Studies of the processing of animal blood plasma

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STUDIES OF THE PROCESSING OF ANIMAL BLOOD PLASMA

AUTHOR: G. O. E. SHOFOLU

SUPERVISOR: PROFESSOR J. MANN

A DOCTORAL THESIS SUBMITTED IN PARTIAL
FULFILMENT OF THE REQUIREMENTS FOR THE
AWARD OF DOCTOR OF PHILOSOPHY

JUNE 1978
ACKNOWLEDGMENTS

The author wishes to thank Prof. J. Mann, Dr. J. Selman and the staff of the Department of Chemical Engineering for their guidance, advice and suggestions throughout the period of work for this thesis. The co-operation of Dr. Peter Rice is gratefully acknowledged.

Appreciation is extended to Mrs. C. M. Lincoln of the Information Services of the University Library for her assistance.
I certify that neither my thesis nor my original work contained therein has been submitted to this or any other institution for consideration of a degree.

G. O. E. SHOFOLU.
SUMMARY

Even though the demand for protein is increasing with the increasing population, some reliable and cheap sources of protein have remained largely untapped for human food. One such neglected source of food protein is animal blood.

The production of spun protein fibres from bovine blood plasma and the conversion of the accompanied blood cells into a dry, free-flowing powder is a stride forward in the economic disposal of huge amounts of animal blood unused daily in the U.K.

The effects of altering environmental conditions of the plasma solution during ultrafiltration have been studied. Adjusting plasma solution to pH 9.5 was found to improve ultrafiltration rate significantly. Moderate acidification of the feed solution also showed improved results. Changing the ionic strength of the plasma solution also gave favourable results. 40°C was found to be optimum temperature for the ultrafiltration of plasma solution in relation to the processing quality of the concentrated product.

Concentrated and de-salted bovine blood plasma solution, of less than 15% protein content, has been turned into a meat-like product.
The rheological characteristics of spinning dopes, on the other hand, are similar to that of a Bingham plastic at certain pH values whilst exhibiting pseudoplasticity plus yield stress at other pH values, and are generally of a time-dependent nature. The flow curve alone is insufficient to characterise completely the rheological behaviour of spinning dopes.

Effect of alkali-treatment of plasma proteins has been observed to increase the length of the protein molecules which become entangled in one another in such a way as to bring an increase in viscosity. Extent of denaturation due to processing treatments, configurational and structural changes due to alkali treatment, and the Scanning Electron Microscope view of the finished products, were studied.

Physical properties of spun fibres were measured mechanically. The major physical change was colour which changed from pale yellow to milky white as a result of processing.
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<th>to</th>
<th>multiply by</th>
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<tr>
<td>angstrom</td>
<td>nm, nanometre</td>
<td>0.1</td>
</tr>
<tr>
<td>micron</td>
<td>m, micrometre</td>
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</tr>
<tr>
<td>inch</td>
<td>mm, millimetre</td>
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<tr>
<td>centistoke</td>
<td>mm²/s, square millimetre per second</td>
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<tr>
<td>ton (short, 2000 pounds)</td>
<td>Mg, megagram</td>
<td>0.9072</td>
</tr>
<tr>
<td>ton (metric) tonne</td>
<td>Mg</td>
<td>1.0</td>
</tr>
<tr>
<td>ton (long, 2240 pounds)</td>
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<tr>
<td>dyne per square cm.</td>
<td>Pa, pascal</td>
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<tr>
<td>psi, lbF/in²</td>
<td>kPa, kilopascal</td>
<td>6.895</td>
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<tr>
<td>kgF/cm²</td>
<td>kPa</td>
<td>98.0665</td>
</tr>
<tr>
<td>Centipoise</td>
<td>mPa.s, millipascal sec.</td>
<td>1.0</td>
</tr>
<tr>
<td>poise</td>
<td>Pa.s, pascal sec.</td>
<td>0.1</td>
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<tr>
<td>cubic foot</td>
<td>m³, cubic metre</td>
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<tr>
<td>litre</td>
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<tr>
<td>gallon</td>
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INTRODUCTION

Increasing concern about environmental pollution hazards and the search, in the last decade, for more animal proteins from unconventional sources that are suitable for human consumption has led to the processing of animal blood plasma for some type of reuse.

Halliday (1973) found that the main proportion of blood withdrawn from slaughtered animals is allowed to go into the drainage system except for small quantities collected by local sausage manufacturers and possibly butchery businesses while 90% of the blood in continental Europe (not the U.K.) was being passed through the processing system. The total amount of blood available from animals dressed in U.K. slaughter houses is about 90.72 megagram per annum representing some 3.5% of the weight of live carcasses (Ranken, 1977). Direct discharge of such huge amount of animal blood to municipal drainage system, without any pre-treatment, is a potential health risk to both industry and municipality.

According to Dart (1967) one of the Chief sources of polluting matter in waste waters from slaughter houses is blood. He emphasised that blood contributes considerably to the polluting load of waste waters and if efficient collection of blood is not carried out, the polluting load from a slaughter house
might be increased by as much as 40%. Separation techniques such as chemical fractionation (Tybor, Dill and Landmann, 1973), freeze concentration, (Young and Lawrie, 1974), ultrafiltration (Dulaney, 1977) have been suggested as ways of utilizing the large quantities of blood which are available annually as a slaughter house by-product.

In order to save the enormous quantities of blood that are allowed to go into the drainage system annually, there is, an urgent need for industries to utilize this valuable by-product of meat industry. It is the aim of this study to make use of the animal blood plasma, by way of its processing into spun protein fibres that may eventually be used as meat substitutes or extenders.

The processing of animal blood as suggested above can then serve as an alternative to the accepted idea of allowing blood to drain to waste thereby reducing the double economic loss associated with its conventional disposal practices.
1.0 MANUFACTURE OF EDIBLE FOOD PROTEIN

The constituents of a manufactured food can be grouped into five principal categories: macronutrients, micronutrients (vitamins, minerals) aesthetic substances (flavours, colours and texturising materials), preservatives and processing ingredients.

The majority of the substances in the last four categories are now technologically being produced they are manufactured and sold without problem. But when the world food supply is considered from the standpoint of nutrition the substances most needed are vitamins and protein. Many vitamins can be produced in quantity at a price acceptable to all but the poorest. With protein, however, the situation is quite different. In many countries the total supply of protein is inadequate in both quantity and quality. There is a shortage of high quality protein such as the protein of milk, eggs, and meat. In many of these countries it would not be possible, at least under the present conditions, to produce enough of these animal proteins to meet the needs of children, lactating and pregnant women.
It is generally recognised that there is sufficient protein available in the world, indeed frequently available in many countries where malnutrition is rampant. Ignoring the economic problem, the problems of education, particularly in terms of the value of a mixed diet, and the development of an acceptable low cost food of high protein quality which is stable in storage and distribution which might be used as an adjunct to the normal diet.

There are three possible steps - one that is already in use and two that are capable of future development.

The first source is through the production of high quality protein by ungrading the nutritionally incomplete proteins of cereal grains and oil seeds by the addition of the limiting amino acids, made synthetically or biosynthetically by the addition of small quantities of foods rich in the missing limiting amino acids, and/or by educating the consumer to accept a more varied diet.

A second source is through the synthetic production of the eight (or ten) essential amino acids and their incorporation in the diet in the proportion found in animal protein. The third possibility is the total manufacture of protein from simple, cheap, abundant raw materials. This last step has the most to offer.
1.1 Unusual Sources for Food Protein

Improved technology in the past has made available many protein-rich concentrates from vegetable sources (maize, groundnuts, cotton seed) which, unfortunately, are not readily acceptable, often through unfamiliarity in the form in which they are normally produced. Examples are soya bean curd and protein rich residues from seed oil manufacture. Residues from seed oil manufacture after oil extraction have long been used for animal feed but are now assuming greater importance for human consumption.

Fishmeal and soya protein isolates are both solvent extracted from their respective raw material. Fishmeal protein isolate is one of the earliest novel proteins which was first produced in America, but since its development, its acceptability has been challenged, and production cost also prevented its wide use. Soya protein isolate, in contrast, has been made to be more accepted by further steps in technology, which helps to convert it into spun or textured product.

Animal proteins such as casein and keratin and single cell protein have been spun into textured materials.
However, one source of high quality protein as yet untapped, at least, to any reasonable extent, and the source is slaughter house by-products. This source is a reliable pool of unrecognised or neglected materials of moderate protein contents. Such materials include animal by-products like blood, bones, hoofs, horns, inedible meat and other parts which can be turned into edible form which can prevent them from being completely wasted.

Of greater interest in this study is the animal blood. Unlike other animal by-products it represents a double economic loss. The main bulk of blood withdrawn from slaughtered animals - cattle, pigs, sheep etc. has been allowed to pass into the drainage system. Only a small proportion of the blood has been prevented from flowing to waste by drying to highly concentrated granular powder containing between seven and 12 percent moisture.

Halliday (1973) reported that small percentages of animal blood (1 to 2) have been and are still, at least in the U.K. used by manufacturing industries specifically engaged in its conversion to products of a pharmaceutical nature for therapeutic use, and for occasional inclusion in sausage type products in the U.K. and more prevalent use on the continent.
Apart from its protein, there is another important economic consideration for animal blood to be used by the manufacturing industries. If the blood is not used, it may be a source of health risk if allowed to go into the drainage system where it clots and perhaps seals the drains or even decomposes to give off unpleasant odour, and more importantly being a good medium for bacterial growth, it may turn to be a 'microbial den' within the slaughter house and its environment. If processed, however, it may represent an additional course of income which may be used toward the maintenance of the slaughter house or reduce the cost of slaughtering operations.

An investigation through the co-operation of Severn-Trent Water Authority revealed that a large modern West Midland abattoir uses about 0.523 m$^3$ per cattle unit, a large public abattoir 2.07 m$^3$ and a small private abattoir 0.54 m$^3$ of water per cattle unit, 20 to 40% of the above figures depending on the size of abattoir, being the amount for flushing the blood down the drain (1 cattle unit = 1 beef animal = 2 pigs = 5 sheep). Dart (1967) gave a figure of about 5 to 15 m$^3$ water per 1.008 Mg. live weight. Calculation from the above stated figures gave an average quantity of water used in the slaughter houses in the U.K. during the period 1965 to 1975 to be about 2.325 million m$^3$ per annum out of which 0.316 million m$^3$ per annum was used to flush the blood down the drain.
An urgent need for the processing ways of transforming animal blood into valuable food resources has long been due if not for the above reasons, at least, for the quantity of water involved in doing the unnecessary. Some slaughter houses even use steam, chemicals and a 4-hour-man working time to discard what need not be thrown away. This simply boils down to double economic loss.

One economic method of disposal is the recovery of valuable protein from blood plasma by pressure assisted fractionation systems into a high quality protein concentrate and a salt containing filtrate. The high quality protein concentrate can be either dried or directly incorporated into food products and even the conversion of the salty filtrate into clear water of sufficient potability for re-use is possible.

1.2 Animal Blood

Animal blood comprises of a pale yellowish or straw-colour fluid called plasma in which are suspended red and white blood cells with smaller bodies termed platelets. The haemoglobin molecule of the red blood cells gives its characteristic colour. The amount of fresh blood recoverable by slaughtering live stock varies but is of the order of 3.5% of the liveweight for cattle and somewhat less for other species.
Plasma is that fraction of the blood after the removal of the cells from the blood by centrifugation. It is a clear viscous fluid and accounts for some two-thirds of the total wet weight of whole blood. Table 1 (Page 12) shows the approximate composition of blood and plasma from different species of animal. Tables 2a (Page 13) and 2b (Page 13) show the protein content practically recoverable, of Bovine blood fractions, while Table 3 (Page 13) compares plasma composition to that of the whole Bovine Blood.

The Blood Plasma Proteins

Blood plasma is a pale yellow, viscous fluid after mechanical separation of the red blood cells from whole blood. Depending on the handling conditions of the blood and the degree of separation, this fluid part of the blood can be tinted red in colour. Once this happens, the red colouration remains permanently with the fluid. It contains on the average 8.5% total solids about 7% being protein in nature.

The plasma is a valuable source of different types of proteins. Among these are albumin, immunoglobulins. Others are conjugated proteins such as lipo-proteins, glycoproteins and metal binding proteins. According to Davidsohn and Nelson (1974) albumin is the smallest and most abundant of the plasma proteins, normally constituting slightly half of the total protein.
The albumin molecule has a diameter of about 3.8nm. and a length of roughly 15nm. Because of its size, albumin is the most osmotically active of the plasma proteins accounting for 75 - 80% of their total osmotic effect. Plasma albumin is generally believed to be a single polypeptide chain stabilised in three dimensions by 17 disulphide bonds. While albumin is a homogenous molecule all the globulin fractions consist of a number of different proteins of similar electrophoretic mobility but otherwise, unrelated chemically.

The alpha fraction, for example, includes a number of gluco-proteins, lipo-proteins and several other components. Fibrinogen, on the other hand, is thought to be a dimer of subunits containing three peptide chains joined through disulphide link. Putman (1960) reported that fibrinogen is responsible for the viscous property of the plasma fluid.

Apart from the complex mixture of proteins, bovine plasma comprises amino acids, hormones, enzymes, carbohydrates, mainly sugars, lipids, and salts. The albumin fraction is water soluble, whilst the globulins and the fibrinogen are considered salt-soluble proteins. In the liquid plasma, the three proteins constitute 8% of the weight, the balance consists of 90 - 91% water, plus small amounts of fat, minerals and vitamins.
The plasma proteins on the whole act at blood pH as if they were weak acids (Putman, 1960). Major bovine plasma proteins are summarised in Table 4 (Page 14) and their proportion and approximate dimensions in Table 5 (Page 14).
Approximate composition of blood and plasma

**TABLE I**

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<th>% Component</th>
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<td>Cattle</td>
<td>Pig</td>
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<tr>
<td>Moisture</td>
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<td>78</td>
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<tr>
<td>Haemoglobin protein</td>
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<td>14</td>
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<tr>
<td>Other protein</td>
<td>7</td>
<td>6</td>
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<tr>
<td>Non-protein solids</td>
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</tbody>
</table>

* CSIRO Division of food research

**TABLE 2a**

Protein content (Total N x 6.25) of major fractions of bovine blood

<table>
<thead>
<tr>
<th>Sample</th>
<th>Milligrams Protein/ml.  VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole Blood</td>
</tr>
<tr>
<td>1</td>
<td>175.25</td>
</tr>
<tr>
<td>2</td>
<td>160.80</td>
</tr>
<tr>
<td>3</td>
<td>165.45</td>
</tr>
<tr>
<td>4</td>
<td>169.20</td>
</tr>
<tr>
<td>5</td>
<td>159.50</td>
</tr>
<tr>
<td>6</td>
<td>176.85</td>
</tr>
<tr>
<td>7</td>
<td>162.75</td>
</tr>
<tr>
<td>8</td>
<td>172.00</td>
</tr>
<tr>
<td>9</td>
<td>167.00</td>
</tr>
<tr>
<td>10</td>
<td>170.50</td>
</tr>
</tbody>
</table>

‡ Mean of at least two duplicates
### TABLE 2b

<table>
<thead>
<tr>
<th></th>
<th>Milligrams Protein/ml.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±</td>
<td>Range</td>
</tr>
<tr>
<td>Whole Blood</td>
<td>167.93 ± 5.90</td>
<td>159.5 - 176.85</td>
</tr>
<tr>
<td>Blood Cells</td>
<td>308.89 ± 11.5</td>
<td>295.75 - 327.25</td>
</tr>
<tr>
<td>Blood Plasma</td>
<td>65.83 ± 4.83</td>
<td>60.4 - 74.38</td>
</tr>
</tbody>
</table>

### TABLE 3

Compositional analysis of bovine blood and plasma

<table>
<thead>
<tr>
<th>Component</th>
<th>Bovine Blood</th>
<th>Blood Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>80.5</td>
<td>90.2</td>
</tr>
<tr>
<td>Protein (N x 6.25)</td>
<td>16.25</td>
<td>6.75</td>
</tr>
<tr>
<td>NPN</td>
<td>1.88</td>
<td>1.05</td>
</tr>
<tr>
<td>Carbohydrate (as sugars)</td>
<td>0.10</td>
<td>0.12</td>
</tr>
<tr>
<td>Ash*</td>
<td>0.85</td>
<td>0.92</td>
</tr>
<tr>
<td>Fat (including other constituents)</td>
<td>0.42</td>
<td>0.96</td>
</tr>
</tbody>
</table>

* including Anticoagulant

† Average of ten samples
**TABLE 4**

Molecular weights of different fractions of bovine blood plasma proteins

<table>
<thead>
<tr>
<th>Plasma protein</th>
<th>Molecular weight*</th>
<th>Molecular weight †</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>69,000</td>
<td>67,000</td>
</tr>
<tr>
<td>Globulins (alpha)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipoproteins</td>
<td>41-82,000</td>
<td>45,000</td>
</tr>
<tr>
<td>Glycoproteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-Globulins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gamma-globulins</td>
<td>150,000</td>
<td></td>
</tr>
<tr>
<td>(Fibrinogen)</td>
<td>341,000</td>
<td>330,000</td>
</tr>
<tr>
<td>Transferrin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipoproteins</td>
<td>195,000 - 5 x 10⁶</td>
<td></td>
</tr>
<tr>
<td>Prothrombin</td>
<td></td>
<td>62,000</td>
</tr>
</tbody>
</table>

* Davidsohn and Nelson (1974)
† Putman (1960)

**TABLE 5**

Proportion and approximate dimension of Beef plasma protein components

<table>
<thead>
<tr>
<th>Amount (%)‡</th>
<th>Dimension of molecule (nm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length</td>
</tr>
<tr>
<td>Albumin</td>
<td>50</td>
</tr>
<tr>
<td>Globulins</td>
<td>23 - 27</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>17 - 23</td>
</tr>
</tbody>
</table>

* Harkness (1971)
‡ Pals (1970)
### TABLE 6

Some of the functional properties of animal blood proteins

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>Globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility</td>
<td>Good, little effect of salt.</td>
<td>Good, but only pH 6.0 Insoluble with 2% salt</td>
</tr>
<tr>
<td>Emulsifying capacity</td>
<td>Good</td>
<td>Very good</td>
</tr>
<tr>
<td>Emulsion Stability</td>
<td>Good, poorer with salt</td>
<td>Good below pH 6.0, better with salt</td>
</tr>
<tr>
<td>Foaming</td>
<td>Good</td>
<td>Very good</td>
</tr>
<tr>
<td>Gel formation</td>
<td>Very good</td>
<td>None (cream)</td>
</tr>
</tbody>
</table>

‡ Ranken (1977)

### TABLE 7

Analysis of Ox Meat and Blood

<table>
<thead>
<tr>
<th>% Component</th>
<th>Ox meat</th>
<th>Ox blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>70.72</td>
<td>80.85</td>
</tr>
<tr>
<td>Protein</td>
<td>20.59</td>
<td>18.00</td>
</tr>
<tr>
<td>Fat</td>
<td>5.33</td>
<td>0.18</td>
</tr>
<tr>
<td>N-free Extract</td>
<td>0.66</td>
<td>0.05</td>
</tr>
<tr>
<td>Ash</td>
<td>1.22</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Hirschberg (1957)
TABLE 8

Protein content of animal blood and hen eggs

<table>
<thead>
<tr>
<th>Animal Blood</th>
<th>Hen Eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td>Swine</td>
</tr>
<tr>
<td>Protein (%)</td>
<td></td>
</tr>
<tr>
<td>17.7</td>
<td>19</td>
</tr>
</tbody>
</table>

Hirchberg (1957)
1.3 **Blood Plasma - As a Fibre Forming Material**

Bovine blood plasma contains a number of proteins having adequate fibre forming properties. Table 4 (Page 14) shows these proteins to have high molecular weights in the range of about 40,000 to $5 \times 10^6$ which is one of the conditions of fibre-forming materials because they are linear macro-molecules (Lundgren, 1949). However, Lundgren (1949), and Balmaceda and Rha (1974) observed that due to differences in polypeptide chain length, molecular size, shape, chemical nature and disposition of amino acids residues along the chain, differences in their fibre forming properties do exist and vary with protein source. In view of this and because bovine plasma contains the valuable coagulable proteins namely albumin and the globulins its extensive use by the meat product manufacturers as protein fibres or as an aid in the stabilising and fortification of many of their fresh and canned products cannot be over-emphasised.

The properties of the blood plasma proteins have been reported to be closely related to those of egg white (Ranken, 1977) and one would, therefore, expect dried blood plasma to replace egg white particularly in the baking industries.
But the problem of off-flavour associated with dried plasma and possible lipid oxidation may seriously restrict its wide use in this respect. Table 6 (page 15) summarises some of the functional properties of blood proteins.

4 Blood Cells

Unlike the blood plasma proteins, the globulin protein is not similar to that of any other commonly used food ingredient and this may be expected to find new applications when produced on a large scale and at a cheaper cost.

The globulin protein is only effective under acid conditions, it forms viscous creams on heating but does not form gel, it readily absorbs water in the cold (Ranken, 1977).

The outlets for the use of red blood fractions are as fertilisers, animal feeding supplement etc. but its present production cost prohibits its extensive use in these products. The dark colour and strong flavour of the cell fraction, like those of the whole blood are major dis-advantages to its use in food products. Haemoglobin is well known to be highly susceptible to oxidation and this requires special careful and hygienic handling of the whole blood. Small quantities of whole animal blood, despite this drawback, have been used by local sausage manufacturers (Ranken 1977).
In direct contrast to animal blood, the use of whey derivatives as substitutes for powdered milk in bread has been thoroughly explored and incorporation procedures established by Webb (1970) and Guy (1972). Practical means of transforming animal blood into valuable food resources are, therefore, long overdue. Bovine blood contains reliable amounts of proteins which have potential as a nutrient in human foods (Table 7 Page 15) and Table 8 (Page 16) only the processing know-how is lacking.

Satterlee et al (1973) found that protein concentrates prepared from whole blood are excellent emulsifiers. The whole blood proteins exhibit emulsification capacities and emulsion stabili­ties equal to or greater than that of the proteins of other organ and tissue concentrates including muscle proteins (Ranken, 1977). Tybor et al (1973) observed that plasma and globulin protein isolates prepared from slaughter blood are excellent emulsi­fiers under optimum conditions of pH and protein concentration.
**FIGURE 1**

SUGGESTED FLOW DIAGRAM FOR BLOOD UTILIZATION.
Breyer and Goodwin (1962) also reported that bovine blood proteins exhibit many functional properties of utility in food product formulations, particularly the ability of the serum proteins to act as emulsifiers. Figure 1 (Page 20) shows the suggested flow diagram for blood utilisation.

1.5 Blood Yield

The total quantity of blood in bovine species amounts to between 7 and 9% of the live weight but the whole quantity cannot be possibly recovered from the body due to losses by spilling, type of collection method used, state of health of the animal and more important is the adverse effect that complete removal of blood has on the carcass. The amount of blood, therefore, recoverable per animal for processing varies between 3 and 5% of the live weight. Depending on the degree of clotting and system of mechanical separation, between 50% and 65% liquid plasma can be obtained from the whole blood.
1.6 Availability of Blood for Processing into Edible Food Products

The attraction of animal blood and bovine plasma, in particular, as raw material for protein fibre production is mainly due to its abundance and cheapness. Availability alone has not been the only attraction for blood plasma protein, the other important considerations have been those of high functionality of its proteins. Its high proportion of nutritive albumin/globin fraction has made blood an important factor in the diet of civilised peoples (Porter and Michaels, 1971).

