text{max}} = 1/2\delta\), in which case the condition \(\theta_{\text{max}} = 1\) does not adequately predict the time to the peak maximum. The resulting equations for the peak maximum concentration given by Levenspiel and Smith can be
written as

$$C_p = \frac{\nu(\pi \delta)^{-\frac{1}{2}}}{\delta} \left( \delta^2 + 1 \right)^{\frac{1}{2}} \exp \left( -\frac{\delta^2 + 1}{2 \delta} \right)$$

Equation 3.25

and

$$C_{C(\theta_{\max} = 1)} = \frac{\nu(\pi \delta)^{-\frac{1}{2}}}{\delta}$$

Equation 3.26

where concentration is expressed in dimensionless units.

Equation 3.25 is the general form for all values \( \delta \), whilst equation 3.26 is the form for very small values of \( \delta \).

3.3.3 Applications of the Diffusion Model

The diffusion model - whether derived from the work of Taylor or Levenspiel and Smith - is one of the most widely used models in FIA.

Ruzicka and Hansen [15] described dispersion in a single-line flow injection manifold using the diffusion model for low values of \( \delta \) (equation 3.23). Following Levenspiel [35], equations were given for peak variance, peak height and peak width in terms of the longitudinal dispersion number. It should be noted that the equation for \( C(\theta_{\max} = 1) \) (\( C_{\text{max}} \) of equation 6(b) of reference 15) is incorrect as published, the exponent having the wrong sign (c.f. equation 3.26). Subsequently [57], these equations were used to derive an expression for the dispersion coefficient in terms of different manifold parameters. In order to take into account a finite sample volume, equation 3.26 was modified by considering the dilution of a volume \( V_s \) of sample of concentration \( C_0 \) injected into a manifold of volume \( V_r \), i.e.

$$C_p = C_0 \cdot \frac{V_s}{V_r} \cdot 2(\pi \delta)^{\frac{1}{2}}$$

Equation 3.27

It should be noted that this modification is only valid
for the case when $V_s \ll V_r$. When $V_s$ is significant in comparison to $V_r$, the differential equation describing the model must be solved for the appropriate boundary conditions (see later). Equation 3.27 yields the result

$$D = C_0/C_p = 2V_r \langle \pi \delta \rangle^{1/2} / V_s$$

(3.28)

Since for low values of $\delta$, $t_\pi \approx L/\bar{u}$ and $V_r = \pi a^2 L$, equation 3.28 can be rearranged to give

$$D = 2\pi a^2 t_p \frac{\langle \delta \rangle}{a} / V_s$$

(3.29)

(equation 10 of reference 57). Ruzicka and Hansen found that the diffusion model fitted an experimentally obtained curve better than the tanks-in-series model. They also compared experimental values of $D$ with those calculated using equation 3.29 by calculating values of $\delta$ from experimental values of $\sigma^2$ (the peak variance). Results were obtained for a flow-rate of about 1.2 ml min$^{-1}$, sample volumes of 26 and 30 µl, and tube volumes from 74 to 314 µl. (The tube volume was calculated from the flow-rate and the time to the peak maximum). Errors of less than 10% in the value of $D$ calculated from equation 3.29 were obtained for conditions giving rise to $D>4$. The failure of equation 3.29 for values of $D \leq 4$ was attributed to the large sample volume and short tube lengths used to generate such values. Under the conditions used by Ruzicka and Hansen, values of $D \leq 4$ were obtained for sample volumes of more than 25% of the tube volume. These authors later applied equation 3.29 to their discussion of the miniaturisation of flow injection manifolds [70].

Pungor et al [111] used the diffusion model to modify the response equation derived for a manifold containing a well-stirred mixing tank when the length of tubing between the tank and the point of injection was greater than 20 cm. This was to take into account the distortion of the injected sample zone produced by diffusion-convection within the manifold.
Tijssen [37] and Van den Berg et al [38] have used the diffusion model to compare dispersion in straight and coiled tubular reactors. Van den Berg et al assumed conditions for which $\delta < 0.01$, and combined the expression for peak variance with that derived from the tanks-in-series model in order to obtain an equation for peak height in terms of the plate number, $N$. (Ruzicka and Hansen [15] also combined these models in the same way). Van den Berg et al also made use of the modified equation for $D_a$ derived by Aris [41] (equation 3.16). However, these authors found that for the conditions used in their calculations, equation 3.12 could be used. Tijssen also assumed conditions for which $\delta < 0.01$. This is implicit in the equation used for the peak variance (equation 6 of reference 37). Both Tijssen and Van den Berg et al compared straight and coiled reactors with packed-bed reactors, although these authors reached different conclusions as to which reactor gave the best performance in terms of mixing and peak broadening (see reference 38 for a discussion of the reasons for this).

The simplified form of the diffusion model obtained for $\delta < 0.01$ has been used by many other authors. This is probably because it is much easier to derive expressions for peak height, width and variance from this form than from the general form (equation 3.20). Examples include the work of Poppe [43], Reijn et al [51], Betteridge et al [64], Olsson et al [88] and Harris [105]. Examples of the use of the general form of the diffusion model (sometimes referred to as an exponentially-modified Gaussian function) can be found in the work of Meschi and Johnson [52, 53] and Leclerc et al [89]. It should be noted that the assumption that $\delta < 0.01$ (which yields a normal error function) is often not explicitly stated, but is implicit in the exact form of the equations used. This is important since, as demonstrated by Ruzicka and Hansen [57], significant errors can be introduced by applying this form of the diffusion model to conditions for which the above assumption is not valid.
3.3.4 Discussion and Conclusions

The diffusion model - whether it be referred to as diffusion-limited dispersion or axially-dispersed plug flow - may, with some caution, be used to describe dispersion in a single-line flow injection manifold. The model assumes that (a) $D_m$ is independent of solute concentration, (b) that longitudinal diffusion is negligible, (c) that dispersion occurs in an ideal manifold, and (d) that processes of convection and radial diffusion may be treated as being analogous to one-dimensional molecular diffusion (Fick's second law). This leads to equation 3.22, which is most commonly solved for a delta injection function. It is often further assumed that the value of $\delta$ ($=D_m/\bar{U}L$) is very small resulting in the normal error curve described by equation 3.23 for which [36]

\[ \theta_{\text{max}} = 1 = t \frac{Q}{V} \frac{1}{r} \]  
\[ \frac{C}{C_0} = \frac{1}{4\pi} (\pi \delta)^{-\frac{1}{2}} \]  
\[ \sigma^2 = 2\delta \]  

The conditions for which the general diffusion model is valid may be summarised as (see sections 3.1.2 and 3.3.1)

\[ \tau > 0.8 \]  
\[ P > 200 \]  

For the simplified solution to equation 3.22 there is the further condition

\[ \delta < 0.01 \]
Assuming values of \( a = 0.25 \text{ mm} \) and \( D_m = 1.00 \times 10^{-4} \text{ m}^2 \text{ s}^{-1} \), equations 3.31 and 3.32 yield the conditions \( t > 50.0 \text{ s} \) and \( u_o > 8.00 \times 10^{-4} \text{ m s}^{-1} \) \( (Q > 0.0094 \text{ ml min}^{-1}) \) respectively. Assuming that \( t_p = L/u \), then for \( L = 100 \text{ cm} \) a flow-rate of less than 0.24 ml min\(^{-1}\) must be used to satisfy equation 3.31. Similarly, for a flow-rate of 1.00 ml min\(^{-1}\) a tube length of greater than 4.25 m would be required. For the simplified dispersion model, equation 3.33 yields the condition \( t > 130.2 \text{ s} \). For the two cases described above \( (L = 100 \text{ cm}, Q = 1.00 \text{ ml min}^{-1}) \), values of \( Q < 0.091 \text{ ml min}^{-1} \) and \( L > 11.07 \text{ m} \) are required respectively. These calculations were performed using Taylor's equation for \( D_a \) (equation 3.12), since it can easily be shown that Aris's modification does not introduce any significant difference in the value of \( D_a \) for flow injection conditions.

A further limitation to the applicability of the diffusion model to FIA is the requirement that \( V_s \) should be less than \( V_r \) for equation 3.27 (and those derived from it) to be valid. Ruzicka and Hansen [57] found that this condition was met for \( V_s < 0.25 V_r \). For a 1.00 m length of 0.5 mm i.d. tubing this yields the condition \( V_s < 50 \mu \text{l} \). These calculations highlight the need to exercise caution in applying the diffusion model to dispersion in single-line manifolds.

The model was compared with experimental data using the diffusion coefficient as the model parameter (Table 3.1). In general, it was found that although similar peak heights and shapes could be obtained for low flow-rates and long tube lengths \( (Q < 1 \text{ ml min}^{-1}, L > 1 \text{ m}) \), the model consistently predicted that the peaks should appear earlier in time than was found experimentally (Fig. 3.11). The differences between the observed and predicted values of \( t_a \) (the appearance time) were too large to be explained by the experimental error associated with the measurement of \( t_a \) and tube length. Careful examination of the experimental conditions used showed that in some cases the discrepancy could be explained by boundary conditions for the model being exceeded. However, this was not always the case.
Fig. 3.11 Fit of the diffusion model (-----) to experimental peak shapes (——). For the experimental conditions for peaks A-D see table 3.1 refs. 1, 5, 12 and 13 respectively.
<table>
<thead>
<tr>
<th>Ref.</th>
<th>Q ml/min⁻¹</th>
<th>L/cm (b)</th>
<th>V_s/µl (c)</th>
<th>D_m /10⁻⁹ m² s⁻¹</th>
<th>Fit? (d)</th>
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<td>113.0</td>
<td>1.5</td>
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<td>113.0</td>
<td>3.0</td>
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</tr>
<tr>
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<tr>
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</tr>
<tr>
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</tr>
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</tr>
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<td>205.0</td>
<td>1.2</td>
<td>X</td>
</tr>
</tbody>
</table>

(a) 1-11, 0.2 µl cell, 12-17, 8.0 µl cell

NOTES: (b) d = 0.58 mm

(c) 1-11, slug injection and 12-17, time injection

(d) X = no fit, ? = partial fit, Y = good fit

Table 3.1 Results obtained for the fit of the diffusion model.

When values of $\tau_p$ were calculated from the experimental data assuming $D_m=1.00 \times 10^{-9}$ m² s⁻¹, it was found that good agreement between experimental and theoretical peak shapes was obtained only for peaks for which $\tau_p>0.8$. Values of $D_m$ used in the model calculations were $0.5 \times 10^{-9}$ to $5.0 \times 10^{-9}$ m² s⁻¹.

In summary, the diffusion model can be used to predict peak shape in FIA provided the conditions for which it is valid are met. The main difficulty with the model is the problem of obtaining reliable values of the
diffusion coefficient for the solute under investigation. One way around this problem is to calculate values of $D_m$ from the peak variance [57]. The model is valid for conditions under which dispersion is limited by the diffusion process. Clearly, the model will not apply for conditions under which secondary dispersion mechanisms make a significant contribution (coiling, confluencing, etc.). Neither will the model be valid when the sample volume accounts for a large part of the total volume of the manifold.

3.4 THE TANKS-IN-SERIES MODEL

3.4.1 Description of the Model

The tanks-in-series (TIS) model represents a different approach to that of the convective flow and diffusion models described in the previous sections. Whereas the convective flow and diffusion models can be directly related to real manifolds, the TIS model is derived by assuming that dispersion in a real manifold can be represented by a purely hypothetical situation. In order to fit such a model to experimental data, it is therefore necessary to derive an empirical relationship between the parameters of the model and the experimental conditions.

The TIS model assumes that dispersion in the manifold or reactor under consideration may be represented by flow through a number $N$ of well-stirred tanks of equal volume connected in series (Fig. 3.12). Levenspiel [35] gives the exit age distribution (or E-curve) for this model as

$$E = \frac{1}{t_i} \left( \frac{t}{t_i} \right)^{N-1} \frac{1}{(N-1)!} \exp\left(-\frac{t}{t_i}\right) \quad (3.34)$$

where $t_i$ is the mean residence time of a single tank, and is equal to $V/Q$. Equation 3.34 may also be expressed in units of reduced time, $\theta$, defined as
Fig 3.12 The TIS model. See text for details.

\[ \theta = t/\bar{t} = t/N\tau_i \]  

(3.35)

where \( \bar{t} \) is the mean residence time in the \( N \) tank system.

Substituting for \( t/\bar{t} \) in equation 3.34 gives

\[ E_\theta = (N\tau_i)E = N(N\theta)^{N-1} \exp(-N\theta) \]  

(3.36)

Levenspiel states that the E-curve is the equivalent of the C-curve, which is the response curve obtained for a delta injection function normalised so as to have unit area under the curve. Therefore, the C-curve can be written as either

\[ C' = \frac{1}{\bar{t}_i} \left( \frac{t}{\tau_i} \right)^{N-1} \frac{1}{\tau_i} \exp(-t/\bar{t}_i) \]  

(3.37)

or

\[ C_\theta = \frac{N(N\theta)^{N-1} \exp(-N\theta)}{(N-1)!} \]  

(3.38)

where \( C_\theta = N\tau_i C' \). Some typical results obtained using equations 3.37 and 3.38 are shown in Fig. 3.13.

It should be noted that the maximum value of \( C_\theta \) increases with \( N \) for \( N>1 \), whilst for \( N=1 \) both equations
reduce to a simple exponential decay curve. Because of the way in which the E- and C-curves are defined, C' above represents the instantaneous "concentration per unit area under the curve" at the outlet of the N tank system. This can be verified by considering the response equation for N tanks in series derived for the case of the first tank filled with solute of concentration $C_m$ at time $t=0$ (equivalent to delta injection) [108].

