METHODS OF PLASMA PROTEIN FRACTIONATION

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The CSVM Fractionation Process

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I. Introduction

Since the first cold-ethanol preparation of human albumin in 1942 (Palmer 1976) the fractionation of human blood plasma has become established as a major biochemical industry throughout the world. It was the wartime need for albumin which provided the initial impetus and, although a whole range of different products can now be prepared from human plasma (Heide *et al.*, 1977), it has been the ever-increasing demand for albuminoid products which has defined plant capacities and stimulated change in the industry (Surgenor 1978). The raw material, human plasma, is a scarce and valuable resource and to meet the clinical requirements for blood products (Barker 1976, Jeffrey 1976) the efficiency of fractionation procedures must come under careful scrutiny.

The preparation of plasma fractions is based almost exclusively on Method 6 of Cohn *et al.* (1946) whereby proteins are separated from one another according to their solubility behaviour in the presence of ethanol. Albumin is recovered at the fifth, in a sequence of fractionation stages (F V + VI),* with insoluble protein being removed at each stage by centrifugation. Losses of albumin can occur at each of these earlier stages in three ways:

(1) As solids precipitated along with the preceding fractions; this is most serious in F IV-4 which is removed immediately prior to albumin precipitation.
(2) As occluded liquor removed along with the preceding fractions, the composition of centrifuged protein precipitates being 60-70% mother liquor.
(3) As line-losses; that is, as house-keeping losses due to spillage and to residual material left in equipment and flow lines.

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*F together with roman numerals refers to the Cohn Fractions (Cohn 1946).

The problem of improving albumin yield has been tackled largely from a standpoint of fractionation chemistry. For example, Cohn's Method 6 has been modified to reduce the number of stages (Kistler *et al.*, 1962) or to produce a less pure final product, Stable Plasma Protein Solution, prepared from F IV-4 + V rather than F V + VI (Hink *et al.*, 1957). Important though these contributions have been, emphasis has continued to centre on albumin yield, particularly as the collection of plasma from paid donors is being discouraged (WHO 1975). Now further strategies are being proposed whereby immunoglobulin and other fractions are discarded in the interest of albumin recovery (Schneider *et al.*, 1975, Hao 1979).

It is apparent that further improvement in albumin yield requires reappraisal not only of fractionation chemistry, but also of the technology by which this chemistry is applied.

The modern fractionation industry incorporates many complex and sophisticated operations and services other than the actual separation of plasma proteins. Such features include plasma procurement, engineering services, a multitude of finishing and packaging operations and quality control. To be effective in these circumstances the fractionator, whether on a commercial or a state basis, must process relatively large quantities of plasma; throughput being measured in 1000's of litres per week. This scale of operation has been met by carrying out the various manipulations with plasma held in large tanks of 2000 to 6000 litres in capacity. It is in this practice of large-volume batch processing that major albumin loss can be located.

The even distribution of added reagents, the removal of the heat of solution of ethanol and the rapid cooling of the bulk solution to the desired temperature all demand efficient agitation. Yet the high degree of turbulence required to achieve efficient mixing of such large volumes (Holmes *et al.*, 1964, Blakebrough 1972) cannot be tolerated by protein solutions without foaming and denaturation (Charm *et al.*, 1970, Thomas *et al.*, 1978). Inevitably, therefore, agitation is inadequate and as a consequence high local concentrations of ethanol and pH reagents and poor temperature control occur, resulting in precipitation of undesired components (Foster *et al.*, 1976, Foster 1978), sometimes irreversibly (Mitra *et al.*, 1978). It is this over-precipitation which is responsible for the loss mechanism (1) above. There is also a risk of denaturation because of poor heat transfer and many hours of process time are required simply to cool the solution to the desired temperature.

Efficient mixing and heat transfer can be achieved by means of continuous processing. In this mode of operation relatively small volumes are handled at any point in time but, because processing continues over extended periods, plant capacity can be easily maintained. This is the

essence of the continuous, small volume mixing (CSVM) process (Watt 1970).

