FRACTIONATION, PLASMA

1. Introduction

Human blood plasma contains >700 different proteins (qv) (1). Some of these are used in the treatment of illness and injury and form a set of pharmaceutical products that have become essential to modern medicine (Table 1). Preparation of these products is commonly referred to as blood plasma fractionation, an activity often regarded as a branch of medical technology, but which is actually a process industry engaged in the manufacture of specialist biopharmaceutical products derived from a natural biological feedstock (see PHARMACEUTICALS).

2. History

Methods for the fractionation of plasma were developed as a contribution to the U.S. war effort in the 1940s (2). Following publication of a seminal treatise on the physical chemistry of proteins (3), a research group was established that was subsequently commissioned to develop a blood volume expander for the treatment of military casualties. Process methods were developed for the preparation of a stable, physiologically acceptable solution of albumin [103218-45-7], the principal osmotic protein in blood. Early preparations, derived from equine and bovine plasma, caused allergic reactions when tested in humans and were replaced by products obtained from human plasma (4). Process studies were

Table 1. **Pharmaceutical Plasma Derivatives**[a]

Product	Clinical application	Molecular weight $\times 10^3$	Normal plasma concentration, g/L
	Albumin[b]		
human serum albumin	protein and volume replacement	68	31–33
plasma protein fraction	volume replacement	68	36–40
	Coagulation proteins[c]		
Factor VIII	hemophilia A treatment	300	3×10^{-4}
Factor IX complex	treatment of hemophilia B and other coagulation disorders	57	5×10^{-3}
antiinhibitor coagulant complex[d]	hemophilia A treatment where Factor VIII antibodies are present		
	Inhibitors[c]		
α-1-proteinase inhibitor	emphysema treatment	52	1.5
antithrombin III	antithrombin III deficiencies treatment	58	0.1
	Immunoglobulins[e]		
immune globulin intravenous (normal)	immunoglobulin (IgG) replacement; treatment of immune disorders	150	12.5
immune globulin intravenous	treatment of cytomegalo-virus (CMV) infection in immune-suppressed individuals	150	
immune serum globulin (normal)	prevention of hepatitis A and rubella infections	150	12.5
hepatitis B immune globulin	prevention of hepatitis B infection	150	
pertussis immune globulin	prevention of whooping cough infection	150	
rabies immune globulin	prevention of rabies infection	150	
rho(D) immune globulin	prevention of hemolytic disease of the newborn	150	
tetanus immune globulin	treatment or prevention of tetanus infection	150	
vaccinia immune globulin	prevention of small-pox infection	150	
varicella immune globulin	prevention of chicken-pox infection	150	

[a] U.S. Licensed.
[b] Active component is albumin.
[c] Active component is indicated product.
[d] Active component is not known.
[e] Active component is IgG.

Table 2. **2004 Plasma Fractionators**

Organization	Capacity, m^3/yr	Plant/location
	Commercial	
CSL Ltd (Commonwealth Serum Laboratories)	6700	Melbourne, Australia; Berne, Switzerland[a]; Kankakee, Illinois, USA[b] Marburg, Germany[c]
Baxter Healthcare Corp	3500	Glendale, California, USA[d]; Vienna, Austria[e]
Probitas Pharma (Inst. Grifols SA)	3200	Barcelona, Spain; Los Angeles, California, USA[f]
Bayer Healthcare AG	2500	Clayton, N Carolina, USA[g]
RAAS Blood Products Co Ltd	2000	Shanghai, China
Octapharma GmbH	1700	Vienna, Austria; Stockholm, Sweden[h] Strasbourg, France[i]; Mexico City, Mexico[j]
Kedrion S.p.A	1500	Lucca, Italy[k]; Naples, Italy
Korean Green Cross Corp	600	Seoul, Korea
	Not-for-profit	
Lab. Fractionation & Biotechnol. (LFB)	900	Paris, France[l]
Bio Products Laboratory (BPL)	750	Elstree, UK[m]
Sanquin Red Cross (CAF-DCF)	750	Amsterdam, The Netherlands[n] Brussels, Belgium[o]
Japanese Red Cross	500	Chitose City, Japan
Korean Red Cross	500	Chungbuk, Korea
Massachusetts Biologic Laboratories	250	Boston, Mass., USA
Scottish National Blood Transfusion Service (SNBTS)	150	Edinburgh, UK
National Bioproducts Institute	150	Pinetown, South Africa[p]
Finnish Red Cross	120	Helsinki, Finland