Fig. 3.13 Typical curves predicted by the TIS model. Calculated for $Q = 16.7 \, \mu l \, s^{-1}$ and $V = 1 \, \mu l$. N = 1 (A, B), 5 (B, E) and 10 (C, F). Curves A-C were calculated using equation 3.37 and curves D-F using equation 3.38.
\[ C = C_m \left( \frac{t}{t_i} \right)^{N-1} \frac{1}{(N-1)!} \exp\left(-\frac{t}{t_i}\right) \]  

(3.39)

On integration over the range \( t=0 \) to \( t=\infty \), this yields the result

\[ \int_0^\infty C \, dt = C \cdot \frac{t}{m} \]  

(3.40)

so that dividing equation 3.39 by the area under the concentration-time curve gives equation 3.37. The relationship between equations 3.38 and 3.39 is given by

\[ C_\theta = NC/C_m \]  

(3.41)

The differences between \( C' \), \( C_\theta \) and \( C \) are important and should be borne in mind when considering the application of the TIS model to FIA. From equations 3.37-3.39, the following relationships may be derived

\[ \bar{t} = \bar{t}' = N\bar{t_i} = NV/Q \]  

(3.42)

\[ \bar{\theta} = 1 \]  

(3.43)

\[ \sigma^2 = \langle \sigma^2 \rangle' = N\bar{t_i} = \bar{t}^2/N = \sigma^2_\theta \cdot \bar{t}^2 \]  

(3.44)

where \( \bar{t}' \) and \( \langle \sigma^2 \rangle' \) are the mean and variance of equation 3.37, \( \bar{\theta} \) and \( \sigma^2_\theta \) refer to equation 3.38, and \( \bar{t} \) and \( \sigma^2 \) refer to equation 3.39. The time to the peak maximum predicted by equations 3.37-3.39 may be found by differentiation and solving for \( t_p \) when \( \frac{dC}{dt}=0 \). This results in the relationship

\[ t_p = t'_p = \theta_p \cdot \bar{t} = \bar{t_i} \cdot (N-1) \]  

(3.45)

which, on substitution into equations 3.37-3.39 gives
The simplest case of the TIS model is that of a single tank (N=1), for which

\[ C_p = C_m \cdot \ln \cdot \frac{C_p}{N} = C_m \cdot (N-1)^{N-1} \cdot \exp(1-N) \]  

The differences between this case and the well-stirred tank model will be discussed in section 4.1.3.

3.4.2 Applications of the TIS Model

The TIS model was used by Ruzicka and Hansen in their study of the theory of dispersion in FIA [15]. For a sufficiently large number of mixing stages (N>10), the TIS model gives rise to peak profiles that closely approximate Gaussian error curves. Ruzicka and Hansen used this fact to derive an expression for \( \delta \) from the diffusion model for Gaussian peak shapes (\( \delta<0.01 \)) by comparing the expressions for peak variance derived from the models (equations 3.30 and 3.44). This gave

\[ \delta = \frac{t^2}{2N} \]  

It was concluded from this that symmetrical peak profiles would be obtained for manifolds corresponding to a large number of identical mixing stages (rule 2 of reference 15). However, since symmetrical peak profiles were
assumed in order to derive equation 3.49, this represents a circular argument.

It was also stated that, for a constant value of $\bar{t}$, the dispersion would decrease with increasing $N$. At first sight, this seems contradictory to what might be expected. However, it must be remembered that $\bar{t}$ is a function of $N$ (equation 3.42). For a constant flow-rate, if the number of tanks is doubled then the volume of each tank must be halved in order to keep $\bar{t}$ constant, which reduces the peak variance by a factor of 2. If $N$ is taken as being a measure of the mixing efficiency, then it can be concluded that for two different flow injection manifolds having the same mean residence time, narrower peaks can be expected for the manifold characterised by the highest value of $N$, which will also show the most symmetrical peak profiles.

Van den Berg et al [38] reached a similar conclusion in their study of coiled and packed-bed reactors for FIA. They also applied both the diffusion and TIS models to dispersion in FIA and gave the expression for a finite injection volume

$$\frac{C_p}{C_o} = \frac{V_s}{(2\pi)^{\frac{1}{2}}} N^{\frac{1}{2}} \sqrt{\frac{Q}{t}}$$

This differs from a similar equation presented by Ruzicka and Hansen [22] by the $t$ term in the denominator (equation 3.26 of reference 22), although it is not clear where the discrepancy arises from.

Reijn et al [42] have used the TIS model to discuss the influence of sample loop volume on peak shape. The peak shape was characterised in terms of the peak maximum concentration, the time to the peak maximum and the mean and variance of the concentration distribution. Calculations were performed for delta, time and ramp injection functions. The authors concluded that peak broadening could be limited by minimising the sample volume.

Other uses of the TIS model include the model for on-line dialysis units of Van der Linden [66], the study by
Reijn et al [68] of kinetics in single bead string reactors and the work of Dyke and Fernando [112] on deconvolution techniques for FIA.

Painton and Mottola [110] briefly discussed the TIS model, but were critical of its application to FIA for situations represented by a small number of tanks, except for the special case of a single tank. However, the TIS model for \( N=1 \) has not been widely used in FIA (this will be discussed in chapter 4).

Ramsing, Ruzicka and Hansen [57] used this model to describe the dispersion of the sample zone associated with the injection process. Equation 3.48 was fitted to the falling curve of peak profiles obtained for a manifold comprising of 23 cm of 0.5 mm i.d. tubing and a sample volume of 30 \( \mu \)l. Values of the tank volume, \( V_m \), were calculated for peak profiles recorded at flow-rates of 0.37 to 2.05 ml min\(^{-1}\). The authors found the mean value of \( V_m \) to be 73 \( \mu \)l with a relative standard deviation of 2.0\% and further noted that this was equal to the reactor volume \( V_r = 55 \mu l \) plus half of the sample volume, i.e.

\[
V_m = V_r + \frac{1}{2}V_s
\]  

(3.51)

When \( V_m \) was measured as a function of \( V_s \) for the same manifold at flow-rates of 1.15 and 1.75 ml min\(^{-1}\), this same relationship was observed for sample volumes giving values of \( D<2 \). It was also noted that the sample volume to give \( D=2 \) was 54 \( \mu \)l, i.e.

\[
V_{1/2} = V_r
\]  

(3.52)

These findings require some comment.

Firstly, equation 3.48 is valid only if the sample volume is small in comparison to the tank volume. \( V_m \) is the parameter used to fit the model to the falling part of the peak profile). Comparison of the results obtained by the same authors for the diffusion model (section 3.3.3) suggests that the criterion \( V_s < V_r / 4 \) might be appropriate. Examination of the conditions used by these authors shows that sample volumes of between 14\% and 360\% of the reactor
volume were used. Thus although a single exponential function could be fitted to the falling part of the peak profiles, it is not clear whether any special significance can be attached to the values of $V_m$ obtained in terms of the contribution of sample injection to dispersion. Secondly, equations 3.51 and 3.52 were derived for a limited range of conditions and cannot be considered as generalised expressions. During the experimental work described in this thesis, neither equation was found to be valid for the conditions used.

The remaining usage of the TIS model for $N=1$ of special interest is the work of Harris [105]. In this case, the model was combined with the diffusion model to give an exponentially modified Gaussian (EMG) function to describe dispersion in a simple flow injection manifold. EMG functions have also been described for liquid chromatography [113, 114] and demonstrate the use of combined models to provide descriptions of dispersion in different manifolds and reactors.

3.4.3 Discussion and Conclusions

Because the TIS model is essentially a descriptive model - that is, it describes dispersion using a hypothetical system - a different approach must be used when fitting the model to experimental data to that used for theoretical models. With theoretical models, such as the diffusion and convective flow models, values of the relevant experimental parameters can be substituted directly into the model equations. (It should be noted, however, that the diffusion model can also be treated as a descriptive model with parameter $D$.) With the TIS model, the model parameters ($N$ and $V$) need to be varied so as to match the response predicted by the model with experimental data. Once this has been achieved, the model can be used to predict the effect of changing experimental parameters by varying the model parameters.

Methods for fitting flow models to experimental systems have been described in the chemical engineering
literature [35,97]. These are based on the stimulus-response technique. Briefly, the response of the system is investigated using a suitable tracer material and detection method for a specific input function (e.g. delta injection, step function, etc.) and the result expressed as a probability distribution function, $f(x)$. The statistical moments of this function can then be used to compare the experimental data with the moments of the model and so obtain values for the model parameters. For a normalised probability distribution Wen and Fan [97] give mean, variance and skewness of $f(x)$ for positive values of $x$ as

$$
\mu = \int_0^\infty xf(x)dx \quad (3.51)
$$

$$
\sigma^2 = \int_0^\infty (x-\mu)^2f(x)dx \quad (3.52)
$$

$$
\gamma^3 = \int_0^\infty (x-\mu)^3f(x)dx \quad (3.53)
$$

As has been seen already, expressions for $\mu$ and $\sigma^2$ (and hence $\gamma^3$) can be readily derived for different flow models, whilst values of $\mu$ and $\sigma^2$ may be obtained from experimental peak profiles.

This approach was adopted to examine the fit of the TIS model to experimental peak profiles. For the TIS model, the mean value of the concentration-time curve is given by equation 3.42, whilst the mean residence time for a single-line manifold is given by $Vr/Q$. Therefore, values of $\bar{t}$ were calculated from the experimental conditions and used to calculate $V$ for different values of $N$. For preliminary investigations, the "best fit" was determined visually by comparing the peak heights and shapes of the theoretical and experimental profiles.

Some typical results are shown in Fig. 3.14. It was found that the model consistently predicted lower values of $t_p$ than those measured experimentally (Table 3.2). Differences of up to 76% of the observed value were found
Fig. 3.14 Fit of the TIS model (-----) to experimental peak shapes (——). For the conditions for curves A-D see table 3.2 refs. 1, 4, 7 and 15 respectively.
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<th>(L/cm(^{(b)})</th>
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\(\text{(a) 1-11, 0.2 \mu\text{l cell, 12-17, 8.0 \mu\text{l cell}}\)

\(\text{NOTES: (b) } d = 0.58 \text{ mm}\)

\(\text{(c) 1-11, slug injection and 12-17, time injection}\)

\(\text{(d) } X = \text{ no fit, } ? = \text{ partial fit, } Y = \text{ good fit}\)

**Table 3.2 Results obtained for the fit of the TIS model.**

for the value of \(t_p\) predicted by the model. The best results in terms of peak shape and the value of \(t_p\) were obtained for values of \(T>10 \text{ s and } V_s/V_r<0.2\). Better fits for these conditions could not be obtained because of limits imposed by the computer program used to calculate the theoretical peak profiles, as will be discussed later (section 5.3.2).

Good fits in terms of peak shape were also obtained for values of \(t<10 \text{ s and } V_s/V_r>0.5\) under certain conditions.
\( (L<100 \text{ cm}, \; Q>1.00 \text{ ml min}^{-1}) \), although these showed the poorest agreement between the theoretical and experimental values of \( t'_p \).

These results confirm that the TIS model is only valid for a single-line manifold when the sample volume is small compared to the manifold volume. When \( V_s \) is large, the differential equations describing the model cannot be solved assuming a delta injection function with \( C_m = C_o \cdot V_s / V \), but must be solved for the appropriate boundary conditions. An alternative to this might be to model the flow injection manifold using a transport and a mixing stage (Fig. 3.15), thus "correcting" the predicted value of \( t'_p \). (This approach will be discussed further in chapter 5). The TIS model is more widely applicable to manifolds containing more efficient mixers than open tubes, such as packed-bed and single bead string reactors and dialysis units [38, 66, 68].

Fig. 3.15 Suggested modification of the TIS model to include sample transport.
Chapter Four

Further Models in FIA
4.1 MODELS BASED ON A SINGLE TANK

4.1.1 Introduction

In the previous chapter the convective flow, diffusion and tanks-in-series models were described and their applicability to single-line flow injection manifolds discussed. It was shown that no single model was applicable to the full range of conditions used in FIA. This was either because of limiting assumptions made in the derivation of the model, or because the model does not accurately predict the appearance time and shape of peak profiles for all experimental conditions. These findings suggest that a range of different models is required to describe dispersion, especially if more complex manifolds are to be considered.

In this chapter, some new models for dispersion based on the single well-stirred tank (WST) model of Tyson [58, 59] are described and their performance assessed in terms of the criteria outlined in chapter 1. Before discussing these models, however, it is important to consider the application of models based on mixing tanks that have been described in the flow injection literature.

4.1.2 Mixing in a Single Tank

The response equations for a single mixing tank have already been described (section 2.2). However, it is helpful at this point to consider the theoretical basis of these equations. It is assumed that the tank behaves as an ideal mixer, i.e. the contents of any element of fluid entering the tank are instantaneously and homogeneously distributed throughout the entire volume of the tank. Under these conditions, the rate of change of concentration in the tank is given by

$$\frac{dC}{dt} = \frac{dC_{\text{in}}}{dt} - \frac{dC_{\text{out}}}{dt}$$

(4.1)
where $C_{\text{in}}$ is the concentration of material entering the tank and $C_{\text{out}}$ is the concentration of the tank effluent. The concentration of effluent from the tank will be equal to the concentration in the tank, i.e.

$$
\frac{dC}{dt} = \frac{dC_{\text{in}}}{dt} - \left( \frac{Q}{V_m} \right) C
$$

(4.2)

where $Q$ is the volumetric flow-rate and $V_m$ the tank volume. For a delta injection function, $dC_{\text{in}}/dt=0$ and $C=C_m$ at $t=0$, which gives

$$
C = C_m \exp\left(-\frac{Qt}{V_m}\right)
$$

(4.3)

Equation 4.3 is identical to that for the TIS model for $N=1$ (equation 3.47). For a step function ($C=0$ to $C=C_m$ at $t=0$), equation 4.2 has the solution

$$
C = C_m \left[ 1 - \exp\left(-\frac{Qt}{V_m}\right) \right]
$$

(4.4)

The reverse step function ($C=C_m$ to $C=0$ at $t=0$) is equivalent to the delta function and so yields the same result as equation 4.3. If a plug of volume $V_s$ and uniform concentration $C_o$ is allowed to enter the tank starting at time $t=0$, then the concentration-time distribution at the tank outlet can be calculated by considering the combination of two step functions and is described by equations 4.6 to 4.8 (p. 172). The peak profiles obtained for these different input conditions are illustrated in Fig. 4.1.

4.1.3 Single Tank Models in FIA

The most obvious application of single tank models in FIA is to manifolds incorporating a real mixing chamber. Such systems have been described for performing flow injection "titrations" [14,15,115], on-line sample dilution [116] and calibration [60,61,62]. For example, Pungor et al [111] found good agreement between
Fig. 4.1 Peak profiles for a well-stirred tank for different input conditions. (A) delta injection, (B) step input, (C) finite plug (time injection).

experimental and theoretical data for their flow-through system. The kinetic model of Pardue and co-workers [54,55,84] also describes a flow injection manifold incorporating a real mixing tank.

Single tank models may also be applied to situations which can be approximated by a real mixing chamber. Some examples of this are FIAAS systems [58,59], certain types of detector [104] and various gradient-forming devices [118]. Both the WST model and the TIS model for $N=1$ have been applied in this way.