Other attributes of continuous-flow processing include compatibility with automatic process control systems, a reduction in unit process time, less material at risk, smaller and less expensive process facilities and a high degree of flexibility in terms of fractionation chemistries, work routines and plant capacity (Dunnill *et al.*, 1967, Watt 1972). It was for all of these reasons that investigations into the feasibility of continuous-flow fractionation were begun in the late 1960's at the Blood Products Unit of the Scottish National Blood Transfusion Association, where a new fractionation plant was due to be designed.

As a result of this work a complete computer-controlled, continuous-flow plasma fractionation process was brought on-stream at the newly built Scottish Protein Fractionation Centre (PFC) on 7 January 1976.

II. Design and operation of the CSVM process

Design strategy

To researchers the arguments for the introduction of new technology may appear overwhelming but, with a relatively safe and satisfactory product at their disposal (Tullis 1977), fractionators and legislators alike are reluctant to accept radical change. This dilemma can be resolved by applying new technology to the tried and trusted cold-ethanol chemistry. The CSVM design strategy therefore began by adopting the chemistry of Cohn for process design and control specification. However the concept of process flexibility was also introduced by placing major investment emphasis on a process control facility which could be adapted easily to encompass new chemistries or new technologies whenever they became attractive.

The process consists of a number of unit operations imposed on a flowing stream of plasma, Fig. 1. These include:

(1) A pH adjustment system whereby the hydrogen ion concentration of the plasma is adjusted to a desired value before contact is made with the ethanol stream.
(2) A flow metering system which accurately controls and monitors the flow of ethanol, plasma and the precipitated mixture.
(3) A mixing and cooling device in which alcohol and plasma are brought together and mixed in the desired proportion such that the heat of mixing can be quickly dissipated.
(4) An ageing system where precipitated solutions are conditioned for centrifugation.