[a] Formerly: Swiss Red Cross Central Laboratory.
[b] Aventis Behring, Centeon, Armour Pharmaceuticals.
[c] Aventis Behring, Behringwerke AG.
[d] Baxter Travenol, Hyland Therapeutics.
[e] Immuno AG.
[f] Alpha Therapeutics.
[g] Cutter Biologicals.
[h] Pharmacia & Upjohn, Kabi Pharmacia, Kabivitrum AB.
[i] Strasbourg Regional Blood Transfusion Centre.
[j] Probifas SA.
[k] Farma Biagini S.P.A.
[l] National Centre for Blood Transfusion.
[m] Blood Products Laboratory.
[n] Central Laboratory of the Netherlands Red Cross.
[o] Belgian Red Cross Fractionation Centre.
[p] Natal Bioproducts Institute.

still being carried out in the pilot-plant laboratory at Harvard in December 1941 when the small supply of experimental product was rushed to Hawaii to treat casualties at the U.S. naval base at Pearl Harbor. On January 5, 1942 the decision was made to embark on large-scale manufacture at a number of U.S. pharmaceutical plants (4,5).

Full details of this work were published (6) and the processes, or variants of them, were introduced in a number of other countries. In the United States, the pharmaceutical industry continued to provide manufacturing sites, treating plasma fractionation as a normal commercial activity. In many other countries, processing was undertaken by the Red Cross or blood transfusion services that emerged following World War II. In these organizations plasma fractionation was part of a larger operation to provide whole blood, blood components, and specialist medical services on a national basis. These different approaches resulted in the development of two distinct sectors in the plasma fractionation industry; ie, a commercial or for-profit sector based on paid donors and a noncommercial or not-for-profit sector based on unpaid donors.

In 2004, there were ~80 organizations undertaking plasma fractionation worldwide, having plant capacities ranging from 5 to 2900 m^3 plasma/year. Virtually all of these plants use methods based on those originally devised, but with additional purification using modern bioprocess technologies. Table 2 lists the major commercial and noncommercial manufacturers and indicates the restructuring that has place in the industry.

3. Manufacturing and Processing

Plasma fractionation is unusual in pharmaceutical manufacturing because it involves the processing of proteins and the preparation of multiple products from a single feedstock. A wide range of unit operations are utilized to accomplish these tasks. They are listed in Table 3; some are common to a number of products and all must be closely integrated. The overall manufacturing operation can be represented as a set of individual product streams, each based on the processing of an intermediate product derived from a mainstream fractionation process (Fig. 1).

4. Principal Unit Operations

Figure 2 shows the principal unit operations involved in a typical fractionation operation.

5. Protein Precipitation

The separation of proteins according to differences in solubility plays a significant role in plasma fractionation; a number of precipitation steps are used in the processes for albumin, immunoglobulin (immune globulin), and Factor VIII [9001-27-8] manufacture. Solubility behavior, a unique property of a protein, is determined by size, composition, and conformation, as well as by the environment in which the molecules are located. The protein surface can be regarded as mostly hydrophilic and the protein interior as largely hydrophobic. These properties are determined by the nature and distribution of the amino acid

Table 3. **Plasma Fractionation Unit Operations**[a]

Unit operation	Method/technology	FVIII	FIX	IgG (im)	IgG (iv)	PPF	Albumin
	Protein separation[b]						
fractional precipitation[c]	cold-ethanol precipitation		++	+	+	+	+
fractional extraction[d]	from cold-ethanol ppt			+	+	+	+
solid–liquid separation	centrifugation	+	++	++	++	++	++
	depth filtration			++	++	++	++
selective adsorption	depth filtration			+	+	+	+
selective adsorption/ desorption[e]	ion-exchange chromatography	+	+		++		++
	immuno-affinity chromatography	++	++				
	Virus inactivation, in-process						
heat treatment	carbohydrate stabilized	++	++		++		
	fatty acid stabilized					++	++
chemical treatment[f]	solvent–detergent treated	++	++		++		
	Formulation and finishing[g]						
selective adsorption	depth filtration			+	+	+	+
membrane filtration	cross-flow filtration	++	++	++	++	+	+
	dead-end filtration	+	+	+	+	+	+
stabilization	chemical additives	+	++		+	+	+
dispensing	aseptic-dispensing	+	+	+	+	+	+
drying	freeze drying	+	+	++	++		
	Virus inactivation, terminal						
heat treatment	pasteurization					+	+
	dry heating	++	++				