The TIS Model for $N=1$

As described earlier (section 3.4.2), this model was used by Ramsing et al [57] to discuss the contribution of the injection process to dispersion in FIA. In their work, the dispersion behaviour of a short, single-line manifold was considered to be analogous to the behaviour of a single well-stirred tank for a delta injection function. The falling portions of peak profiles recorded for different flow-rates were used to calculate values of the (hypothetical) tank volume from equation 4.3. The
results obtained by these authors have already been discussed. However, the validity of the model has not yet been examined.

It was mentioned earlier (section 3.4.1) that, provided the sample volume was smaller than the tank volume, equation 4.3 could be written as

\[
c = c_0 \exp\left(\frac{-Qt}{V_m}\right)
\]

which is the form of the TIS model used by Ramsing et al. However, it has already been shown that the conditions employed by these authors do not fully support such an assumption. It must also be stated that equation 4.5 does not fully describe the peak profiles observed by these authors (see Fig. 4 of reference 57). This is because the TIS model for a single tank as it is conventionally derived predicts only a single exponential decay curve, arising from the washout of the tank. In contrast, careful consideration of the flow manifold suggests a two-stage process; washout of the sample loop followed by mixing in the manifold tubing.

Although Ramsing et al found that the falling portion of the peak profiles they obtained could be fitted by a single exponential decay curve, the use of the TIS model for N=1 to describe dispersion in a short single-line manifold is not satisfactory. Therefore, the conclusion of these authors that such a manifold consisted of a single mixing stage is open to some criticism (see also section 3.4.2). From the above discussion, it seems that a two-tank model might be more appropriate for the manifolds described. Such a model will be described later.

The WST Model

The WST model of Tyson [58, 59] differs from the TIS model for N=1 in two important respects. These are (a) the fundamental equation describing the model (equation...
(4.1) is solved for a finite sample volume by assuming two step functions, rather than a delta injection function; (b) a single line manifold is represented by flow through a length of tubing in which it is assumed that no dispersion occurs prior to entry into a well-stirred mixing tank (Fig. 4.2). It is further assumed that the distribution is given by

\[ C = C_0 [1 - \exp(-Qt/V_m)], \quad 0 < t < t_p \]  
\[ C_p = C_0 [1 - \exp(-V_s/V_m)], \quad t = t_p \]
where $t=0$ is the time at which sample first enters the tank (given by $\pi a^2 L'/Q$) and $t_\text{p}$ is the time for the peak maximum to be reached (given by $V_s/Q$). The WST model thus predicts peak profiles having exponential rising and falling curves and is not limited by assumptions concerning the sample volume. The input conditions assumed by the model correspond to those for time, rather than slug injection.

The WST model was first applied to FIAAS, where the response characteristics of the AA spectrometer combine with the dispersion of the manifold to give peak profiles that are more nearly true exponentials than those encountered in FIA [58-60]. The model was used to derive equations for a flow injection standard additions method for low and medium dispersion systems, and to develop a continuous-dilution calibration method for flame AAS based on a real well-stirred mixing tank [60-62]. A later development was the application of the model to predicting peak width for specific concentration (or absorbance) values as a means of extending the working calibration range of a flame AA spectrometer [71]. Tyson showed that the peak width $t'$ at a concentration $C'$ on the peak profile was given by

$$
\frac{t'}{m} = \left( \frac{V_m}{Q} \right) \ln \left( \frac{C_o - 1}{C'} \right) - k'
$$

where $k' = (V_m/Q) \ln (D-1)$. This predicts a linear relationship between $t'$ and $\ln(C_o/C'-1)$, which was confirmed experimentally. In this respect it is interesting to note that not all the peak profiles recorded were pure exponential functions (Fig. 2 of reference 71). Following this paper, Bysouth and Tyson [117] described the use of this method for the determination of chromium, nickel and magnesium. It was found that equation 4.9 broke down for very high concentrations, for which the peaks were broader than expected. These findings suggest that the WST model has a
limited applicability to the prediction of peak shape in FIAAS. (Compare this with the results described in section 2.10). In a later paper [82], equation 4.9 was extended to cover the situation where a chemical reaction was involved and the equations derived applied to manifolds using solution spectrophotometric detectors. Tyson further extended equation 4.9 in developing a "doublet peak" method for flow injection titrations [118]. Doublet peaks are obtained when the sample volume and concentration are such that reagent depletion occurs in the centre of the sample zone. The validity of the equation derived for the time interval between the two peak maxima was investigated for a range of gradient-forming devices. It was found that real mixing chambers gave the best results, followed by gradient tubes (short tubes having a large internal diameter) and coiled tubes. Straight tubes gave the poorest results.

The EST Model

The extended single tank (EST) model was discussed briefly in section 2.10 and was developed by Appleton and Tyson [79] to describe dispersion in FIAAS. Although this model will not be discussed in any detail in this thesis, a brief description is included here for completeness.

The dispersion associated with the nebuliser/spray chamber of a flame AA spectrometer (including both physical mixing and the time response of the system) is assumed to be represented by a well-stirred tank incorporating a diluent stream. Only a fraction $f$ of the sample stream is dispersed throughout the tank, the remainder flowing to waste (Fig. 4.3). The rate of change of concentration in the tank for a step change in concentration ($C=0$ to $C=C_0$ at $t=0$) is

$$\frac{dC}{dt} = \left(\frac{fQ_s}{V_m}\right)C_0 - \left(\frac{Q_r}{V_m}\right)C$$  \hspace{1cm} (4.10)
Fig. 4.3 The EST model for flame AAS as described by Appleton and Tyson [79].

which has the solution

\[ C = C_0 f \cdot \frac{Q_S}{Q_T} \left[ 1 - \exp \left( -\frac{Q_T t}{V_m} \right) \right] \]  \hspace{1cm} (4.11)

If it is assumed that an ideal detector is situated at the tank outlet for which absorbance is directly proportional to the rate at which analyte enters the detector, then

\[ A = k C_0 f \cdot Q_S \left[ 1 - \exp \left( -\frac{Q_T t}{V_m} \right) \right] \]  \hspace{1cm} (4.12)

where \( k \) is a constant. It can further be shown that, for the introduction of a volume \( V_S \) of sample of concentration \( C_0 \), the absorbance at the peak maximum is given by

\[ A_p = k C_0 f \cdot Q_S \left[ 1 - \exp \left( -\frac{Q_T V_S}{Q_S V_m} \right) \right] \]  \hspace{1cm} (4.13)

from which the response dispersion coefficient can be derived as
\[ D_r = \left[ 1 - \exp\left( -Q_s V_m \right) \right]^{-1} \]  

(A full derivation of these equations may be found in chapter 9 of reference 108).

4.1.4 The Performance of the WST Model

The application of the WST model to the description of rise curves to the steady-state in FIAAS was discussed in Chapter 2. It was found that the model could be applied to the experimental data only for short manifolds (L<40 cm) and flow-rates close to the natural aspiration rate of the AA spectrometer. The application of the WST model to the prediction of peak shape in FIA will now be discussed.

Examination of the peak shape predicted by the WST model (see for example Fig. 2.7, p. 43) shows that the model is not applicable to conditions giving rise to skewed Gaussian or humped peaks. Therefore, the model was compared only with exponential, tailed and triangular peaks. Values of \( V_m \) were calculated from the rising and falling portions of the peak profiles using the method outlined in section 2.10. Values of \( V_m \) were also calculated from the peak maximum concentration via equation 4.7. The results obtained are summarised in table 4.1. Results for both time and slug methods of injection were examined.

Agreement between the values of \( V_m \) calculated from the rise, peak and fall of each profile was found to vary considerably with the different conditions used. For slug injection, the values of \( V_m \) calculated from the falling curves were generally higher than those from the rising curve, reflecting the greater extent of dispersion experienced by the rear of the sample zone for this method of injection.

In order to assess the performance of the model, the values of \( V_m \) derived from the peak maxima were used to
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NOTES:

(a) 1-26, 0.2 µl cell, 27-36, 8.0 µl cell

(b) d = 0.58 mm throughout

(c) 1-20, slug injection and 21-36, time injection

(d) X = no fit, ? = partial fit, Y = good fit

Based on \( Vₘ^{\text{PEAK}} \)

(e) calculated assuming \( C_p/C_o = 0.999 \)

Table 4.1 Results obtained for the fit of the WST model.
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(a) 1-26, 0.2 μl cell, 27-36, 8.0 μl cell

NOTES:
(b) d = 0.58 mm throughout
(c) 1-20, slug injection and 21-36, time injection
(d) X = no fit, ? = partial fit, Y = good fit
(e) Based on \((V₃)_{\text{PEAK}}\)

\(\text{Table 4.1 (cont.)}\)
calculate peak profiles which were than compared with the experimental data. It was found that the assumption that the appearance time, $t_a$, was given by $V_r/Q$ was not valid. Therefore, the tube length of the model manifold ($L'$) was calculated from the observed appearance time in order to allow a direct comparison between the predicted and actual peak shapes. The "goodness of fit" of the model to the experimental peak profiles was assessed qualitatively by comparing the shape of the rising and falling curves and the value of $t_p$. Some typical results are shown in Fig. 4.4.

For slug injection, a poor fit of the model to the experimental peak profiles was obtained whenever the sample volume was significantly greater than the manifold volume ($V_s / V_r > 1.5$). Good fits were obtained only for the shortest length of tubing examined and small sample volumes ($L=19.3$ cm, $V_s = 30.1$ and 53.6 $\mu$l). For this tube length, the agreement between the model and experimental peak profiles appeared to be independent of flow-rate. For longer tube lengths ($L=50.0$ and 70.4 cm), partial fits could only be obtained for sufficiently high flow-rates.

Better results were obtained for time injection peak profiles. For a tube length of 20 cm and the low volume flow cell (F3), good agreement between the observed and predicted rise curves and the value of $t_p$ were obtained for all sample volumes. However, the model consistently predicted a less rapid fall curve from the peak maximum than was found experimentally. One possible explanation for this is that, with the valve in the "inject" position, the additional backpressure introduced by the sample loop causes the flow-rate to drop. When the valve is returned to the "fill" position, this additional backpressure is removed, so that the tail of the sample zone passes through the manifold at a higher flow-rate than the front.

This problem was not encountered with results obtained using a 50.1 cm length of tubing and an 8 $\mu$l flow cell (F2). In contrast to the results obtained for slug injection, agreement between model and experimental peak profiles was obtained for large sample volumes ($V_s / V_r$...
Fig. 4.4 Comparison of the WST model (-----) with experimental peak shapes (———). For the experimental conditions for curves A-D see table 4.1 refs. 32, 23, 7 and 11 respectively.
The best results were obtained for sample volumes giving rise to peak heights close to the steady-state $(V_s/V_r > 1.0)$. It should be noted that high flow-rates were not investigated for time injection since the sample volume is dependent on flow-rate, making it difficult to obtain small values of $V_s$ at such flow-rates.

The different results obtained for time and slug injection reflect the fundamental difference between these two techniques. For slug injection, the bigger the sample loop then the greater the extent of dispersion experienced by the tail of the sample zone. Since the conditions assumed by the WST model are equivalent to time injection, it follows that the model will only fit when dispersion of the sample zone within the sample loop is limited (low sample volumes). For time injection, very small sample volumes are obtainable which approximate to the delta injection function. Once again, this departs from the conditions assumed by the model, resulting in little or no agreement with the experimental data. For long tube lengths and/or low flow-rates, there is an increased contribution from radial diffusion to the dispersion of the sample zone. This gives rise to non-exponential peaks regardless of the method of injection (see section 2.7), which obviously cannot be described by the WST model.

4.1.5 Summary and Conclusions

The WST model may, with some caution, be applied to the prediction of peak shape in FIA. The advantages of this model are that it is described by simple equations, is easily modified, and can be used to predict peak height and peak width given the value of $V_m$ which most closely represents a particular manifold. The model is applicable to single line manifolds for which (a) the right type of peak profile is obtained and (b) the injection conditions used are the same as, or approximate to, time injection for a finite sample volume. The disadvantages of the model are that these two requirements are met only for a limited range of experimental conditions, whilst the model
does not perfectly predict observed peak shapes, particularly at the initial rise and peak maximum of the profile.

For the results obtained using both time and slug injection and the low volume flow cell, the WST model was found to be valid for the conditions $0 < \tau < 0.11$, $1 \times 10^4 < P'_{e} < 1 \times 10^5$ and $0 < X_R < 0.03$ (assuming $D_m = 1 \times 10^{-5} \text{cm}^2 \text{s}^{-1}$). These correspond to tube lengths of less than 50 cm for 0.58 mm i.d. tubing and flow-rates of greater than 1 ml min$^{-1}$. The further condition that $V_s / V_r < 1$ for slug injection and $V_s / V_r > 0.3$ for time injection were also derived from this work. (It should be noted that these conditions are only approximate). The different conditions for which good agreement between the model and the experimental peak profiles was obtained are shown in Fig. 4.5.

![Fig. 4.5 Experimental conditions for which the WST model was found to be valid. (D) time injection, 0.2 µl cell. (O) slug injection, 0.2 µl cell. (X) time injection, 8.0 µl cell.](image-url)
The TIS model for a single tank (N=1) is not as applicable to the description of dispersion in FIA as the WST model because it only predicts the falling portion of the peak profile. A further disadvantage of the TIS model is that it assumes a delta injection function, which restricts the range of sample volumes that can be accommodated by the model. The WST model is essentially the TIS model solved for a single tank and time injection conditions.

The EST model demonstrates the ease with which the basic single tank model can be modified to take into account different effects. However, this model will not be discussed further for the reasons outlined in section 2.10.

4.2 THE TWO-TANKS-IN-PARALLEL MODEL

4.2.1 Description of the Model

The two-tanks-in-parallel (TTP) model was first proposed by Appleton and Tyson [79] to describe the action of the nebuliser/spray chamber of a flame AA spectrometer, and was discussed briefly in chapter 2. In this model, it is assumed that the flow is split between two well-stirred tanks and then recombined before entering the detector (Fig. 4.6).