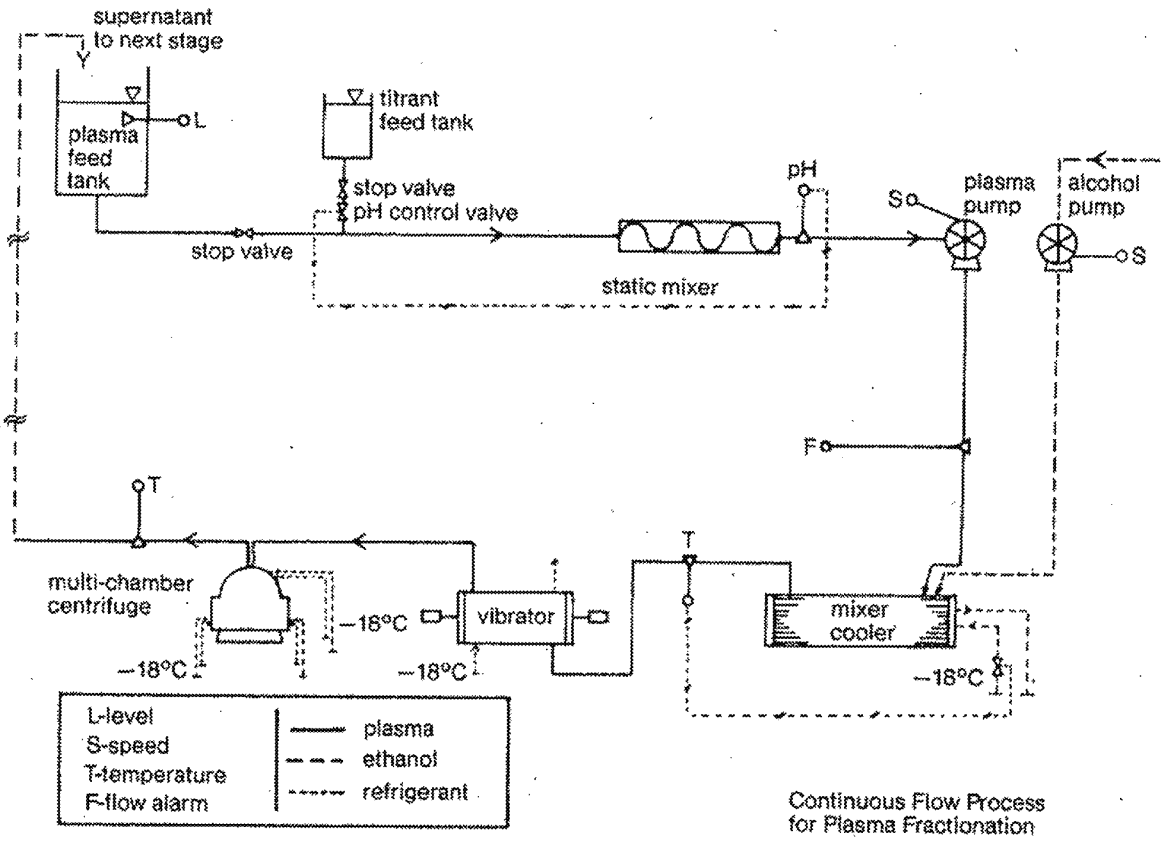

Fig. 1. Equipment flow sheet for the CSVM process.

Design and development of the system elements

The design and development of a pH adjustment and control system involved the study of a number of problems. Firstly a combination pH electrode had to be developed for robust operation in ethanolic protein solutions at temperatures below 0°C (Watt 1972), then a number of interacting features had to be resolved. For in-line pH measurement the electrode response is influenced by the ethanol and protein concentrations of the solution and when precipitation occurs the latter is partly determined by the degree of precipitation taking place. These points had to be defined so that in-line measurements could be related to the conventional batch values determined externally in a warm dilute solution (Cohn *et al.*, 1946). The in-line solids content is particularly interesting; a function of precipitation kinetics, it increases progressively along the flow-stream. Hence both the site of the electrode and the plasma flow-rate can contribute to the pH value actually measured.

Before pH is sensed the reagents must be fully mixed cleanly and quickly to minimise the time lag in the control loop. This has been achieved using an in-line Kenics static mixer (Chen *et al.*, 1973) sized to give com-

plete mixing with a process dead-time of about 10 seconds. Other aspects that required attention were the design of the control valve, by which titrant was added, and the design of the valve actuator (Watt *et al.*, 1978) so that the system was mechanically capable of both fine control and rapid response to a step change. Control performance is also influenced by the concentration of titrant used.

Finely machined gear pumps (Slack and Parr Limited, Leics., U.K.) have been adapted to provide accurate, pulse-free, positive displacement flow (Watt 1970). Fluid flow is determined by the speed of the pump which is sensed by a proximity detector, each pump being controlled independently to provide the desired throughput and plasma/ethanol composition. Other flow metering devices were rejected at an early design stage because of insufficient accuracy or suspect cleanliness. To achieve the desired control performance the mixing/cooling unit had to incorporate rapid mixing and cooling within a small hold-up volume to minimise process dead-time in the control loop. The unit also had to be robust and easily cleaned. This posed something of a contradiction. Efficient mixing is best achieved by agitating a small bulk volume, but rapid cooling requires fluid to flow in thin films over a large surface area. The problem was finally resolved in 1973 by combining Sulzer static-mixing elements (Richarz *et al.*, 1972) with a stainless steel shell and tube heat exchanger. With this arrangement mixing and removal of the heat of solution take place across the mixing elements and further cooling is rapidly achieved as the fluid flows over the tube bundle. The factors limiting control performance have been the response time of the temperature sensor (Ross 1978) and the sensitivity of the refrigerant control valve and valve actuator.

In batch processing it is necessary to age protein precipitates for a number of hours to achieve efficient recovery by centrifugation. Early CSVM designs included an ageing vessel for this purpose, however this was later replaced by an acoustic conditioning chamber (Jewett 1974) inserted directly into the flow-stream (Foster 1978) so that solids, comparable to those of the batch process, could be recovered without interruption of the process flow-stream.

Separation of the various components takes place within the centrifuge and it is important that all of the desired fractionation parameters be attained at the solid-liquid interface. Temperature control is crucial here; if too warm, solids may redissolve and if too cold unwanted protein may leave solution.