[a] +, method in common use; ++, optional method, depending on procedures used by different manufacturers; im, intramuscular; iv, intravenous.
[b] Size exclusion by gel filtration is an optional method for FVIII.
[c] Charge reduction (pH, temperature) and other precipitation are common methods of FVIII fractionation.
[d] Extraction from other precipitates is an optional method for FVIII fractionation.
[e] Affinity chromatography is an optional method for FIX.
[f] Optional chemical treatments include potassium thiocyanate [333-20-0] for FIX and acid/enzyme treatment for IgG (iv).
[g] Selective proteolysis by acid/enzyme treatment is an optional method for IgG iv.

residues that make up the protein (7) (see AMINO ACIDS). The ionizable and polar amino acids are involved in charge repulsion, which plays an important role in preventing the aggregation of protein molecules. It is for this reason that proteins normally display a solubility minimum at their iso-ionic pH.

The solubility of a protein also is determined by the physical and chemical nature of its environment. Properties of the solution that influence protein solvation and protein–protein interactions are particularly important. These interactions, believed to be predominantly electrostatic in nature (8), are therefore

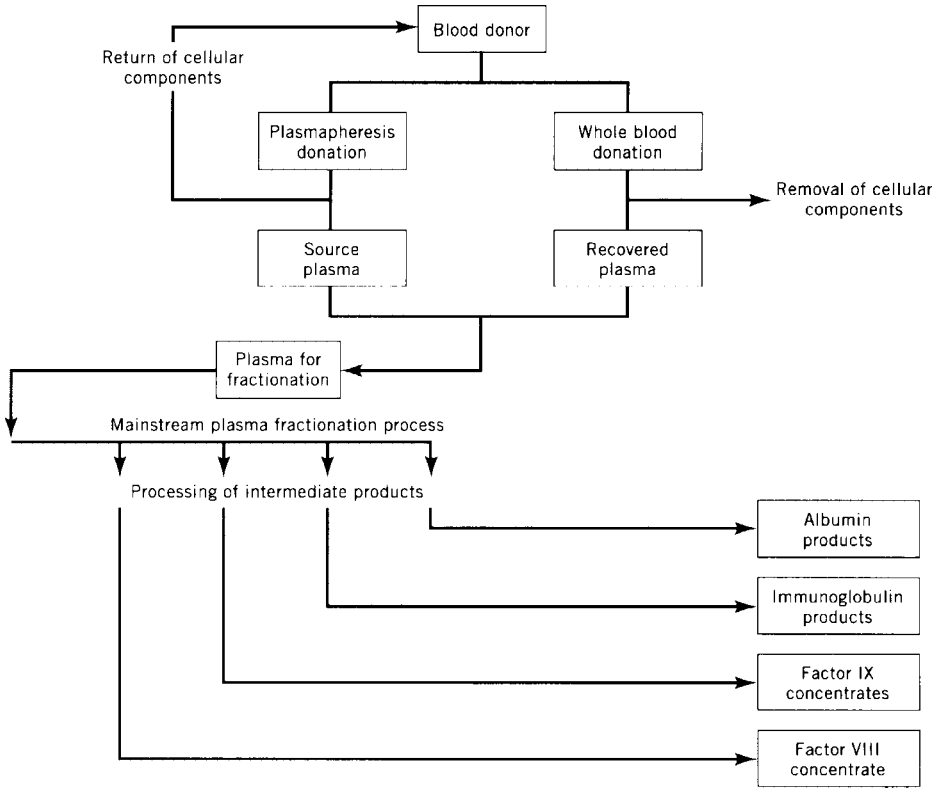

Fig. 1. Organization of the supply and fractionation of human plasma.

influenced by the temperature, dielectric constant, and ionic strength of the solution. The presence of substances that compete preferentially for water molecules reduces protein solubility; a number of substances of this type, such as neutral salts, organic solvents, and nonionic polymers, have been used to precipitate proteins (9). Other available precipitation reagents function by interacting directly with the protein, ie, either changing the surface charge or linking protein molecules together to form aggregates that exceed the solubility limit. Reagents in this category include metal ions, organic dyes, and polyelectrolytes (9). A large number of parameters are potentially available for the manipulation of protein solubility and many of these have been applied to the separation of plasma proteins (10,11).