Blood plasma, of course is low in protein content (5-6%) compared with soya bean (35%), groundnuts about 25% and maize (10%). But thanks to modern technology this is no longer a constraint on its large scale use because it can be ultrafiltered to give at least four to six times the original protein content. Amount of animal blood available for processing in the U.K. is depicted in Figure 2 (page 25). The total amount of blood available from animals dressed in U.K. slaughter houses is about 90.72 mega gram per annum representing some 3.5% of the weight of live carcasses (Ranken, 1977). He also reported that further smaller quantities are processed into petfoods, animal feeding stuffs and fertilisers.
The remainder, thought to amount to about two-thirds is turned to waste in slaughter house effluent where it contributes significantly to the burden and cost of subsequent effluent treatment (Patel, 1976). Halliday (1973) also found that the main proportion of blood withdrawn from slaughtered animals is allowed to go into the drainage system except for small quantities collected by local sausage manufacturers and possibly butchery businesses while 90% of the blood in continental Europe (not the U.K.) was being passed through the processing system.

Ranken (1977) also reported that substantial quantities of blood are used directly or further processed for incorporation into meat products in Sweden and Germany while in Northern Ireland a single company claims to be processing at least three quarters of the blood available from all the slaughter houses in that province.

Contrary to the situation in the U.K. it is interesting to note that over 95% of the 0.45 million Mg. of blood generated by the animal industries in the U.S.is recovered with an attendant reduction of 42% in waste effluent from packing plants (Garner, et al 1971) but only a negligible quantity is similarly utilised up to date as a human food ingredient.
In order to reduce drastically the health risk involved in the slaughter house waste disposal practices, rapid progress needs to be made in the utilisation processes involving animal blood on a large scale.
FIGURE 2

Avg. ($m^3 \times 10^3$)

- Cattle + Calves = 45.93 ± 6.14
- Pigs = 24.18 ± 2.03
- Sheep + Lambs = 11.04 ± 0.9

AMOUNT OF ANIMAL BLOOD WASTED PER YEAR IN THE U.K. [★ obtained by calculation from production figures].
1.7 Collection of Animal Blood for Edible Purposes

Of all the huge amount of animal blood available as slaughter house by-product only a small quantity has been and can still be used as human food because of the collection method commonly used. Blood must of necessity be maintained clean, fresh and in its original liquid if it is to be used for food or industrial purposes and must only come from animals which have been certified to be fit for human consumption. The collection of blood from animals should therefore be done with the minimum possible contamination. Since the production of high quality products depends on a high standard of hygiene, immediate processing and use of low temperatures, it is therefore essential to adopt an efficient, speedy method of collecting blood.

In some abattoirs where an attempt is made to collect blood, open vessels are still in use. This method of collection, not surprisingly, lends itself to a very high degree of contamination from bacteria, both air borne and shed from the skin of the animal during pre- and post slaughter treatments (Akers, 1973).

All satisfactory blood collection techniques require that the stick wound area be prepared in such a manner that contamination of the blood is avoided. In swine, this is often done by applying a gas torch and shaving, in bovine animals, a portion of the skin is grasped by
hand and removed by a single stroke with a sterile knife thus exposing an area of tissue having minimal contamination. A hollow-bladed sticking knife is then inserted, severing the blood vessels. The blood is conveyed through tubing to a holding tank. It is generally batched either on the basis of animal numbers, or elapsed time. The anticoagulant is usually applied at a point close to the sticking knife.

At high slaughtering rates, vacuum equipment is generally used to speed up bleeding. The animals can on the other hand, be hung after stunning to a suspension rail on which they travel in a semi-circle around the blood collection point. On completion of bleeding they are so transferred to a standard dressing rail system. But while this is an improvement on the open pan method, contaminants can still be drawn from the area of the animal's skin covered by the cup at the base of the blade. The more recent method (German Patent, 66a-1-2 035019, 1971) incorporates anticoagulant into the head of the sticking knife, thus achieving the earliest possible mixing with the blood flow. Another feature of this latest method is the total enclosure of the system, preventing the introduction of external contaminants. With a suitable vacuum applied to the system, high rates of blood recovery are attained with an improvement in the blood yield of 30 to 40% over the open pan methods (Wakefield, 1963).
Public health considerations demand that whatever system of blood collection is used the blood remains identifiable with the carcass until it has been passed to be fit for human consumption. The best practical solution has been to bulk the blood from a batch of animals whose carcasses are carried consecutively on the dressing line. Should any of these carcasses be rejected as unfit for human consumption, all the blood recovered from that group must be rejected or diverted to another use other than human, as stated earlier on. In practice, it is possible for the blood of about five cattle, or 20 pigs to be so treated. In order to maintain a high level of hygiene throughout the line, it is desirable that the entire collecting equipment be cleaned at frequent intervals after each group of animals has been bled. While metal parts can be cleaned effectively by live steam, such treatment cannot be applied to the plastic tubes used to carry the blood or plastic holding containers. For such items the following treatment may be applied, rinsing in cold running water for a short time then soaking or washing with a 5% formalin or any suitable sterilising agent. They may be washed with detergent solution and then disinfected with a hypochlorite solution (1:2 dilution) followed by a hot water wash (80°C) before being rinsed.
1.8 Chemical Preservation of Blood

It is customary from clinical and biochemical studies to use chemical preservation to keep the blood in liquid state. Blood clots only in the presence of calcium and if calcium is removed by reacting with oxalates or citrates, clotting would not take place. This has been achieved by stirring the fresh blood, as it is withdrawn in a sterile vat with calculated amount of anticoagulant such as sodium or potassium citrate/oxalate.

Chemical preservation of blood is essential in order to deliver the blood in its original liquid form to the processing industries even though this treatment is not desirable since it adds to the properties of the blood. More important is the fact that such preservation is only for a short period. Addition of 3 to 5% (v/v) of 5% sodium citrate or other salts to the blood before it is stored cold at $-4^\circ\text{C}$ has been observed to increase significantly its keeping quality. Blood treated as above and kept under refrigerated conditions deteriorated after 24 hours.

Of the usual anticoagulants the tri-sodium citrate (G.P.R) and oxalate are cheaper than heparin (a polysaccharide sulphuric acid ester). The former, at £1.94/kg. is the most cost effective, and has been used throughout this work.
1.9 Objective and Purpose of Study

Various protein materials have been spun into edible protein fibres in the past, vegetables notably soya (Boyer, 1954), animal proteins such as casein and Keratin (Boyer, 1956), soya protein (Anson, 1957), peanut protein (Giddey, 1960), single cell protein (Huang And Rha, 1972) and zein and single cell protein (Balmaceda, 1973).

Application of membrane processing to cheese whey has received much attention during the past decade (Fenton-May (1971), Donnelly (1971), Dunkley (1971), McDonough (1971). Pilot scale studies have been reported by McDonough and Mattingly (1970) and Fenton-May et al. (1971). In sharp contrast so little has been published concerning membrane fractionation of a valuable source of proteins such as animal blood plasma.

Porter & Michaels (1971) have concentrated whole blood and blood plasma by ultrafiltration. Blood plasma has also been freeze-dried and spun into fibres (Young and Lawrie, 1974). Plasma and globulin protein isolates were prepared from slaughter animal blood and powdered into white, free-flowing isolates (Tybor, 1974). Delaney & Donnelly (1973), Delaney, Donnelly and Bender (1975) have reported on the use of ultrafiltration for the concentration and purification of porcine blood plasma and on the subsequent conversion of the concentrated plasma solutions into powders by spray drying.
Erikson & von Bockelmann (1975) have reported on the ultrafiltration of animal blood serum prepared from pooled porcine and bovine blood.

This research was proposed to study the ultrafiltration of bovine blood plasma and to investigate how such concentrated and demineralised solution could be converted into edible fibrous product of adequate strength.

1.10 **Analytical Methods:**

**Total Solids**

Total solids content of samples was determined by the method of Jacobs (1951). Approximately 15 - 20 ml. duplicated portions of sample were transferred into tared dishes, then dried in a vacuum oven for 12 hours at 110°C, removed from the oven, cooled in a dessiccator, reweighed and the drying and cooling procedure repeated until constant weight was achieved.

**Ash**

Ash was determined by drying in an oven at 110°C and heating in an electric muffle furnace at 550°C to ash.

**Total Protein**

Total protein by the semi micro-Kjeldahl procedure (Protein N x 6.25).

.../32.
Non-Protein Nitrogen (NPN)

NPN was determined as soluble nitrogen in 15% TCA filtrate.

Protein Solubility

Protein solubility was determined according to the method of Nitrogen Solubility Index (NSI) with a modified technique for the removal of insoluble proteins. The centrifugal force was 10,000g for 30 min. The amount of protein in the solution after centrifugation, relative to the total amount of protein in the solution gives the solubility index.

Titratable Chloride

Titratable chloride by method of Schales and Schales (1946).

Total Phosphorus

The total phosphorus of plasma was determined as inorganic phosphate after wet ashing with 60% perchloric acid by a modified procedure of Delsal & Manhouri (1958). The equation for the standard curve was $Y = 0.0221 \pm 10.62X$, where $Y$ and $X$ were absorbance at 880 nm. and mg. Total inorganic phosphorus/ml. respectively.

Total Reducing Sugars

Total hexoses was estimated by the phenolic-sulphuric acid reaction as originally described by Dubois et al (1954). The equation for the standard curve using galactose was $Y = 0.0077 \pm 10.52X$ where $Y$ and $X$ were absorbance at 490 nm. and mg. Total
Total sugars/ml. respectively.

**pH Determination:**

All pH determinations were made at 20°C with the glass electrode standardised at pH 4.0 and 9.5 with standard buffer solutions.

**Statistical Methods**

The data, where necessary, was subjected to analysis of variance to detect significant effects among means, and differences between means were analysed by Turkey's w-procedure* (Steel & Torrie 1960) to separate means.

\[ w = q_x(p, n_2) \times \frac{s}{x} \text{ where } q_x \text{ is obtained from table for } x = 0.05 \text{ or } 0.01, p \text{ is the number of treatments and } n_2 \text{ equals error degrees of freedom}. \]
CHAPTER 2

LITERATURE SURVEY AND RESEARCH BACKGROUND

2.0 ANIMAL BLOOD PROTEINS

Proteins isolated from slaughter animal blood are a potential source of food proteins (Tybor et al., 1973) and attempts have, therefore, been made to produce dried protein concentrates from whole blood (Vickery, 1968).

Gordon (1971) suggested that blood plasma could be included in meat products and meat analogues derived from soya protein to improve binding quality and nutritional value. It has, however, been known that spray-dried bovine plasma rapidly acquired such a fishy flavour that it could not be used as a possible substitute for dried egg albumen (Brooks & Ratcliff, 1959) and it was found that the change was primarily an oxidative one. Brooks & Ratcliff (1959) also adopted freeze-concentration, using the apparatus and technique developed by Gane (1968) to increase the solids content of liquid plasma to 26% with an 87% recovery of plasma solids.

2.1 PRESSURE-ASSISTED MEMBRANE SEPARATION

Membrane separation is a pressure-assisted technique based on the ability of semi-permeable membranes to separate components of a solution on the basis of molecular size (Erikson, 1974).

Separation can be effected between solvent and solute or between
different solutes in a multi-component solution. The separating agent is a thin membrane which can be viewed as a molecular sieve, characterised by a pore size which will allow transport of solvent and lower molecular weight species, and prevent passage of higher molecular weight solutes or macromolecules. The membrane often consists of an active, very thin layer (0.01 - 0.02nm) and a porous support (about 10nm) (Erikson, 1974).

According to Lonsdale (1972) pressure driven membrane separation processes have been known for over a century and until the mid-1950's the term ultrafiltration was generally used to describe such processes when applied to liquids. At about that time it was recognised that at pressures in excess of the osmotic pressure, water could be demineralized by passage through a semi-permeable membrane, and the term reverse osmosis came into use to describe this application. The concept of molecular retention or separation effected by permselective membranes had obvious appeal as a non-denaturing method of fractionation since no change in species state is occasioned during partition (Blatt, 1976). Unfortunately, the early membranes, amorphous in composition, were plagued by extremely low flow rates, tendencies toward clogging and relative non-selectivity (Blatt, 1976). Accordingly, a new membrane technology resulted and led to the development of the anisotropic cellulosic structures by Loeb and Sourirajan (1962).
2.2 REVERSE OSMOSIS (RO) AND ULTRAFILTRATION (UF)

Reverse osmosis and ultrafiltration are similar and related membrane processes since both use a semi-permeable membrane as the separation medium and applied hydraulic pressure as the driving force to achieve separation. Both processes utilize the ability of certain membranes to discriminate between molecules on the basis of size, shape and/or chemical structure (Donnelly et al, 1974). According to Perry and Chilton (1973) reverse osmosis, or ultrafiltration, separates a solute from a solution by forcing the solvent to flow through a membrane by applying a pressure greater than the normal osmotic pressure. The reverse osmosis is generally used to describe the processes in which the solute molecules are of about the same size as that of the solvent molecules, whereas ultrafiltration is used for the separations involving solutes whose molecular dimensions are ten or more times larger than those of the solvent and are below 0.05 nm (0.5 µ) size. Such a separation process based on pressure difference across a membrane, according to Perry and Chilton (1973), combines technical simplicity with versatility. Unlike distillation and freezing processes, it can operate at ambient temperature without phase change.

There are important differences between reverse osmosis and ultrafiltration which lead to different equipment process conditions and applications for each of these two membrane processes. The
basic difference being the type of membrane used — specifically, the permeability of the membrane. The property governs the size of particle which will be allowed to permeate through the membrane, and those which will be retained or rejected by the particular membrane (Donnelly et al, 1974).

Figure 3a (page 41) is diagramatic representation of osmosis and two membrane separation processes of reverse osmosis and ultrafiltration. The left hand illustration is a typical osmosis system while the right hand section (top) represents both Reverse osmosis and ultrafiltration. The right hand section (middle) illustrates a typical reverse osmosis and it is based on the use of a 'small pore' or tight membrane. According to Donnelly et al (1974) such a tight or retentive membrane has pore sizes of about 0.4 nanometre, is impermeable to even small molecules or solutes (e.g. salts, sugars, simple acids etc.) and only allows the passage of the solvent normally water, through the membrane is described as the permeate or hyperfiltrate whilst the material which is retained or rejected by the membrane is referred to as the concentrate or retentate. In reverse osmosis applications, both macromolecules and solutes (e.g. salts) are retained in the concentrate. Hence, reverse osmosis is a term applied to the membrane separation of low molecular weight solutes with molecular weights generally less than 500, from water.
The right hand section of Figure 3a (bottom) shows a typical ultrafiltration application, based on the use of an 'open pore' or 'loose' membrane with pore sizes, according to Donnelly et al (1974) above 2 nanometre. Figure 3b (page 42) shows the diagramatic principle of vertical flow (i) and tangential flow (ii) during ultrafiltration. This loose membrane allows the permeation of small solute molecules (e.g. salts) as well as water, but retains or rejects relatively high molecular weight solutes or macromolecules (e.g. proteins, natural gums, polymers) and colloidally dispersed substances, such as clays, pigments etc. Again, the material retained by the ultrafiltration membrane is called the retentate, while the material passing is either termed the permeate or, more specifically, the ultrafiltrate. Ultrafiltration is thus applied to the membrane separation of relatively high molecular weight solutes, with molecular weights generally above 1,000 from the solvent, which is normally water.

By proper membrane selection, it is possible to concentrate, purify and/or fractionate any solution, the sole energy requirement being the compression energy of the feed solution. Because all solutes are retained by the tight (reverse osmosis) membrane, the osmotic pressure of the feed solution becomes an important parameter. Not alone does one have to overcome the osmotic pressure of the initial feed solution during reverse osmosis, but this initial osmotic
pressure will increase greatly during the concentration process. In practice, operating pressures of $28 - 84 \text{ kg/cm}^2 (400 - 1,200 \text{ psi})$ are normal for reverse osmosis, depending on both the osmotic pressure of the feed material and the degree of concentration required. Conversely, ultrafiltration is associated with very low operating pressures in the range of $0.7 - 17.5 \text{ kg/cm}^2 (10 - 250 \text{ psi})$.

In reverse osmosis based on the use of tighter membranes than ultrafiltration, practically all the substances in solution are rejected or retained by the membrane and thus concentrated. Hence, reverse osmosis can be used for concentration applications. In practice, there is a very small loss of salts and other low-molecular weight compounds, such as non-protein nitrogen, simple acids (e.g. lactic acid) and even some sugars (e.g. lactose). A final operational difference between reverse osmosis and ultrafiltration is that the permeation or flux rate increases linearly with pressure in reverse osmosis, whereas it is pressure-independent in the case of ultrafiltration (Donnelly et al, 1974). Table 9 (page 40) summarises the differences between reverse osmosis and ultrafiltration.
TABLE 9
Differences between reverse osmosis and ultrafiltration  *Data from Delaney, Donnelly & O'Sullivan (1973)

<table>
<thead>
<tr>
<th>Property</th>
<th>Reverse Osmosis</th>
<th>Ultrafiltration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size of solute retained</td>
<td>Molecular weights generally less than 500</td>
<td>Molecular weights generally over 1,000</td>
</tr>
<tr>
<td>Osmotic pressure of feed solutions</td>
<td>Important; can range to over 70 kg/cm² (1,000 psi)</td>
<td>Generally negligible.</td>
</tr>
<tr>
<td>Operating pressures</td>
<td>Greater than 7 kg/cm² (100 psi) up to 84 kg/cm² (1,200 psi)</td>
<td>Low – 0.7 kg/cm² (10 psi) to 7 kg/cm² (100 psi).</td>
</tr>
<tr>
<td>Nature of membrane retention</td>
<td>Diffusive transport barrier; possibly molecular screening</td>
<td>Molecular screening</td>
</tr>
<tr>
<td>Chemical nature of membrane</td>
<td>Important in affecting transport properties</td>
<td>Unimportant in affecting transport properties, as long as proper pore size and its distribution are obtained.</td>
</tr>
</tbody>
</table>
FIGURE 3a

OSMOSIS

Water passes through the semipermeable membrane to cause dilution of solution until osmotic equilibrium is achieved.

DIAGRAMATIC PRINCIPLE OF REVERSE OSMOSIS (RO) AND ULTRAFILTRATION (UF).

PRESSURE IS USED TO DRIVE WATER FROM SOLUTION. IN MANY CASES SELECTED SOLUTES ARE ALSO DRIVEN FROM THE SOLUTION.

RO.
~ 3450 - 10345 kPa

UF.
~ 70 - 690 kPa
FIGURE 3b

(i.)

(ii.)

DIAGRAMATIC PRINCIPLE OF VERTICAL FLOW (i) AND TANGENTIAL FLOW (ii) DURING ULTRAFILTRATION.
Ultrafiltration is emerging as an attractive method for concentrating biologicals on a large scale. It has been used for concentrating proteins in solutions as well as fine particles of protein aggregates which could only be separated by ultracentrifugation (Charm and Lai, 1971). Muller (1972) outlined the approach being taken in the collaborative research programme and emphasised the potential role of reverse osmosis and ultrafiltration. Among relevant references to reverse osmosis and ultrafiltration, Sourirajan (1970) reviews the history and properties of cellulose acetate membranes. The book edited by Lacey and Loeb (1972) includes Chapters on theory and practice, costs and applications. Fenton - May (1971) and Fenton - May et al (1971) give a good account of the basic principles and behaviour of reverse osmosis and ultrafiltration systems as well as describing their potential applications to whey. General accounts of developments in this field and of potential applications to whey are given by Donnelly (1971), Dunkley (1971), McDonough (1971), O'Sullivan (1971) and Porter and Michaels (1971-72).

Reports on their applications to animal blood are given by Chicago Daily News (1969), Delaney and Donnelly (1973), Porter and Michaels (1971), Delaney et al (1975); and Nelsen (1969) reported that the UM-10 and PM-30 membranes are completely retentive for blood
proteins, while completely permeable to known blood toxins like inulin of urea.

Osmosis. Reverse and Ultrafiltration membrane systems have been used commercially for a number of years to profitably process cheese wheys (Schitzer, 1972; Horton, 1974; Crocco, 1975; Anon, 1975) simultaneously recovering marketable products and reducing pollution loads (Lawhon et al., 1977). Investigations into the use of these techniques for processing both Soy wheys (Goldsmith et al., 1972) and cotton seed wheys have also been conducted (Lawhon et al., 1973, 1974, 1975, 1976).

2.4 MODELS FOR MEMBRANE TRANSPORT

There are two models that can be considered (e.g., Banks and Sharples, 1966; Brian, 1966; Lonsdale, 1972) viz:-

i. diffusion through a non-porous layer and

ii. passage through pores or channels in an otherwise impermeable layer.

It is possible that transfer may occur by a combination of (i) and (ii) (Evans and Glover, 1974).

According to solution - diffusion model, the only way that molecules can pass through the membrane is for each molecular or ionic species to dissolve in the material of the membrane and to be carried through
it by diffusion. The components of the solution will have different solubilities in the membrane, and the membrane will be selective. Diffusion through the membrane will also be a property of each component of the solution relative to the material of the membrane; this again affects selectivity.

The pore model on the other hand, assumes that the membrane is porous - there are continuous channels, though extremely fine ones, through the filtration layer. The membrane must be selective towards the solvent - it must not allow the solution to flow through it undiluted. It may be selective on molecular or ionic size while permeation through certain synthetic polymers may involve chemical affinities between the membrane, the solute and the solvent. In both cases there will be flow of liquid through pores and additionally diffusion of solute through the solvent within pores, so that:

\[
\text{Solute flux} = \text{Solute carried by solvent and solute diffusing through solvent in pores.}
\]

A solution-diffusion mechanism is favoured by (Lonsdale et al, 1965, 1966, 1967, Riley et al, 1967), whose transport equations are apparently limited to their concepts of 'perfect' membranes, which are presumably those which have a completely nonporous surface structure (Riley et al, 1967). Banks and Sharples (1966a, 1966b, 1966c) also consider that the mechanism of RO is one of diffusive flow through the pore-free layer on the membrane surface. According to Michaels

2.5 DIAlFILTRATION

Addition of fresh deionised water or solvent (dialysate) to feed solution from which solvent and micro-constituents are ultrafiltered, is referred to here as diafiltration. This in essence, effectively washes the retentate. In contrast, to conventional dialysis the rate of microspecies removal is independent of their concentration but is simply a function of the UF rate relative to solution volume.

2.6 CONCENTRATION POLARISATION

According to Blatt et al, (1969) concentration polarisation is the accumulation, at the upstream surface of an ultrafiltration membrane, of solute molecules which are rejected or retained by the membrane in the course of ultrafiltration. At the outset, concentration polarization always operate to reduce the efficiency and/or rate of an ultrafiltration process. He observed that membranes which display the capacity to retain rather small solute molecules (i.e. those of molecular weight under about 500, or of molecular diameters under about 1 nanometre) generally transmit solute and solvent mole-
molecules via molecular diffusion within the membrane matrix. The solvent flux through this "diffusive" type of membrane is directly proportional to the "effective pressure difference" across the membrane. On the other hand, the rejection efficiency of the membrane increases hyperbolically with increasing pressure difference. If the solute-permeability of the membrane is sufficiently small relative to its solvent-permeability, the rejection co-efficient reaches values approximating unity at quite low pressures (Blatt et al, 1969).

Ultrafiltration membranes which display retention for only relatively large solute molecules (those of molecular weight in excess of 500, or of molecular diameters above 1 nanometer \(10^{-9}\) ) appear to function as molecular sieves or screens, solvent flowing in viscous flow through micropores in the membrane, and solute molecules being carried convectively with solvent only through the pores large enough to accommodate them. For such membranes, the solvent flux is thus linear in the hydraulic pressure difference, and the rejection co-efficient is essentially constant and pressure-independent (Blatt et al, 1969).

Concentration polarisation arises in both reverse osmosis and ultrafiltration separations but the higher solute concentrations normally encountered make the problem more acute with ultrafiltration (Brener et al, 1976). This behaviour is, therefore, typical of solu-
tions of macromolecules. In membrane separation systems, solvent is being taken out of solution preferentially at the membrane surface, so that the layer of solution adjoining the membrane may be much more concentrated than the feed solution in bulk (Blatt et al, 1969).