At the CSVM design flow-rate of 15 litres/h (Watt 1970) the temperature within a tubular bowl centrifuge (Sharples-6P) was unsatisfactory and multi-chamber centrifuges (Westfalia BKA-6 and BKA-25), incorporating direct refrigeration of the rotating bowl (Hemfort 1978), were developed

instead. In this design refrigerant flows between the source of heat, the outer bowl wall and the solids held within the bowl, thereby providing security of product as well as good temperature control.

Control

All of the sensing elements are linked to a process control computer which calculates the necessary actions and drives the respective control valves such that many parameters can be automatically controlled simultaneously over long periods of time. The process is operated from a remote control room (Fig. 2) where the status of the process can be observed on a visual display unit (Fig. 3). An alarm system sounds automatically if a parameter moves outside its defined limits and the operator can modify or change control actions if necessary. Alarm situations are automatically recorded on a special alarm log which also records all actions initiated by the operator; thus providing an independent product security system.

The value measured by each sensing element is displayed and recorded at 2 minute intervals throughout the process period (Watt *et al.*, 1978) to provide a running log, a detailed process record and information for research purposes. The control performance that has been consistently achieved over three years of operation is shown in Table 1, while in Fig. 4 the mean deviation from the set point for each of 90 consecutive days is

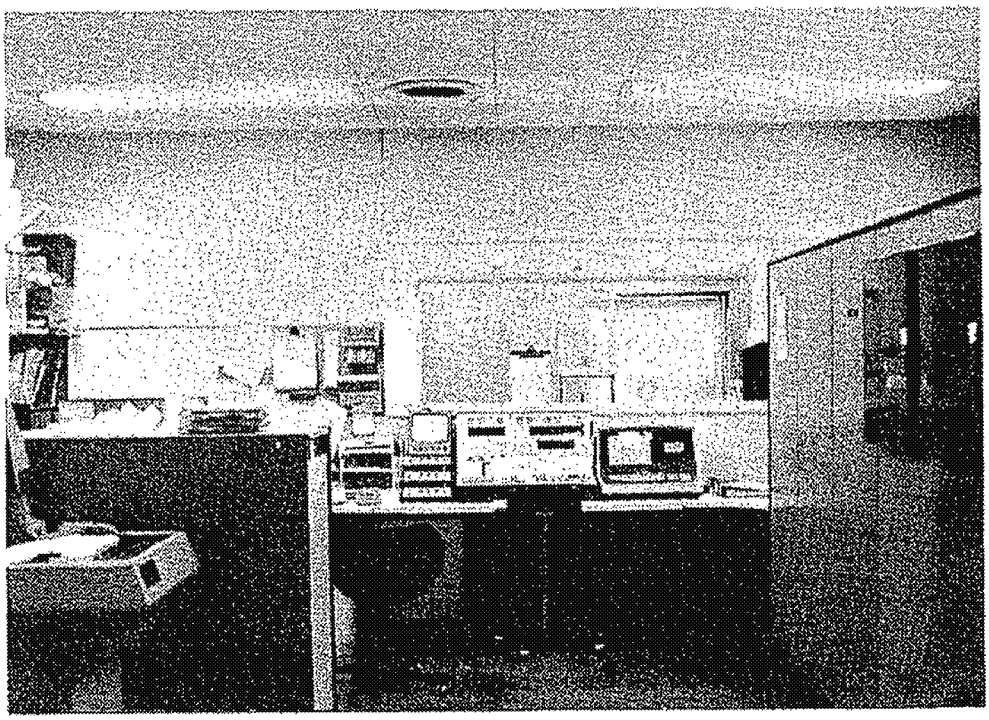

Fig. 2. CSVM process control room.