Precipitation was the principal protein separation technology initially chosen for the development of an industrially suitable fractionation process. Ethanol [64-17-5] (qv) was selected as the precipitation reagent because of its volatility. It can be subsequently removed using available drying technology (12).

In designing this fractionation scheme (6), known properties of the most significant plasma proteins were utilized to establish a set of process parameters. The solution was adjusted to a pH close to the iso-ionic point of the proteins to be precipitated. Small increases in ionic strength were used to specifically

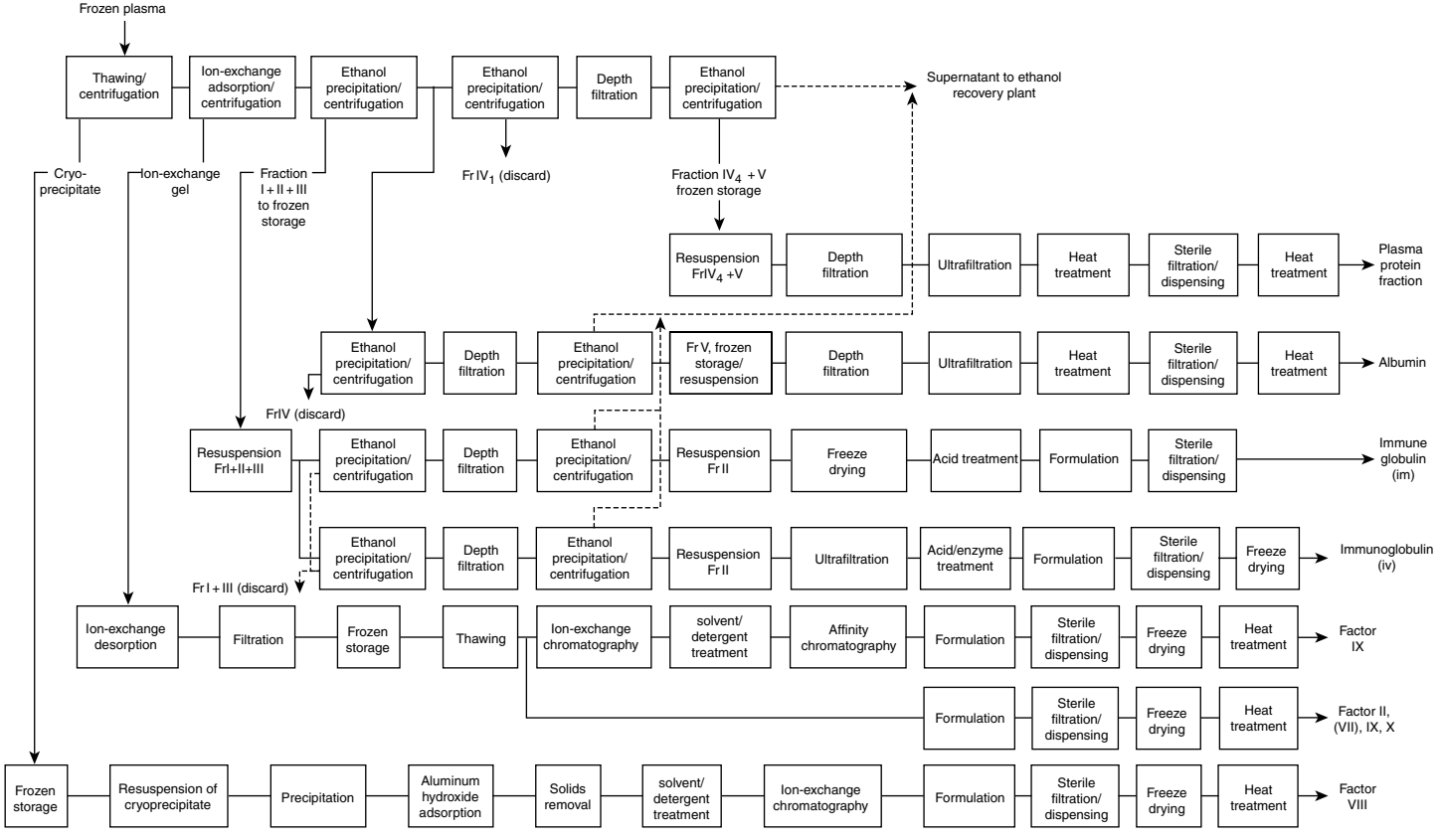

Fig. 2. Typical plasma fractionation operation, where Fr represents fraction. Based on processes at the Protein Fractionation Centre, Scottish National Blood Transfusion Service, Edinburgh.