Considering the flux of solvent, the difference in osmotic pressure is increased over the value for the bulk feed solution, so that either solvent flux is reduced if pressure is maintained constant, or pressure (and hence the power required) is increased to maintain the flux. Solute flux is also increased. Both effects are working to reduce the efficiency of the process, causing the permeate to have higher than optimum solute content (Evans & Glover, 1974). The increased concentration of solute sets up a diffusion process by which solute is carried away from the membrane.

According to Evans & Glover (1974) whether flow conditions are turbulent or laminar, concentration polarisation may be reduced by:

(a) increasing the feed flow rate
(b) decreasing the tube or module diameter or the separation of plates
(c) using short flow paths - use of shorter tubes, channels etc.
(d) increasing the temperature of the feed to decrease viscosity and increase the rate of diffusion of solute away from the membrane
In summary, solute polarization is a natural consequence of ultrafiltration through a membrane displaying solute-retention. The effects of polarization upon "diffusive" and "microporous" membranes - despite the differences in their inherent transport mechanisms - are to reduce flux and/or reduce solute-rejection efficiency. Minimization of polarization is thus a necessary and important objective to achieve maximum performance in ultrafiltrative separation.

2.7 POWER REQUIREMENTS IN REVERSE OSMOSIS AND ULTRAFILTRATION

According to Evans and Glover (1974), there are three main forces to overcome in reverse osmosis:

(i) the opposing osmotic pressure, and due to concentration polarization the effective osmotic pressure is much greater than that in the main body of the solution

(ii) viscous forces in the flow of solution over the membrane surface

(iii) forces required to push the solvent through the membrane.

Concentration polarization may be reduced by creating high rates of shear or turbulence which reduces the osmotic pressure but involves higher viscous forces (Evans and Glover, 1974). At low feed velocities concentration polarization tends to be excessive, so that the effective osmotic pressure is large and hence the pumping power
required to achieve the specified permeate flux will be high. If higher feed velocities are employed to reduce the concentration polarization more power is required to maintain these velocities. There is therefore an optimum set of conditions for any flux rate, membrane and geometrical arrangement, such that power requirements are a minimum.

2.8 ECONOMICS AND ADVANTAGES OF MEMBRANE SYSTEMS

There are still a number of uncertainties in trying to pinpoint long term capital and operating costs of these membrane systems. It can be said, however, that RO and UF, even at this stage of their development, have an economic advantage over competing unit operations for both the concentration and fractionation of whey (Fenton-May et al, 1972). For whey concentration, the competing operation is thermal evaporation. RO can only be used to concentrate whey to 20 to 24% solids. Consequently, to concentrate from this level to that required for spray drying (usually 40 - 55% solids), thermal evaporation will have to be used. Up to 24% solids, RO definitely appears to be more economical than evaporation (McDonough and Mattingly, 1970), even if one takes into account the high membrane replacement costs prevailing today (Fenton-May et al, 1972).

For whey fractionation, competitive unit operations are electrodialysis or ion-exchange (for demineralization), followed by concentration and
lactose crystallization, or gel permeation chromatography, followed
by concentration of the products thus obtained. While both these sets
of operations have their proponents, ultrafiltration would appear to
have the advantage of simplicity and probably too, an advantage in
operating costs (Fenton-May et al, 1972)

Harrison (1970) listed the advantages of membrane systems as:
(a) No net phase change occurs.
(b) No heat is required hence heat sensitive materials remain
unharmed and lower energy costs will be incurred.
(c) No large quantities of condensate cooling water are required
as with distillation.

Some other advantages of RO, according to Harrison (1970) over
traditional methods in food processing include the following

(i) **Product improvements possible:**
(a) Thermal damage to delicate flavouring compounds can be
eliminated.
(b) Storage life and quality increased.
(c) Removal of contaminants such as salts.
(d) Sterilisation of permeate solutions by micro organism retention.
(e) Sweetness enhancement by acid removal.
(f) Clarification or colour reduction enhances appearance of per­
meates.
(ii) **Product handling improvements**

(a) Reduction of liquid volume yields lower packing, freezing, storing, handling and transportation costs.

(b) Multiple processes may be replaced by a single process type.

(c) Continuous automated processing.

(d) Lower Energy requirements in comparison with thermal evaporative processes.

(iii) **Water Treatment:**

(a) Low grade water made suitable for process water.

(b) Low pressure boiler-feed water can be softened directly without chemicals.

(c) Effluent water can be recovered.

(d) Effluent water impurities may be concentrated for easier disposals.

(e) Effluent sludges may be thickened in some cases.

In summary, the main advantage of membrane fractionating systems over the conventional processes is that no heating and no phase change in product are involved. Another feature of UF is that it has lower operating costs (with the critical limitation of a 25 per cent solids concentration ceiling) than say evaporation, dehydration or drying processes (Donnelly et al, 1974).
2.9 FRACTIONATION OF BLOOD PLASMA PROTEINS BY ULTRAFILTRATION

Van Oss and Bronson (1970) reported that the majority of human blood serum (non lipoprotein) proteins exist in only three discrete sizes: roughly 70 per cent are of the size of serum albumin, with a molecular weight around 70,000 (sedimentation coefficient 4S); some 25 per cent, the globulins, have a molecular weight of 160,000 (7S); and about 5 per cent, the macroglobulins, have a molecular weight of 860,000 (19S). There are also small amounts of microglobulins (smaller than albumin), some globulins intermediate in size between globulins and macroglobulins (m = 3000,000; 10S), and some supermacroglobulins of a molecular weight of several millions (23S, 28S, 33S etc.).

Van Oss and Bronson (1970) observed that membranes that stop all proteins have been routinely available, since at least the late 1940's, in the form of cellophane sheets and tubing. These (homogenous) regenerated cellulose membranes are quite reproducible, have a uniform pore size almost exactly calculated to stop all blood serum proteins, are generally available in large quantities, and are quite cheap. They noted, however, that their sole drawback is the flow rate that can be attained with them, which is about 3 ml/hour/100 cm² membrane/207 kPa, when ultrafiltering a 0.7 per cent protein solution.
The discovery by Loeb and Sourirajan of anisotropic ("shinned") membranes in 1962 was a great advance because, although these membranes were initially mainly intended to retain salt by reverse osmosis, their principle was soon extended to more porous membranes which could ultrafilter the solvent of diluted protein solutions at flow rates of several hundred ml/hour/100 cm$^2$ membrane/207 kpa. Very high flux anisotropic cellulose acetate membranes can, now be made which retain all normal plasma blood proteins. A membrane of this type makes the concentration of about a liter of a dilute protein solution down to only a few milliliters a matter of only a few hours without any protein denaturation (Van Oss and Bronson, 1970).

It was found that the optimal pressure for concentrating proteins with this membrane is close to 207 kpa. The optimal thickness of the membrane is 0.15mm and under the scanning electron microscopy the thickness of the actual protein stopping skin is of the order of 1 micrometre supported by the very porous coarse bottom. The membrane always has the highest flow rate when it has never been dried and is conserved in cold water from its inception on (Van Oss and Bronson, 1970).

2.10 EFFECT OF OPERATING PRESSURE ON UF

An ideal ultrafiltration membrane would give 100% retention of proteins and no retention of lower molecular weight solutes. Fenton-May (1971), Fenton-May et al (1971), Pepper (1971) have shown that increasing
pressure can increase retention of the lower molecular weight solutes and that, as pressure increases, a limiting value for permeation rate is reached because of the effects of pressure on concentration polarization or formation of deposits which change membrane characteristics. Goldsmith et al (1970) referred to a gel layer of a high molecular weight solute on the membrane acting as a "dynamic rejecting membrane" for lower molecular weight solutes and recommended operation at low pressure to improve fractionation efficiency.

2.11 EFFECT OF OPERATING TEMPERATURE ON ULTRAFILTRATION

Fenton-May et al (1971), and Lacey and Loeb (1972) have shown the relationship between water permeation (flux) rates and temperature to correspond to an Arrhenius – type activation energy of 21–25 kilojoules per mole representing about 3% change in flux per °C over the temperature range investigated. Forbes (1972) points out that for hydrophilic solutes the effects of temperature can be greater as a result of the decrease in viscosity. This is borne out by the results of O'Sullivan (1971) who reported a doubling of flux rate for whey as temperature increased 20°C. To avoid microbial spoilage in experiments of long duration it would be desirable to operate either at low temperature or a temperature high enough to be unfavourable to most species. An upper temperature limit would be imposed by membrane stability and by possible denaturation of proteins. Forbes (1972) suggested 45–55°C as suitable compromise.
Evans and Glover (1974) reported that water flux increases as the operating temperature increases and that three parameters are involved, solubility, diffusion and viscosity, the effects of which may be summarised as:

(i) an increase in the solubility of solvent and solute in the membrane, and of solute in the feed
(ii) an increase in the diffusion of solvent and solute in the membrane and feed,
(iii) a decrease in the viscosity of the solvent and of the feed;

(i) leads to an increase in permeation rate due to increased uptake in the membrane, less possibility of fouling of the membrane due to precipitation by exceeding solubility limits, but an increase in concentration polarisation due to increased solute in solution;

(ii) causes increased membrane transport and also a reduction in concentration polarisation since solute will diffuse more easily away from the membrane.

A reduction in viscosity (iii), facilitates pore flow and also the flow of the feed solution and hence the dispersion of material away from the membrane.
2.12 TEXTURISING PROTEIN SOLUTION

Animal blood plasma, like any other protein solution can be texturised using technological processes such as thermosetting, extrusion or spinning. The sole aim in these processes is to achieve some desired textural characteristics.

In thermosetting, heat is generally applied to cause irreversible thermal coagulation. The rate of heat addition is always greater than the rate of thermal diffusivity within the system and the difference must be sufficient to provide for heat of coagulation. In this process, temperature, time and pH seem to be the main processing variables so that the degree of manipulation is somewhat restricted. In thermoplastic extrusion, however, protein solution is fed to a cooker-extruder, where it is subjected to heat and pressure for a pre-determined time. That is, thermal energy and shear is applied at the same time to enhance the intermolecular alignment by stretching and bringing the molecules together and supplying the heat of fusion.

An additional processing variable is the rate of shear in extrusion compared with thermosetting.

Most interesting as far as this study is concerned is spinning and, in particular, "wet-spinning" in the case of edible protein. Here a moderately viscous protein solution is passed through the fine holes of a spinnerette immersed in a coagulating medium where the fresh plastic
filaments are stretched after coagulation to obtain well orientated, and partially elastic fibres. The processing variables involved in this latter process can be complex but in addition to temperature and shear through the fine holes, temperature, composition and concentration of the coagulating solution, extent of stretching and residence time in precipitating solution, and viscosity of the spinning dope are important. These large numbers of processing variables make it a very flexible process although the mechanisms involved are rather complicated.

2.13 DEVELOPMENT BACKGROUND OF TEXTURED MATERIALS

The development of textured protein materials is based on modifying the physical characteristics of the protein to be used in order to resemble those of meat. According to Wrenshall (1951), modifications needed depend on the characteristics of the original proteins, their method of extraction or isolation and treatment before use and the characteristics desired in the final product. Much work has been done in the production of textured materials and the types of protein materials used from various sources have included soya, casein, milk powder, peanut and wheat glutens (Anson & Pader, 1957, 1958, 1959; Kende & Ketting, 1959; MacAllister & Finucane, 1963; Wrenshall, 1951) as well as safflower, zein and keratin.
The first step in the production of such simulated meat products is the preparation of fine filaments from the protein materials. These filaments are then treated with binders, shaped and sized to desired meat products. Wrenshall (1951), used skim milk solids as the basic matrix in the simulation of several types of comminuted meat preparations such as country sausage, bologna, salami, and hamburger. The process involved mixing texturising agents which were mainly of cereal origin, with an aqueous suspension of skim milk solids and heating until coagulated. The product was reported to possess the texture, flavour and appearance of comminuted meat.

Anson & Pader (1957, 1958, 1959), used the gelling properties of proteins extracted from such materials as soya, casein, and peanut to simulate meat. The steps included separating the protein, adjusting the pH, adjusting the protein content of the suspension to form a gel precursor and, by subsequent appropriate treatment, usually including controlled heating, forming discrete particles of chewy gel. It is claimed that when pieces of substantial size are chewed in the mouth they have the physical properties of resilience, elasticity and resistance to shear.

Kende & Ketting (1959), used pH to control the characteristics of casein so as to approximate to those of meat. The product which they called "milk meat" is prepared by using 30 to 40 parts of cereals,
fat, flavouring and adjusting the pH to 4.9 - 5.2 and moisture content to 55 - 60%. MacAllister and Finucane (1963), used soya flour and wheat gluten together with minor amounts of albumen, starch and meat flavour to form dough which is extruded and dried to the desired shape. When cooked in water the granules develop a flavour and chewy texture which is characteristic of cooked meat. These granules are said to be suitable for such entrees as meat patties, hamburgers and sausages.

2.14 PRODUCTION OF FIBROUS PROTEIN BY "WET-SPINNING"

The general method of producing protein fibres from any protein source by wet-spinning can be briefly summarised as follows:-

(a) extraction or isolation and purification of the protein material
(b) dissolving the protein suspension in a strong alkaline medium to yield a more viscous dispersion.
(c) extrusion of the viscous material through fine holed spinnerettes into a coagulating bath
(d) applying a stretching force from the pulling action of the take-off reel in order to improve orientation and strength of the newly formed filaments
(e) setting up of fibres and final processings
2.15 MODES OF FIBRE FORMATION

In regenerating peanut, Zein, and casein protein fibres, the mechanism is believed to be that globular proteins are denatured to random coil and then transformed into extended Beta-configuration by stretching (Brown and Menkart, 1963).

In studying fundamental flow behaviours during spinning, Ziabicki (1959) applied the following scheme of orientation mechanisms as a model for his mathematical treatment, namely, streaming orientation in elongational flow, and deformation orientation of elastic network. He believed that fibre orientation is developed gradually in the course of free jet flow, rather than being predetermined during extrusion. Such an orientation can be due to either of two further mechanisms: Streaming orientation in the elongational flow, or deformational orientation. Furthermore, Ziabicki (1967) stated that the extent of streaming orientation of spinning fluid emerging from the spinnerette would increase with the output rate, reciprocal radius of the channel and channel length.

Kelly and Pressey (1966) suggested a schematic diagram for the fibre formation of the soybean protein.
2.16 Production of Texturised Protein Products from Various Protein Sources

The spinning process was first used to convert globular protein into fibrous form by Boyer (1953) using a modification of the system for spinning textile fibres. The purpose of this fabrication was to make food materials more appealing to the consumers (Pyke, 1970). To convert natural, non-fibrous protein into fibrous form, a series of treatments must be made. The treatments change the intramolecular stereochemistry and develop intermolecular structural arrangement of the polypeptide in the protein chains. Unfolding of peptide chains followed by reorganising to more aligned and cross-linked state, which imparts higher degree of physical strength, is the basic principle of the process (Huang and Rha, 1974).

Boyer (1954) obtained a dispersion or colloidal solution of spinnable protein by dispersing an oilseed protein isolate of 95 to 98% purity in an aqueous alkaline solution. The latter was a 5 to 10% by weight solution of NaOH and the preferred concentration of the protein was 10 to 30% by weight. The pH was between 9 and 13.5. He observed the viscosity of the spinnable dope to be between 10,000 and 20,000 mPa.s and the temperature between 35 and 45°C. He devised in 1954 a continuous process in which "maturing" of the protein solution occurred in 10 minutes instead of 60 minutes in
the previous experiment. He found that by controlling certain variables it was possible to form a spinnable solution in the viscosity range of 2.5 – 40 Pa.s, in a continuous stream. Boyer used a spinnerette with approximately 5,000 – 15,000 holes, each of the order of 0.01182 mm. in diameter and an acid-salt bath of between 0.5 and 10% by weight of acid and salt concentration in between 0.5 and 12%.

Giddey (1960) invented a method of producing artificial fibres consisting predominantly of protein and at least one polysaccharide having acidic side groups such as carrageenans, alginates etc. The resulting viscous solutions (0.05 – 2 Pa.s) were then spun horizontally through a glass spinnerette, having 400 holes of 0.2 mm. diameter, into a coagulating bath consisting of an aqueous solution of 100g/litre CaCl₂·6H₂O adjusted to pH 1.3 with hydrochloric acid, the coagulating bath being at room temperature.

Westeen and Kuramoto (1964) prepared texturised protein product using an extrusion device through a small die having 1000 to 16,000 holes each of the order of 0.051 – 0.15 mm. in diameter. They employed a continuous process for producing fibres from proteins and the solids content of the protein solution was in the range of 10 to 35% by weight.
Dechaine and Callaghan (1967) invented an apparatus for processing protein fibre from protein sources such as soya, corn, peanut, casein and keratin. The viscosity and temperature of such dispersions was within the range of about 10 to 20 Pa.s and about 35 to 45°C respectively.

Okumura and Wilkinson (1970) texturised a defatted concentrate of vegetable proteins from seeds such as soya bean, sesame, cotton seed, alfalfa etc. by a freezing step into a fibrous, sponge-like protein mass lacking the taste of soya or other starting material.

Heden et al (1971) reported on the spinning of heat-denatured bacterial protein (heating by a microwave unit at 80°C) through capillary tubes in the range 0.1 to 0.6 mm. diameter. They observed that capillary tubes gave better fibres than spinnerettes of various kinds in a coagulating bath of a mixture of water and 0.7% propionic acid.

Anker and Burchill (1972) prepared fibrous protein products by extruding plastic masses comprising keratin protein directly into a gaseous medium and then elongating the resulting extrudate.

Young and Lawrie (1974) produced edible protein from blood plasma by forcing the viscous dope (about 15 Pa.s) through a stainless steel
spinnerette immersed in the coagulating solution and containing 40 holes, each of 0.008 cm. diameter. The coagulating solution was 11% Na$_2$SO$_4$ or 20% NaCl in 1N acetic acid.

Rosenfield (1974) prepared fibrous protein products by suspending soya protein isolate in water to a solids content of about 20% by weight followed by raising the pH to a desired level and precipitating the extremely viscous mass obtained through the spinnerette immersed in a coagulating bath at its isoelectric point (pH 4.6). He used spinnerettes containing 15,000 holes each of 0.1016 mm. diameter.

Jaynes and Asan (1976) extruded concentrate of whey protein into the coagulating bath through 20 stainless steel hypodermic needles with a squared tip. The composition of the coagulating solution was 12% glacial acetic acid and 12% NaCl in distilled water. They worked with spinning solutions containing 1:1 ratio of whey protein concentrate and detergents in the concentration range of 20 to 30% total solids, 0.5% 2-mercaptoethanol and a pH of 9.0–11.0.

2.17 Rheological Properties of Protein Dispersions prepared as Spinning Dopes

The rheological properties of spinning dopes are one of the most important physical parameters to be considered in the spinning process and quite often, the viscosity is a critical factor in spinning
(Huang and Rha, 1971). This view was also held by Kelley and Pressey, (1966). They reported that the suitability of a dope solution for spinning fibres is determined by its viscosity, which increases rapidly after alkali and protein are mixed to form a spinning mass. Lundgren (1949) cited the necessary minimum chain length or degree of polymerization of the molecules for fibre formation.

Thompson (1946) found that the highest viscosity short of gel formation gives the best spinning conditions. Rha (1971) observed that it is fundamentally necessary for a dope to have some minimum, internal resistance to deformation in order to remain as a distinct and continuous filament without complete dispersion or breakage after emerging from the spinnerette.

Studies on rheology of the alkaline protein dispersion have been concerned mainly with their fluidity and conditions suitable for non-gelation which may occur at protein concentrations higher than 12% and at high alkaline pH's above 12.0. The apparent viscosities for the spinning dopes used mainly for spinning by various workers included 18-45 Pa. s reported by Preston (1953), 10-20 Pa. s, 5-200 Pa. s and 5-100 Pa. s noted by Dechaine and Callaghan (1967); - Morgan (1962), and Elmquist (1965) respectively.
Circle et al (1964) noted that the viscosity of unheated soy protein dispersions rises exponentially with increase in concentration—a behaviour typical of macromolecular dispersions. The same result holds for the heated gels. They observed that the irreversible heat-setting of sodium soy proteinate dispersions to a gel state is a heat-denaturation phenomenon practically by definition. At concentrations of 6% and lower, gelation does not occur, although there is a small increase in viscosity, while the gel rigidity on heating is primarily dependent on concentration.

To bring about the increase in viscosity of spinning solution Ferry (1948) observed that gels of denatured proteins involve first an unfolding or extension of the globular protein into a more asymmetric shape, the protein backbone chain assuming a more or less extended and irregular form, and exposing reactive groups and also non-polar groups during the unfolding, making them less hydrophilic. Association of the chains by cross-links spaced some distance apart, and also by localized and nonlocalized attractive forces (including hydrogen bonding), lead to the formation of a three-dimensional network throughout the sample, in which the "gel-fraction" approaches unity, and in which rigidity is enhanced by further cross-linking taking place during the heating. The irreversibility of the soy proteinate heated gels, however, indicates that primary covalent bonds are involved in the cross-linking.
2.18 Coagulation by "Wet-spinning"

The formation of fibres by wet-spinning proceeds by a very complex mechanism, which involves a combination of rheological and diffusional phenomena that are not altogether understood individually (Paul, 1968b). Studies of the wet-spinning process have been carried out (Craig et al., 1962; Knudsen, 1963). Fitzgerald and Craig (1966) discussed the extrusion of the spinning solution through the spinnerette holes and mentioned briefly the behaviour of the filament in the bath.

During wet-spinning a very viscous polymer solution is extruded through the small holes of a spinnerette immersed in a liquid bath. Paul (1968a) studied the mechanism of coagulation and explained that a diffusional interchange between the freshly formed fluid filaments and the bath solution causes the polymer to solidify. It follows that during coagulation one or more of the bath components diffuse into the filament, while the solvent diffuses out of it. The polymer precipitates or crystallises as a consequence of this exchange, because it is rendered insoluble by chemical reaction of the polymer or by an excessive build up of non-solvent or by both.
2.19 Mechanical Treatment of Fibres

In the spinning process, pressure and shearing forces are introduced to enhance the temperature effect and to align the extended chains for the formation of crystalline regions in filament (Peters, 1963; Hearle and Peters, 1963; Lundgren, 1949). Increasing shear is believed to be favourable to the disentanglement of polymeric coils (Ram, 1967). Moreover, it is believed that, by extruding soybean protein dope through fine spinnerettes, the polypeptide chains can be brought close together favouring the hydrogen and ionic bonding (Brown and Menkart, 1963; Kelley and Pressey, 1966). However, the flow created by extruding the spinning mass through the capillary is believed (Roberts, 1960) to lead only to a preliminary fibre structure. Further treatment, such as stretching is necessary to produce a fibre with more desirable properties.

Mechanical orientation by stretching the fibres formed is believed to lead the filament to a more uniform, organized alignment in the direction of fibre axis (Lundgren, 1949), or to increase the crystallinity in a polymer (Odian, 1970). This type of orientation often brings changes in mechanical or physical properties to the fibre.
Controlling the Texture of Spun Fibres

According to Ziemba (1969) textures are varied according to the degree the protein's molecules are oriented during processing. Some orientation stems from molecular alignment while spinning dope is being forced through the spinnerette's orifices. He also reported that additional orientation is by roll-stretching the plastic-like extruded protein fibres. He observed that highly oriented fibres have a more meat-like texture and chewiness than those less oriented. Fibre's texture is also controlled by pH-adjustment of coagulating solution (U. S. Patent 2,730,448). Stretching and heating imparts tensile strength, toughness, some elasticity and overall contributes to the needed chewiness (Kinsella, 1977). The texture of fibre according to (Kinsella, 1977) is influenced by composition and purity of protein, modification if any, extrusion pressure, acidity of coagulating solution, diameter of fibre, degree of stretching of the fibre and tow, the nature of binders and fat, extent of heating. It has been shown in the prior art of producing man-made textile fibres that physical properties such as strength and elasticity are greatly influenced by the degree of molecular orientation, and similarly found that the toughness or tenderness of the synthetic meat product is also affected by an orientation of the protein molecules (Boyer, 1954). Another method for controlling toughness is by the use of hardening or insolubilizing chemicals. For instance, soaking the fibre in a brine of sodium chloride of any concentration up to a saturated
solution will have a toughening effect (Boyer, 1954).