In order to increase the versatility of the model, it is further assumed that the fractional flow-rates entering each tank are independent of the tank volumes. The resulting peak profile predicted by the model is described by the equations

\[
C = C_0 \left[ 1 - f_1 \exp \left( - \frac{f_1}{V_1} Q t \right) - f_2 \exp \left( - \frac{f_2}{V_2} Q t \right) \right],
\]

for \( 0 < t < t_p \) \quad (4.15)
Fig. 4.6 The TTP model. Distances A-A' and B-B' are assumed to be negligible.

\[ C_p = C_0 \left[ 1 - f_1 \left( \frac{\exp(-f_2 V_s)}{V_1} \right) - f_2 \exp\left( \frac{-f_2 V_s}{V_2} \right) \right], \]
\[ t = t_p = \frac{V_s}{Q} \]  \hspace{1cm} (4.15)

\[ C = C_0 \left[ f_1 \beta_1 \exp\left( \frac{-f_1 Q(t-t_p)}{V_1} \right) + f_2 \beta_2 \exp\left( \frac{-f_2 Q(t-t_p)}{V_2} \right) \right], \]
\[ t > t_p \]  \hspace{1cm} (4.17)

where

\[ \beta_n = \exp\left( f_n \frac{V_s}{V_n} \right) - 1 \]

It should be noted that if the fractional flow-rates are defined as functions of the tank volumes, i.e.

\[ f_1 = \frac{V_1}{(V_1+V_2)}, \quad f_2 = \frac{V_2}{(V_1+V_2)} \]  \hspace{1cm} (4.18)

then equations 4.15 to 4.17 reduce to the equations for a single tank with volume \( V_m = V_1 + V_2 \). A full derivation of
these equations is given in appendix C. Some typical results obtained using the TTP model are shown in Fig. 4.7. This model is similar to the multiparameter model described by Levenspiel [35] ("model k" of reference 35), although the latter has not been used in FIA.

![Graph](image)

**Fig. 4.7** Typical curves predicted by the TTP model, calculated for \( Q = 1.00 \text{ ml min}^{-1} \) and \( V_s = 50.0 \mu l \). Values of \( V_1 (\mu l) \), \( V_2 (\mu l) \) and \( f_1 \) were (A) 10.0, 90.0, 0.5, (B) 50.0, 50.0, 0.5, (C) 30.0, 70.0, 0.7 and (D) 30.0, 70.0, 0.9 respectively.

### 4.2.2 Performance of the TTP Model

The application of the TTP model to FIAAS was discussed in chapter 2. It was found that this model was more widely applicable to the prediction of growth curves to the steady-state than the WST model. However, both models were only applicable to short manifolds (\( L<50 \text{ cm} \)).

In predicting peak shape, the TTP model has the same
limitation as the WST model, namely that it predicts peak profiles having a sharp discontinuity at the peak maximum and no points of inflection on either side of the peak maximum. It was concluded from this that the TTP model was only likely to be valid for the same conditions as the WST model. Therefore, the same peak profiles were examined as for the WST model.

Unlike the WST model, the parameters of the TTP model \((V_1, V_2, f_1)\) cannot easily be derived from the experimental data. A similar approach to that outlined in section 2.10 was adopted. Values of \(V_1\) and \(V_2\) were calculated from the slopes of graphs of \(-\ln(1-C/C_0)\) and \(-\ln(C/C_0)\) against \(t\) for the rising and falling parts of the peak profiles respectively. A value of 0.5 was assumed for the flow fraction through each tank. These values were then used as the initial estimate for a SIMPLEX optimisation routine. (The program written to perform these calculations is described in appendix D). The routine used the root mean square of the residuals between the experimental and theoretical concentration values as the response function. The results obtained are summarised in Table 4.2. The "goodness of fit" of the model to the experimental peak profiles was assessed in the same way as for the WST model, although this was complicated by the fact that the SIMPLEX routine did not necessarily result in the best fit. Some typical results are shown in Fig. 4.8.

The results obtained for time injection using the low volume flow cell showed good agreement between the model and the experimental profiles. It is possible that the results obtained could be improved further by using a different initial estimate for the SIMPLEX routine, or by using a finer tolerance for determining when the "best fit" has been achieved (see appendix D for details). Poorer agreement was found for the results obtained using the 8 \(\mu l\) cell. For low sample volumes (<20.5 \(\mu l\)), the model could not be fitted to the experimental data as the experimental conditions gave rise to a different peak shape. For intermediate sample volumes (41.0 to 82.0 \(\mu l\),
Fig. 4.8 Comparison of the TTP model (---) with experimental peak shapes (----). For the experimental conditions for A-D see table 4.2 refs. 5, 11, 32 and 23 respectively.
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(a) 1-26, 0.2 µl cell, 27-36, 8.0 µl cell
NOTES: (b) d = 0.58 mm throughout
(c) 1-20, slug injection and 21-36, time injection
(d) X = no fit, ? = partial fit, Y = good fit
Asterisks indicate visual fittings

Table 4.2 Results obtained for the fit of the TTP model.
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(a) 1-26, 0.2 μl cell, 27-36, 8.0 μl cell

NOTES: (b) $d = 0.58$ mm throughout

(c) 1-20, slug injection and 21-36, time injection

(d) $X =$ no fit, $? =$ partial fit, $Y =$ good fit

Asterisks indicate visual fittings

Table 4.2 (cont.)
the fitting procedure gave values of \( V_1, V_2 \) and \( f_1 \) which accurately predicted the rise curve, but not necessarily the peak height. Where the model peak height was too low, a good fit was obtained by reducing the tank volumes by between 5 and 10 \( \mu l \) each. No agreement was found for large sample volumes (>82.0 \( \mu l \)), although this may be due to the failure of the fitting procedure rather than the model itself.

Similar results were obtained for the slug injection data. Good fits were obtained for the same conditions as the WST model, it being possible to improve the agreement between experimental and theoretical peak profiles by adjusting the values determined by the fitting procedure. It is possible that, where poor fits were obtained, this could be improved by using the methods suggested above.

4.2.3 Summary and Conclusions

The TTP model has been successfully applied to the description of the dispersion generated by the nebuliser/spray chamber of a flame AA spectrometer. The model may also be applied to the description of single-line flow injection manifolds under certain limiting conditions. From the results obtained and consideration of the peak shapes predicted by the model, it has been concluded that these are the same as for the WST model, i.e. tube lengths of less than 50 cm and flow-rates greater than 1 ml min\(^{-1}\) (see Fig. 4.9).

Although the TTP model offers greater flexibility than the WST model, this is achieved at the cost of greater mathematical complexity. Since values of the model parameters cannot be derived from the experimental data readily, this makes it difficult to relate the model to the experimental conditions. An attempt was made to overcome this problem by adopting a SIMPLEX optimisation procedure to fit the model to experimental peak profiles. The results obtained suggest that some modification of the methods of obtaining the initial estimates of \( V_1, V_2 \) and \( f_1 \) and of the criterion used for assessing "goodness of
Fig. 4.9 Experimental conditions for which the TTP model was found to be valid. (O) slug injection, 0.2 μl cell. (O) time injection, 0.2 μl cell. (X) time injection, 8.0 μl cell.

"fit" is required to improve the performance of this approach. (This will be discussed further in chapter 5).

4.3 THE TWO-TANKS-IN-SERIES MODEL

4.3.1 Description of the Model

The two-tanks-in-series (TTS) model is a development of the WST model and is shown in Fig 4.10. This model
Fig. 4.10 The TTS model. Distances A-A', B-B' and C-C' are assumed to be negligible.

differs from the TIS model for N=2 in two important respects. Firstly, it is derived for a finite sample volume and time injection. Secondly, the tanks are assumed to be of different, rather than equal, volume.

The rate of change of concentration in the second tank is given by

\[
\frac{dC_2}{dt} = \left( \frac{Q}{V_1} \right) C_1 - \left( \frac{Q}{V_2} \right) C_2
\]  \hspace{1cm} (4.19)

Substituting for \( C_1 \) from equations 4.6 to 4.8 and solving for \( C_2 \) gives

\[
C_2 = C_0 \left[ 1 - \nu_1 \cdot \exp \left( -\frac{Qt}{V_1} \right) + \nu_2 \cdot \exp \left( -\frac{Qt}{V_2} \right) \right],
\]  \hspace{1cm} (4.20)

\[
C_2 = C_0 \left[ 1 - \nu_1 \cdot \exp \left( -\frac{V_s}{V_1} \right) + \nu_2 \cdot \exp \left( -\frac{V_s}{V_2} \right) \right],
\]  \hspace{1cm} (4.21)

\[ t = t' = \frac{V_s}{Q} \]
\[ C_2 = C_0 \left[ \nu_1 \alpha_1 \exp\left(\frac{-Qt}{\nu_1}\right) - \nu_2 \alpha_2 \exp\left(\frac{-Qt}{\nu_2}\right) \right], \]

\( t > t' \) \hspace{1cm} (4.22)

where

\[ \nu_1 = \frac{V_1}{V_1 - V_2}, \quad \nu_2 = \frac{V_2}{V_1 - V_2} \]

\[ \alpha_1 = \exp\left(\frac{V_s}{V_1}\right) - 1 \]

\[ \alpha_2 = \exp\left(\frac{V_s}{V_2}\right) - 1 \]

(A full derivation of these equations is given in appendix C). Since the time to the peak maximum will always be greater than \( t' \), \( t_p \) may be obtained by differentiating equation 4.22 and equating to zero:

\[ t = \frac{1}{p} \left( \frac{V_1 V_2}{Q(V_1 - V_2)} \right) \ln\left( \frac{\alpha_1}{\alpha_2} \right) \]

(4.23)

Substituting for \( t_p \) in equation 4.22 gives

\[ C_p = C_0 \left[ \frac{\nu_1 \alpha_1 \exp(-\nu_2) - \nu_2 \alpha_2 \exp(-\nu_1)}{\alpha_2} \right] \]

(4.24)

If it is assumed that \( V_1 = V_2 \), then equation 4.19 may be simplified and leads to the form of the TIS model with \( N=2 \) for a step, rather than a delta, input function. It should be noted that equations 4.20 to 4.22 are not valid for \( V_1 = V_2 \). A general form of these equations valid for all positive values of \( V_1 \) and \( V_2 \) may be derived [119], but is considerably more complicated than either of the alternative forms described above. Some typical results obtained using the TTS model are shown in Fig. 4.11. The TTS model has not been described in the literature.
Fig. 4.11 Typical curves obtained for the TTS model for $Q = 1.00 \text{ ml min}^{-1}$ and $V_s = 50 \mu l$. Values of $V_1$ ($\mu l$) and $V_2$ ($\mu l$) were (A) 10.0, 90.0, (B) 40.0, 60.0, (C) 50.0, 100.0 and (D) 15.0, 35.0 respectively.

4.3.2 Performance of the TTS Model

Examination of the peak profiles calculated for the TTS model (Fig. 4.11) suggested that the model was applicable to a wider range of conditions than either the WST or TTP models, since a wider range of peak shapes can be obtained. Therefore, both skewed Gaussian, triangular and exponential peak profiles were used to evaluate the TTS model. The model was fitted to the experimental peak profiles using the same SIMPLEX procedure as for the TTP model. The initial estimates of $V_1$ and $V_2$ were chosen such that $(V_1 + V_2)$ was of the order of the tank volume predicted from the peak maximum for the WST model. It was found that varying the initial estimates of $V_1$ and $V_2$ did
not have a significant effect on the results obtained. The results are summarised in table 4.3. The "goodness of fit" was assessed in the same way as for the TTP model (section 4.2.2). Some typical results are shown in Fig. 4.12.

Excellent agreement was found between the model and the results for time injection using the 8 µl flow cell for sample volumes greater than 20 µl. Poorer agreement was found for the time injection results obtained using the low volume flow cell. This may be due to the effect of the sample loop on flow-rate discussed earlier (see section 4.1.4). The best results for slug injection were found for a sample volume of 113 µl, 110 cm of 0.58 mm i.d. tubing and flow-rates of 0.37 to 5.57 ml min^{-1}. Partial agreement between the model and the experimental data was obtained for tube lengths of 50 and 70 cm and flow-rates greater than 2 ml min^{-1}. Partial fits were also obtained for a tube length of 19.3 cm, flow-rates of 5.2 and 1.1 ml min^{-1} and sample volumes up to 77 µl.

The results in table 4.3 marked with an asterisk were obtained by varying the values of \( V_1 \) and \( V_2 \) returned by the fitting routine and judging the "goodness of fit" by a visual comparison. The results obtained in this manner appeared to provide a better fit than those obtained by the fitting routine, particularly in terms of the peak height. However, careful examination of the model and experimental peak profiles showed that the manual method gave rise to a higher value for the root mean square of the residual concentration values. Thus a "good fit" in terms of a visual comparison does not necessarily mean a minimum difference in the concentration values at different times between the model and the experimental data.

Examination of the data in table 4.3 shows that the TTS model is more widely applicable than either the WST or TTP models. The results obtained suggest that the model is valid for values of \( V_s/V_r \geq 0.15 \), although one result lies outside this condition. The results also suggest that the fit of the model is not so dependent on the
Fig. 4.12 Comparison of the TTS model (-----) with experimental peak shapes (---). For the experimental conditions for curves A-D see table 4.3 refs. 4, 12, 41 and 45 respectively.
<table>
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<th>Ref. (a)</th>
<th>Q/ml min$^{-1}$</th>
<th>L/cm (b)</th>
<th>$V_s/\mu l$ (c)</th>
<th>$V_1/\mu l$</th>
<th>$V_2/\mu l$</th>
<th>Fit? (d)</th>
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(a) 1-35, 0.2 \(\mu l\) cell, 36-45, 8.0 \(\mu l\) cell

NOTES: (b) \(d = 0.58\) mm throughout
(c) 1-29, slug injection and 30-45, time injection
(d) \(X = \text{no fit}, \ ? = \text{partial fit}, \ Y = \text{good fit}\)
Asterisks indicate visual fittings

Table 4.3 Results obtained for the fit of the TTS model.
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(a) 1-35, 0.2 µl cell, 36-45, 8.0 µl cell  
NOTES:  
(b) \(d = 0.58\) mm throughout  
(c) 1-29, slug injection and 30-45, time injection  
(d) \(X = \) no fit, \(? = \) partial fit, \(Y = \) good fit

Asterisks indicate visual fittings

Table 4.3 (cont.)
method of injection as the WST model, since the model could be fitted to peak profiles for short tube lengths and both slug and time injection.

4.3.3 Summary and Conclusions

The conditions for which the TTS model was found to be valid are shown in Fig. 4.13. For slug injection,
these conditions were $5 \times 10^3 < P_e < 1 \times 10^5$, $0.003 < X_R < 0.3$ and $0.01 < \tau < 0.7$. In general, these correspond to the conditions for the "intermediate" peak shapes as defined in chapter 3. The results for time injection were obtained for $0.05 < \tau < 0.3$, $X_R = 0.016$ and $0.038$ and $P_e = 10.7 \times 10^3$ and $22.5 \times 10^3$.