increase the solubility of the more soluble proteins (12,13), thereby increasing the degree of discrimination between different proteins. A progressive increase in ethanol concentration was used to generally decrease protein solubility. Concentrations ranged from 8 to 40%, with the more soluble proteins, eg, albumin, being precipitated at the final stage. The temperature of the solution was held close to the freezing point to avoid protein denaturation, and the use of very dilute solutions was avoided, as proteins are known to be more stable at higher concentrations.

The ability of ethanol to precipitate proteins was believed to result from a reduction in the solution dielectric constant (14). However, the difference between water and ethanol–water mixtures is relatively small at $-5^{\circ}C$, compared to values at $20^{\circ}C$ (15), leading to questions about the original interpretation (16,17). From a surface-thermodynamic analysis, it has been proposed that the relative surface tension values of the different components favor the binding of water to ethanol rather than the plasma proteins, enabling ethanol to have a dehydrating effect (17). This alternative mechanism of cold-ethanol precipitation, which has yet to be confirmed, appears to be similar to theories developed in the 1970s concerning the precipitation of proteins by salting-out (18–20).

As of this writing, cold-ethanol precipitation remains the dominant separations technology used in plasma fractionation (6,21–24). The methods have been reviewed in detail elsewhere (10,11,25,26). The process of cold-ethanol fractionation was originally established as a batch operation. However, the use of continuous-flow processing under automatic control was subsequently suggested;

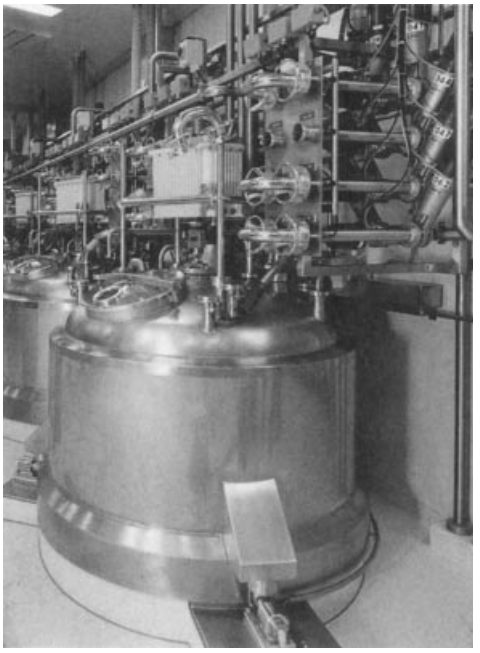

Fig. 3. Example of stirred batch vessels for ethanol fractionation. Courtesy of Swiss Red Cross, Bern.

details of these proposals are available (27–29). With the benefit of a mainframe computer for process control (qv), continuous-flow processing was introduced successfully into routine use in 1976 (30,31), but was eventually replaced by batch processing in 1998 because of concern that the continuous-flow equipment may have been contaminated with the agent resposible for the transmission of variant Creutzfeldt-Jakob disease (vCJD). Despite interest in continous-flow processing (32,33) the stirred batch tank has remained the dominant mode of operation in the industry (Fig. 3).

6. Solid–Liquid Separation

The separation of proteins by precipitation technology is accomplished when the solid and liquid phases have been separated from one another. Centrifugation, using either tubular bowl or multichamber centrifuges, is used for this purpose (9,34) (see CENTRIFUGAL SEPARATION). The machines must be refrigerated to ensure that the correct temperature for precipitation is obtained at the point of separation. Protein precipitates consist of large numbers of small particles, typically 0.1–1.0 μm in diameter, which aggregate together to form a larger particle or floc. This aggregate can be broken down by the shear forces experienced in some types of pump and in the entry region to centrifuges (35). Consequently, performance of the centrifugation operation is influenced by the shear characteristics of the process equipment as well as by the size, density, and strength of the particles (36). Both of these types of centrifuge function by retaining the solids within the rotating bowl, while the feed suspension and resultant supernatant flow continuously. One consequence of this mode of operation is a relatively small solids holding capacity per machine and the need to use a large number of centrifuges to process the contents of a large fractionation vessel. Some manufacturers have introduced depth filtration (qv) for the removal of the solids at some of the precipitation stages. However, protein precipitates consist of highly compressible particles, and filter aids (37) must be used if blinding of the filter surface is to be avoided.