2.21 Measurement of Textural Properties

The response of fibres to stresses and strains can be a major factor in determining their usefulness in products made from them. Hence, a method for the quantitative measurement of plasma fibre texture is needed in order to understand and control the variables involved in texturising plasma proteins.

Measurement of the textural properties of foods is essentially an exercise in determining the strength (i.e., tenderness) of the materials from the force-distance/time curve obtained under controlled conditions, although, perhaps, weakness of materials might be a better description since most foods are preferred when in a structurally weakened state (Bourne et al., 1966). The texture Profile Analysis originally developed for the General Foods Texturometer (Szczesniak, 1963; Szczesniak et al., 1963) has been a useful method for the evaluation of textural parameters for a wide range of foods (Peleg, 1976).

Bourne (1968) derived a technique by which the texture profile parameters could be evaluated from the force-deformation curves obtained by the Instron Universal Testing Machine. Though the magnitude of these parameters is influenced by experimental variants (such as the deformation rate) they can provide objective and comparative information on the textural properties if obtained
under standardised conditions (Peleg, 1976). The simplicity of carrying out the test procedure by the Instron and other universal testing machines made this texture profile analysis procedure widely used.

Several investigators have employed an Instron Universal Testing Machine. In observations on spun soya fibres, Stanley et al (1972a) reported that the break elongation of spun soya fibres was much higher than that of cooked meat and that this parameter could be an important determinant in soya fibre texture. The same investigators (Stanley et al, 1972b) showed a relationship between tensile strength of the fibrous structure and temperature of extrusion. Breene and Barker (1973) also utilising the Instron, developed a type of texture profile capable of separating soya protein products into different texture classes. A critical review of the applications of texture profile analysis and the experimental conditions under which it has been carried out can be found in the work published by Breene (1975).

2.22 Tensile Strength of Fibres

Among the various physical properties of fibrous materials, the mechanical properties are the most important from a technological point of view (Mohanaradhakrishnan et al, 1970). The obvious and
indeed the only available method for the evaluation of the mechanical stability of a fibre is the determination of its tensile strength (Hightberger, 1947). It is known, however, that various factors like fibre length, diameter, rate of loading, humidity and mechanical conditioning alter considerably the breaking strength, as also the extension (Morgan and Mitton, 1960).

Stanley et al (1971) (Tables 12 and 13, Page 76) noted that their results on the breaking strength of beef muscle did not resemble that of spun soya fibres (Stanley, unpublished data) and attributed the difference to the fact that the stress-strain curve for muscle reflects other constituents than the fibrous proteins, perhaps the sarcolemma. Cumming et al (1972) employed thermoplastic extrusion to produce texturised soya bean product. Break elongation was calculated as a percentage of the original 2 cm length. The Instron machine was used to apply longitudinal force and to examine tensile properties of the fibres. They found that as temperature increased, breaking strength increased from 425 to 660g/g between 148°C and 175°C followed by a sharp decrease between 175°C and 192°C. Break elongation did not show significant changes due to process temperature. They attributed their observation to the fact that although dramatic changes are taking place in the secondary structure of aligning of fibres, break elongation is more a reflection of primary structure, possibly related to the strength of interpeptide bonds.
Jaynes and Asan (1976) produced fibrous protein from cottage cheese whey. Examination of the physical properties reveals that stretching during formation of fibre reduced fibre diameter and improved tensile strength (Table 10 Page 75) while the unstretched samples showed better elongation (Table 11). The dry finished fibres, when subjected to boiling in water for 30 minutes, retained their fibrous character and absorbed water to the extent of 1.25 times their dry weight.
TABLE 10

Tensile strength of fibrous protein from cottage cheese whey. +

<table>
<thead>
<tr>
<th>Fibre type</th>
<th>Detergent extractant</th>
<th>Conc. of detergent &amp; WPC (%)</th>
<th>Age of spinning solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Stretched</td>
<td>60% Acetone</td>
<td>12</td>
<td>25900a</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td></td>
<td>23200a</td>
</tr>
<tr>
<td></td>
<td>60% Acetone + 5% KCl</td>
<td>14</td>
<td>18700a</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td></td>
<td>25200a</td>
</tr>
<tr>
<td>Unstretched</td>
<td>60% Acetone</td>
<td>12</td>
<td>8400b</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td></td>
<td>9200b</td>
</tr>
<tr>
<td></td>
<td>60% Acetone + 5% KCl</td>
<td>14</td>
<td>8200b</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td></td>
<td>9100b</td>
</tr>
</tbody>
</table>

a Means of two measurements on 10 fibres from each of two replicate lots.

b Means followed by the same letter are not significantly different at the 5% level by Tukey's w-procedure.


TABLE 11

Elongation of fibres. +

<table>
<thead>
<tr>
<th>Fibre type</th>
<th>Detergent extractant</th>
<th>Conc. of detergent &amp; WPC (%)</th>
<th>Age of spinning solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Stretched</td>
<td>60% Acetone</td>
<td>12</td>
<td>1.80a</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td></td>
<td>1.79a</td>
</tr>
<tr>
<td></td>
<td>60% Acetone + 5% KCl</td>
<td>14</td>
<td>1.50a</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td></td>
<td>1.55a</td>
</tr>
<tr>
<td>Unstretched</td>
<td>60% Acetone</td>
<td>12</td>
<td>2.95b</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td></td>
<td>2.68b</td>
</tr>
<tr>
<td></td>
<td>60% Acetone + 5% KCl</td>
<td>14</td>
<td>3.28b</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td></td>
<td>2.15b</td>
</tr>
</tbody>
</table>

+ Jaynes & Asan (1976)
TABLE 12

Effect of muscle type, ageing and post-mortem treatment on physical properties of beef muscle. +

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Breaking strength (g/g)</th>
<th>Break elongation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shank</td>
<td>414</td>
<td>28</td>
</tr>
<tr>
<td>Tenderloin</td>
<td>155</td>
<td>20</td>
</tr>
</tbody>
</table>

+ Stanley et al (1971): Breaking strength of a sample was determined by mounting it in the jaws of the Instron, initially 3.5 cm. apart, and applying force by the downward movement of the crosshead; break elongation or strain required to rupture the sample as a percentage of the original 3.5 cm. sample between the jaws. Diameter of sample less than 0.5 mm.

TABLE 13

Effect of muscle type, ageing and post-mortem treatment on physical properties of muscle. +

<table>
<thead>
<tr>
<th>Ageing period</th>
<th>Post-mortem treatment</th>
<th>Breaking Strength (g/g)</th>
<th>Break Elongation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 hr.</td>
<td></td>
<td>156</td>
<td>34</td>
</tr>
<tr>
<td>1 day</td>
<td>restrained</td>
<td>89</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>unrestrained</td>
<td>115</td>
<td>31</td>
</tr>
<tr>
<td>8 days</td>
<td>restrained</td>
<td>72</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>unrestrained</td>
<td>109</td>
<td>33</td>
</tr>
<tr>
<td>1 day</td>
<td>restrained</td>
<td>133</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>unrestrained</td>
<td>260</td>
<td>21</td>
</tr>
</tbody>
</table>

+ Stanley et al (1971)
2.23 Solubility of Blood Plasma Proteins

Solubility is a very useful index of overall functionality because soluble proteins possess superior functional attributes for most applications (Hermansson, 1973). Several processes such as the use of alkaline processing, application of heat etc. result in some destruction of functional properties. According to Tybor et al (1970) factors affecting the functional properties of proteins are processing treatment and presence of mono- and disaccharides. These factors primarily affect solubility and may have a possible relationship to emulsifying capacity (Tybor et al, 1973).

The solubility of the serum proteins shows only a slight dependence on pH as indicated by the 7% decrease in protein at the point of minimum solubility, pH 4.8; while the solubility of the globin concentrate demonstrates a minimum at pH 6.5, with a 64% difference between the minimum and maximum points of solubility (Tybor et al, 1973). Pennell (1960) studied the solubilities of plasma proteins and observed that the proteins of plasma solution vary widely, not only in their relative solubilities in the isoelectric state but also in the pH region in which they are isoelectric. He also noticed that albumins, and some of the \( \alpha \)-globulins have isoelectric points somewhat below pH 5.0 while \( \alpha \)-globulins are isoelectric above pH 7. There is, indeed, evidence to show that albumin precipitated at pH 4.8 dissociates from some of the small molecules for which it serves as a carrier at normal pH of plasma (Antoniades et al., 1957).
2.24 Heat Denaturation of Proteins

The evaluation of the heat treatment to which proteins may be subjected during processing is essential in indicating their suitability for subsequent utilization. Advantage is usually taken of the heat labile proteins in monitoring heat effects in liquid form and concentrate, by measuring the extent of denaturation of the proteins (e.g. plasma and whey proteins).

According to O'Sullivan (1971) protein denaturation involves any modification of the native protein structure excluding primary covalent bond hydrolysis i.e. changes in primary structure. Thus the denaturation process is confined to alterations in the secondary and tertiary structure of the protein molecule. In the case of globular proteins, this change in molecular configuration involves the unfolding of the $\alpha$-helix structure into a random coil formation. The denaturation process may be reversible (e.g. enzyme reactivation), but is usually irreversible if the treatment is prolonged. Heat is probably the most important of the various physical and chemical denaturing agents. Thus, when a protein molecule is irreversibly heat denatured, it does not assume its original native configuration. Because of this alteration in protein structure on denaturation, many changes occur in the properties of the protein. The major changes manifested in the properties of whey proteins and of milk due to heat – denaturation are depicted in Table 14.
TABLE 14

Major changes in properties of whey proteins and milk on heat denaturation*

1. Decreased solubility at isoelectric point
2. Increased reactivity of sulphydryl groups giving cooked flavour formation, reduced oxidation – reduction potential development of anti-oxygenic properties.
3. Reduced protein digestibility.
4. Resistance to milk clotting by rennin.
5. Lower curd tension
6. Loss of capacity to form cream layer.
7. Increase in reflectance
8. Increased heat stability of milk following concentration.
9. Reduced colloidal stability of milk when frozen.


Among the proteins of milk, the whey proteins, as opposed to the caseins, are very heat-labile and undergo conventional heat-induced denaturation (O'Sullivan, 1971). Denatured whey proteins will coprecipitate with the caseins when the latter are removed from solution by one of two common procedures. The classical method is isoelectric precipitation (Rowland, 1938) at pH 4.6 with acetate
buffer and Kjeldahl determination of the nitrogen content of the filtrate. The resultant non-casein nitrogen value is expressed as a percentage of the total milk nitrogen. The alternative common procedure for co-precipitation of denatured whey proteins with the caseins involves salting out with saturated NaCl at 40°C (Harland and Ashworth, 1945).

O'Sullivan (1971) observed that Kjeldahl nitrogen values for undenatured whey proteins obtained by isoelectric and saturated NaCl precipitations differ. This was recognised by Harland and Ashworth (1945), who found that their saturated salt procedure resulted in 17.3 percent less whey protein nitrogen than Rowland's method (Rowland, 1938). Harland et al. (1955) confirmed this difference and suggested that the saturated salt precipitated a protein fraction not precipitable by acid. Similar differences of larger magnitude in denaturation level were found when these two methods of precipitation of denatured proteins were used to monitor the denaturation status of porcine plasma concentrate proteins by Delaney (1975b). Larson and Rolleri (1955) observed that heating of skim milk at 70°C for 30 min. denatured 29% of the total whey proteins and was due to the specific denaturation of 89% of the immunoglobulins; 52% of blood serum albumin, 32% of \( \beta \)-lactoglobulin and only 6% of \( \alpha \)-lactalbumin.
2.25 Effects of Alkali Treatment of Proteins

Alkaline treatment is employed to facilitate extraction of proteins from soya, single cell, leaf, rapeseed, fish etc. and is also commonly used in the food industry for improving solubility of proteins. Exposure of proteins to alkali is increasingly applied in technological treatment of foods and feeds, e.g. for dissolving proteins in the preparation of concentrates and isolates (Sullivan, 1943); for obtaining proteins with specific properties such as foaming, emulsifying or stabilising (Circle and Johnson, 1958); for inactivation of enzymes and destruction of toxins e.g. destruction of aflatoxin in groundnuts (Screenivasamurthy, 1967), and for obtaining protein solutions suitable for spinning fibres (Soya fibres, 1967). Alkali treatment improved the extractability and solubility of fish protein concentrate (Tannenbaum et al, 1970). Hydrolysis was limited, and the major effect of the alkali was attributed to partial depolymerisation of the protein (Tannenbaum et al, 1970).

Alkali depending upon concentration, temperature and duration may merely cause loosening, deaggregation and depolymerisation or cause limited hydrolysis of proteins to yield polypeptides. Under intense conditions free amino acids are released (Hermansson, et al, 1974; Tannenbaum et al, 1970b). Hence different products are obtained depending upon reaction conditions and therefore functional
properties of alkali treated proteins can be quite variable. With regard to biological value, several authors have observed that alkali treatment of wool, enzymes, and serum albumin may induce chemical changes in these proteins which lead to the formation of new amino acids: lysinoalanine (Bohak, 1964), lantionine (Horn et al, 1941), and ornithinoalanine (Ziegler et al, 1967). These modifications involve the amino acids cystine, lysine, arginine and possibly serine. Thus by causing racemisation, destruction of some limiting amino acids (serine, arginine, threonine, lysine, isoleucine, methionine) and by causing cross linking, alkali treatment reduces biological value of proteins (Tannenbaum et al, 1970; DeGroot and Slump, 1969; Provensal et al., 1975). Effects of alkali on the amino acids in protein have also been studied in connection with alkaline hydrolysis (Hill, 1965).

Provensal et al (1975) carefully determined the conditions under which alkali caused formation of undesirable compounds. Increasing alkali concentration, temperature and duration of hydrolysis caused a progressive destruction of the labile amino acids, and formation of lysinoalanine, alloisoleucine. While conditions used in normal processing are less intense, undesirable alterations may be induced. Thus, alkaline treatments of food proteins should be mild, and careful assessment of the benefit must be made when using this method for improving functional properties.
2.26 Configuration of Plasma Proteins after Alkali Treatment

Considering the molecular structure of plasma proteins, Putnam (1960) reported that fibrinogen has a molecular weight of about 330,000 and it is thought to be a dimer of sub-units containing three peptide chains joined through disulphide linkages. Plasma albumin, on the other hand, is believed to be a single polypeptide chain stabilised in three dimensions by 17 disulphide bonds (Cecil and Wake, 1962). Hunter and McDuffie (1959) reported that the molecular weight of bovine serum albumin is unchanged by reduction of all the disulphide bonds estimated to be 17 in number by Cecil and Wake (1962). They concluded that the molecule must, therefore, consist of a single peptide chain and the disulphide bonds must all be intra-chain. Astbury et al. (1935) hypothesised that the globulins have spherical or coiled molecules which unwind or unfold to long chains on reaction with caustic soda, and if the reaction proceeds far enough, breakdown to small molecules. This view has also been recognised by Tannenbaum et al., (1970) who reported that alkali depending upon concentration, temperature and duration may merely cause loosening and depolymerisation or cause limited hydrolysis of proteins to yield polypeptides and under intense conditions free amino acids are released.
2.27 Blood Plasma Solution Rheology

Tanford and Duzzell (1956) measured the viscosity of solutions of bovine serum albumin as a function of concentration, pH and ionic strength. They found that there was no appreciable increase in viscosity between pH 4.3 and 7.3 at all ionic strengths above 0.01. Only above pH 7.3 – 10 does a small increase in intrinsic viscosity take place. Young and Lawrie (1974) using a modification of the falling sphere technique obtained a stable protein dope of 25.0 Pa. s which they found suitable for the spinning of alkali-treated bovine blood plasma solution. Delaney (1977) made viscosity determination on porcine red cell and concentrates with a Brookfield Model RVT synchro-electric viscometer. He obtained a viscosity of about 30 mPa.s at 21.5 °C for a red cell concentrate of about 10.0% total solids and the viscosity of RBC at 30.0% total solids was about 400 mPa.s at the same temperature. He went further to determine the viscosity of rehydrated diafiltered red cell concentrates and he reported that the viscosity of the latter increased from about 10 mPa.s at about 5% total solids to about 600 mPa.s at 40% total solids. Several workers have shown that the increased viscosity of blood plasma relative to water owes 1 to 2 percent to the small molecular components (inorganic salts, urea, sugar, cholesterol etc.) and 98 to 99 percent to the proteins (Markness, 1952, Lawrence, 1961; Pavey, 1968; Skovborg, et al., 1966). Hence the study of
changes in the rheological parameters of ultrafiltered blood plasma solution can be entirely interpreted as the changes in its protein, as a good approximation.
CHAPTER 3

ULTRAFILTRATION (UF) OF BOVINE BLOOD PLASMA SOLUTION

3.0 INTRODUCTION

Latest attempts have been to reduce or eliminate some of the costly steps involved in the production of textured materials for food uses. It is with this intention of producing bland-tasting, low-costing spun protein products from bovine blood plasma proteins that ultrafiltration was chosen as a means of concentrating and fractionating the low protein content of plasma solution. The UF experiments were, therefore, designed with attention paid to the filtration rate and to the solute retention both of which are important for the economics of the UF system.

The experiments were studied to reveal the influence of temperature, pH and ionic strength treatments on filtration rate and solute retention.

3.1 Experimental

Bovine blood was collected during slaughtering time from a local abattoir and treated with anti-coagulant. Ultrafiltration of such treated blood plasma was carried out mainly with an Amicon ultra-
filtration unit (Model TCF 10). The unit is a self-contained, internally circulating system. It has an integral peristaltic pump which re-circulates the liquid through a thin spiral channel. A selector valve of special design permits solvent addition or sampling during each run, and also provides a simple means of retentate withdrawal.

Equipment specifications are as follows:

- **Membrane type**: Diaflo XM 50 (Mol. Wt. cut-off = 50,000)
- **Effective membrane area**: 40 cm²
- **Membrane diameter**: 90 mm
- **Maximum internal volume**: 600 ml
- **Minimum recirculating volume**: 10 ml
- **Maximum hold-up volume**: 3 ml
- **Maximum operating pressure**: 276 kPa
- **Channel depth**: 0.4 mm

Figure 5 (Page 88) shows the set-up of the Amicon unit as used during this study. The unit was connected to a coil placed in the water bath and the temperature of the feed solution was constantly monitored throughout the experimental period. For individual experiments blood plasma solution previously frozen, stored until required at −15°C and thawed, since freezing and thawing has no adverse effect on plasma proteins (Brooks and Radcliff, 1959).
After pre-filtering, plasma solution was allowed to attain the UF temperature and 500 ml. portions were pumped to the cell and pressurised as recommended by the manufacturer (172 kPa). Feed temperatures and pH values were measured throughout the processing cycle of each run. Three runs were carried out for each experimental treatment and the results expressed as averages.

Experiments were also carried out using Sartorius UF system (Model SM 16525) and this is shown in Figure 4 (Page 88). This is an ultrafiltration apparatus in which the plasma solution flows through the thin-channel plates over the membrane where it is separated under pressure into ultrafiltrate which passes through the membrane and retentate, which becomes increasingly concentrated in the channel before returning to the reservoir. Recirculation proceeds until the required amount of ultrafiltrate has been collected or the retentate has reached the desired concentration level.

A complete Sartorius system consists of the UF apparatus (made of plates and pressure valve) and the membrane pump. The system can be used with filtration areas of 170 cm$^2$ to 2550 cm$^2$, in steps of 170 cm$^2$. The plates have V-shaped grooves 0.7 mm wide running parallel to each other.
The blood plasma solution was pumped into the distributing channel of the inlet plate. From there it flowed into the grooves and across the membrane into the collecting channel and was fed back into the storage container. The ultrafiltrate which passed through the membrane flowed through the channel on the outlet plate into the receiver vessel.

A pressure of 150 kPa was used in combination with the pressure valve throughout the experiment. Figure 4 (Page 89) shows the set-up of the Sartorious apparatus as used in the author's laboratory. After a few runs the cost of membrane prevented further experimentation.

Operating conditions are as follows:

Feed volume : 7.0 l.
UF. - temperature : 25°C
Applied pressure : 150 kPa
Membrane type : SM 12136
Membrane Mol. wt. cut-off : 10,000
Effective membrane area : 1700 cm²

In order to observe the true effect of pH and ionic strength each of the three experiments for one pH value or ionic strength was carried out with a new membrane and after a thorough clean-up of the appar-
atus. The pH or ionic strength of the plasma solution was adjusted immediately before the start of each run and its temperature raised to $25\pm 1^\circ C$. This temperature was subsequently selected for experimental convenience.

Samples of the feed solution, concentrated plasma solution and successive filtrates were analysed for protein content by the semi-micro-Kjeldahl method, total solids and ash of the initial and final feed solutions, total reducing sugars, total phosphorus and titratable chloride. Kinematic viscosity of the concentrated feed solution was also determined with calibrated capillary viscometers.
TABLE 15

Reflection Coefficients (RC) for blood plasma components
during ultrafiltration at 25°C using Diaflow XM 50; 172 kPa

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>MOLECULAR WEIGHT</th>
<th>Avg. REFLECTION COEFFICIENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloride</td>
<td>35.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Phosphate</td>
<td>95.0</td>
<td>0.98</td>
</tr>
<tr>
<td>Glucose</td>
<td>180.0</td>
<td>0.045</td>
</tr>
<tr>
<td>Protein</td>
<td>41,000 - 580,000</td>
<td>0.99</td>
</tr>
</tbody>
</table>

+ (Reflection coefficient is defined as \[
\frac{\text{Retentate content}}{\text{Feed content}}\] )
3.2 **Results and Discussion**

As would be expected the protein fraction was retained to a much greater degree than smaller salt and sugar molecules. Filtration rate decreased with increase in protein content of retentate. The reason for the decreased filtration rate with time is that in normal plasma solution pH the protein molecules are sufficiently separated to move almost independently of each other while in concentrated solution the molecules are extensively intertwined with an increase in osmotic pressure. Filtrability of the microspecies and low molecular weight substances seemed to be dependent mainly on the volumetric flow through the membrane, their nature and conditions prevailing as ultrafiltration progressed (Figures 11, 16 and 17).

Filtration rates vary with temperature, pH and ionic strength of the plasma solution. pH of the feed solution fell sharply and that of the filtrate increased correspondingly under high transmembrane flow while there was no appreciable change in pH under low transmembrane flow (Figure 12, Page 102). Table 15 (Page 92) shows the average reflection coefficients for blood plasma constituents ultrafiltered at 25°C using Diaflo XM 50.

3.2.1 **Effect of Temperature on UF**

The data in Figure 6 (Page 94) show that filtration rate was linearly
RELATION BETWEEN TEMPERATURE & FILTRATION RATE FOR ULTRAFILTERED BOVINE BLOOD PLASMA SOLUTION [pH=7.5; T.solids=8.75%; Amicon TCF 10 with XM 50].
Figure 7

\[ \frac{dV}{dt} = K_0 - K_1 V - K_2 T. \]

PLOT OF $\frac{dV}{dt}$ AGAINST V, VOLUME OF FILTRATE DELIVERED IN TIME $t$ (min.) AT A GIVEN TEMPERATURE $T$ ($^\circ$C) FOR ULTRAFILTERED BLOOD PLASMA SOLUTION [pH 7.5, 8.75% TOTAL SOLIDS.]
Figure 8

Influence of Temperature on the Feed Protein level of ultrafiltered blood plasma solution of fixed pH.
related to temperature. The slope of the curve is 0.98 ml.