Although the TTS model is described by relatively complex equations, it is easier to apply to the description of peak shape than the TTP model. The model also has the advantage of being applicable to conditions not readily described by either the diffusion or WST models.
Chapter Five

Discussion and Conclusions
5.1 INTRODUCTION

The aims and objectives of the work described in this thesis were outlined in chapter 1. These may be summarised as being (a) the assessment of existing models for dispersion in FIA, and (b) the development of new flow models. In order to achieve this, an extensive investigation of the factors affecting physical dispersion was undertaken, the results of which were discussed in chapters 2, 3 and 4. From this work, several results were obtained having important implications for practical FIA which were discussed in the relevant sections. The results were also used to assess the applicability of both existing and new flow models in terms of their ability to describe such factors as peak height and peak shape.

The findings of this work raise some important questions. These are:

(a) What are the implications of this work for the design of flow manifolds?
(b) How can sample dispersion be described in such a way as to take into account all the relevant variables?
(c) Which flow models should be used (if any), and how may they be related to the physical parameters of real manifolds?
(d) How can these flow models be developed to describe more complex situations?
(e) How do reaction kinetics contribute to dispersion?

An attempt has been made to provide answers to some of these questions in the preceding chapters. These questions will now be discussed in more detail.

5.2 PRACTICAL IMPLICATIONS OF THE WORK

5.2.1 The Role of the Detector System

In chapter 2, the contribution of the flow cell used with spectrophotometric detectors was examined both theoretically and experimentally. It was found that both the volume and construction of the flow cell contributed
to the observed dispersion behaviour of a flow injection manifold, and the reasons for this were discussed (see section 2.2).

Since the work described in this thesis was primarily concerned with the dispersion occurring within the manifold rather than the detector, a low volume flow cell was constructed to minimise the contribution of the detector to the observed dispersion. A review of the literature showed that such an approach had not been adopted by all authors describing studies of sample dispersion in FIA; cells having 8, 18 and 80 µl internal volumes had all been used. This can make the comparison of results obtained by different authors and the application of such results to the design of manifolds for practical analyses difficult.

A further problem is presented by the kinetic response of the detector system as a whole. This is exemplified by the results obtained for flame AA spectrometers (see section 2.10), although other types of detector can have a significant effect on observed sample dispersion. The contribution of the detector system to dispersion can be broken down into contributions from the way in which sample enters (or passes through) the detector, the way in which the analytical measurement is performed, and the response time of the detector and recorder electronics [42,43,79,80]. Some of these represent "real" dispersion processes (additional mixing or disruption of the sample stream), whilst others represent "apparent" dispersion processes (distortion of the observed peak profile by the detector and recorder electronics). Therefore, careful consideration must be given to the detector used for dispersion studies in FIA and to the interpretation of the results obtained. The need for this became particularly apparent during preliminary studies (not reported in this thesis) in which various detectors and flow cells were used. These studies showed that large flow cells could give a misleading impression of the way in which sample dispersion varied with such factors as flow-rate and tube length.
The situation for practical analyses is, in many ways, a lot less demanding. Here, it is not essential that the contribution of the detector to dispersion should be minimised, provided that it is reproducible and does not adversely affect either the sensitivity or the sampling frequency of the method. In general, most applications employing spectrophotometric detection described in the literature make use of 8 or 18 μl flow cells and these appear to be adequate for such purposes.

5.2.2 The Role of the Injection Process

Two methods of injection – time and slug injection – have been described and investigated (see section 2.7). These two methods result in different peak shapes for the same sample volume and flow manifold. The reasons for this and the conditions for which differences between the methods are significant have already been discussed. Time injection gives rise to peaks showing a lesser degree of tailing than slug injection for the same peak height, especially for low dispersion manifolds. Thus it is possible to obtain higher sampling frequencies whilst maintaining sensitivity simply by changing from slug to time injection.

The theoretical concept of delta injection has also been described (see chapter 3). Delta injection may be approximated to by either time or slug injection, but only when the sample volume is small in comparison to the manifold volume ($V_s / V_m \to 0$). This becomes particularly important when considering the application of flow models to dispersion in FIA, since both the TIS and diffusion models assume delta injection functions. This means that the method of injection must be considered carefully when applying flow models to dispersion in FIA.

For practical analyses, slug injection is easier to implement than time injection, whilst the injection valve may be readily automated using pneumatic actuators, d.c. motors or stepper motors. However, an automatic valve of the type described in section 2.7.1 for time injection
offers the advantage of providing a range of sample volumes from one sample loop without the need for recalibration of the loop volume. The main disadvantage of time injection is the dependence of the sample volume on flow-rate. Thus the flow-rate must be constant to ensure reproducible sample volumes and low enough to ensure that the minimum sample volume required may be obtained with the valve being used. Reproducible timing of the valve switching sequence may be obtained using a computer or electronic timer.

5.2.3 The Role of the Solute

The effect of the solute on the observed sample dispersion has been investigated (see section 2.8). Since one of the principal dispersion mechanisms is that of diffusion, different results are to be expected for solutes having different diffusion coefficients. The diffusion coefficient of the solute has an effect on both peak height and the variation of dispersion with factors such as flow-rate and tube length. This means that any tracer material used to optimise a flow injection manifold should have a similar diffusion coefficient to the analyte being determined. A further implication of this is that caution needs to be exercised when comparing results obtained using different solutes for studies of physical dispersion, especially if these results are to be used in drawing up guidelines for the design of flow injection manifolds. Similar considerations apply to the nature of the solvent/carrier stream as well.

The use of tracer materials for the optimisation of flow injection manifolds is an attractive proposition since it means that the physical mixing of the sample can be investigated without the complication of a chemical reaction occurring at the same time. This is particularly important if a flow model is to be applied to the manifold under study. In the light of the above discussion, it can be seen that the variety of different sample types examined in FIA requires a range of different tracer
materials. Such materials should be (a) of a similar size and charge to the analyte, (b) readily soluble in the same solvent systems, (c) give a linear relationship between concentration and detector response, (d) be highly stable and free from chemical impurities and (e) be free from pH effects or chemical interferences. For solution spectrophotometric detection, a further requirement is that the tracer material should not give rise to any significant refractive index effects. Solutes used during the course of the work described in this thesis that meet these requirements are potassium nitrate, potassium permanganate, tartrazine, green S, ponceau S and vitamin B\textsubscript{12}. (Tartrazine and green S are the two components used in Langdale's Sap Green food colouring, which has been recommended as a liquid absorbance standard for absorption spectrometry [120].) For larger molecules of biological interest, the use of cytochrome C as a tracer is suggested.

5.2.4 Construction of the Manifold

Various factors relating to the construction of flow injection manifolds have been considered. These are connections (section 2.1), coiling (section 2.3) and confluencing (section 2.9). All of these factors have some effect on the measurement of dispersion in FIA. For example, the breaking and making of connections within a manifold introduces a degree of uncertainty into the value of D obtained for that manifold. This must be taken into account when studying the effect of, for example, tube length in both optimisation and dispersion studies. Coiling of the tubing can also introduce differences in dispersion behaviour for high flow-rates, long tube lengths and/or low coil aspect ratios (coil to tube diameter). Since coiled tubes are often used in practical FIA, either for convenience or to minimise peak broadening and promote mixing, flow models must be able to take this into account. Confluence points also need to be considered carefully since these do more than simply
achieve the dilution of the sample zone, and are widely used in practical analyses.

Another important factor is tube internal diameter, which does not seem to have been much investigated in the flow injection literature. It was found that this had a strong influence on the dispersion behaviour of a flow injection manifold (see section 2.6), in contrast to the findings of Ruzicka and Hansen [15]. Practical considerations have meant that narrow bore tubing (<0.3 mm i.d.) has not been recommended for FIA applications [22]. The use of short lengths of wide bore tubing has been proposed for flow injection titration and gradient systems [57, 115], as these may be used to generate exponential peak profiles. However, the results obtained in this work show that humped (or double) peaks are also obtained for wide bore tubing and so some care needs to be taken if such gradient devices are to be used. In general, 0.5 or 0.6 mm i.d. tubing is recommended for practical FIA.

5.3 THE USE OF FLOW MODELS

5.3.1 The Description of Peak Shape in FIA

The description of peak shape in FIA has been discussed in sections 1.2 and 3.1. The peak shape obtained for the injection of a volume of sample material into a flow injection manifold is important for several reasons. These are:
(a) it is the source of analytical information;
(b) it reflects the extent of mixing (and hence reaction and dilution) of sample with reagent achieved by the manifold;
(c) it determines both the sensitivity and sampling frequency of the analytical method;
(d) it provides a source of information for fitting a suitable model to the flow manifold.
Therefore, it is important to derive suitable parameters for the description of peak shape in FIA.

In section 3.1, the different peak shapes that were
observed were divided into five categories: skewed Gaussian, triangular, humped, exponential and tailed. When all the experimentally recorded peak profiles were characterised by the dimensionless parameters $X_R$, $\tau_p$ and $P_e$, considerable overlap was found between the triangular and humped and exponential and tailed categories (see Figs. 3.1 and 3.2). This led to the simplified definitions of convection, intermediate and diffusion peak shapes, the names being derived from the dominant dispersion process occurring under the relevant conditions. Some overlap was also found between these three broad categories, particularly between the convection and intermediate peak shapes (see Fig. 3.2). A further problem encountered was that none of the parameters used took into account either the method of injection or the sample volume. Thus the parameters $X_R$, $\tau_p$ and $P_e$ may be used to provide some indication of peak shape, but are not entirely satisfactory in this respect.

The problem of accounting for the contribution of sample volume to peak shape also applies to the discussion of dispersion generally. For example, neither the dispersion coefficient ($D$), the time to peak maximum ($t_p$) nor the baseline peak width ($\Delta t_b$) take sample volume into account. Yet all three parameters are strongly influenced by the sample volume. This has led Ruzicka and Hansen to define the "dispersion factor", $V_{V_s}/V_r$ where $V_s$ is the sample volume required to achieve a dispersion of $D=2$ [70]. This is similar to the sample-manifold volume ratio, $V_s/V_r$, but requires the experimental determination of $V_{V_s}$ for any manifold to which it is applied.

The advantage of the ratio $V_{V_s}/V_r$ is that it can be used to determine whether or not a significant difference in peak shape will be obtained for different methods of injection. For example, the conditions for which similar peak shapes were obtained for time and slug injection (see section 2.7.3) may be expressed as $V_{V_s}/V_r \leq 0.25$. It is interesting to note that this is the same condition for which Ruzicka and Hansen found that slug injection could be approximated to by a delta injection function with a
sample concentration equal to $C_0 V_s/V_r$ (see section 4.1.3).

Several different equations were tested to see if the sample volume could be incorporated into the dimensionless parameters described earlier. These were

\begin{align*}
\tau_p^* &= t_p D_m V_r/a^2 V_s \\
\tau_p^* &= t_p Q/V_s \\
\tau_p^* &= t_p Q (V_s + V_r)
\end{align*}

Values of $\tau_p^*$ and $X_R^*$ were calculated from the data obtained for the effect of sample volume on dispersion (section 2.7). None of the equations for $\tau_p^*$ gave rise to constant values of $\tau_p^*$ for the same manifold for different sample volumes. This meant that there was no particular advantage in using $\tau_p^*$ rather than $\tau_p$. However, when the results were plotted as $\tau_p^*$ against $X_R^*$ (Fig. 5.1), the effect of increasing sample volume for the same conditions could be seen clearly. It is therefore suggested that equation 5.4 may provide a suitable means of characterising peak shape taking sample volume into account, although the full implications of equation 5.4 have not yet been examined.

5.3.2 The Comparison of Different Flow Models

The models discussed in this thesis fall into two categories, namely theoretical and descriptive models. Theoretical models have been defined as those derived from consideration of the dispersion processes known to occur in a manifold, making simplifying assumptions. Descriptive models have been defined as those which represent the dispersion processes by a hypothetical manifold having a known relationship between time and
Fig. 5.1 Use of $X^*_R$ to correct experimental data for the effect of sample volume. All data points are for the 0.2 μl cell. (+) slug injection, $L = 69.3$ cm, $Q = 1.1$ ml min$^{-1}$. (X) slug injection, $L = 169.3$ cm, $Q = 5.2$ ml min$^{-1}$. (□) time injection, $L = 20.0$ cm, $Q = 1.1$ ml min$^{-1}$. (O) time injection, $L = 170.0$ cm, $Q = 1.1$ ml min$^{-1}$.
concentration for specific input functions. The convection and diffusion models fall into the first category. The tanks-in-series, well-stirred tank, two-tanks-in-parallel and two-tanks-in-series models all fall into the second category. These models were discussed in chapters 3 and 4.

The conditions for which these models have been found to be valid are summarised below in table 5.1. The conditions for the convection and diffusion models cited are those for which the underlying assumptions of the models are valid (see chapter 3). Insufficient results were available for these two models to establish the conditions for which they could be applied to time injection peak profiles. The data for the TIS model was also insufficient for boundary conditions to be established. This was due in part to the problems encountered in fitting the model to the experimental results (see later).

It should be noted that the values of $\tau_p$, $X_R$ and $P_e$ were calculated assuming a value of $1.0 \times 10^{-9} \text{ m}^2 \text{s}^{-1}$ for the diffusion coefficient of tartrazine in water. This means that some caution must be exercised if these results are to be applied to other solutes. However, the results obtained do provide a basis for the comparison of the different models examined with each other.

Of the six flow models considered, the convection model is probably the least useful for FIA. Although mathematically simple, this model is only valid under restrictive conditions which are not normally encountered in FIA.