7. Protein Adsorption

The selective adsorption (qv) of a protein or group of proteins to a solid-phase reagent, followed in most cases by some form of selective desorption, also constitutes a principal form of protein separations technology in plasma fractionation. Solid-phase reagents can be categorized according to the forces responsible for adsorption; eg, charge interactions [ion-exchange (qv)], biochemical interactions (affinity), and highly bioselective interactions (immunoaffinity). Some adsorbents have been less well characterized, eg, kaolin–silica composites [1335-30-4] used in depth filters for the adsorption of lipoproteins from albumin and immunoglobulin solutions, and aluminum hydroxide [21645-51-2] used for the removal of coagulation factor contaminants from Factor VIII solutions.

The forces involved in the separation of proteins by ion-exchange adsorption are believed to be similar to those associated with protein precipitation (18).

However, separation is usually achieved by manipulation of pH and ionic strength alone. Ion-exchange chromatography (qv) has been used in the preparation of Factor IX [9001-28-9] concentrates since the early 1970s (38–40) and is also used in the preparation of Factor VIII concentrates (41–43). A number of manufacturers have utilized ion-exchange adsorption in immunoglobulin (44–46) and albumin (47–49) manufacture, usually as an adjunct to cold-ethanol fractionation. Some procedures have been developed to replace cold-ethanol precipitation by ion-exchange chromatography, an approach initially confined to relatively small-scale plants (50); but which now forms the basis for a 500-m^3/year plant operated in Melbourne by CSL Bioplasma Ltd. that opened in 1994 (51). Ion-exchange chromatography also has a role in the preparation of newer plasma products such as α-1-proteinase inhibitor [9041-92-3] (52).

Affinity chromatography is used in the preparation of more highly purified Factor IX concentrates (53–55) as well as in the preparation of products such as antithrombin III [9000-94-6] (56,57). Heparin [9005-49-6], a sulfated polysaccharide (58), is the ligand used most commonly in these applications because it possesses specific binding sites for a number of plasma proteins (59,60).

Immunoaffinity chromatography utilizes the high specificity of antigen–antibody interactions to achieve a separation. The procedure typically involves the binding, to a solid phase, of a mouse monoclonal antibody that reacts either directly with the protein to be purified or with a closely associated protein that itself binds the product protein. The former approach has been applied in the preparation of Factor VIII (43) and Factor IX (61) concentrates. The latter method has been used in the preparation of Factor VIII (42) by immobilization of a monoclonal antibody to von Willebrand factor [109319-16-6] (62), a protein to which Factor VIII binds noncovalently. Further purification is necessary downstream of the immunoaffinity step to remove traces of mouse protein that may leach from the solid phase (63), as well as the toxic chemicals required to disrupt the strong antigen–antibody bond for product desorption (43,61). Ion-exchange chromatography, affinity chromatography, and ultrafiltration (qv) have all been used for this purpose.

8. Membrane Separations

The availability and development of microporous synthetic membranes and associated process technology has had a significant impact on the manufacture of plasma derivatives (see MEMBRANE TECHNOLOGY). Two very different areas of application exist: (*1*) the concentration of protein solutions and the removal of low molecular weight solutes use membrane systems that retain macromolecular substances, ie, ultrafiltration; (*2*) the removal of bacteria utilizes membranes sized to retain particles and larger microorganisms while allowing the macromolecular proteins to pass through, ie, sterile filtration.