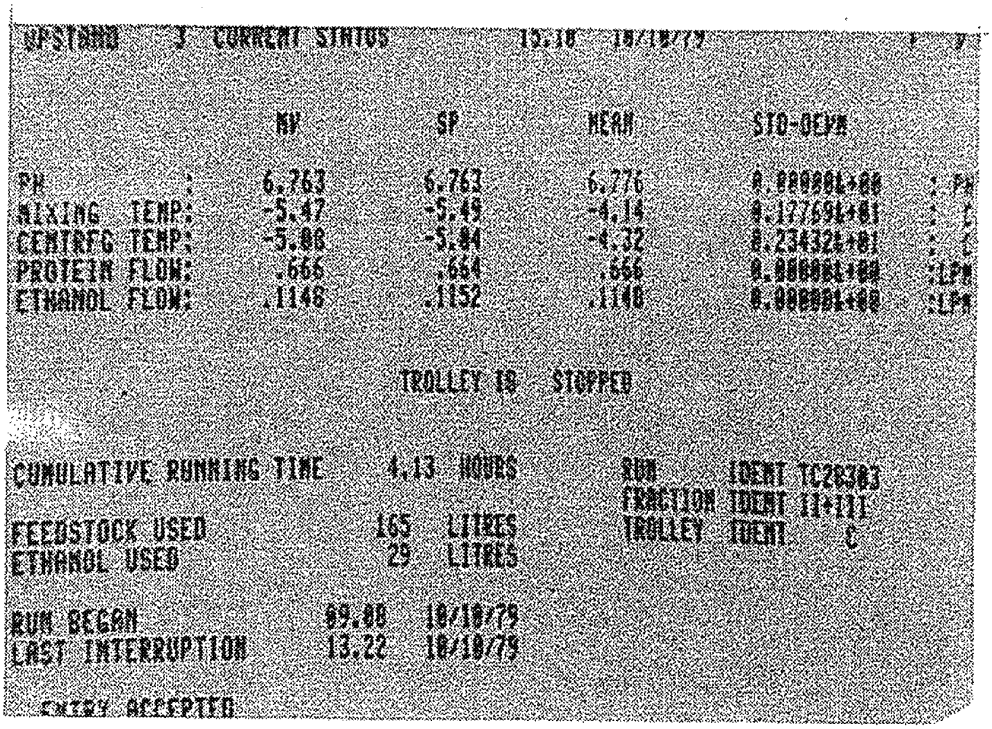

Fig. 3. CSVM process monitor.

plotted for pH control of F II + III, F IV-1 and F IV-4 + V. In this figure each point has been calculated from data collected at 2 minute intervals over 6-7 hours of processing.

TABLE 1

Design for Fractionation Control Parameters

Control element	Design specification	Performance achieved
pH (pH Units)		
F II + III	±0.05	±0.03
F IV-1	±0.05	±0.04
F IV-4 + V	±0.05	±0.04
Temperature (°C)	±0.5	±0.5
Flow (l/h)		
Ethanol	±0.03	±0.01
Plasma	±0.15	±0.06