The diffusion model has been used widely in FIA, usually in the form for which the equations reduce to a normal (or Gaussian) error curve ($\delta<0.01$). The boundary conditions for which this model is valid were not met for the majority of the peak profiles examined. This may explain why it proved difficult to match the model and experimental peak profiles and why a range of values of $D_m$ were needed to obtain similar peak shapes. Mathematically, the model is relatively simple provided
<table>
<thead>
<tr>
<th>MODEL</th>
<th>( t_p )</th>
<th>( X_R )</th>
<th>( F_a )</th>
<th>( V_e / V_r )</th>
<th>( t_p )</th>
<th>( X_R )</th>
<th>( F_a )</th>
<th>( V_e / V_r )</th>
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</thead>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&gt;1000</td>
<td>-</td>
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<tr>
<td>DIFFUSION</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&gt;0.01</td>
<td>&gt;0.01</td>
<td>&gt;200</td>
<td>&lt;0.25</td>
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<tr>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>&lt;1 (c)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&lt;1 (c)</td>
</tr>
<tr>
<td>WST</td>
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<td>&gt;0.01</td>
<td>&gt;1x10^4</td>
<td>&gt;0.3</td>
<td>&gt;0.01</td>
<td>&gt;0.01</td>
<td>&gt;1x10^4</td>
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<td>&lt;0.11</td>
<td>&lt;0.03</td>
<td>&lt;1x10^5</td>
<td></td>
<td>&lt;0.01</td>
<td>&lt;0.03</td>
<td>&lt;1x10^5</td>
<td></td>
</tr>
<tr>
<td>TTP</td>
<td>&gt;0.05</td>
<td>(0.015)</td>
<td>(1x10^4)</td>
<td>&gt;0.3</td>
<td>&gt;0.02</td>
<td>&gt;0.03</td>
<td>&gt;1x10^4</td>
<td>&gt;0.5</td>
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<td>&lt;0.3</td>
<td>(0.364)</td>
<td>(2.2x10^4)</td>
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<td>&lt;0.02</td>
<td>&lt;1x10^5</td>
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<tr>
<td>TTS</td>
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<td>(0.015)</td>
<td>(1x10^4)</td>
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<td>&gt;5x10^3</td>
<td>&gt;0.05</td>
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<td>&lt;0.3</td>
<td>(0.038)</td>
<td>(2.2x10^4)</td>
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<td>&lt;0.7</td>
<td>&lt;0.3</td>
<td>&lt;1x10</td>
<td></td>
</tr>
</tbody>
</table>

(a) Calculated from \( t_p \) assuming \( t_p = 2L/u_0 \)
(b) Based on Remsing, Ruzicka & Hansen [57]
(c) From consideration of the model.

Figures in brackets indicate that boundary conditions could not be properly established.

Table 5.1 Boundary Conditions for Different Models.
that a delta injection function is assumed. However this imposes a further condition on the application of the model to real flow manifolds. The main problem with this model is that of obtaining values of the diffusion coefficient for the solute of interest. Ramsing, Ruzicka and Hansen [57] used the peak variance to calculate values of $D_m$ from the experimental peak profiles. It would be of interest to calculate values for solutes of known diffusion coefficient in this way to see how reliable the method is. (Other flow injection methods for calculating diffusion coefficients have been described in the literature [64, 98]). However it is not strictly necessary to know the correct value of $D_m$, since the model can be used as a descriptive model with the apparent dispersion coefficient, $D_a$, as parameter.

The tanks-in-series model has also been used widely in FIA, particularly for manifolds containing efficient mixing devices such as packed-bed reactors. As with the diffusion model, the TIS model is relatively simple and can be characterised by it's statistical moments. The TIS model also assumes a delta injection function, but the equations describing the model can be solved using other input functions (see appendix C). If delta injection is assumed, then the concentration of sample in the first tank at time $t=0$ is related to the actual sample volume and concentration by

$$C_m = C_o \cdot \frac{V_s}{V_r} \quad \text{(5.5)}$$

Equation 5.5 is only valid when $V_s$ is small compared to $V_r$. By comparison with the diffusion model, it is suggested that the condition for this is $V_s/V_r < 0.25$. Whether or not this proves to be true, it is clear from equation 5.5 that the TIS model with delta injection cannot be applied to manifolds for which $V_s/V_r > 1$. (This condition applies to any model that assumes delta injection). As with the diffusion model, some difficulty was experienced in fitting the TIS model to experimental peak profiles. The fitting procedure adopted was to use
the mean residence time to determine the mean of the concentration-time distribution predicted by the model, i.e. \( \bar{t} = \frac{V_r}{Q} \) (see section 3.4.3). Since for the model \( \bar{t} = \frac{N}{V/Q} \), it was therefore possible to calculate a series of curves having the same mean residence time and compare these with experimental peak profiles. However, even when peak height and shape could be matched by a subjective comparison, the model predicted peaks occurring too early in time. This may be because of the finite sample volume used in the experimental work (i.e. \( V_s/V_r \) too large), or because \( \bar{t} \) is not the best parameter for relating the model to the experimental peak profile. A further problem experienced with this model related to the mainframe computer used to calculate the model profiles. Because of the way in which numbers were stored internally by the computer, there was a limit on the values that could be used in the calculations. For the TIS model, this meant that the number of tanks had to be less than 12. A further restriction imposed by the model was that \( N \) had to have a positive integral value greater than zero. However, it is possible to derive a form of the TIS model for non-integral values of \( N \) [121], so this restriction could be relaxed.

The well-stirred tank model has been applied to FIAAS, flow injection titrations and other peak width methods. This model is applicable to low dispersion manifolds, whereas the diffusion model is applicable to high dispersion manifolds. One big advantage of the WST model is its mathematical simplicity. The model can be used to predict both peak height and peak width. The model does not perfectly describe peak shape, since it is impossible in practice to obtain the abrupt rise and sharp discontinuity at the peak predicted by the model. However, it provides a good approximation to experimental peak shapes for the conditions listed in table 5.1, especially when time injection is used.

The two-tanks-in-parallel model is in principle more versatile than the WST model, having three parameters \((V_1, V_2 \text{ and } f_1)\) rather than one. However, this results in more
complex equations for the model and presents difficulties in fitting the model to experimental data. The fitting procedure used (see section 4.2.2) often resulted in "best fits" that were not "good fits" when assessed on a subjective basis. This may be because the objective criterion used (the root mean square of the residual concentrations between model and actual peak profiles) was not sufficiently sensitive to differences in peak shape, or because the SIMPLEX method was not the best optimisation procedure to use. Alternative approaches would be to derive expressions for the mean, variance and skewness of the model and use these to match model and experimental peak profiles.

The two-tanks-in-series model was found to be applicable over a wide range of conditions. Although more complicated than the WST model, it is mathematically simpler than the TTP model having only two parameters ($V_1$ and $V_2$). Similar problems were experienced in fitting the model to experimental data as for the TTP model, although generally the fitting procedures worked well. One particular advantage of the TTS model is the range of conditions covered. If $V_1 \ll V_2$, the model approximated to the WST model, whilst other values of $V_1$ and $V_2$ give rise to the convection and intermediate peak shapes not covered by the diffusion model.

5.3.3 The Development of Further Flow Models

The flow models discussed in this thesis have all been applied to the simplest case of a single-line flow injection manifold. The application of some of these models to other situations (coiled tubular, packed-bed and single bead string reactors and dialysis units, for example) has been described in the flow injection literature (see chapter 1). The modification of some existing models has also been suggested. However, it is clear that further models are required if more complex flow manifolds are to be described. In particular, there is a need to take into account the effect of stream
confluencing in FIA.

In developing such models, there will inevitably have to be some degree of compromise between flexibility and mathematical complexity. The greater the number of parameters a model has, the more widely applicable it will be, but the greater the difficulty in fitting the model to experimental data. Such a problem was encountered with the TTP and (to a lesser extent) TTS models.

In chemical engineering, the approach usually adopted is to describe the experimentally recorded exit age distribution (EAD) in terms of its statistical moments [97]. The EAD can then be approximated to by a suitable probability density function (such as the normal, Poisson, gamma or binomial distributions), and an appropriate flow model constructed on this basis. For example, for small values of $\delta < 0.01$ the diffusion model approximates to a normal error curve, whilst the TIS model is a form of the Poisson distribution. For more complex flow systems, combined models are used. The TTS, TTP and EST models are all examples of simple combined models. Wen and Fan [97] and Levenspiel [35] have described various combined and multiparameter models. Some examples are shown in Fig. 5.2. Similar models were described in section 3.1.3 to illustrate a modelling (descriptive) approach to the discussion of peak shape in FIA. Since such models may be related to the non-ideal behaviour of real manifolds, they may provide significant advantages over those discussed in this thesis.

The problem of confluencing was discussed in section 2.9. From the results obtained, it was apparent that stream confluencing could not be achieved without some degree of mixing, but that this mixing was far from perfect. Therefore, a confluence point cannot be modelled by simple dilution of the sample stream in direct proportion to the flow-rates of the streams being confluenced. The most likely form of model would be a combined model, in which the effect of confluencing is represented by a dilution stage and a mixing stage (Fig. 5.3). Experimental results for a range of different
Fig. 5.2 Examples of different combined models. (A) Perfect mixing with a dead volume. (B) Perfect mixing with bypassing. (C) Perfect mixing with bypassing and a dead volume.

Confluence points and conditions would be needed in order to develop this.
5.4 THE DESIGN OF FLOW MANIFOLDS

5.4.1 Guidelines for Dispersion

Ruzicka and Hansen [15, 22] have proposed several guidelines for the design of single line manifolds in FIA. These were summarised in chapter 1 (see page 12). Of particular interest to the work described in this thesis are those guidelines relating to flow-rate, tube length and sample volume. These were discussed briefly in the relevant sections of chapter 2.

Ruzicka and Hansen have reported that the dispersion coefficient increases with flow-rate. This is not in full agreement with the results described in section 2.4 and possible reasons for this have already been discussed. However, the results obtained suggest that Ruzicka and Hansen's "rule" may be applied under certain conditions, namely tube lengths greater than 1 m, flow-rates up to about 4 ml min⁻¹ and an 8 µl flow cell.

The variation of the dispersion coefficient with tube length has also been discussed (section 2.5). Ruzicka and Hansen have suggested the relationship $D=KL^k$. However, this was found to be unsatisfactory because it did not accurately fit the experimental data available. Neither did it predict the condition that $D=1$ when $L=0$. Appleton and Tyson [79] have suggested the alternative relationship, $(D-1) = KL^k$ (equation 2.12). This was found
to be more applicable to FIAAS than conventional FIA. The results presented in sections 2.5 and 2.10 suggest that equation 2.12 could be applied to single-line manifolds for flow-rates above about 5 ml min\(^{-1}\), especially when a flame AA spectrometer is used as the detector.

The variation of the dispersion coefficient with sample volume was discussed for both time and slug injection in section 2.7. Ruzicka and Hansen [15,57] have given the relationship \(D^{-1}=1-\exp(-KV_s)\) where \(K=0.693/V_s\) (equation 2.13). This equation was found to be valid for time injection (but see section 2.7.3), and for slug injection provided that \(V_s/V_r<1\). It should be noted however that equation 2.13 was not found to be valid for results obtained using a tube length of 20 cm for either time or slug injection. Equation 2.13 breaks down for sample volumes giving rise to peak heights near the steady-state value for the reasons discussed in section 2.7.2. This may well explain the failure of the equation for the results obtained using short tube lengths. The results obtained show that equation 2.13 may be used to relate \(D\) to the sample volume for single-line manifolds provided that \(V_s/V_r<1\) for slug injection and that the value of \(D\) is sufficiently greater than unity.

From the above discussion it is clear that the "guidelines for dispersion" may be applied to the design of single-line manifolds, provided that the conditions for which these guidelines are valid are met. Other factors affecting dispersion in single-line manifolds have also been discussed (see section 5.2).

5.4.2 The Dispersion of a Single-line Manifold

Ramsing, Ruzicka and Hansen [57] have derived an equation for the dispersion coefficient of a single-line manifold. The derivation of this equation was discussed briefly in section 3.3.3 and will now be considered further. The equation is derived from the diffusion model by making several assumptions. These are (a) \(\delta<0.01\), (b) \(V_s/V_r\) and (c) \(t_p=V_r/Q\). Because the equation is

-218-
derived from the diffusion model, the further conditions that \( \tau_p > 0.8 \) and \( P_e > 200 \) also apply. Given these conditions, the dispersion coefficient is given by equation 3.29 (see p. 150),

\[
D = 2\pi a t_p D_a \delta / V_s
\]

Since \( \delta = D_a / \bar{u} L \), this can be written as

\[
D = 2\pi a t_p \delta \bar{u} L / V_s \quad (5.6)
\]

This predicts that the dispersion coefficient increases with the square root of the residence time and tube length and is inversely proportional to the sample volume. It also predicts that \( D \) increases with the square of the tube radius. However, these predictions are not in agreement with the way in which \( D \) was found to vary with these parameters as reported in this thesis. This disagreement may be explained to some extent by a consideration of the conditions for which equation 5.6 was derived. These conditions are only met for long manifolds having high residence times and small sample volumes. Such manifolds will be characterised by large values of \( D \), for which the approximation \( (D-1) \approx D \) may be applied. Furthermore, for small sample volumes, \( [1 - \exp(-K V_s)] \approx K V_s \) so that \( D a V_s \). These approximations can be used to explain the disagreement between the predictions outlined above and the findings presented elsewhere in this thesis (see the preceding section and section 5.2). However, the conditions for which equation 5.6 and these approximations are valid are extremely limiting. It is therefore concluded that equation 5.6 cannot be generally applied to the design of single-line flow injection manifolds.

Equations such as the one discussed above are attractive because they bring together the various physical parameters of a flow injection manifold (flow-rate, tube length, etc.) into a single expression for some function of dispersion. However, it is difficult to
derive such equations because of the complexity of the relationships between these parameters and dispersion. A further problem is that there is no single function \((D, \Delta t_b, \text{etc.})\) that fully describes sample dispersion. This means that any "dispersion equation" must be derived for several different functions for dispersion if such an approach is to be used in the design of flow manifolds. Thus, in the example given above, dispersion may be defined in terms of the peak variance or the mean residence time (see section 3.3). The derivation of such equations is discussed further in the following section.

5.4.3 The Derivation of Dispersion Equations

In deriving equations for dispersion, three different approaches may be adopted. These are (a) the use of calculations based on exact theoretical equations; (b) the use of flow models; and (c) an entirely empirical approach.