As Figure 8 (Page 96) shows, an increase in temperature led to a corresponding increase in protein content of retentates. Increased temperature improved the filtration rates by reducing the viscosity of the concentrated solution, and altering the constitution of the ions in solution.

The relationship between filtration rates and temperature within the temperature range studied for blood plasma solution can be represented by the following equation (Figure 7 Page 95):

\[ -\frac{dV}{dt} = K_0 - K_1 V - K_2 T \]

where 
- \( t \) = ultrafiltration time in min,
- \( V \) = volume (ml) of filtrate delivered per given time,
- \( T \) = ultrafiltration temperature (°C) and
- \( K_0, K_1, K_2 \) are constants, and equal to \(-0.011, 0.0166, 0.0005\) respectively.

This relationship between temperature and filtration rates was observed to correspond to an average of 2.5% change in filtration rates per °C.

From the above results it is evident that temperature is an important driving force during UF. and it is, therefore, desirable to adopt the highest temperature compatible with the ultrafilter and the solution to
be ultrafiltered. For bovine blood plasma solution, an operating temperature around 40°C was found to be suitable. Temperatures higher than the latter while still improved filtration rates gave concentrated plasma solutions that performed poorly during storage and processing.

3.2.2 Effect of pH of the Medium on UF

Figure 10 shows filtration rates achieved while processing blood plasma solutions of different pH values by ultrafiltration at 25±1°C. The filtration rates of blood plasma adjusted to pH 9.50 and 5.0 was increased by 10% and 9.5% respectively compared with normal plasma pH. There was in each case a rapid improvement in filtration rates followed by a steady fall with time.

The mechanism of the action of acid pH is thought to be due to decreased hydration of the proteins with the resultant increase in water flow rate across the membrane. At pH 5.0, most of the proteins are likely to exist in their least soluble state while the solubility of the globulin fraction is probably increased. At pH 9.50, the macromolecules are most likely to exist in dispersed state and their denaturation at this pH is believed to be responsible for the improved filtration rates.
Figure 9

VOLUMETRIC CONCENTRATION RATIO (25° C) OF ULTRAFILTRATED BLOOD PLASMA SOLUTION AGAINST FEED PROTEIN LEVEL (IN % 6.25) AS A FUNCTION OF IONIC CHARGE & STRENGTH. (* = CONTROL; a = 2.5% K₂SO₄, b = 5.0% (w/v) K₂SO₄).
VARIATION IN FILTRATION RATE OF BOVINE BLOOD PLASMA SOLUTION 
(25°C) AS A FUNCTION OF pH AND IONIC STRENGTH WITH THE FEED PROTEIN LEVEL.
FIGURE 11

(a)

mg. Cl⁻/100ml.

500

-400

-300

-200

-100

V. C. R. (25°C)

1.4

1.8

2.2

2.6

pH 5.0

pH 7.50 (Control)

pH 9.50

Fil.

Feed

(b)

mg. Cl⁻/100ml.

400

300

200

100

V. C. R. (25°C)

1.4

1.8

2.2

TITRATABLE CHLORIDE (mg./100ml.) OF ULTRAFILTERED BLOOD PLASMA SOLUTION AGAINST VOLUMETRIC CONCENTRATION RATIO (V.C.R.) AS A FUNCTION OF (a) pH AND (b) IONIC STRENGTH. (* = 2.50%; ** = 5.0% (w/v) K₂SO₄).
VARIATION OF FEED & FILTRATE pH DURING ULTRAFILTRATION (25°C) OF BLOOD PLASMA SOLUTION DUE TO THE EFFECT OF IONIC CHARGE. (* = Control).

VARIATION OF FEED & FILTRATE pH DURING ULTRAFILTRATION (25°C) OF BLOOD PLASMA SOLUTION DUE TO THE EFFECT OF IONIC STRENGTH. (A = 2.5% and B = 5.0% (w/v) K₂SO₄).
3.2.3 Effect of Ionic Strength on UF

The addition of 2.5% \((\text{w/v})\) \(K_2\text{SO}_4\) to blood plasma solution gave a significant increase in filtration rates (Figures 9 and 10). This is probably due to the increased solubility of some of the proteins or a conformational change in protein which improved ultrafiltration rates in relation to the control. This view is also supported by a comparatively sharp fall in pH of the feed solution (Figure 12b, Page 102).

But at 5% level filtration rates fell sharply and there was no appreciable change in feed pH and viscosity. It is more probably that association followed by precipitation of protein molecules did occur which led to the formation of a secondary membrane barrier. Hence, the poor ultrafiltration results obtained during this treatment.

Considering the effect on the nature of the protein fractions, little effect is likely on the albumin. Globulin fraction is likely to be more insoluble with about 2% salt while fibrinogen is salted out readily by this concentration.

3.2.4 Diafiltration of Blood Plasma Solution

Figures 14, 15, 16 and 19a show the results obtained during diafiltration of blood plasma solution at 25°C and variation in pH is
depicted in Figures 18 and 19b. The progressive dilution of concentrated feed solution during diafiltration results in an almost constant flow rate per given time and consequently an increase in overall filtration rates when compared with direct concentration. This increased filtration rate is thought to be due to a significant decrease in feed viscosity and density with a simultaneous reduction or elimination of concentration polarisation. Although diafiltration is effective in reducing the salt content of blood plasma solution it is also associated with an unusual increase in filtrate protein level (Figure 19a Page 112). This might be due to improved filtration rate which remains high or to repeated dilution of retentate leading to protein denaturation. It is also noted that only the removal of salts of simple monovalent type was improved appreciably.

Analysis of successive filtrates indicated only minor changes in composition during the period of UF. Total solids varied slightly, reflecting increased total solids of retentates. Nitrogen compounds of the filtrates that are soluble in 15% TCA decreased slightly with UF time. Nitrogen content of filtrates increased with ultrafiltration temperature but remained almost constant throughout UF at a particular temperature. Total inorganic phosphorous determinations indicated that transmembrane flow of this ion was limited as expected.
owing to its close association with the macromolecules which, of course, are 100% retained. The same behaviour is suspected of calcium ions. Filtrates from process feed solution adjusted to pH 9.5 were detectably lower in total phosphorous than filtrates obtained from pH 7.5 and the latter lower than the filtrates obtained from pH 5.0. Phosphate ion being less soluble at higher pH values in its ionic form, would tend to associate more strongly with the protein. Increased phosphate retention would, therefore, be likely.

The reflection coefficient of chloride ions was almost constant during ultrafiltration of blood plasma solution. It, therefore, follows that the filtrability of the ions does not depend on concentration. The almost constant transmembrane flow of the ions as time progressed indicates that chloride filtrability or perhaps that of any similar ion depends upon the volume of the hydrated ion. The reason for this is likely to be that salt ions when present in solution are in hydrated state and they attach to themselves a certain number of water molecules. Since the attractive force operates at a distance the ions mutually dehydrate themselves, the effect being more pronounced the closer they are together or the greater the concentration. Hence, the slight change in the pH value of successive filtrates.

The reason for the poor filtration rates obtained for 5% salt is that the interaction between water and protein was reduced as the interaction between water and salt ions increased. Consequently,
FIGURE 13

VARIATION OF FEED VISCOSITY AT 25 ± 0.1°C AGAINST VOLUMETRIC CONCENTRATION RATIO OF ULTRAFILTERED BLOOD PLASMA SOLUTION AT DIFFERENT pH VALUES: (a = 2.5% (w/v) $K_2SO_4$, b = 5.0% (w/v) $K_2SO_4$).
Figure: 14

DIAFILTRATION (25°C) OF BOVINE BLOOD PLASMA SOLUTION (pH 7.90)
Figure: 15

DIAFILTRATION (25°C) OF BOVINE BLOOD PLASMA SOLUTION (pH 7.90).
DIAFILTRATION (25°C) OF BOVINE BLOOD PLASMA SOLUTION (pH 790).
Figure 17

UF of Blood Plasma & one-stage Diafiltration.
VARIATION IN pH OF ULTRAFILTERED [BOVINE] BLOOD PLASMA SOLUTION.
Ultrafiltration (25°C) of Blood Plasma Solution (pH 7.90) with one-stage Diaphragm. Figure 19a: Volume Reduction (%) vs. Filtration Time [Hr.] and Protein Level. mg. Protein (N x 6.25)/ml. vs. mg. Titratable Chloride/100 ml.
Figure: 19b

VARIATION OF BLOOD PLASMA pH DURING ULTRAFILTRATION [pH 7.90, 25°C]

![Graph showing the variation of blood plasma pH during ultrafiltration.](image-url)
ULTRAFILTRATION (25°C) OF BOVINE BLOOD PLASMA SOLUTION [pH 7.95] WITH THE SARTORIUS UF SYSTEM [MODEL SM 16525/SM 16896/SM 12136].
increased interaction between protein molecules was induced which resulted in association and precipitation of the protein molecules as to form a secondary membrane barrier. The effect of pH might be due to the reversible denaturation of proteins which occurred as a result of the rupture of hydrogen bonds. This is likely to lead to a progressive increase in retention with increased deviation from the normal plasma pH because of an increase in molecular size to the extent that the protein is more easily retained by the membrane.

3.3 **Viscosity and Volumetric Concentration Ratio**

Blood plasma solution being a viscous fluid, not surprisingly, exhibited a sharp decrease in filtration rates (Figures 17 and 20, Pages 110 and 114). Viscosity of concentrated plasma solutions is plotted against volumetric concentration ratio and the differences in the slopes of the curves reflect the differences in experimental treatments (Figure 13, Page 106). As ultrafiltration progressed plasma feed viscosity rose almost linearly as the volumetric concentration ratio and this increased feed viscosity was considered to be partly responsible for the sharp decrease in filtration rates. This view could be substantiated with already known fact in membrane processes that solvent flow is highly dependent on the prevailing viscosity for microporous ultrafilters.
It is interesting to note that bovine blood plasma solution with many different fractions of proteins of high molecular weight of several thousands gave concentrated solutions of relatively low increase in viscosity. The reason for this might be that the polypeptide chains still retained their folded, compact configuration. If this is the case, it can be inferred that the protein molecules are not denatured during ultrafiltration. If, on the other hand, they have lost their tertiary structure, that is, the original folding of the polypeptide chains during ultrafiltration would have resulted in concentrated solutions of relatively high viscosity. It follows, therefore, that plasma proteins maintained their original conformation during ultrafiltration hence a small increase in feed viscosity. Another reason for the relatively low increase in the viscosity obtained for the ultrafiltered plasma solution, might be probably for the fact that the rate of shear in the capillary viscometer is so high say, several hundred reciprocal seconds as to minimise non-Newtonian characteristic and/or for the fact that the rate of shear is not constant across the capillary.
CHAPTER 4

SOLUBILITY OF PLASMA PROTEINS

4.0 The protein is of key significance to the physical and functional properties of the final product. Hence its solubility in the processing medium is highly desirable. The main factors affecting solubility are temperature and pH, and these are examined here.

4.1 Experimental

The solubilisation process was determined by dissolving a known quantity of ultrafiltered plasma solution in a measured quantity of aqueous caustic soda and shaking the mixture at constant temperature mechanically for the desired time period (20 min.). The time period was chosen to reflect an acceptable solubilisation level. Five samples were analysed from five batches of blood collected during the experiment from the same abattoir and the results expressed as average.

Twenty and 40 parts of ultrafiltered plasma solution of known concentration was dispersed in 80 and 60 parts (duplicates) of 0.1N NaOH respectively to give a final reaction pH of 12.75. The total amount of soluble nitrogen was determined by a micro-Kjeldahl procedure on the supernatant obtained after centrifugation at 10,000G for 30 min.
Solubility tests were made at pH 4.0 to 12.0 in steps of 0.5. Using the method outlined by Mattil (1971), known quantities of blood plasma solution were adjusted with 1N NaOH or 1N H$_2$SO$_4$ to desired pH. Solutions were shaken mechanically for 30 minutes at 40°C. The solution was centrifuged at 10,000G for 30 minutes, and the supernatant (filtered through Whatman No. 1 filter paper) analysed for total nitrogen by the semi micro-Kjeldahl method. The results were expressed as percent plasma protein soluble at the given pH.

4.2 Results and Discussion

The effect of pH and temperature on solubility of ultrafiltered blood plasma solution was determined using the Nitrogen Solubility Index (NSI) method. While variations in solubility within and between batches are negligible within the limit of experimental error of samples studied, other possible variations, for example, seasonal were not studied and might, therefore, need further investigation. The effect of pH on solubility is presented in Figure 21 (Page119). The solubility curve of the plasma solution shows a sharp dependence on pH as indicated by the 55% decrease at pH around 4.0 which represents the point of minimum solubility within the range of pH 4.01 - 12.0; a slight increase in solubility within the pH range of 7.0 - 9.5 followed by a decrease at pH values higher than 9.5.
FIGURE 21
EFFECT OF pH ON THE SOLUBILITY OF AN ULTRAFILTERED BOVINE BLOOD PLASMA SOLUTION [10.85% PROTEIN] AT 40°C.
FIGURE 22

ALKALINE SOLUBILITY OF ULTRAFILTERED BOVINE BLOOD PLASMA SOLUTION AS A FUNCTION OF TEMPERATURE.
The response of plasma proteins to pH indicates the potential use of ultrafiltered plasma solution at such a wide pH range of 5.5 to 10.5 for the processing technique employed in this study. At the alkaline pH range four distinct phases can be recognised from the electron microscopy studies of the samples; the solubility phase (pH 7.0 - 9.5), deaggregation phase pH 11.5. These phases indicate how sensitive are plasma proteins to change in pH.

The effect of temperature on alkaline solubility of ultrafiltered plasma proteins is presented in Figure 22 (Page120). The results show strong dependence of plasma proteins on temperatures above 50°C. The solubility curves decrease with increasing heat treatments above 50°C and the reason for this is probably due to heat-induced denaturation followed by aggregation of some of the heat sensitive plasma proteins. The coagulable fractions, notably fibrinogen and the globulins would be rendered insoluble by such heat treatments. The solubility of the ultrafiltered blood plasma solution is, therefore, dependent on pH and the processing temperature.

4.3 Denaturation of Blood Plasma Proteins

Introduction

Protein denaturation in the general sense involves any modification of the native protein structure excluding primary covalent bond
hydrolysis, that is, changes in primary structure. Thus the denaturation process is confined to alterations in the secondary and tertiary structure of the protein molecule. In the case of plasma globular proteins, the change in molecular configuration will involve the unfolding of the $\alpha$-helix structure into a random coil formation. Because of the alteration in protein structure on denaturation, many changes will be expected to occur in the properties of the protein. Since blood plasma is aimed to be a nutritive supplement such as meat extenders it is desirable that any processing treatment should not significantly alter nutritive value of the protein.

Plasma proteins like other proteins possess functional properties in their native state. However, during centrifugation, ultrafiltration, storage etc. denaturation occurs to varying extents. Denaturation in the present context denotes loss of functional properties and it is usually (for practical purposes) measured by determining the solubility of the protein. Solubility is a very useful index of overall functionality because soluble proteins possess superior functional attributes for most applications (Hermansson, 1973). Several processes such as the use of alkaline processing, application of heat etc. result in some destruction of functional properties.
4.4 **Experimental**

Undenatured protein values were determined in 9.66 mg. TPN/ml. (0.7 mg. NPN/ml.) normal and ultrafiltered blood plasma (20.3 mg. TPN/ml.) solutions by micro-Kjeldahl method on saturated NaCl (Harland and Ashworth, 1945) and hydrochloric acid (Rowland 1938) filtrates using centrifugation and Whatman No. 42 filter paper. The filtrates were obtained by adjusting the pH of 100 ml. portions of ultrafiltered or original plasma solution to desired pH values with 50% (w/v) caustic soda solution in 150 ml. conical flasks. The flasks were shaken mechanically for 60 min. at 40 ± 1°C and samples for NaCl and HCl filtrates were withdrawn at 15 minute intervals. The acid filtrates were obtained by adjusting the pH of the alkali-treated plasma solutions to 4.0 using one-fold diluted conc. HCl. The NaCl filtrates were obtained after the mixed NaCl-plasma solutions were allowed to stand at 40°C for 30 minutes.

4.5 **Results and Discussions**

Denaturation susceptibility was expressed as percentage denaturation relative to the control sample. Kjeldahl nitrogen values for undenatured plasma proteins obtained by isoelectric precipitation are higher than that of saturated NaCl filtrates throughout the pH range investigated. The data obtained are presented in Figure 23 (Page 124) and Table 16 (Page 126).
FIGURE 23

EXTENT OF DENATURATION OF NORMAL (a) AND ULTRAFILTERED (b) BLOOD PLASMA SOLUTION (a) ADJUSTED TO DESIRED pH (b)

*Control

% Denatured Protein

Total Solids

Denaturation Time (min.)

HCl Filtrate

NaCl
Despite the differences, however, the two methods of precipitation did show similar trends of denaturability of plasma proteins due to alkaline processing at 40°C. The percentage denaturation of the total plasma proteins obtained in this experiment varied from 4 to 38 percent. The plasma proteins in concentrated form were found to be more susceptible to denaturation than the less concentrated and normal plasma solutions at a given pH. It was observed that for heating times above 15 minutes the variation in percentage denaturation had no effect comparable to the holding time except for the concentrated plasma solution.

The higher the pH the more susceptible are plasma proteins to denaturation (Figure 23, Page 124). The plasma proteins of normal plasma solution are also found to be more susceptible than their counterparts in ultrafiltered plasma solution for HCl filtrates whilst the latter appear to be more susceptible at higher pH values for saturated NaCl filtrates. Among the plasma proteins, the globulins and fibrinogen are the fractions likely to be affected much by the disrupting effects of pH. Increased denaturability with rise in pH points to the fact that increased disrupted polypeptide chains are present and precipitable. The discrepancies between the two methods of precipitation might be due to the fact that some of the disrupted and extended polypeptide chains or probably undenatured proteins are not
precipitable by HCl at pH 4.0. The globulins, for example, are likely to be more soluble in acid medium.

Denaturation was minimal under the conditions of processing employed at pH 10.0. On the whole, denaturation levels even at pH 12.0 obtained in this experiment are less than those obtained for sprayed-dried porcine blood cells by Delaney (1975b).

**TABLE 16**

<table>
<thead>
<tr>
<th>pH</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>15</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heating time (min.)</td>
<td>15</td>
<td>30</td>
<td>45</td>
<td>60</td>
<td>15</td>
</tr>
<tr>
<td>NaCl filtrate</td>
<td>10.30</td>
<td>12.26</td>
<td>19.38</td>
<td>22.64</td>
<td>Gel</td>
</tr>
<tr>
<td>HCl filtrate</td>
<td>13.41</td>
<td>15.37</td>
<td>20.18</td>
<td>30.76</td>
<td>-</td>
</tr>
</tbody>
</table>
CHAPTER 5

SCANNING ELECTRON MICROSCOPY (S. E. M.) OF PLASMA PROTEINS

5.0 INTRODUCTION

Alkaline-treatment of blood plasma solution was carried out to effect functional changes in the protein configuration, for example, re-organisation of the polypeptide chains. That this step is necessary was realised when spun dispersions of non-alkali treated plasma solution even at 15% protein level did not form filaments whereas spun dispersion of denatured proteins at lower protein concentration did so.

The structure of a protein is currently pictured as compact with the polypeptide chains folded in a unique and highly organised fashion. Since the molecular chains of proteins tend to assume more or less randomly coiled configuration in an undisturbed state, appropriate treatments must be applied to overcome this natural tendency in order to form a desirable product. The objective here is to study the influence of alkaline pH on the ability of the proteins to rearrange to new configuration.

5.1 Experimental

Freshly ultrafiltered blood plasma solution was adjusted in equal
volumes to various pH values from 8 to 12 at 0.5 intervals with 50% caustic soda. The flasks were allowed to stand in a constant temperature (40°C) bath for 60 min. and samples were withdrawn at desired time and carefully spread onto double-sided sticky tape on mounting studs for scanning electron microscopy. Plates 1 to 7 were prepared after 30 minutes. The technique of photomicroscopy was employed to measure globule diameters within the pH range of 7 to 12. After photographing the samples, each sample slide was introduced into a slide projector and the image projected onto a vertical surface covered with white paper. The projector was then positioned in such a way that the reading meter of the instrument equalled 1 mm. The longest axis of globules was measured to the nearest 1 mm, each globule being marked on the paper as it was measured and the final figure was obtained as average for each sample.

5.2 Results and Discussion

Since proteins are polyelectrolytes, the dispersion state of protein treated with alkaline solution may be expected to vary with pH of the medium. Figure 24 (Page 129) shows pH dependence of globule size and two distinct phases can be recognised according to the mean globule diameter of the alkali-treated, ultrafiltered blood plasma proteins - deaggregation phase which exists between pH 8.0 and 10.0 and aggregation phase between 10.00 and 12.0.
FIGURE 24

INFLUENCE OF pH ON THE MEAN GLOBULE DIAMETER (micrometer) OF ALKALI-TREATED, ULTRAFILTERED BLOOD PLASMA PROTEINS.
SEM photographs revealed that while the native proteins are but little hydrated and isolated, (Plate 1, Page 133), the denatured proteins are markedly hydrated, closely packed together with loss of individuality (Plates 2 to 20). It is evident that alkaline treatment seemed to effect a change in the intramolecular configuration leading to intermolecular structural arrangement of the polypeptide chains in such a way as to bring about in the dispersed state some minimum internal resistance to deformation in order for the dispersion, when spun, to remain as a distinct and continuous filament, without complete disintegration as observed with non-alkali treated plasma solution. Unfolding or reduction in molecular size occurred between pH 8.0 and 9.50. Apparent disruption of the structural arrangement of the protein bodies occurred at a pH around 10.0. From structural viewpoint only the globulins could be greatly affected by treatment with caustic soda.

With every increase in pH especially at high pH's (≥ 10), the length of protein molecular chain increases and becomes so great at pH 12.0 suggesting that more and more acidic groups must have been taken part in aggregation reaction with rise in pH. The ultimate effect of this observation on spinning would be cross-linking the long molecules to become longer which could prevent the molecules sliding over each other when closely packed together. During alkaline treatment of
plasma proteins, cross-linking is believed to have taken place and this view is supported by the occurrence of gelation at some point in the aggregation reaction phase. Conversion of hydrophilic into hydrophobic groups is also thought to have occurred and since hydrophobic bonds tend to be stronger as temperature increases this emphasises the importance of the application of appropriate heat treatment to the spinning solution. It is believed that alkaline treatment promotes bond formation such as covalent (e.g., disulphide) bond and non-covalent (hydrogen, ionic, and hydrophobic) bond and interaction between the protein molecule and the solvent.

It is not true as is generally believed that denatured protein dispersions such as that of blood plasma consist of long chain-like molecules capable of being oriented into a continuous filament system as the photomicrographs reveal. Even though the molecules are somehow linked it is believed that this linkage is not generally an end-on-end type.

Partially unfolded polypeptide chains aggregated probably due to hydrophobic and ionic ions. Individual subunits although reduced in size associated to form larger particles. Because of different nature of various chains, unfolding or structural rearrangements was not uniform.
The tendency to gel at pH around 12.0 is evident from the conformational arrangement of the extended molecules (Plates 19 and 20). Molecules assumed amoebid shaped structure with the probably exposure of their active end groups which would promote further polymerisation reactions; even though alkaline treatment effected the breakdown of the plasma proteins into their structural subunits it is viewed that only subunits linked solely by simple bonds such as hydrogen could be so affected by this treatment. This view can be supported by the fact that some molecules still remained largely intact even at pH 11.0 and above. Increased viscosity with rise in pH of blood plasma solution is not, therefore, solely due to the unfolding of the macromolecules to elongated forms but rather increased interaction of protein-protein and/or protein-solvent caused perhaps by the presence of free alkali or its effect to bring to the surface more reactive groups of the protein molecules.