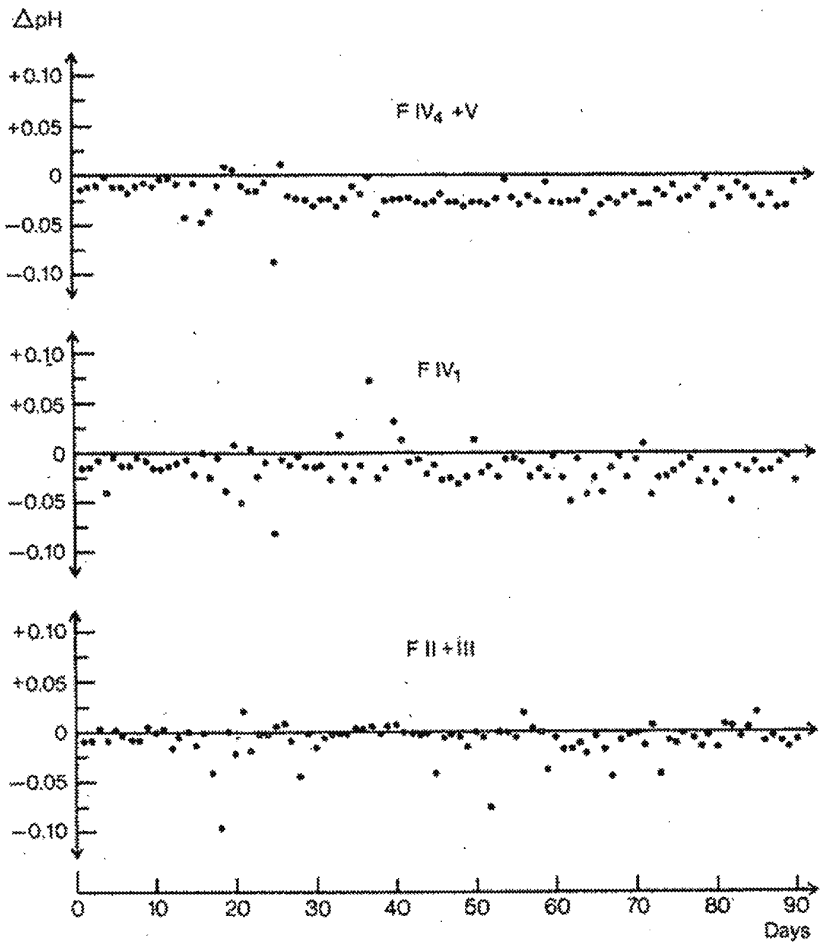

Fig. 4. pH control achieved at different fractionation stages. Mean deviation from the set-point on each of 90 consecutive days.

Process operation

The flow sheet (Fig. 1) shows the elements required for one fractionation stage. For ease of working these are mounted within a mobile unit which can be removed from the cold process area for cleaning or maintenance. A series of modules linked together forms a complete fractionation unit with the supernatant from one trolley providing the source material for the next (Fig. 5). In this way an element of plasma can be fractionated to albumin paste within 2½ hours.

Although designed for a throughput of 15 litres/h, flow-rates of up to 45 litres/h have been tolerated but routine processing is usually carried out at about 30 litres/h. The immediate limitation here is the surface area of the shell and tube heat exchanger. However, the ultimate limitation will probably be associated with precipitation kinetics or centrifuge residence time.

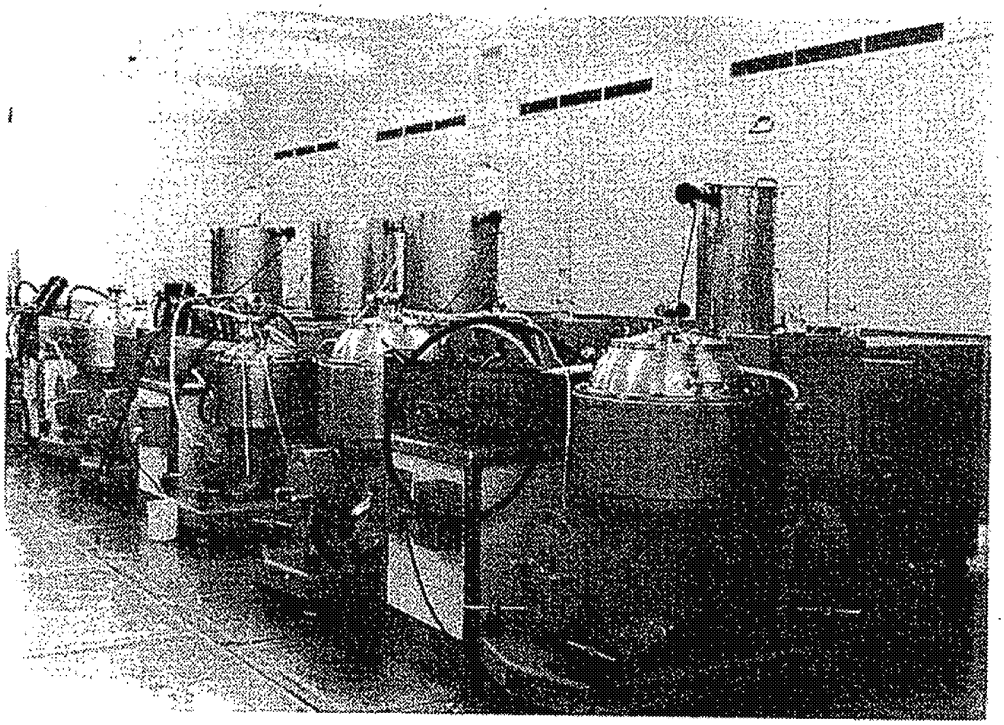

Fig. 5. CSVM modules operating in series to provide a complete fractionation process.