The first approach has been described by Vanderslice et al. [44, 47, 81]. Numerical techniques were used to solve the diffusion-convection equation for a single-line manifold and the results used to derive expressions for the appearance time \(t_a\) and baseline peak width \(\Delta t_b\) in terms of the flow-rate, tube length, tube radius and diffusion coefficient of the sample. The resulting equations could be applied to the prediction of \(t_a\) and \(\Delta t_b\) for single-line manifolds under certain restrictive conditions. A similar approach was adopted by Betteridge et al. [74], who used a simulation method to calculate peak profiles for single-line manifolds both with and without a chemical reaction. The advantage of such procedures is that they can be applied easily to a range of manifold parameters and can accommodate the presence of a chemical reaction. The main disadvantages are that they can only be applied to manifolds that closely resemble the ideal (pure laminar flow, etc.) and cannot be adapted to more complex situations. Since few manifolds for practical FIA approximate to the ideal manifold, the theoretical
A purely empirical approach to the derivation of equations for dispersion has been described by Gomez-Nieto et al [122, 123]. This method uses multiple regression analysis on experimentally determined data to derive expressions for $t_a$, $\Delta t_b$, $t_p$ and $D$ for the manifold under study. In this respect, the method is similar to that employed by Vanderslice et al [44]. However, because the data is obtained by performing a series of experimental measurements for different flow-rates etc., the non-ideal behaviour of the manifold is taken into account. The main disadvantage of this method is the large number of experimental measurements required. Thus, if the effects of tube length, flow-rate and tube diameter are to be considered, some 45 separate measurements are required. Furthermore, since these measurements are made for a specific range of conditions and specific manifold components, the equations thus derived cannot be applied to similar manifolds made using different components or conditions outside those previously used.

The modelling approach to the derivation of equations for dispersion represents a compromise between the theoretical and empirical approaches. (The equation discussed in section 5.4.2 is an example of this type of approach). Once a suitable model has been identified, this can then be used to derive expressions for dispersion in terms of the model parameters. If these can then be related to the relevant manifold parameters, the model can be used to predict dispersion (as $D$, $t_a$, or $\Delta t_b$, for example) and peak shape. Clearly this will require some experimental measurements to be made. However, if different manifold components can be characterised by specific flow models (such as the type of detector, coiled reactor, packed-bed reactor, etc.) then these could be combined to aid the design of further manifolds. For example, if for a specified range of conditions a single-line manifold could be characterised by the TTS model and a flame AA spectrometer was characterised by a specific value of $V_m$ for the WST model, these could be combined to
predict the effect of using the manifold with that particular detector. Such an approach would have the advantage that, as more data was accumulated from the design of different flow manifolds, fewer experimental measurements would be required for the design of further manifolds. Some problems would need to be overcome before such an approach was practical, such as the development of methods for relating model and manifold parameters. A further problem would be developing a suitable method for determining the dispersion characteristics of specific manifold components. Such a method would probably have to be based on the use of a standard "reference" manifold, into which such components could be incorporated. The combination of different models would also need to be investigated, since this would inevitably lead to greater mathematical complexity. In this respect, it may well be that there is a practical limitation to the complexity of manifold that could be designed in this way. It must further be recognised that this approach will not provide exact predictions of dispersion and peak shape. However, if a method could be developed that was capable of predicting such factors to within 10%, this would be of great benefit in the design of manifolds for FIA.

5.5 SUGGESTIONS FOR FURTHER WORK

It is clear from sections 5.3 and 5.4 that further work will be required in order to develop the use of flow models for the description of dispersion in, and design of, manifolds for FIA. In particular, there is a need to develop methods for fitting flow models to experimental peak profiles. A method based on the SIMPLEX optimisation procedure has been described for models having two or more parameters. It is envisaged that other methods would be based on a comparison of the statistical moments (mean, variance and skewness) of both experimental peak profiles and theoretical concentration-time distributions. A related problem is that of finding ways of relating flow injection variables to the parameters of descriptive
models such as the TIS and WST models.

Although preliminary studies of the effect of confluence points and tube internal diameter have been made, these remain to be studied in detail. Other areas that could be investigated are the use of bypass loops on sample injection valves and the dispersion properties of packed-bed and single bead string reactors. These have all become widely used in FIA and so are of particular importance in the development of design strategies for flow injection manifolds.

The work described in this thesis has concentrated on the conditions most commonly used in FIA. Thus investigations have centred on simple manifolds employing spectrophotometric detection, with some preliminary work on the use of confluence points and flame AAS detection (see sections 2.9 and 2.10). However, the use of enzyme reactors, preconcentration columns and other packed reactors is becoming more widespread. This is resulting in an increasing overlap between FIA and HPLC. It is therefore proposed that future work should reflect this and that the development and application of flow models to both techniques should be carried out simultaneously. This should include the study of the contribution of reaction kinetics to dispersion.
Appendix A

Bibliography
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Appendix B

List of Symbols
Symbol | Definition
---|---
a | tube internal radius
$A_0', A_p$ | initial and peak absorbance values
C | instantaneous concentration, normalised concentration
$C_A', C_A, C_B$ | apparent and real concentration of species A and B
$C_m$ | maximum attainable concentration
$C_o$ | injected or initial sample concentration
$C_{obs}$ | apparent or observed instantaneous concentration
$C_p$ | concentration at the peak maximum
$C', C'_p$ | $C_p$ normalised by the area under the C-t curve for the TIS model
$C_{θ}, \langle C_{θ} \rangle_p$ | normalised $C_p$ for the TIS model
d | tube internal diameter
D | dispersion coefficient, $=C_o/C_p$
\(D_a\) | apparent diffusion coefficient
\(D_{chem}\) | value of D arising from chemical reaction only
\(D_{eff}\) | effective value of D for heterogeneous samples
\(D_m\) | (molecular) diffusion coefficient
\(D_{phys}\) | value of D arising from physical dispersion only
\(D_{phys+chem} = \frac{D_{phys} + D_{chem}}{D_{phys}}\) | reagent dispersion coefficient \([=H_o/H = A_o/A_p]\)
\(D_p\) | response dispersion coefficient \([=H_o/H = A_o/A_p]\)
\(D^R\) | reagent dispersion coefficient \([=D/(D-1)]\)
E, $E_θ$ | exit-age distribution, normalised E.A.D.
$f_n$ | fraction of flow through nth tank, $n=1,2$
$G_n$ | gradient of $-\ln(1-C)$ vs. t associated with the nth tank, $n=1,2$
H, $H_o, H_p$ | detector response due to $C, C_o, C_p$
$K_{AB}$ | selectivity coefficient for A in the presence of B
K, $K', k'$ | constants
L | tube length (between injection valve and detector)
$L'$ | hypothetical tube length
L_s  length of initial sample zone
M    mass of sample injected for a delta function
N    number of loops in a helical coil; number of tanks in TIS model
P    pressure drop across a length of tubing
Pe   Peclet number (dimensionless)
Q    volumetric flow-rate
r    radial distance from tube axis
R    volume of carrier mixed with sample in unit time
Re   Reynold's number (dimensionless)
t    time
t_a   appearance time of a peak
Δt_b  baseline peak width
t_d   residence time of fluid in the detector
t_p   time at the peak maximum
tr    mean residence time of fluid in a manifold
()̅t   mean residence time of the TIS model
ti    mean residence time in a single tank
()̅t'   normalised value of ()̅t
u_o, u_r linear flow velocities at r=0, r=r
()̅u    mean linear velocity [=Q/πa^2]
V    tank volume for the TIS model
V_CELL flow cell volume
V_d   detector volume
V_n   volume of the nth tank, n=1,2
V_m   volume of a single well-stirred tank
V_r   manifold volume
V_s   sample volume
V_½ sample volume giving rise to D=2
x    longitudinal distance
x_l   = x-½. u_o t
X_R   reduced length (dimensionless)
X_R^*  X_R corrected for sample volume
z    reduced radial distance [=r/a]
γ^3   skewness of a probability distribution
δ    longitudinal dispersion number [=D_a/()̅UL]
\( \eta \) fluid viscosity
\( \tilde{\theta}, \theta_p \) reduced \( t, \tau_p \) for the TIS model
\( \lambda \) coil aspect ratio [coil diameter/tube diameter]
\( \mu \) mean value of a probability distribution
\( \pi \) numerical constant
\( \rho \) fluid density
\( \sigma \) standard deviation of a probability distribution
\( \sigma_t^2 \) peak variance
\( \sigma^2, \langle \sigma^2 \rangle \) variances for the TIS model
\( \sigma^2_\theta \)
\( \tau_t \) reduced time (dimensionless)
\( \tau^*_p \) \( \tau_p \) corrected for sample volume
Appendix C

Mathematical Derivations
C.1 THE CONVECTIVE FLOW MODEL

For conditions of laminar flow, the Poiseuille equation gives

\[ u_r = \frac{p(a^2 - r^2)}{4\eta L} \]  \hspace{1cm} (C.1)

If the sample zone starts moving at time \( t=0 \), then the appearance and peak maximum times will be given by

\[ t_a = \frac{L}{u_0} \]  \hspace{1cm} (C.2)

\[ t_p = \frac{(L + L_s)}{u_0} \]  \hspace{1cm} (C.3)

The relative concentration of solute within the plane at \( x=(L+L_s) \) at any time will be given by the relative cross-sectional area \( A \) of the sample zone lying in that plane. For \( t_a < t < t_p \), the radius of this area may be calculated from equation C.1 by making the substitution \( u_r = L/t \) and rearranging to give

\[ r^2 = a^2 - \left( \frac{4\eta L^2}{pt} \right) \]  \hspace{1cm} (C.4)

\[ A_1 = \frac{\pi r_1^2}{\pi a^2} \]

\[ A_1 = 1 - \left( \frac{4\eta L^2}{pa^2 t} \right) \]  \hspace{1cm} (C.5)
For \( t > t_p \), the trailing edge intersects the leading edge so that

\[
A_2 = \left( \pi r_1^2 - \pi r_2^2 \right) / \pi a^2
\]

\[
\therefore A_2 = A_1 - \left[ 1 - \frac{4\eta L (L + L_s)}{pa^2 t} \right]
\]

\[
\therefore A_2 = 4\eta L L_s / pa^2 t \quad (C.6)
\]

Now \( Q = \frac{1}{2} \pi a^2 u_0 \) so that substituting into equation C.1 and rearranging gives

\[
Q = \frac{\pi a^4 p}{8\eta L} \quad (C.7)
\]

which on substitution into equations C.5 and C.6 gives

\[
A_1 = 1 - \left( \frac{\pi a^2 L}{2Qt} \right) \quad (C.8)
\]

\[
A_2 = \frac{\pi a^2 L_s}{2Qt} \quad (C.9)
\]

Now, \( V_r = \pi a^2 L \) and \( V_s = \pi a^2 L_s \), so we can write the concentration-time profile as

\[
0 < t < t_a, \quad C/C_0 = 0 \quad (C.10)
\]

\[
t_a < t < t_p, \quad C/C_0 = 1 - \left( V_r / 2Qt \right) \quad (C.11)
\]

\[
t > t_p, \quad C/C_0 = V_s / 2Qt \quad (C.12)
\]

Furthermore, since \( t_p = (V_r + V_s) / 2Q \) it can be shown that substituting for \( t = t_p \) in equation C.11 gives rise to the equation
\[ \frac{C_p}{C_o} = \frac{V_s}{(V_s + V_r)} \quad (C.13) \]

The condition for laminar flow is that the Reynold's number, \( R_e < 1,000 \). Now

\[ R_e = \frac{2\bar{u}a\rho}{\eta} \quad (C.14) \]

For water at 20°C, \( \rho = 998 \text{ kg m}^{-3} \) and \( \eta = 1.00 \times 10^{-3} \text{ N s m}^{-2} \) which means that for laminar flow if \( a = 0.25 \text{ mm} \), \( Q < 23.65 \text{ ml min}^{-1} \) and if \( Q = 2.00 \text{ ml min}^{-1} \), \( a > 0.021 \text{ mm} \).
These conditions are not normally exceeded for practical FIA.

C.2 THE WELL-STIRRED TANK MODEL

For a single well-stirred mixing tank, the rate of change of concentration in the tank is given by

\[ \frac{dC}{dt} = \frac{\text{in} - \text{out}}{dt} \quad (C.15) \]

If a plug of sample of uniform concentration \( C_o \) and volume \( V_s \) starts to enter the tank at time \( t = 0 \) (i.e. \( t_s = 0 \)), then the time to the peak maximum will be the time at which the trailing edge of the sample zone just enters the tank, i.e.

\[ t_p = \frac{V_s}{Q} \quad (C.16) \]

So for \( 0 < t < t_p \),

\[ \frac{dC}{dt} = \frac{C_o \cdot \frac{Q}{V_m} - C \cdot \frac{Q}{V_m}}{V_m} \]

\[ \therefore \frac{dC}{(C_0 - C) V_m} = \frac{Q}{V_m} dt \quad (C.17) \]
Integrating both sides of equation C.17 gives

\[-\ln(C_o - C) = \frac{Qt}{V_m}\]

\[\therefore C_o - C = K \cdot \exp\left(-\frac{Qt}{V_m}\right)\]  \hspace{1cm} (C.18)

But when \(t=0\), \(C=0\) and so \(K=C_o\) which on substitution into equation C.18 gives

\[C = C_o \left[1 - \exp\left(-\frac{Qt}{V_m}\right)\right]\]  \hspace{1cm} (C.19)

When \(t=t_p\), \(C=C_p\) and from equation C.19

\[C_p = C_o \left[1 - \exp\left(-\frac{Qs}{V_m}\right)\right]\]  \hspace{1cm} (C.20)

For \(t>t_p\), equation C.15 becomes

\[
\frac{dC}{dt} = 0 - \frac{C \cdot Q}{V_m}
\]

\[\therefore \frac{dC}{C} = -\frac{Q \cdot dt}{V_m}
\]

\[\therefore C = K \cdot \exp\left(-\frac{Qt}{V_m}\right)\]  \hspace{1cm} (C.21)

But when \(t=t_p\), \(C=C_p\) and so

\[K = C_p \cdot \exp\left(\frac{Q t_p}{V_m}\right)\]

\[\therefore C = C_p \cdot \exp\left[\frac{Q(t - t_p)}{V_m}\right]\]  \hspace{1cm} (C.22)

If a length \(L'\) of tubing is assumed to exist between the injection valve and the mixing tank, and if it is further assumed that no dispersion occurs within this length of tubing, then \(t_a = \pi a^2 L'/Q\) and the concentration-time curve may be calculated by substituting \((t-t_a)\) for \(t\) in
equations C.19, C.20 and C.22.