It can be concluded from the above that the use of suitable base is highly desirable in the spinning of globular proteins and the base must be able to dissociate in order to attain a critical alkalinity, capable of causing the reduction of molecular weight of the constituent protein molecules in order to raise the viscosity of the solution.
PLATE 1
Ultrafiltered blood plasma solution.
X 4500
pH 7.10

PLATE 2a
X 4500
pH 8.0
(Edge)

PLATE 2b
X 4500
pH 8.0
(Centre)
PLATE 3a
X 4,000
pH 8.50
(Edge)

PLATE 3b
X 4,200
pH 8.50
(Centre)

PLATE 4a
X 8,000
pH 9.0
(Edge)
PLATE 7b
X 5000
pH 10.5
(Centre)

PLATE 8
X 4500
pH 11.0
(After 5 min.)

PLATE 9
X 4500
pH 11.0
(After 30 min.)
PLATE 10
X 4500
pH 11.0
(45 min.)

PLATE 11
X 4500
pH 11.0
(60 min.)
PLATE 12
X 5000
pH 11.5
(5 min.)

PLATE 13
X 5000
pH 11.5
(10 min.)

PLATE 14
X 4500
pH 11.5
(30 min., Edge)
PLATE 15
X 4500
pH 11.5
(30 min., Centre)

PLATE 16
X 4500
pH 12.0
(After 2 min.)

PLATE 17
X 4500
pH 12.0
(After 5 min.)
PLATE 18
X 4500
pH 12.0
(10 min.)

PLATE 19
X 1800
pH 12.0
(15 min.)

PLATE 20
X 1800
pH 12.0
(20 min.)
Specimen prepared with minimum possible disturbance to the plasma solution.

ULTRAFILTERED [BOVINE] BLOOD PLASMA SOLUTION [pH 7.5, 13.2% TS, 11.5% PROTEIN, 0.48% ASH].
Plate 21 adjusted to pH 11.9 with 50% NaOH solution & aged at 20°C.

Effect of adding alkaline solution to ultrafiltered blood plasma solution.
Plate 23 sheared ($\gamma = 28 \text{s}^{-1}$) at $20^\circ \text{C}$ for 10 min.

Plate 25

EFFECT OF SHEARING TIME ON THE DEFORMATION OF STRUCTURE IN SPINNING DOPE.
CHAPTER 6

PLASMA SOLUTION RHEOLOGY

6.0 Introduction

A fluid undergoes continuous deformation when subjected to shear stress. The resistance offered by the fluid to such deformation is termed its consistency. For simple (or Newtonian) food liquids or dispersed systems the consistency is approximately the same irrespective of the rate of shear which is applied. For such materials the consistency is called the viscosity. In non-Newtonian flow the viscosity changes as the rate of shear is altered. Thus, for the complete characterisation of non-Newtonian materials the influence of rate of shear on consistency needs, therefore, be studied over a wide range of shear rates. Knowing the latter, one can relate the rate(s) of shear the materials will be subjected to during usage (processing and consumption of foods) to experimentally obtained data. Furthermore, in pumping spinning dope there will be an internal friction or resistance to relative motion of the particles present. Viscous liquids and dispersions such as the spinning dope (alkali-treated protein solution) will, therefore, tend to increase the horsepower required by a pump, to reduce the pump efficiency, head and capacity, and increase friction in tubing.
Measuring the consistency of alkali-treated blood plasma solution over a range of shear, there will exist a region where a stable viscosity prevails. This limiting viscosity will be an important rheological parameter which can be helpful in interpreting spinnability. Not only that, the viscosity of a colloidal solution is known to be very sensitive to changes in the degree of aggregation or polymerisation of the colloidal particles. So, this index can be used as a measure of deaggregation such as that produced in the disruption of globular proteins during alkaline treatment.

In addition, high shear rates are reached by forcing a viscous dispersion through a fine multi-hole spinnerette and a knowledge of the viscosity behaviour of the spinning dope at high as well as low shear rates is most valuable.

For the reasons above, it was decided to study the shear stress-shear rate relationship of the spinning dope and the influence which factors such as total solids content, shearing time, ageing time and temperature and the pH of the medium might have on such shear stress-shear rate curves.

6.1 Measurement of Viscosity

Viscosities were measured with a Haake Rotovisko (RV2) viscometer. It has both coaxial cylinder and plate and cone viscosity sensor systems
that allow viscosity measurement of Newtonian and non-Newtonian substances over an extremely wide range of shear stress and shear rate.

The test material is contained in the annular space between a rotating cylinder (rotor) and a fixed cylinder (cup or beaker) in both of which the flow regimes are mathematically defined. The rotor rotates at a defined r.p.m. and the resulting shear stress is a measure of the viscosity of the substance. Since the test material is sheared within a fixed gap distance and the geometry is of such a nature that shear stress and shear rate can be calculated, the absolute viscosity, is obtained from the relationship:

\[
\text{Viscosity (Pa.s)} = \frac{\text{Shear Stress (N/m²)}}{\text{Shear Rate (s⁻¹)}}
\]

6.2 Experimental

The flow characteristics of ultrafiltered blood plasma solutions of various concentrations and spinning dopes (alkali-treated plasma solutions) were studied at 20 ± 0.1°C and 22 ± 0.1°C with a Haake Rotovisko viscometer (Model RV2) by measuring the torque developed at shear rates ranging from 0.44 to 1958 s⁻¹. Some tests were studied at temperature of 25, 35 and 45 ± 1°C. Samples were allowed to be sheared for 1 minute at a given shear rate. Three to
five tests were performed under each experimental condition and the results are expressed as mean values. Continuous shear that can be closer to the actual usage of the material was adopted in most of the tests. The power law equation was used, where applicable, to describe the flow curves.
6.3 Results and Discussion

Flow behaviour curves were constructed using shear stress and shear rate data obtained from the viscometric test. The shear stress-shear rate characteristics of many non-time-dependent materials may be expressed by: \( T = b \dot{\gamma}^s + C \) where \( T \) = shear stress; \( \dot{\gamma} \) = shear rate; \( b, s \) are power law constants and \( C \) represents the yield stress.

The power law equation relates the shear stress to the shear rate over any range of shear rates for which \( s \) is constant. \( b \) is constant at a fixed temperature and is a measure of the viscosity of the fluid or semi-solid material hence called consistency coefficient. The higher the value of \( b \), the more viscous is the material. \( s \), the flow behaviour index, is a measure of the degree of non-Newtonian behaviour. When \( s \) equals 1 the material is said to be Newtonian. The further \( s \) deviates from unity, the more non-Newtonian the material.

\( b \) and \( s \) are, therefore, regarded as the flow parameters in addition to \( C \), the yield stress or value where it exists. The values for \( C, b \) and \( s \) are shown in Tables 17 - 19 (Page 147).

As Table 19 (Page 147) shows, fresh ultrafiltered blood plasma solution was characterised by a yield stress. The yield stress or strength represents the stress at which appreciable flow starts and/or at which the structure begins to be broken down.
### TABLE 17
Values of parameters in flow pattern of ultrafiltered (bovine) blood plasma solution at a fixed pH (9.2% Protein; pH 12.2; Ageing temperature 25°C)

<table>
<thead>
<tr>
<th>Curve</th>
<th>Ageing Time (HR)</th>
<th>Yield stress C, (Pa)</th>
<th>Consistency Coefficient b</th>
<th>Flow behaviour Index, S</th>
<th>Range of (sec(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>0.6864</td>
<td>0.0138</td>
<td>1.2723</td>
<td>43.28-1958.42</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>0.5625</td>
<td>0.0477</td>
<td>1.1078</td>
<td>43.28-1384.96</td>
</tr>
<tr>
<td>3</td>
<td>2.0</td>
<td>0.5100</td>
<td>0.0624</td>
<td>1.0608</td>
<td>43.28-1384.96</td>
</tr>
<tr>
<td>4</td>
<td>24.0</td>
<td>0.8370</td>
<td>0.0312</td>
<td>1.0582</td>
<td>43.28-2769.92</td>
</tr>
</tbody>
</table>

### TABLE 18
Values of parameters in flow pattern of ultrafiltered blood plasma solution at various pH values

<table>
<thead>
<tr>
<th>Curve</th>
<th>pH</th>
<th>Yield strength C (Pa)</th>
<th>b</th>
<th>S</th>
<th>Range of (sec(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.0</td>
<td>0.1669</td>
<td>0.0512</td>
<td>1.0184</td>
<td>122.27-2769.92</td>
</tr>
<tr>
<td>2</td>
<td>10.0</td>
<td>0.6661</td>
<td>0.0354</td>
<td>1.1049</td>
<td>86.56-1958.42</td>
</tr>
<tr>
<td>3</td>
<td>11.0</td>
<td>0.9488</td>
<td>0.0352</td>
<td>1.2344</td>
<td>43.28-979.21</td>
</tr>
</tbody>
</table>

### TABLE 19
Values of parameters in flow model of ultrafiltered (bovine) blood plasma solution

<table>
<thead>
<tr>
<th>Curve</th>
<th>Protein (%)</th>
<th>C (Pa)</th>
<th>b</th>
<th>S</th>
<th>Range of (sec(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>0.538</td>
<td>0.0339</td>
<td>0.9929</td>
<td>344.24-3916.84</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>0.915</td>
<td>0.0355</td>
<td>0.9952</td>
<td>344.24-3916.84</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>1.295</td>
<td>0.0363</td>
<td>0.9960</td>
<td>344.24-3916.84</td>
</tr>
</tbody>
</table>
TABLE 20

Effect of Temperature on App. Viscosity-Shearing Time relationship of spinning dope (Shear Rate: 28.16 s\(^{-1}\))

<table>
<thead>
<tr>
<th>Ageing Temp. (°C)</th>
<th>Ageing time (Min.)</th>
<th>pH</th>
<th>Peak viscosity attained (Pa.s)</th>
<th>Viscosity (Pa.s) after shearing for 10 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>60</td>
<td>11.5</td>
<td>3.37</td>
<td>1.003</td>
</tr>
<tr>
<td>35</td>
<td>35</td>
<td>11.5</td>
<td>3.92</td>
<td>1.641</td>
</tr>
<tr>
<td>45</td>
<td>17</td>
<td>11.5</td>
<td>5.19</td>
<td>2.461</td>
</tr>
</tbody>
</table>

TABLE 21

Variation in apparent Viscosity - Ageing Time Relationship for ultrafiltered blood plasma solutions of different protein concentration at fixed pH. (Shear rate: 28.16 s\(^{-1}\))

<table>
<thead>
<tr>
<th>Total solids %</th>
<th>Protein %</th>
<th>NaOH/Protein Ratio</th>
<th>pH (20°C)</th>
<th>Peak viscosity (Pa.s) at 20°C</th>
<th>M *</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.95</td>
<td>15</td>
<td>0.060</td>
<td>11.5</td>
<td>6.08</td>
<td>1.08</td>
</tr>
<tr>
<td>14.75</td>
<td>13</td>
<td>0.046</td>
<td>11.5</td>
<td>5.10</td>
<td>1.81</td>
</tr>
<tr>
<td>12.65</td>
<td>11</td>
<td>0.040</td>
<td>11.5</td>
<td>3.65</td>
<td>1.55</td>
</tr>
</tbody>
</table>

* m = time dependency index (T. = C. t\(^{-m}\))

TABLE 22

Effect of pH on Apparent Viscosity-Shearing Time Curve of an ultrafiltered bovine blood plasma solution stored frozen for one day.

<table>
<thead>
<tr>
<th>% Total solids</th>
<th>Ash %</th>
<th>Protein %</th>
<th>pH (20^\circ)</th>
<th>Alkali/Protein Ratio</th>
<th>Peak viscosity (Pa.s) at 20°C **</th>
<th>Ageing time (min. to attain peak viscosity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.5</td>
<td>0.49</td>
<td>11.70</td>
<td>11.9</td>
<td>0.0453</td>
<td>12.40</td>
<td>60</td>
</tr>
<tr>
<td>13.5</td>
<td>0.49</td>
<td>11.70</td>
<td>12.2</td>
<td>0.0513</td>
<td>14.40</td>
<td>13</td>
</tr>
<tr>
<td>13.5</td>
<td>0.49</td>
<td>11.70</td>
<td>12.5</td>
<td>0.0684</td>
<td>17.32</td>
<td>9</td>
</tr>
</tbody>
</table>

** Shear Rate: 28.16 s\(^{-1}\)
### TABLE 23

Effect of pH on Apparent Viscosity–Shearing Time Curve of an ultrafiltered bovine blood plasma solution stored frozen for a week (Shear rate: 28.16 s\(^{-1}\))

<table>
<thead>
<tr>
<th>% Total solids</th>
<th>Ash %</th>
<th>Protein %</th>
<th>pH (20°C)</th>
<th>Alkali/Protein Ratio</th>
<th>Peak viscosity (Pa.s) at 20°C **</th>
<th>Ageing time (min) to attain peak viscosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.5</td>
<td>0.49</td>
<td>11.7</td>
<td>11.9</td>
<td>0.0479</td>
<td>9.845</td>
<td>78</td>
</tr>
<tr>
<td>13.5</td>
<td>0.49</td>
<td>11.7</td>
<td>12.2</td>
<td>0.0598</td>
<td>13.309</td>
<td>9</td>
</tr>
<tr>
<td>13.5</td>
<td>0.49</td>
<td>11.7</td>
<td>12.5</td>
<td>0.0650</td>
<td>15.497</td>
<td>9</td>
</tr>
</tbody>
</table>

** Shear rate: 26.16 s\(^{-1}\)

### TABLE 24

Effect of pH on Apparent Viscosity–Shearing Time Curve of an ultrafiltered bovine blood plasma solution stored frozen for two weeks

<table>
<thead>
<tr>
<th>Total solids %</th>
<th>Ash %</th>
<th>Protein %</th>
<th>pH (20°C)</th>
<th>Alkali/Protein Ratio</th>
<th>Peak viscosity (Pa.s) at 20°C **</th>
<th>Ageing time (min) to attain peak viscosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.5</td>
<td>0.49</td>
<td>11.7</td>
<td>11.9</td>
<td>0.0479</td>
<td>8.751</td>
<td>90</td>
</tr>
<tr>
<td>13.5</td>
<td>0.49</td>
<td>11.7</td>
<td>12.2</td>
<td>0.0684</td>
<td>12.215</td>
<td>30</td>
</tr>
<tr>
<td>13.5</td>
<td>0.49</td>
<td>11.7</td>
<td>12.5</td>
<td>0.0895</td>
<td>14.403</td>
<td>15</td>
</tr>
</tbody>
</table>

** Shear rate: 28.16 s\(^{-1}\)
From the values of $s$, the flow behaviour index, ultrafiltered blood plasma solution is non-Newtonian (pseudoplastic plus yield value) and its flow behaviour almost approached Newtonian behaviour as evidenced by the slight deviation of $s$, from unity, and the tendency to approach Newtonian behaviour is increased with higher protein concentration.

The viscous behaviour of ultrafiltered blood plasma solutions appeared to be that of a non-Newtonian liquid when it was expected to be a true Newtonian behaviour. Then how will a spinning solution obtained from it behave? Results show that viscosity behaviour of ageing plasma spinning dopes vary with the pH, concentration of plasma proteins, the temperature and time of ageing and also found to be shear dependent.

6.4 Effect of pH on Rheological Behaviour

All alkali-treated plasma solutions studied in this investigation showed a non-Newtonian characteristic (i.e. pseudoplastic plus yield stress) and time-dependency; the pseudoplastic flow being more complex with increase in pH. The apparent viscosities at different shear rates as a function of pH are presented in Figures 25 and 26.

It was found that an increase in pH up to about 12.5 at a given plasma concentration and under comparable conditions resulted in a higher peak viscosity for a given ageing time and temperature (Table 22,
FIGURE 25a

EFFECT OF pH OF THE MEDIUM ON THE FLOW CURVES OF SPINNING DOPE.

Shear Rate (sec\(^{-1}\) \times 10\(^3\))

<table>
<thead>
<tr>
<th>pH 9</th>
<th>pH 10</th>
<th>pH 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>12</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Stress (Pa)
EFFECT OF pH OF THE MEDIUM ON THE FLOW CURVES OF SPINNING DOPE.
EFFECT OF pH OF THE MEDIUM ON VISCOUS BEHAVIOUR OF SPINNING DOPE.
And increase in pH significantly reduces the time taken to reach peak; viscosity. The effect of pH values up to about 10.5 for a given plasma solution was found to increase the solubility (Chapter 4) of the plasma proteins without any appreciable change in viscosity or characteristic colour. Between pH values of 10.5 and 11.5 there was a gradual change in both colour and viscosity within a period between 2 and 6 hours depending on the total solids and ageing temperature.

Stable and spinnable dopes were found in the pH range of 11 to 11.75. Between pH 11.75 and 12.5 rapid change in viscosity and colour of the solution occurred within a matter of minutes (1 to 10) for protein concentrations about 11% and increased solvation for concentrations below. At the latter pH range, ageing time and temperature are less important. pH of 11.50 was noted to be the most suitable (for a given set of variables) for manufacturing purposes and to give the best stable and spinnable dope over the time needed to spin the whole dope through the spinnerette.

On the whole, pH was found to exert the greatest influence on the rheological behaviour of plasma solutions; the pseudoplastic behaviour not only increased but became more complex to interpret especially with increased ageing temperature.
The increased apparent viscosity of ultrafiltered blood plasma solution as the pH of the medium was increased was thought to be largely due to the interaction between the disrupted protein chains and the solvent of the medium, probably free alkaline solution. Such interaction was observed to be accelerated as the pH was raised owing to the immobilisation of free alkaline medium between the aggregates of the protein molecules. Viscosity increases during ageing of a spinning solution as a result of denaturation which is brought about by the complete or incomplete unfolding of the polypeptide chains. Where the incomplete unfolding occurs as a result of minimal alkali treatment application of heat to such maturing solution may enhance increased viscosity. But where complete unfolding occurs as a result of maximal alkali treatment, application of heat will certainly promote gel formation.

There is an initial rise in apparent viscosity of an alkali-treated plasma solution which may or may not be followed by a fall as a result of hydrolytic processes taking place after the initial rise. For the latter to occur depends essentially on the protein concentration. Where the consistency of the spinning solution is too thin for spinning it might be due to partial solubility of the plasma proteins or the nature of their aggregation in solution. The drop in viscosity at pH's values lower than 11.0 with time and even in the heated
dispersions might be indicative of some hydrolytic action.

6.5  Effect of Protein Concentration And Total Solids On Rheological Behaviour

The apparent viscosity of the samples increased with increasing protein concentrations and total solids as shown in Figures 27, 28 and 30. Low total solids or protein concentration caused a rapid fall in apparent viscosities after the initial rise even in the presence of sufficient alkalinity. Hence it is important for destructed protein molecules to be present at a sufficient concentration to bring about an appreciable increase in viscosity (Giddey, 1960).

An increase in protein concentration or total solids was found to increase the magnitude of the peak viscosity attained to increase the viscosity obtained for a given ageing temperature and time. (Table 21, Page 148). The influence of protein concentration on the ageing time taken to attain peak viscosity was not as pronounced as that of pH and indeed not great. Concentrations below 11% resulted in too thin a consistency to be of interest in fibre production or where structure is desired. Figure 28 (Page 159) shows that viscosity rises linearly with increase in concentration, a behaviour known to be typical of macromolecular dispersions (Circle et al. 1964).

An increase in protein concentration of spinning dopes resulted in a corresponding increase in consistency and shear stress. At the right pH value (~ 11.0) plasma solution containing 15% protein is
FIGURE 27a
FLOW BEHAVIOUR CURVES OF ULTRAFILTERED BLOOD PLASMA SOLUTION AS A FUNCTION OF PROTEIN CONCENTRATION.
FLOW BEHAVIOUR CURVES OF ULTRAFILTERED BLOOD PLASMA SOLUTION AS A FUNCTION OF PROTEIN CONCENTRATION.
APPARENT VISCOSITY - SHEAR RATE RELATIONSHIP OF ULTRAFILTERED BLOOD PLASMA SOLUTION AS A FUNCTION OF PROTEIN CONCENTRATION.
sufficient to produce protein fibres of satisfactory texture and strength. Although a high protein concentration enables lower pH's (10.5 to 11) to be used when spinning the resulting fibres demand longer residence time in the coagulating solution. Such dispersions are easy to spin since they do not form gel throughout the spinning period and require less heat treatment during the ageing period.

6.6 Effect of Ageing Time on Rheological Behaviour

The very high molecular weights of most of the plasma proteins will greatly prolong the time necessary for appreciable configurational changes and this was somehow reflected in the data obtained under the effect of ageing time on viscous behaviour of spinning dopes at different pH values and ageing temperatures (Figures 35 - 45). The increasing apparent viscosities with ageing time reflects the increasing response to stress by configurational rearrangements.

Ageing time of spinning dopes (at a fixed temperature) was found to influence the rheological data and it could, therefore, equally affect textural parameters of the spun products. Ageing time was found to be only critical at pH below 11.0 and low protein concentrations (< 11.5%). Figure 35 (Page 169) shows how ageing time affected apparent viscosities of spinning dopes of such low protein content. There is a decrease in apparent viscosity after 2.0 hours.
This might be due to hydrolytic effect at the pH of ageing. Increase in viscosities during the first hour can be attributed to molecular reduction in polypeptide chains and their subsequent aggregation. The slight change between the first two hours can be traced to the fact that different protein chains are progressively disrupted at different rates under the operating conditions. After 2 hours of ageing period there is a fall in apparent viscosities as the breakdown reaction progresses. From the relation \( T = c \cdot t^m \) where \( T \) = shear stress, \( t \) = ageing time (minutes) and \( c \) is a constant, \( m \), time dependency index, equals 1.55, 1.81 and 1.08 for 11%, 13% and 15% protein respectively suggesting that ageing time becomes less important for concentrations above 13%.

6.7 The Effect of Temperature on Rheological Behaviour

The effect of temperature can be attributed to changes brought about by the more heat-sensitive globulins and fibrinogen. Although the concentrations of protein fractions are normally in the ratios of albumin : globulin : fibrinogen at 4.0 : 2.5 : 0.3 approximately but their contributions to viscosity increase over that of the ultrafiltrate are as 36 : 41 : 22 percent according to Lawrence (1961).

An increase in temperature reduced significantly the time taken to reach peak viscosity Table 20 (Page 149) and accelerated gel formation at pH (\( > 11.75 \)) and increased solvation at pH (\( < 10.5 \)).
FIGURE 29

EFFECT OF TEMPERATURE ON APPARENT VISCOSITY – SHEARING TIME RELATIONSHIP OF SPINNING DOPE.
Stable and spinnable dopes were obtained at temperatures between 35 and 45°C other variables being appropriate. Figures 29, and 35 to 45 show how temperature could influence viscosity behaviour of ageing solutions. Raising the temperature rapidly increased the apparent viscosities of ultrafiltered plasma solutions adjusted to pH 10.5 and 11.0 thereby reducing the 'ripening' time. Raising the temperature of solution adjusted to pH 11.9 and 50°C as above formed gel within 5 minutes. However, temperature was used to reduce the ageing time, but such heat treatment did cause greater difficulties in spinning.