Ideally, the process should be operated continuously over many hours to minimise the incidence of start-up and shut-down procedures. For administrative reasons the Scottish unit has operated on a day-time basis, processing approximately 250 litres of plasma to F IV-4 + V per 3-trolleys in 6-7 hour cycles. Many modular variations are possible though and we have operated variously with sequences of 3-, 4-, 5- and 6-trolleys. Facilities are available to accommodate 9-trolleys and by operating these under fully industrial conditions the estimated PFC plant capacity is in excess of 9000 litres of plasma per week. Stable Plasma Protein Solution represents almost 90% of PFC albuminoid production and routine CSVM fractionation is therefore based on the method of Hink *et al.*, (1975). However, during our development studies the methods of Kistler *et al.*, (1962) and Krijnen *et al.*, (1970) were applied successfully.

III. CSVM Products

Albumin yield

In plasma fractionation albumin is lost in three ways, by precipitation into other fractions, as occluded liquor and as line-losses. Major loss usually occurs in the first category, and study of pH limits, using a CSVM module, has shown that an error of 0.1 pH units at the F IV-1 stage will

substantially reduce albumin recovery (Mitra *et al.*, 1978). A standard batch fractionation process has been reported to give a F IV paste containing 3.7 g albumin/litre plasma (Hanford *et al.*, 1978), over 10% of the initial plasma albumin.

The albumin content of F IV-1 prepared by the CSVM process is shown in Table 2. Here the figure of 0.37 g albumin/litre of original plasma represents the mean loss over the same 90 day period shown in Fig. 4 for pH control.

TABLE 2

Albumin Loss into Fraction IV-1 Over 90 Consecutive Days

	Albumin loss (g/l plasma)	
	Mean	Standard deviation
n = 90	0.37	0.27

The yield of albumin from the CSVM process, averaged over three years operation, is 25 g albumin/litre plasma. However, because of the mode of operation at PFC, both occlusion and line-loss are significant. Almost 50% of the plasma received at PFC is "fresh frozen" and is therefore used for recovery of coagulation factors VIII and IX as well as immunoglobulin and albumin. Hence, there are five stages of occlusion loss prior to albumin precipitation. Line-loss occurs each time the process is shut-down and this is exaggerated by "office hours" working. For example, the 90-day period shown above involved a total of 270 shut-down procedures. Based on a 60-hour cycle the same quantity of plasma would be processed with only 27 shut-down procedures, giving a 90% reduction in line-loss.

Albumin purity

Albumin solutions have a relatively safe clinical history (Tullis, 1977). However, their implication in hypotensive reactions (Harrison *et al.*, 1971, Bland *et al.*, 1973) has caused some concern. The problem was linked initially to the presence of bradykinin-like material (Izaka *et al.*, 1974) but is now thought to be due primarily to the presence of prekallikrein-activator, PKA, (Alving *et al.*, 1978). PKA can be assayed against a reference solution, Ref. I (USA Bureau of Biologics), and a limit

of 25% Ref I has been recommended (Alving, 1979). Excessive levels of PKA have been reported in albumin prepared by various cold-ethanol fractionation schemes and by ion-exchange chromatography (Horowitz *et al.*, 1979).

Seventy-five CSVM SPPS lots have so far been assayed for PKA content and all have been found to be within the recommended limit. The mean PKA content of SPPS lots prepared from plasma fractionated over the 90-day period of Fig. 4 and Table 2 is 10% Ref. I (Table 3). The PKA content of F IV has been reported to be influenced by the quality of the plasma used (Horowitz *et al.*, 1979) and CSVM SPPS quality may therefore be related to the proportion of fresh frozen plasma fractionated at PFC. Nevertheless, to-date two lots of SPPS (Hink *et al.*, 1957) and two lots of Salt Poor Albumin (Kistler *et al.*, 1962) have been found to exceed 25% Ref. I (Turnbull 1979). These were prepared by a routine batch procedure operated independently but in parallel with the automated process line.