C.3 THE TWO-TANKS-IN-PARALLEL MODEL

In this model, the flow is split between two tanks and then recombined. The input conditions are the same as those for the WST model (preceding section), i.e. a volume $V_s$ of sample of concentration $C_0$ which starts passing points A' at time $t=0$. If the concentrations at the tank outlets B are $C_1$ and $C_2$, then the equations for each tank are derived in the same way as for the WST model so that for $0<t<t_p$,

$$C_1 = f_1 C_0 [1 - \exp(f_1Qt/V_1)] \quad \text{(C.23)}$$

$$C_2 = f_2 C_0 [1 - \exp(f_2Qt/V_2)] \quad \text{(C.24)}$$

$$\therefore C = C_1 + C_2$$

$$\therefore C = C_0 \left[1 - f_1 \cdot \exp\left(-\frac{f_1Qt}{V_1}\right) - f_2 \cdot \exp\left(-\frac{f_2Qt}{V_2}\right)\right] \quad \text{(C.25)}$$
The peak maximum will be reached after \( t = \frac{V_s}{Q} \) so that \( C_p \) may be obtained by substituting for \( t = t_p \) in equation C.25

\[
C_p = C_0 \left[ 1 - f_1 \exp\left( -f_1 \frac{V_s}{V_1} \right) - f_2 \exp\left( -f_2 \frac{V_s}{V_2} \right) \right] \quad (C.26)
\]

For \( t > t_p \), by analogy with the WST model,

\[
(C_1)_p = f_1 C_0 \left[ 1 - \exp\left( -f_1 \frac{V_s}{V_1} \right) \right] \quad (C.27)
\]

\[
(C_2)_p = f_2 C_0 \left[ 1 - \exp\left( -f_2 \frac{V_s}{V_1} \right) \right] \quad (C.28)
\]

and therefore

\[
C_n = C_0 f \beta_n \exp\left( -f_n \frac{Q(t - t_p)}{V_n} \right) \quad (C.29)
\]

where

\[
\beta_n = \exp\left( f_n \frac{V_n}{V_s} \right) - 1 \quad (C.30)
\]

so that

\[
C = C_0 \left[ f_1 \beta_1 \exp\left( -f_1 \frac{Q(t - t_p)}{V_1} \right) + f_2 \beta_2 \exp\left( -f_2 \frac{Q(t - t_p)}{V_2} \right) \right] \quad (C.31)
\]

The TTP model may be readily extended to any number of tanks, since it follows from the preceding equations that for \( 0 < t < t_p \),

\[
C = C_0 \sum_{i=1}^{n} f_i \left[ 1 - \exp\left( -f_i \frac{Q t}{V_i} \right) \right] \quad (C.32)
\]
and for $t > t_p$

$$C = C_0 \sum_{i=1}^{i=n} B_i \exp\left(-Q(t - t_p)/V_i\right)$$  \hspace{1cm} (C.33)

The TTP model may be extended to include a length $L'$ of tubing between the valve and point A in the same way as the WST model.

**C.4 THE TWO-TANKS-IN-SERIES MODEL**

**Fig. C.3 The Two-Tanks-in-Series Model**

This model assumes the same conditions as for the WST and TTP models. The concentration at the outlet of the first tank is the same as for the WST model for the same tank volume. However, because the effluent from this tank passes into the second tank, the time to the peak maximum is no longer given by $t = V_s/Q$. The rate of change of concentration in the second tank is given by

$$\frac{dC_2}{dt} = C_1 \left(\frac{Q}{V_1}\right) - C_2 \left(\frac{Q}{V_2}\right)$$  \hspace{1cm} (C.34)

For $0 < t < V_s/Q$, this becomes

$$\frac{dC_2}{dt} = C_0 \left(\frac{Q}{V_2}\right) \left[1 - \exp\left(-Qt/V_1\right)\right] - C_2 \left(\frac{Q}{V_2}\right)$$  \hspace{1cm} (C.35)

Equation C.35 may be solved by multiplying both sides by
the factor \( \exp\left(\frac{Qt}{V_2}\right) \) and rearranging to give

\[
\frac{dC_2}{dt} \left(\frac{Q}{V_2}\right) \exp\left(\frac{Qt}{V_2}\right) + \frac{dC_2}{dt} \exp\left(\frac{Qt}{V_2}\right) = C_0 \left(\frac{Q}{V_2}\right) \exp\left(\frac{Qt}{V_2}\right) \left[1 - \exp\left(-\frac{Qt}{V_1}\right)\right]
\]

(C.36)

The left hand side of equation C.36 is the differential of the product \( C_2 \cdot \exp\left(\frac{Qt}{V_2}\right) \). Therefore, integrating both sides and solving for the condition that when \( t=0, C_2=0 \) yields, on simplification,

\[
C_2 = C_0 \left[1 - V_1 \cdot \exp\left(-\frac{Qt}{V_1}\right) + V_2 \cdot \exp\left(-\frac{Qt}{V_2}\right)\right]
\]

(C.37)

where \( V_1 = \frac{V_1}{V_1 - V_2} \)
\( V_2 = \frac{V_2}{V_1 - V_2} \)

It should be noted that this derivation makes the simplifying assumption that \( V_1 \neq V_2 \). When \( t=V_s/Q (=t') \), the value of \( C_1 \) reaches its maximum value. The corresponding value of \( C_2 \) can be obtained by substituting for \( t \) in equation C.37, which gives,

\[
C_2 = C_0 \left[1 - V_1 \cdot \exp\left(-\frac{V_s}{V_1}\right) + V_2 \cdot \exp\left(-\frac{V_s}{V_2}\right)\right]
\]

(C.38)

For \( t>t' \), equation C.34 becomes

\[
\frac{dC_2}{dt} = C' \left(\frac{Q}{V_2}\right) \exp\left[-Q\left(t - t'\right)/V_1\right] - C_2 \left(\frac{Q}{V_2}\right)
\]

(C.39)

where

\[
C' = C_0 \left[1 - \exp\left(-\frac{V_s}{V_1}\right)\right]
\]
Equation C.39 can be solved by rearranging as before to give

\[
C_2 \left( \frac{Q}{V_2} \right) \exp \left( \frac{Qt}{V_2} \right) + \frac{dC_2}{dt} \exp \left( \frac{Qt}{V_2} \right) = C_0 \left( \frac{Q}{V_2} \right) \left[ \exp \left( \frac{V_s}{V_1} \right) - 1 \right] \exp \left( -\frac{Qt}{V_1} \right) \exp \left( \frac{Qt}{V_2} \right)
\]

(C.40)

On integration, solving for the condition that \( C_2 = C' \) when \( t = t' = \frac{V_s}{Q} \) and simplification, this results in the expression

\[
C_2 = C_0 \left[ \frac{V_1}{V_2} \alpha_1 \exp \left( -\frac{Qt}{V_2} \right) - \frac{V_2}{V_1} \alpha_2 \exp \left( -\frac{Qt}{V_2} \right) \right]
\]

(C.41)

where

\[
\alpha_n = \exp \left( \frac{V_s}{V_n} \right) - 1
\]

Equations for \( t_p \) and \( c_p \) can be derived as explained in chapter 4. The model may be extended to include a length \( L' \) of tubing in the same way as the WST and TTP models.
Appendix D

Computer Programs
D.1 CALCULATING THE EFFECT OF A FINITE DETECTOR VOLUME

The effect of a finite measuring volume was modelled by the WST model with a mean-value detector situated immediately at the tank outlet.

![Well Stirred Tank with Mean-Value Detector Diagram]

**Fig. D.1** Well Stirred Tank with Mean-Value Detector

As explained in section 2.2.2, this results in the relative observed concentration at a point in time $t$ being given by the equation

\[ C_{\text{obs}} = \frac{1}{t_d} \int_{t-t_d}^{t} C(t) \, dt \quad \text{(D.1)} \]

The subroutine SMCINT was written to calculate values of $(C_{\text{obs}}, t)$ for a given $(C, t)$ profile using a six-strip Simpson's rule procedure to calculate the integral term in equation D.1.

This results in the expression

\[ C_{\text{obs}} \approx \frac{1}{t_d} \frac{h}{3} \left( f_1 + 4f_2 + 2f_3 + 4f_4 + 2f_5 + 4f_6 + f_7 \right) \quad \text{(D.2)} \]

which simplifies to
Values of $C_{\text{obs}}$ and $C$ were calculated for 100 values of $t$ over any specified interval using equation D.3 in conjunction with the known equations for the WST model. Values of $t_d$ (and hence $h$) were calculated from the specified detector volume using the expression $t_d = V_d / Q$.

Values of $C_{\text{obs}}$ and $C$ were returned to a main program (PROFILE), which allowed subsequent printing, plotting and storage of the data using additional subroutines. The same main program was also used to call subroutines written for the convective flow, diffusion, TIS, WST, TTP and TTS models.

D.2 ANALYSIS OF EXPERIMENTAL DATA FOR THE FIT OF A SINGLE EXPONENTIAL FUNCTION

The fit of the WST model to FIAAS growth and decay curves and FIA peak shapes was investigated using the program SHAPE. Consideration of the equations describing the model led to the relationships
\[
\ln(1 - C/C_o) = -\frac{Qt}{V_m} + k \quad (D.4)
\]

\[
\ln(C/C_o) = -\frac{Qt}{V_m} + k \quad (D.5)
\]

for the rising and falling curves respectively. Therefore, if the experimental data could be described by the WST model, a plot of \(-\ln(1-C/C_o)\) or \(-\ln(C/C_o)\) against \(t\) should be linear with a slope equal to \(Q/V_m\).

The experimental data was stored as normalised concentration and time values in data files, which were then read by the program. The data was sorted into rising and falling portions, which were then treated in sequence. After calculating values of \(\ln(1-C/C_o)\) or \(\ln(C/C_o)\), it was necessary to extract a linear portion from the data for analysis by a LEASTSQUARES routine. This was done by testing the ratio of the slopes between successive data points:

\[
r_n = \left| \frac{S_1}{S_2} \right| \quad (D.6)
\]

Hence for a linear portion, \(r_n = 0\). Values of \(r_n\) were compared to a "critical value", which was initially set to 0.200. This value could be changed by the operator, or a linear portion selected by eye from a plot of the results on the terminal screen. Once a linear portion had been isolated, a LEASTSQUARES analysis was performed by the National Algorithm (NAG) library routine G02CAF (linear
regression analysis), the Pearson correlation coefficient being used as a measure of the "goodness of fit" of the data to the model. Values of $V_m$ were then calculated from the slope, and the results printed for subsequent evaluation.

D.3 USE OF A SIMPLEX OPTIMISATION PROCEDURE FOR THE FITTING OF MODELS WITH TWO OR MORE VARIABLES

The program MOD2 was written in an attempt to analyse experimental peak shapes for agreement with the TTP and TTS models. Because of the somewhat complex nature of the equations describing these models (see chapter 4), it was not possible to derive simple expressions relating the model parameters to simple functions of the data such as the peak height or the logarithm of the concentration values. A SIMPLEX optimisation procedure appeared to offer a suitable means of varying the parameters of each model until the "best fit" of the model to experimental data was obtained.

The program made use of the NAG library routine E04CCF, which performs such a procedure for any number of variables using a function provided by the user. The routine also requires initial estimates of the values of the parameters to be optimised, and a "tolerance" by which it assesses the "goodness of fit". The function used was the root mean square of the residuals between the actual and predicted concentration values for every data point for the peak shape being analysed. The data were stored in the same files as for the previous program (section D.2).

For each simplex of $N+1$ points (where $N$ is the number of parameters for the model being used), the routine calculates the corresponding value of the function for each point ($f_i$) and evaluates the function
This value is then compared with the "tolerance" supplied by the user, and the "best fit" assumed to be when the value of the function is less than the tolerance. Typical tolerances used were $10^{-4}$ or $10^{-5}$.

The routine will automatically expand, contract, reflect and translate the Simplex until the "best fit" is obtained. This in itself caused a few problems, since this could result in unrealistic values of the model parameters being used, e.g. tank volumes having negative values and fractional flows greater than 1. To this end the routines written to calculate the r.m.s. of the concentration residuals incorporated tests that returned artificially high values of the function for conditions outside those employed by the model. This sometimes resulted in the routine being unable to find a "best fit" inside 100 simplexes (the limit decided on for the maximum number of attempts). It was also found necessary to perform all calculations in double precision mode (16 digit accuracy).

Trials of the program showed that the initial estimates given to the routine for the optimum values of the model parameters did not significantly affect the performance of the routine, provided that they gave a peak height that was in the region of that for the experimental data. The length of tubing assumed to exist in the model (see appendix C) was determined from the appearance time of the experimental peak, in order to directly compare model and experimental peak shapes. The performance of the program was initially assessed using model data, and found to be satisfactory. The actual performance of the program for analysing experimental peak shapes was discussed in chapter 4.
Appendix E

Construction of an Automatic Valve
E.1 CONTROL OF A MOTORISED VALVE

A motorised valve for performing both time and slug injection was described in section 2.7.1. This consisted of a Rheodyne 5020 injection valve coupled to a Philips 31004 stepper motor via a 75:1 reduction drive. This was controlled from an Apple IIe computer using the system shown below:

![Diagram of valve control system]

**Fig. E.1 Valve control system**

The motor control was based on the RS components SAA 1027 driver chip. This has three inputs: a line to control direction (forward/reverse), a line to activate the motor (on/off), and a control line (pulse train to drive the motor). This chip is capable of driving the 31004 motor directly, but not the more powerful 31104 motor as originally used.

The interface card was based on the 6522 Versatile Interface Adaptor (VIA) chip, which complements the processor of the Apple IIe. The VIA was used to provide a programmable "intelligent" interface that could be used to control the valve switching sequence, whilst leaving the computer free to perform other functions. The switching sequence was split into three distinct events: driving the valve, a delay period, and then returning the valve. In order to achieve the first and last of these, it was necessary to provide the motor control with a pulse train of known frequency and duration. The frequency of the pulse train was important as it controlled the rate of turn of the valve, whilst the duration of the pulse train controlled the angle through which the valve was turned.

The essential features of the VIA chip used to
achieve this are a free-running timer and two data ports, which were used to control auxiliary timers on the interface board.

![Diagram of the Interface Board](image)

Fig. E.2 The Interface Board

These were used in conjunction with the data ports and timer to provide the necessary control signals for the motor control. The frequency of the pulse train could be varied by changing the countdown interval for the VIA timer, whilst the duration (in terms of number of pulses) of the drive, delay and return periods were controlled by the auxiliary timers via the VIA data ports. Additional logic gates were incorporated so that the on/off and direction signals were provided from the timers as well:

![Timing Sequence for the Valve](image)

Fig. E.3 Timing Sequence for the Valve

This allowed the switching sequence of the valve to be controlled as outlined in section 2.7.