6.8 Effect of Shear Rate
It is viewed that attractive forces between globules during alkali treatment of plasma proteins result in the building of aggregates and the arrangement of globules in chains. During shearing, these chains are stretched elastically resulting in breakage of bonds between the globules. Consequently, spinning dopes which gradually thickened when allowed to "mature" under appropriate conditions became progressively thinner when sheared. If the shearing rate is sufficiently high so that non-Newtonian viscosity is observed, the nature of the subsequent relaxation depends mainly on shear rate. Apparent viscosity generally decreased with increasing rate of shear. The slope in the log. apparent viscosity vs. log shear rate plot drops
FIGURE 30

VARIATION IN APP. VISCOSITY - AGEING TIME RELATIONSHIP FOR UNFRACTIONATED BLOOD PLASMA SOLUTIONS OF DIFFERENT PROTEIN CONCENTRATION AT FIXED pH (SHEAR RATE: 28.16 s⁻¹).

- 15.95% Total solids
- 14.75% Total solids
- 12.65% Total solids
FIGURE 31

Shear Rate: 2816 s$^{-1}$
Ageing Time: 5 min.
Protein: 11.7%
pH: 12.2

Effect of Shearing Time on Apparent Viscosity of Two Batches of Ultrafiltered Blood Plasma Solution.
Effect of pH on Apparent Viscosity - Shearing Time

Curve of an Ultrafiltered bovine blood plasma solution stored frozen for one day. (Shear rate: $28.16 \text{ s}^{-1}$).
Effect of pH on Apparent Viscosity - Shearing Time

Curve of an Ultrafiltered bovine blood plasma solution stored frozen for a week. (Shear rate: 28.16 s⁻¹).
Effect of pH on Apparent Viscosity - Shearing Time

Curve of an Ultrafiltered bovine blood plasma solution stored frozen for two weeks. (Shear rate: 28. 16 s$^{-1}$).
FIGURE 35a
Effect of Ageing Time on the Flow Curve of an ultrafiltered blood plasma solution at a fixed pH (9.2% protein; 0.15% ash; pH 12.2; ageing temp.: 25°C).
EFFECT OF AGEING TIME ON THE FLOW CURVE OF AN ULTRAFILTERED BLOOD PLASMA SOLUTION AT A FIXED pH [9.2% Protein; pH 12.2; Ageing temp.: 25°C; 0.15% Ash].

FIGURE 35b
FIGURE 36

EFFECT OF AGEING TIME ON APP. VISCOSITY-LOG SHEAR RATE CURVE OF AN ULTRAFILTERED BLOOD PLASMA SOLUTION AT A FIXED pH.
FIGURE 37

EFFECT OF AGEING TIME ON THE FLOW CURVE OF SPINNING DOPE AT FIXED pH AND AGEING TEMPERATURE [11.3% PROTEIN, 12.5 T. SOLIDS].

- pH 10.5
- 0.16 Hr. (20±1)
- 1.0
- 18.0
- 24.0
- 36.0
FIGURE 38

EFFECT OF AGEING TIME ON THE VISCOS BEHAVIOUR OF SPINNING DOPE (Conditions as for Figure 37).
FIGURE 39

Effect of Ageing Time on the Flow Curve & Viscous Behaviour of Spinning Dope
(Conditions as for Figure 37).
FIGURE 40

EFFECT OF AGEING TIME ON THE FLOW CURVE OF SPINNING DOPE (Conditions as for Figure 37)
FIGURE 41

EFFECT OF AGEING TIME ON THE VISCIOUS BEHAVIOUR OF SPINNING DOPE (Conditions as for Figure 37).
FIGURE 42

EFFECT OF AGEING TIME ON THE VISCIOUS BEHAVIOUR OF SPINNING DOPE (Conditions as for Figure 37).
FIGURE 43

EFFECT OF AGEING TIME ON THE FLOW CURVE & VISCOSOUS BEHAVIOUR OF SPINNING DOPE (Conditions as for Figure 37).
FIGURE 44

EFFECT OF AGEING TIME ON THE FLOW CURVE OF SPINNING DOPE (Conditions as for FIGURE 37).
FIGURE 45

EFFECT OF AGEING TIME ON THE FLOW CURVE & VISCOS BEHAVIOUR OF SPINNING DOPE (Conditions as for Figure 37).

pH 12.0
0.16 Hr. (20±1°C)
under high shear rates by a factor of about 2.5. At high shear rates
the molecule is somehow distorted. The sheared sample prepared
for SEM view at even moderately low shear rate lost most of its
structure (Plates 21 to 25). This can be associated with the progres­
ssive breakdown of structures. Flow curves showed that the shearing
samples generally gave a maximum value of stress which decreased
with shearing time. The existence of a maximum stress appears to
be typical for a non-Newtonian viscosity behaviour after a sudden
initiation of flow at a constant shear rate.

The steady fall in stress following the maximum has been observed
by many workers to be due to either slippage at the dope/rotor
contant or to structure deformation. It is in author's view that the
fall in stress during shearing is solely due to the latter case.

6.9 Effect of Shearing Time
Shearing time is as important as the shear rate for time-dependent
viscous material because of the extent to which viscous dopes are
bound to be sheared during spinning. Minimum shear rate and
shearing time compatible with the spinning operations are likely to
promote better reactivity by exposing more amino-acid side chains
to the surface and rendering other reactive groups available for
formation of more linkages during coagulation.
Figure 31 (Page 164) shows the drop in apparent viscosity noted as the spinning solution was subjected to constant time shearing. As figures 29 - 34 show spinning dope is generally time dependent, that is, the stress or the apparent viscosity decreased with time at a particular rate of shear, owing to breakdown of structure. Young and Lawrie (1975a) reported that the flow curves for alkali-treated plasma proteins exhibit non-Newtonian fluid properties of a time-independent nature with the Ferranti-Shirley cone and plate viscometer. Shearing time affects the consistency of spinning dopes to a different degree at various temperatures (Figure 29, Page 162). This structural change and other time dependent changes are bound to influence the textural characteristics of spun products. Using appropriate temperature and shearing time could be helpful in deciding whether a particular dope was spinnable and capable of producing fibre of satisfactory texture.

6.10 Effect of Storage

Different batches of the same ultrafiltered plasma solution showed variation in alkali/protein ratio required for the desired pH during storage (Figures 32 - 34). While the same alkali/protein ratio may give the required pH for different batches of freshly ultrafiltered plasma solution, different alkali/protein ratio was needed to give the same pH when stored for more than 48 hours at -15°C (Tables 22 - 24; Page 148/9). This may be due to intrinsic diffe-
rences between the protein of different batches, and such variations
due to intrinsic factors or pre-handling are, therefore, suggested
for future investigation.

6.11 General Discussion
The addition of few drops of strong caustic soda significantly affec­
ted the flow behaviour and resulted in a honey-like dispersion with
non-Newtonian characteristics. The flow curve for spinning dope
has a yield value and the apparent viscosity falls rapidly with increa­
sing rate of shear. The occurrence of a yield stress means that a
certain amount of stress must be applied before flow is initiated.
Many different factors influenced the flow behaviour of spinning dope
and the principal ones are pH, protein concentration, ageing tempe­
rature and time, shearing time and shearing rate.

pH was noted to be a critical factor in the preparation of a spinnable
dope. The reason for this might be that altering the surrounding
medium of ultrafiltered blood plasma solution affects the structure
and arrangement of different protein chains which eventually results
in rheological parameter changes thereby suggesting an interdepen­
dence with structure. The difference in viscous behaviour is attri­
butable to aggregation of plasma proteins in the presence of alkali
especially at low shear rates. The suspected insolubility or folding
of fibrinogen and globulins at too low pH values would result in monodispersed suspension and the shear dependence of viscosity could then be attributed to the occurrence of shear deformation. At pH around 10.0, where the molecules are associated but probably not bonded there was a slight drop in apparent viscosity after the initial rise but at pH above 11.5 where bondings are more likely to exist, change in apparent viscosity was much more pronounced.

It is interesting to note that onset of flow occurred at a much higher shear rate for pH 9.0 than pH 10.0 and the latter at higher shear rate than pH 11.0 (Figure 25, Page 151). It can be inferred from this observation that the form of the dependency of apparent viscosity on shear rate is strongly related to the presence and distribution of the disrupted polypeptide chain brought about as a result of change in ionic charge; an even distribution throughout the spinning mass will cause a more gradual change of apparent viscosity at low shear rates where the limiting value of consistency is approached.

The appropriate pH was not necessarily the one that offered highest peak viscosity but rather one that allowed best manipulation of the spinning mass. Apart from the formation of gel or dope which could be troublesome during spinning too high a pH (> 11.75) would reduce the nutritional qualities of the final product if it were to be spun.
This latter condition, therefore, limits alkali treatment to a minimum compatible with satisfactory spinning, hence the importance of using a high protein concentration and low pH around 10.5. The protein concentration (or total solids) of a blood plasma spinning mass is also an important factor affecting the conditions of the flow of a fibre-forming mass. This might be due to the fact that concentration, as well as temperature, influence the mobility of the spinning mass and the extended polypeptide chains.
Specimen prepared with minimum possible disturbance to the plasma solution.

ULTRAFILTERED [BOVINE] BLOOD PLASMA SOLUTION [pH 7.5, 13.2% TS, 11.5% PROTEIN, 0.48% ASH].

PLATE 21
X 10,000
(Edge)

X 10,000
(Centre)
Plate 21 adjusted to pH 11.9 with 50% NaOH solution & aged at 20°C.

Plate 22
X 10,000
(After 2 min.)

Plate 23
X 10,000
(After 5 min.)

EFFECT OF ADDING ALKALINE SOLUTION TO ULTRAFILTERED BLOOD PLASMA SOLUTION.
Plate 23 sheared \( (\gamma = 28 \text{s}^{-1}) \) at \( 20^\circ \text{C} \) for 10 min.

Plate 24 \( \times 10,000 \) (For 5 min.)

Plate 25 \( \times 10,000 \) (For 10 min.)

EFFECT OF SHEARING TIME ON THE DEFORMATION OF STRUCTURE IN SPINNING DOPE.
CHAPTER 7

DETERMINATION OF COAGULATION TEMPERATURE

7.0 Introduction

Coagulation temperature of an ultrafiltered bovine plasma solution was determined by the procedure described below with the aim of selecting an appropriate processing temperature.

7.1 Experimental

100 ml. portions (duplicate samples) of an ultrafiltered bovine plasma solution were transferred to 250 ml. conical flasks and placed in a constant temperature bath held at desired temperature for 30 minutes. The temperatures used were from 20 - 70°C with intervals of 10°C. Control samples were kept at 20 ± 1°C during the heat treatment. After the heat treatment the sample was transferred to a centrifuge tube and allowed to stand at room temperature for 30 minutes. The tubes were later centrifuged at 10,000 G for 30 minutes at 0°C. Percentage protein remaining soluble in the supernatant was used as an index of the degree of protein coagulation induced by the heat treatment.

7.2 Results and Discussion

Figure 46 (Page 191) shows the protein solubility of ultrafiltered plasma proteins after 30 min. heat treatment expressed as a frac-
tion of original protein level before heat treatment.

The effect of various heat treatments on the solubility curve of plasma solution can be explained by the difference between the residual proteins and the insoluble plasma proteins at a given pH, which are removed as aggregates by centrifugation at 10,000g during the NSI determination.

There is no appreciable change in solubility with increasing heat treatments up to 55°C. Between 55 and 70°C there is a sharp decrease in solubility. This indicates that ultrafiltered plasma proteins are affected at temperatures around 55°C. The increased protein aggregation in plasma solution at higher heat treatments is the cause of decrease in NSI. Temperature influences the solubility of proteins by its effects on the dissociation of the various free groups of proteins and the forces between molecules. Increase in the effectiveness of electrostatic forces results as the temperature is increased. Hence, solubility is much diminished in concentrated blood plasma solution with temperature. Considering the influence on heat treatments on the physical properties of plasma proteins it is evident that treatments at 55°C are critical in the sense that solubility starts to decrease and any processing conditions at this temperature or above are likely to affect other functional properties to the same extent.
FIGURE 46

INFLUENCE OF TEMPERATURE ON PROTEIN COAGULATION OF AN ULTRAFILTERED BLOOD PLASMA SOLUTION [pH 7.10, 13.96% Protein].
Since the aim of this test was to select the appropriate temperature below the coagulation point, $40^\circ$C was chosen for further processing of plasma proteins.

7.3 Coagulation of Spun Protein Filaments

Fibre formation by wet-spinning involves a process in which a moderately viscous protein solution is spun through the small holes of a spinnerette immersed in an acid-salt bath. During the process of coagulation a diffusional interchange between the freshly formed fluid filaments and the bath solution causes the protein to solidify (Paul, 1968). This means that during coagulation one or more components of the bath solution diffuse into the filament, while the solvent diffuses out of the latter, and as a consequence of this exchange the protein precipitates. Chemical reaction between the protein and the salt further insolubilises the protein filament.

During the diffusional interchange between the two phases, solvent is expected to diffuse out of the protein while acid and salt probably diffuse into the protein phase. After a given length of time no more exchange takes place, hence a state of equilibrium will be established between the two phases. But in view of the nature of the protein and the spinning dope in particular this process may not be described as a true thermodynamic equilibrium. At any rate, for a given spinning dope, the final state would be dictated by the composition of the
coagulating mixture and the temperature at which coagulation occurs. Apart from diffusional exchange, rheological phenomenon is also believed to be involved. Assuming that only diffusional exchange is taking place during coagulation, this process will eventually lead to an increase in the water content of the bath and a change in weight of the coagulated protein filament since the different components will not diffuse at equal and opposite rates. By following the composition of the coagulating solution and the weight of the coagulated fibre one can obtain information on how the water or solvent exchanged for salt and acid at any given time, leads to coagulation.

7.4 Experimental

Experiments were performed on spinning solutions of fixed composition to determine the diffusional changes taking place during coagulation. The composition of the coagulating solution is the same as that described under wet-spinning. The change in bath composition was obtained by measuring the refractive index of the bath and fibre weight changes were simply by weighing different samples that had been in the constant temperature bath for various lengths of time. The spinning dope and the bath were brought to the same temperature before the experiment was started. Care was taken as far as practically possible to prevent moisture from the air or other sources from contacting the coagulated fibres. The time scale was chosen to allow
measurable changes and adequate mixing was provided by mechanical shaker. Results are expressed as percent change in the refractive index of the coagulating solution at 20°C and percent weight loss in fibre as a function of coagulation time.

7.5 Results and Discussion

The results are presented as a function of the time scale and temperature in Figure 47 (Page 195). The data show increased diffusional rates at higher temperatures. When percent changes in bath composition and weight loss in fibres were plotted against the square root of the diffusion time there was an initial linearity which extended for almost the whole length of the curve followed by the peak region where diffusion rate was constant. Where the curve flattened out represents the equilibrium point and determines the optimum residence time for the spinning conditions. From the results of weight loss in fibre, components of the extruded viscous dope leave faster than the rate at which salts and acid enter with the consequence that the liquid component of the dope decreases or the total solids level increases up to a point where the boundary layer is formed. The mechanism eventually leads to the phase change. The high concentration of salts in the coagulating solution sets up an osmotic pressure with the result that there is some decrease in the diameter of the filament so that the fibre finds the penetration of the bath components more difficult along the length of the coagulating tank. The almost constant
FIGURE 47

EFFECT OF TEMPERATURE ON COAGULATION RATE OF SPUN BLOOD PLASMA PROTEIN FIBRES.

Fibre: [Graph showing change in refractive index of coagulating bath over time with different temperatures.] 
Bath: [Graph showing change in weight loss in fibre over time with different temperatures.]
diffusional rates at the later stages can be associated with the finite fibre diameter.

Small changes in bath composition occurred with coagulation time and such changes decreased as the temperature of the coagulating solution increased and this is probably due to the fact that the surface layer of the filament is formed at a faster rate as the temperature is increased. Coagulation may be considered complete after 15 min. under the experimental conditions.
CHAPTER 8

WET-SPINNING OF BLOOD PLASMA SOLUTION

8.0 Coagulating Solution

The precipitating solution consisted of 10% NaCl, 5% Na₂SO₄ and 5% MgSO₄·7H₂O (commercial grade chemicals) adjusted to desired pH of 2.5 or 1.5 with orthophosphoric acid (cf. 1N acetic acid containing 20% (W/v) NaCl, pH 2.3 ——Young and Lawrie, 1975b).

Giddey (1960) used a coagulating bath consisting of an aqueous solution of 100 g. per litre CaCl₂·6H₂O adjusted to pH 1.3 with HCl at room temperature. The solution was composed to increase the binding capacity of the fibres and also to help the fibres to achieve some crystallinity probably through hydrogen bonding. The high salt content of the solution suppressed the swelling of the filaments as a result of high osmotic pressure while the presence of acid exercised a plasticizing effect on the freshly coagulated filaments and facilitated their withdrawal from the spinnerette holes.

Acid concentration around 2% by weight of the coagulating solution was found to be favourable to production of fibres of adequate strength and satisfactory texture. Concentration of acid higher than 5% by weight resulted in poor fibres and this is probably due to rapid coagulation of the surface layer with an uncoagulated inner layer. Phosphoric acid was found to produce fibres of better strength, texture and less acid smell than acetic acid probably due to the presence of
phosphate instead of acetyl radical. Concentrations of salts as used above caused a considerable shrinkage in the diameter of the freshly extruded filaments.

8.1 Spinning Procedures

The apparatus used in the spinning operation was a simple laboratory scale model designed to produce fibres of satisfactory textural qualities. Figure 48 (Page 199) shows the diagramatic layout of the spinning apparatus. The apparatus consists of a glass made reservoir that can withstand applied pressure up to 172 kPa, a Palmer pump with a rotational velocity between 1 and 10 rev/min., a coagulating tank designed to keep the precipitated filaments in straight condition and a spinnerette (Plate 26 Page 200) The spinnerette has a capillary length that is twice the hole diameter and a shape designed to enhance maximum alignment of the disrupted protein molecules.

During the spinning operation, 'mature' dope was forced from the reservoir through the connecting line to the pump with a pressure of 105 kPa. The pump which was maintained a speed of 7.5 rev./min. delivered at a constant rate sheared spinning dope to the spinnerette end that was submerged completely in the coagulating solution. The coagulating solution was maintained at a constant temperature and
FIGURE 48

Mixer/Pressure vessel

Fibre Tow

Winding Reel

Heater/Mixer

APPARATUS:

A Hardening/Neutralizing Solution

B

C

FIBRE SPINNING

PUMP

Spinnerette

SPINNING BATH
PLATE 26 — showing spinnerette A, the conventional type & B, used in this study.
equipped with a circulating pump at the other end of the spinnerette.

Freshly precipitated filaments were led from the solution to the rotating take-away reel maintained at a speed of 400 cm/min. to impart stretch on the filaments. Boyer (1954), Dechaine and Callaghan (1967), claimed that the toughness or tenderness of the spun protein product can be controlled to a degree by the amount of stretch which is imparted to the fibres in the initial forming procedure and that the stretching results in an orientation of the molecules in the protein fibres. Fibres were removed from the reel and transferred to the extended part of the spinning apparatus for further treatment as described under post-treatment of fibres.

8.2 Preparation of Spinning Dope

500 ml. portions of ultrafiltered plasma solution of desired protein and ash content were used for spinning. Spinning dope was prepared by dropwise addition of 50% or 20% (W/V) NaOH to ultrafiltered plasma solution followed by thorough mixing until a desired pH was reached. After adjusting the pH and filtering the alkali-treated solution was allowed to "mature" with constant but gentle stirring in a constant temperature water bath until spinnable viscosity was achieved. The ageing time varied from 0.5 hour to 4.0 hours depending on the protein content and pH of the medium. 20% NaOH was found to be more suitable for plasma solutions with protein concentration above 13%.
Operating conditions must be set during ageing such that the protein is well dispersed and the molecules so aggregated in a manner to enhance their coming close together in an orderly and well-packed way during the process of setting and coagulation. Moderate heat treatment of the spinning dope at this stage was observed to be an essential part in the production of fibrous protein of adequate strength or chewiness. It might be that such heat treatment enhanced complete unfolding of the protein chains and/or increased solubility of the proteins. 40°C was found to be the appropriate temperature for the preparation of stable and spinnable dope.

Complete removal of air bubbles while still in liquid phase was noted to be important, for any air bubbles in the spinning solution would render spinning more difficult or even cause discontinuities in the filaments. Figure 49 (Page 205) shows that spinning dope consistencies increased from fluid at low concentration and pH, to semi-solid at moderate and extremely viscous and/or gel formation at high protein concentrations pH values. It can be said, therefore, that the stability and suitability of dopes for fibre production is markedly dependent on the pH and to a lesser extent on protein concentration. From Figure 47, five regions can be recognised according to the suitability of the resulting dope for fibre production:

A - represents unspinnable viscous dope; dispersed protein chains scattered in the coagulating solution during spinning.
This might be due to low viscosities accompanied by partial unfolding of protein chains at these pH values or for the fact that the protein chains are not well dispersed and/or the molecules are randomly aggregated which perhaps makes their coming in an orderly and well-packed manner impossible.

B - gives viscous and spinnable dope but weak fibres. Coagulated fibres are sensitive to touch and difficult to draw or even handle during subsequent treatments. Dispersed protein chains are probably not well-packed or properly aligned before coagulation. Viscosity can, again, be implicated.

C - yields stable and spinnable dope with fibres of satisfactory properties. Coagulated fibres at these pH values are easy to draw and handle. Fibres tend to float immediately in contact with the coagulating solution and finally settle down at the bottom and other end of the coagulating tank. The resistance of the freshly coagulated fibres against dissolution and deformation is increased. This might be as a result of a more orderly alignment of the dispersed polymeric molecules in fibres.

D - produces the same results as for C above but spinning operations tend to be more difficult. Viscous dope is initially semi-solid which becomes gel as spinning progresses.
At these pH values, more or more reactive groups such as carboxylic groups are perhaps being exposed to form salts. Stabilisation of the unstable dope is needed to render the dope satisfactorily spinnable. 5% Ascorbic acid has been used in this study as a stabilising agent.

E - is another region of unspinnable viscous dope but unlike A due to high viscosity. Gelation sets in after a few minutes of preparation. Stabilisation as for D fails to produce spinnable dope. This is due to high pH values and/or high protein concentrations and formation of gelled structure at these pH values prevents the manipulation of the spinning solution as regards filtration or pumping.

Figure 49 can, therefore, be used to study the relationships between product structure and its rheological characteristics.

8.3 Influence of Ash on Spinning Dope

This was studied by adding back to the ultrafiltered plasma solution 0.5, 1.0 and 2% (W/v) of a mixture of salts thought to have been removed during ultrafiltration (NaCl : MgSO₄ : K₂HPO₄ 2 : 1: 0.5). It seemed from the observation that the level of the total ash in spinning dopes affected the dispersibility of plasma proteins in alkaline medium and, therefore, the suitability of the dopes for spinning. The addition of 0.5% of the mixture of salts to spinning
FIGURE 49

VARIATION IN APPARENT VISCOSITY - pH RELATIONSHIP AS A FUNCTION OF PROTEIN CONCENTRATION FOR ULTRAFILTERED BOVINE BLOOD PLASMA SOLUTION. (SHEAR RATE: 28.16 s⁻¹).
solution had no apparent effect on viscosity while the addition of 1% or 2%, on the other hand, lowered the viscosity and thereby prolonged the ageing time to reach peak viscosity. Heated samples containing 1% or 2% at 40°C resulted in higher viscosity when compared with the control sample. The addition of 2% raised the viscosity of the sample with increased tendency to form gel in the heated sample.