8.1. Ultrafiltration Diafiltration. This application of membrane technology is normally carried out in a cross-flow mode using membranes of a nominal pore size in the molecular weight range 10,000–100,000 Da depending on the size of the protein being processed. Membrane systems are available in a number of formats, including hollow fiber (see HOLLOW-FIBER MEMBRANES), spiral

cartridge, and thin-channel (64,65). The performance of the operation depends on characteristics of the membrane, eg, pore size, porosity, strength, and hydrophobicity; properties of the process solution, eg, the solubility and concentration of protein; fluid mechanics (qv) at the membrane surface (66–68); and pressure difference across the membrane, which acts as the driving force (64,65). Ultrafiltration technology was introduced into plasma fractionation for the removal of residual quantities of ethanol from albumin solutions (69–72), as an alternative to methods such as freeze drying and vacuum distillation. The ability to remove solutes according to molecular size also enables ultrafiltration to be used in the diafiltration mode for the exchange of salts (73,74) and the removal of metal ions, such as aluminum (75,76). Similar technology has been introduced into processes for the manufacture of immunoglobulins (77,78) and coagulation factor concentrates (79–81). Ultrafiltration is generally regarded as the method of choice for the formulation of intermediate and final products with attention directed toward further optimization of conditions (82), and the automation (83) and validation (84) of the operation. Membrane filters with mean pore diameters down to 15 nm have been utilized for the removal of viruses where the molecular size of the product protein is sufficiently small to pass through the membrane (85).

8.2. Sterile Filtration. Plasma derivatives are prepared for infusion into patients and must therefore be free from bacteria at the point of use. This is achieved by filtering the finally formulated solutions, usually through a series of filters, down to a final 0.2-μm membrane filter. Significant advances in the technology include the replacement of filter disks by cartridges of much greater capacity (86) and lower degree of protein adsorption (87,88). The critical nature of this step makes validation of the procedure on a batch-by-batch basis an essential requirement (86). Process operations beyond this point, such as dispensing, freeze drying, and closure of the final container, must all be carried out under sterile conditions (see STERILIZATION TECHNIQUES).

9. Freeze-Drying

Plasma derivatives must have a defined shelf-life, usually for a period of 2 years. The final dosage form must ensure that a product retains its biological activity throughout the period specified. Products that are not stable in solution for this length of time are normally freeze-dried and reconstituted using sterile water for injection at the time of use. Freeze-drying involves the separation of water from nonvolatile constituents by sublimation from a frozen state. To carry out the process effectively it is necessary to specify the operating conditions carefully at a number of stages (89–93).

The product must be formulated and frozen in a manner that ensures that there is no fluid phase remaining. To achieve this, it is necessary to cool the product to a temperature below which no significant liquid–solid-phase transitions exist. This temperature can be determined by differential scanning calorimetry or by measuring changes in resistivity (94,95).

Most of the water is sublimated from the frozen mass by heating the product under reduced pressure. The operating conditions must be such that the product remains in a solid state while sublimation is taking place. The comple-

tion of sublimation can be observed by an increase in product temperature. This increase occurs when the energy being introduced is no longer consumed by the latent heat of sublimation, but is absorbed by the product instead.

When sublimation is complete, further heat is applied and the pressure further reduced in a controlled manner to drive-off residual adsorbed moisture. A final moisture content of $\leq 2\%$ is normally specified. Some moisture can desorb from the closure into the product after the vial has been sealed. This can be avoided by heat treating the closures immediately before use.

The development of freeze-drying for the production of blood derivatives was pioneered during World War II (96,97). It is used for the stabilization of coagulation factor (98,99) and intravenous immunoglobulin (IgG iv) products, and also for the removal of ethanol from intramuscular immunoglobulin (IgG im) solutions prior to their final formulation (Fig. 2).

10. Inactivation and Removal of Viruses

In developing methods of plasma fractionation, the possibility of transmitting infection from human viruses present in the starting plasma pool has been recognized (5). Consequently, studies of product stability encompass investigation of heat treatment of products in both solution (100) and dried (101) states to establish virucidal procedures that could be applied to the final product. Salts of fatty acid anions, such as sodium caprylate [1984-06-1], and the acetyl derivative of the amino acid tryptophan, sodium acetyl-tryptophanate [87-32-1], are capable of stabilizing albumin solutions to 60°C for 10 h (100); this procedure prevents the transmission of viral hepatitis (102,103). The degree of protein stabilization obtained (104) and the safety of the product in clinical practice have been confirmed (105,106). The procedure has also been shown to inactivate the human immunodeficiency virus (HIV) (107).