TABLE 3

Quality of 45 Lots of CSVM Stable Plasma Protein Solution Prepared over 90 Consecutive Days

	Albumin content (%)	Prekallikrein-activator (% BOB Ref. I)
Mean, n = 45	88	10.0
Standard Deviation	2	4.3
Range	85-94	3.3-19.4

For cold-ethanol fractionation a temperature of −10°C at the F IV stage has been proposed to avoid PKA contamination (Horowitz *et al.*, 1979). If it is necessary to adopt this procedure to meet the recommended limit then substantial albumin loss might be expected to occur.

Immunoglobulins

The scale of clinical need for immunoglobulins and the wide range of product specificities used in Scotland are such that it is not practicable to prepare these products other than in small lots which do not justify the use of large-scale systems. Therefore, the CSVM system has not normally been used to prepare immunoglobulins. Lots of normal immunoglobulin have been prepared both from CSVM F II + III by conventional

systems and by utilisation of the CSVM system (Watt *et al.*, 1978). Whilst no attempt has been made to optimise the process from F II + III to F II the experience of optimisation of the other fractionation stages suggests that, for process runs exceeding 300 litres of plasma, little difficulty is to be expected.

Clinical use of CSVM Products

Scotland requires annually approximately 50,000 unit doses of SPPS each comprising 17.2 g of protein with an albumin content not less than 85%. This product is used for all plasma volume support purposes including the replacement fluid used in cell separator techniques. Some 150,000 doses of SPPS of CSVM origin have been distributed for clinical use and have been shown to be safe and satisfactory. One lot has, on two separate occasions, been associated with hypotensive episodes in patients during the progress of cell separator techniques. The explanation for these incidents has not been discovered; the PKA level of the lot being 14% Ref. I.

IV. Further development

The concepts underlying CSVM design have also been applied to the thawing of plasma for factor VIII recovery and a process of continuous thawing (Foster *et al.*, 1979) has given such a marked improvement that all plasma is being thawed routinely by this means even though design studies are not quite complete. This represents an advancement on the original design which promises to open new applications of the basic concept. By allowing the rate of heat addition to the frozen ice mass to be increased to the point where plasma is brought from —40°C to 0°C within 10 minutes and by facilitating the rapid removal of the thawed liquor, conditions can be achieved on an industrially applicable scale similar in some ways to those described by Mason (1978) for single donation factor VIII recovery.

Whilst the original design studies of the CSVM system were related to the organic solvent precipitation systems already widespread in the fractionation industry, it was always intended that the philosophy of process control would be such that change in the basic separation technology could be accepted. Design studies are in progress which will allow the incorporation of automatic gel chromatography separation systems at intermediate and terminal stages of the existing process line (Dickson *et al.*, 1975). This will, for example, allow substantial replacement of

the existing method of removing solvent traces from final product (Smith *et al.*, 1972).

The possibility is also being explored that an extension of the same type of control philosophy can be employed to obtain, by continuous cell culture, several of the plasma proteins now recognised as having clinical value using the waste fractions from the CSVM process as primary feedstock for the culture. This development is seen as being of fundamental importance to the concept of national self-sufficiency in plasma products for clinical use by allowing the manipulation of supply and demand for all products independent of the plasma supply, thus removing the frustration of having over-supply of one product whilst enduring shortage of another which appears to be the experience of all developed nations at the present time.

Considerable data is being amassed from the CSVM process and analysis of this will define the significance of the various parameters more clearly. Re-design of the important elements will then be possible, allowing further improvement to be achieved. In this way the CSVM process does not represent a finished design concept but is, precisely as it set out to be, a system held in a dynamic state of change at any time in its development and allowing a continuous reassessment of its efficiency and effectiveness such that further change to meet the challenge of clinical requirement is comparatively simple.

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