Plasma solution with salt level less than 1.0% dispersed well in alkaline medium and gave better (homogeneous) spinning dope compared with solution of higher salt content. While viscosity rise was somehow retarded in solutions containing 1.0 and 2.0% salt such high salt content was found to be advantageous in preventing or delaying gel formation at 20°C. Apart from raising or lowering the viscosity of spinning dopes, the presence of salts as observed in this experiment also affected the quality of the resulting dope, with soft viscous dope at 0.5% and firm viscous dope at 1% and 2%. Giddey (1960), noted that ageing of spinning solutions may be hindered by the presence of sugars, amino-acids and salts such as those which are to be found in crude protein extracts. Since a high salt content indicates the presence of di- and polyvalent ions, their presence may prevent the complete dispersion of the proteins in alkaline medium by forming complex compounds with the proteins. Considering the quality of the dope samples with 1% or 2% salts the use of ultrafiltration in reducing the ash content of blood plasma solution is justified.
8.4 Post-Treatment of Fibres

One of the most important characterisation which must be controlled during the manufacture of textured protein materials is the texture. While this has been partly achieved by stretching and hydrodynamic flow during the spinning operation it was realised that not only could the taste, odour and appearance of freshly coagulated fibres be improved by altering the pH but also that the degree of toughness of the fibres was a function of the pH and temperature used in the after-treatments of the fibres. Hence, further treatments of fibres after coagulation were carried out in order to improve their texture and to make them satisfactory for food product use.

As the fibres emerged through the spinnerette holes into the coagulating solution they were led out after coagulation via a winding reel at a rate sufficient to keep them in a straight condition into a series of solutions of different composition and pH values:

SOLUTION A: 10% NaCl adjusted to pH 2.5 with 5% ascorbic acid maintained at 30°C.

SOLUTION B: 10% NaCl + 5% Na₂SO₃ adjusted to pH 4.5 with ascorbic acid maintained at 40°C.

SOLUTION C: 10% KCl + 2% Sucrose + 2% Na₂SO₃, pH 6.0, maintained at 50°C.

These neutralizing solutions were maintained at the stated temperature and slowly agitated in order to permit good penetration of the individual fibres. Upgrading the pH of the fibres as described above
did tend to improve the sour taste, appearance and toughness of the freshly coagulated fibres when compared with the control samples. Raising the temperature of the freshly coagulated fibres from 30 to 50°C was found to be beneficial both in appearance and ability to withstand heat treatment during cooking than one-step heat treatment at 50°C, and the latter step better than without such post-treatment.

This treatment is thought to enhance slow and complete coagulation of uncoagulated part of fibres during initial precipitation. The presence of salts during the heat treatments is believed to improve the binding capacity probably through the formation of additional bonds or complexes between protein fibres and salts while the addition of 2% sucrose significantly improved the acid odour and taste as a result of the reaction between reducing sugars and protein fibres.

8.5 Compositional Analysis of Spun Protein Fibres

Fibres after draining off excess solution were dried to constant weight in a vacuum oven at 60°C and analysed for moisture content, protein-N, total sugars, total inorganic phosphorus, titratable chloride, and total ash. Samples of 1.0g dry protein fibre were weighed into 100 ml volumetric flasks and were made to volume with 2N NaOH after shaking vigorously at 50°C. Stock solutions of fibre dispersion so prepared were analysed for the desired components.
Table 25 (Page 210) shows compositional analysis of spun plasma fibres. These results are similar to those reported by Young and Lawrie, Tables 25 and 26 (1974, 1975) with the apparent exception of higher content of protein which is twice their reported figure. The average ash content in this study was also lower and this lower level has been achieved despite the after-treatments of fibres.
TABLE 25

Compositional analysis of spun plasma fibres+

<table>
<thead>
<tr>
<th>Component</th>
<th>Range</th>
<th>Mean\textsuperscript{a}</th>
<th>Mean\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>48.88 - 56.58</td>
<td>54.00</td>
<td>73.30</td>
</tr>
<tr>
<td>Protein (N x 6.25)</td>
<td>28.05 - 39.25</td>
<td>34.58</td>
<td>17.30(20)</td>
</tr>
<tr>
<td>Carbohydrate (as sugars)</td>
<td>3.80 - 5.20</td>
<td>4.17</td>
<td>—</td>
</tr>
<tr>
<td>Ash</td>
<td>5.65 - 6.41</td>
<td>6.06</td>
<td>8.60</td>
</tr>
<tr>
<td>Chloride</td>
<td>3.48 - 4.20</td>
<td>3.88</td>
<td>—</td>
</tr>
<tr>
<td>Phosphate (as total $iP_4$)</td>
<td>0.22 - 0.53</td>
<td>0.25</td>
<td>—</td>
</tr>
<tr>
<td>Other ingredients including Fat.</td>
<td>—</td>
<td>1.19</td>
<td>—</td>
</tr>
<tr>
<td>Fibre pH ($20^\circ{C}$)</td>
<td>5.50 - 6.80</td>
<td>6.20</td>
<td>—</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Mean of five samples from at least two duplicates each.
\textsuperscript{b} Mean analysis obtained by Young and Lawrie (1974). Figure in bracket after a few days storage.

TABLE 26

Analysis of spun protein fibres. +

<table>
<thead>
<tr>
<th>Source of protein</th>
<th>Plasma</th>
<th>Lung</th>
<th>Stomach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (%)</td>
<td>16.50 - 19.7</td>
<td>14.0 - 15.3</td>
<td>11.5 - 14.8</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>72.80 - 75.3</td>
<td>75.1 - 78.9</td>
<td>79.1 - 82.2</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>7.50 - 8.6</td>
<td>8.1 - 8.5</td>
<td>7.7 - 8.4</td>
</tr>
</tbody>
</table>

\textsuperscript{+} Analysis on samples rested in sealed bottles for 24hr. (Young and Lawrie, 1975a).
8.6 Photographs of Spun Fibres:

Plate 27 (X1)

11.5% plasma protein solution spun into an acidified salt solution (pH 2.5, 22°C). Spinning dope (pH 11.5), aged at 20°C to a spinnable viscosity of about 7.5 Pa.s (28.16 sec\(^{-1}\), 20°C).

Plate 28 (X1)

As for Plate 27 but, heat denatured at 40 ± 1°C to viscosity of about 12.5 Pa.s (28.16 sec\(^{-1}\), 20°C).

Plates 29. and 30 (X1)

14% plasma protein solution spun into an acidified salt solution (pH 2.5, 22°C). Spinning dope (pH 11.0) was heat denatured at 40 ± 1°C to viscosity around 10.0 Pa.s (28.16 sec\(^{-1}\), 20°C).

Plate 31 (X1)

As for Plate 28 but coagulating solution temperature raised too 35±1°C.

Plate 32 (X1)

12.5% plasma protein solution (14.56% TS, 1.06% ash) spun into an acidified salt solution (pH 1.0, 22°C) and stretched. Spinning dope (pH 11.5) was heat denatured at 40 ± 1°C to spinnable dope having viscosity around 8.5 Pa.s (28.16 sec\(^{-1}\), 20°C).

Plate 33 (X1)

As for Plate 32 but unstretched.
The most noticeable physical change was colour which changed from pale yellow to milky white as a result of processing. One advantage of coagulating protein fibres using salt solutions is that the colour of the product is whiter and more acceptable than when precipitated by other materials, for example, with acids or alkalis. Even in the presence of increased proportion of acid in coagulating solution gave fibres of poor colour especially when air-dried at room temperature.

With the addition of appropriate colours and flavours to the protein fibres their appearance can be greatly improved to taste which in turn may enhance the acceptability. Being heat stable, spun protein fibres can find suitable uses in various hot dishes. Considering the texture of spun protein fibres after cooking for 15 min. in 1% beef flavour at 80°C and their mechanical properties their chewy property can be expected to be good in any recipe.

8.7 Dried Blood Cells

In order to get rid of whole blood economically, use must be made of the remaining fraction of blood after converting the fluid part into edible fibres. This fraction varies between 30 and 40% of the whole blood depending on the efficiency of separation. This fraction was, therefore, concentrated to increase the total solids, treated with
2 - 5% (w/v) \( K_2SO_4 \) and dried. The resulting dried material contains 9 - 15% nitrogen with an average of 12.5%. This free-flowing powder was stable at room temperature for more than 6 months with about 74.7% recovery. Apart from the nitrogen content, the dried material is believed to be rich in iron, potassium, calcium and phosphorus to mention a few. The cellular fraction of animal blood can be used in two ways. One is to use the concentrated but undried blood cells for improving the binding power and meaty colour of many meat products. The other is not for the edible food industry but in both horticultural and agricultural industries to serve as a supplementary additive in plant or animal feeds, that is, as a fertiliser and live-stock feed.

Blood cells in dried state and in various forms and degrees of purity may be used as a valuable binding medium for plywood, a waterproof glue, a fire-extinguishing foam, a radiator sealing compound and for the manufacture of leather shoes (Hirchberg, 1957b).
CHAPTER 9

PHYSICAL PROPERTIES OF SPUN FIBRES

9.1 Measurement of Tow Linear Density and Diameter

Linear density of each bundle of fibres was determined by weighing on a sensitive balance under the same conditions five 7-cm. long pieces and the results expressed as average linear density.

The diameter of each specimen was found by taking several measurements with a micrometer gauge with minimum pressure.

9.2 Measurement of Tow Tensile strength and Break Elongation

For the determination of tensile strength fibre samples of known linear densities were used. In order to ensure a uniform moisture content, air-dried spun fibres were conditioned in an atmosphere of 70 ± 2% relative humidity at 25 ± 2°C for at least two weeks before they were tested. The fibre length was fixed at 7 cm. and the following conditions were employed during the tests.

- Tension load cell : 50N
- Full scale load : 5N
- Gauge length : 5 cm.
- Crosshead speed : 7 cm/min.
- Paper/Crosshead ratio : 5:1
- Extension : 4% cm. chart travel.

The testing was performed at a relative humidity of 65 ± 2% and a
temperature of 20 ± 2°C on a J. J. Tensile Testing Machine (Model T 5002) and calibrated before each use.

The fibre was placed in position such that it was in straight condition without any undue stretching. One end was fixed to the top clamp of the testing machine and the other to the lower clamp. Tensile strength was determined by mounting a sample with a test length of 5 cm. in the jaws and applying a load through the upward motion of the crosshead. The tension developed was measured by the load-cell to which the upper jaw was attached and recorded on the chart.

Tensile strength and percent elongation were calculated from the stress-strain curves obtained. Elongation was calculated as a percentage of the original test length. Five replicates were done for each experimental sample.
### TABLE 27

Diameter of spun plasma protein fibres.

<table>
<thead>
<tr>
<th>Spinning solution</th>
<th>pH 11.50</th>
<th>Average Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment</td>
<td>% Protein</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>11</td>
<td>0.0648</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>0.0749a</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>0.0752a</td>
</tr>
<tr>
<td>4</td>
<td>11.50</td>
<td>0.0763a</td>
</tr>
<tr>
<td>5</td>
<td>0.0899</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.0808a</td>
<td></td>
</tr>
</tbody>
</table>

+ Average of five measurements for each experimental sample. Means followed by the same letter are not significantly different at 5% level by Tukey's w-procedure. Coefficient of variability = 9.35% Standard error = 0.0023

### TABLE 28

Linear density of spun plasma fibres.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>% Protein</th>
<th>Average Linear density (g/cm.) +</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 11.5</td>
<td>pH 11.0</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>11</td>
<td>0.0069a</td>
</tr>
<tr>
<td>2</td>
<td>0.0072a</td>
<td>0.0059</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>0.0078</td>
</tr>
<tr>
<td>4</td>
<td>0.0076</td>
<td>0.0064a</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>0.0084</td>
</tr>
<tr>
<td>6</td>
<td>0.0081</td>
<td>0.0072</td>
</tr>
</tbody>
</table>
TABLE 29

Tensile strength of spun plasma protein fibres.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>% Protein</th>
<th>Tensile strength (g/cm²)</th>
<th>pH 11.5</th>
<th>pH 11.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>10895</td>
<td>8875</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>11650</td>
<td>9165</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>12748a</td>
<td>9329</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>12865a</td>
<td>9480a</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>13068a</td>
<td>9529a</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>13310</td>
<td>10596</td>
<td></td>
</tr>
</tbody>
</table>

+ Average of five measurements for each experimental sample.

Means followed by the same letter are not significantly different at 5% level by Tukey’s w-procedure.

TABLE 30

Break elongation of spun plasma protein fibres.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>% Protein</th>
<th>% Elongation</th>
<th>pH 11.5</th>
<th>pH 11.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>3.64</td>
<td>4.50</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>3.45</td>
<td>4.20a</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>2.90a</td>
<td>4.00a</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>2.80a</td>
<td>3.81a</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>2.65a</td>
<td>3.80a</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>2.26a</td>
<td>3.60a</td>
<td></td>
</tr>
</tbody>
</table>
9.3 Results and Discussion

Tables 29 and 30 (Page 224) show the mean results obtained on tensile strength and percentage elongation of spun plasma fibres from the stress-strain curves. Tables 27 and 28 (Page 223) are the diameter and linear density values of spun plasma protein fibres. The data showed variations in the mean tensile strength and elongation at break of the different samples examined. The tensile strength and elongation values of blood plasma fibres from samples studied vary from 8875 to 10596 g/cm² for pH 11.0 and 10895 to 13310 g/cm² for pH 11.5; and elongation values of 3.6 - 4.5 percent (pH 11.0) and 2.256 - 3.64 percent (pH 11.5). As the test conditions were the same these results show that there are differences related to the method of production and that even fibres from the same experimental treatment are very dissimilar in their mechanical properties.

It is known that various factors like fibre length, diameter, rate of load, humidity and mechanical conditioning affect considerably the tensile strength, as also the elongation. More important is the fact that the extent of strength, that can be achieved in spun protein fibres depends essentially on the nature of the binding forces within and between the molecules of the fibres, the arrangement of such molecules and the degree of molecular orientation. It is evident from the electron micrographs of spun fibres that they are made up of various components of different shapes, sizes and arrangements. This varia-
tion in the extent of the constitution of the spun fibre may be responsible for the deviation in the values for a given sample. Analysis of variance of the results showed that the samples were significantly different from each other in their mechanical properties at 1% level.

Average breaking load varies because of the variation in the mass per unit length of different fibres in the sample. It is more probable that the forces that caused rupture during fibre breakage are the lateral bonds like hydrogen bonds, salt linkages and other cohesive forces between adjacent units. Breakage might also take place as a result of degradation of intermolecular nature between fibres. It is believed that differences in disrupted protein chains as a function of pH that make up the filaments and which may slip past each other when a fibre bundle breaks, influence the strength of fibres. The differences observed in various samples may also be due to differences in the nature and amount of the constituents. Tensile strength increased with increasing protein concentration and pH of the spinning dope and the observed differences in tensile strength represent variations in the properties of spun blood plasma fibres obtained under different experimental conditions. The occurrence of microscopic imperfections in fibre structure is thought to be responsible for the low tensile strength and poor elongation.
9.4 Water Absorption Capacity of Spun Fibres

Texturized protein products are bound to be rehydrated prior to use and the time and conditions required are important product parameters.

Water absorption capacity was determined by rehydrating an accurately weighed 5.0g. of air-dried spun fibres in solvents at various pH values and a cooking temperature of 80 ± 1°C for 60 minutes. A control sample for each solvent was put in boiling water for zero time, drained and weighed immediately. After rehydration excess water was removed by draining and blotting with filter paper and the sample reweighed. The percent water absorbed was calculated as percentage increase in weight. The water uptake of spun protein fibres after 60 minutes at 80 ± 1°C in solvent of various pH values is represented in Figure 50 (Page 229). Although boiling is not the recommended cooking process, it is easy to control experimentally. It is known that heat processing (boiling or retorting) does not alter fibre textural or water absorption properties. When spun protein fibres are hydrated in water they showed a definite increase in weight and this is an indication that the molecules which make up the fine structure of the fibres have increased in dimensions. While there is no significant change in weight at zero time most of the water absorbed, occurred within the first 10 minutes of cooking period. After 10 minutes of cooking relatively little moisture penetrated the exterior of fibres.
Holding time did not result in increased water uptake as expected. The increase in water uptake with increasing pH is thought to be largely due to changes in ionic charge of the fibres and/or change in fibre density. The increased water uptake may be caused by the changed configuration of the aggregates or by protein denaturation. The high water uptake observed with distilled water at pH 6.3 might be due to increased amount of water bound by denatured proteins favourably at this pH. The relatively low uptake of tap water might be due to the fact that as the molecules of the fibre pack closely together during orientation it becomes increasingly difficult for molecules of water to penetrate between them. Another reason can be attributed to the pH of spun fibres in solution.

Protein fibres in distilled water (pH 6.3) become highly hydrated through the attraction of the water molecules to the various groups present in the protein in order to satisfy the osmotic condition of the solution whereas the degree of hydration in tap water is comparatively reduced owing to the predominance of the basic groups and polyvalent ions which probably reduce diffusion of water molecules into the fibres.

The swelling of protein fibres in water can simply be explained as being due to the attraction of the functional groups of amino-acids
EFFECT OF COOKING TIME AND pH OF THE COOKING MEDIUM ON WATER ABSORBED AT 80±1°C BY SPUN PROTEIN FIBRES [★=0.1 M PHOSPHATE SOLUTION].

FIGURE 50

Water Absorbed (%) vs. Cooking Time (min.)

- Dist. H₂O (pH 6.3)
- Tap (pH 8.3)

pH 10.5 ★
pH 8.5
pH 6.5
pH 4.5
for polar groups and the presence of salts in protein fibres would be expected to promote moisture absorption. The effect of phosphate solution would tend to promote the insolubilisation of protein fibres and this tendency would be increased the lower the pH of the phosphate solution. A variation in the pH of phosphate solution permits a variation in the moisture content of the fibres, the higher pH values permitting the fibre to hold substantially more moisture.

On the whole, the packing nature of the aggregates as well as the enhanced water absorption of the denatured proteins may be the main causes of the increased water uptake with pH.

9.5 Scanning Electron Microscopy (SEM) of Spun Fibres

Photomicrographs of spun fibres were prepared to reveal any alteration in structure as a result of processing and to add support to the belief that globular proteins unfold to long and extended polypeptide chains which lie parallel to the fibre axis during spinning operation.

Sample Preparation for SEM

Samples were air-dried at room temperature for 48 hours and carefully mounted onto double sticky tape on a metal stud. Gold coating was applied to make the sample electrically conducting and enhance emission of secondary electrons.
PLATE 34a
Tow of fibres
x 270
pH 11.50
Ageing Temp.
50 ± 1°C

PLATE 34b
x 60,000

Different sizes of elongated and cylindrical bodies embedded in the spinning mass.
Spinning mass arranged in an orderly pattern.

Despite the processing treatments not all fibre components are in an elongated, fibre-like form.
Plate 35(a)
Single Fibres
× 220
pH 11.50
Ageing Temp.
20 ± 1°C

Plate 35(b)
× 20,000

Plate 35(c)
× 12,000
9.6 Results and Discussion

Plates 34a - 35c show the SEM view of the effect of processing on blood plasma proteins. After spinning the structure of the plasma proteins has been deformed, and the configuration changed forcing protein particles pack closer. As these proteins are denatured and forced through the spinning apparatus, their strands probably become elastic, stretched into fibre-like form and arranged in a peculiar way. Microscopic examination of spun fibres added support to the apparent structural change in protein suggested by alkali-treatment. SEM view also revealed that the fibre is made up of agglomerates of smaller globules of different shapes and sizes that are unrelated to the globules of the native protein. It further showed microscopic imperfections in the fibre make-up such as voids and solid inclusions indicating that fibres would not have a uniform strength along their length. Microscopic examination also revealed that the fibres are not completely parallel to one another.
Ultrafiltration has been used to obtain plasma proteins in a more pure and almost un-denatured form. Based on the data obtained it appears that blood plasma solution can be concentrated and de-salted cheaply and effectively and it can be extended to economic large-scale production. Considerable amounts of salts and non-electrolytes are removed during UF, so that a diafiltration step can be eliminated.

Although elevated temperature has been used to induce higher flux rates with processing fluids such as cheese whey, in the case of blood plasma solution any heating should be avoided, if possible, since the storage and processing quality of plasma solution ultrafiltered at temperatures above 40°C has been found to be degraded. Not only that, reaction of plasma proteins with the reducing sugars is likely to lead to deterioration in the nutritive value of the concentrated products by inactivation of amino acids (particularly lysine) with increased temperature. It is thought that appropriate UF techniques can provide a means for the economic disposal of animal blood at relatively lower cost than it is at present.

The amount of anticoagulant added to the blood to keep it in fluid form before mechanical separation has been observed to affect the storage
life of the resulting plasma solution. Anticoagulant should be added to
give a final plasma pH 7.6, pH higher than 7.6 led to increased inter-
action followed by precipitation of the plasma proteins during cold sto-
rage. The disposal problems of the residual fraction (blood cells)
have been solved by converting the blood cells into a free flowing
powder containing 9 to 15% nitrogen with an average of 12.5%. It is
believed that this would further lower the cost of processing and
encourage the extent of utilization of animal blood.

The processing of concentrated and de-salted plasma proteins was
studied with special reference to the influence of heat treatments and
pH of the medium. The protein solubility as determined by the NSI
decreases rapidly after heat treatment at 55°C and at pH values below
5.0 and above pH 10.0. NSI increases between pH 7.0 and 10.0.
There is a close relationship between the decreasing protein solubility
above pH 10.0 and the extent of denaturation above the pH value.

The rheological properties of the spinning dopes are a function of the
conformational changes in the molecules and the cross-linking within
them. Treatment of ultrafiltered plasma solution with strong sodium
hydroxide at high pH values markedly increases its apparent viscosity.
The effect is accompanied by a rapid change in rheological parameters.
The slope, shape and magnitude of the flow and viscosity curves is a
function of protein concentration or total solids and pH of the solution as well as ageing time and is affected by ageing temperature, rate and time of shearing. The stability and suitability of the plasma dope solution for spinning and production of fibres of satisfactory physical properties is determined largely, if not, solely by its viscosity. From a series of experiment it was found that moderate heat denaturation of the spinning dope at the appropriate pH was an essential part in the production of fibres of adequate texture. The heat treatment of the dope was also found to enhance the elimination of air bubbles which must be removed for a thorough spinning operation.

For the set of conditions used in this thesis the most workable pH was 11.5 with the application of heat at 40 ± 1°C for 30 minutes. The most homogenous spinning dope was obtained after filtration during heat treatment while filtration at the beginning alone was insufficient. Minimal protein concentration and total solids for suitable spinning conditions was 11.5% and 13% respectively. Blood plasma solutions of protein concentrations between 11 and 15% seem to be optimum for the production of fibres of suitable texture. Increasing capillary length of the spinnerette hole gave better fibres. Optimum spinnable viscosity for the blood plasma dope was obtained at pH 11.5. Suitable apparent viscosity (shear rate = 28.16s⁻¹) at 20 ± 1°C was found to be 3 ± 0.5 Pa.s

Microscopic examination of the spun fibres added support to the alteration in configuration suggested by alkali-treatment.
SUGGESTIONS FOR FURTHER WORK

1. Pure protein fibres have been prepared and not a finished fabricated food product. Therefore, further work needs to be done regarding incorporation of other essential ingredients such as binder, flavour, colour and deficient nutrient(s). The finished product then may become meat extenders or substitutes.

2. Nutritional assessment regarding levels of essential amino acids and biological value is highly desirable.

3. The economic aspect of production of protein fibres from animal blood plasma needs thorough examination. It is important that cost comparisons should be done on the whole processes from raw material to the final product.

4. Upgrading the pH of the fibre as described in this research was observed to improve the sour taste, appearance and toughness of the freshly coagulated fibres. This is another important area for further investigation which may have an impact on the overall spinning procedure.

5. Since a high pH was employed in the preparation of spun blood plasma fibres, the attributes of flavour and off-flavour may be more important determinants of acceptability than the textural attributes examined in this study. Consequently, further work needs to be done in this respect.
Comments above and in the text of thesis suggest other areas for further work in addition to those listed.
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