The early immunoglobulin products prepared by cold-ethanol fractionation were found to be free from transmitting hepatitis infection (105,108); this was not the case with products prepared by alternative methods (109). Subsequently, some batches of intravenous immunoglobulin transmitted hepatitis infection (110), emphasizing the importance of establishing validated procedures for dealing with potential viral contaminants (111).

A number of coagulation factor products were introduced for the treatment of coagulation disorders during the 1970s (see BLOOD COAGULATION AND ANTICOAGULANT DRUGS). The risk of infection with hepatitis led to the withdrawal of fibrinogen [9001-32-5] concentrates from clinical use (112) and to the restriction of other products to the treatment of life-threatening conditions such as hemophilia (113). Although some research into the removal (114) or inactivation (115,116) of the hepatitis B virus was underway, the emergence of acquired immune deficiency syndrome (AIDS) in the early 1980s placed the hemophilia population at risk of infection from these products. The impact of HIV cannot be overstated (117–120). Intensive research has led to the development of viral inactivation technologies capable of destroying not only HIV but also viruses responsible for hepatitis B and hepatitis non-A, non-B, eg, hepatitis C. Further methods

Table 4. **Viruses Transmitted by Human Plasma Derivatives**

Virus	Diameter, nm	Nucleic acid[a]	Lipid envelope
HIV-1	100	SS-RNA	yes
hepatitis A virus	27–32	SS-RNA	no
hepatitis B virus	42	DS-DNA	yes
hepatitis C virus	30–60	SS-RNA	yes
hepatitis D virus	35	SS-RNA	yes
hepatitis G virus	50 (approx)	SS-RNA	yes
human parvovirus, B19	18–26	SS-DNA	no
TT virus	32	SS-DNA	no

[a] SS denotes single stranded, DS denotes double stranded.

have been developed for the testing of blood and plasma donations (121). The organization, validation, and control of plasma fractionation operations has been revised to increase the security and reliability of manufacturing procedures (122). These points have been reviewed in detail (122–125).

Viruses that have been transmitted by plasma derivatives are listed in Table 4 together with some of their characteristics. A large number of methods have been studied for the inactivation of viruses (126,127), but not all of these have been found to be fully effective (128). The principal methods, categorized as either in-process or terminal according to position in the process, are listed in Table 5. This distinction is important in the design and organization of manufacturing to ensure that recontamination cannot occur downstream of a virus inactivation step (122,136). The effectiveness of these methods has resulted in plasma products having a high degree of safety with regard to viral infection (122,126,128,137,138). However, further measures may be required to deal with viruses that are resistant to solvent–detergent treatments, eg, hepatitis A virus (139) and parvovirus B19 (140), both of which are small, nonlipid-enveloped viruses. The use of separation technologies such as adsorption chromatography (141,142) or membrane filtration (85,143) has been considered in this context. However, the objective of removing some virus particles at a concentration of 10^5/mL (144,145) requires that a stage efficiency of >99.9999% be guaranteed on every occasion. This level of performance is not normally associated with bioseparations technology and nanofiltration may therefore be less robust than a well designed virus inactivation technology.

10.1. Removal of Prion Agents. The transmission of CJD by pituitary-derived preparations of human growth hormone and other medical procedures has led to concern that this disease might also be transmitted via plasma derivatives. These concerns increased when the disease of vCJD was identified in the United Kingdom as a result of the agent of Bovine Spongiform Encephalopathy (BSE) being transmitted to humans via the food chain. There is no evidence to date of either CJD or vCJD having been transmitted by plasma derivatives (146), however transmission of vCJD via transfusion of red blood cell has been reported (147). Agents responsible for Transmissible Spongiform Encephalopathies (TSEs), or prion diseases, have a low aqueous solubility, form aggregates

Table 5. **Virus Inactivation Methods**

Method	Stabilizers	Temperature, °C	Time, h	Application	References
		In-process solutions			
heating	sodium *N*-acetyl-DL-tryptophanate and/or sodium caprylate	60	10	albumin	(102,105)
	carbohydrate with or without an amino acid[a]	60	10	coagulation factors	129
	carbohydrate and a neutral salt[b]	60	10	coagulation factors	(61,130)
	neutral salts[c]	60	10	antithrombin III	115
acid treatment	carbohydrate	35	20	immunoglobulin	131
solvent–detergent treatment		25	6	coagulation factors, other protein	132
moist heating[d]	lyophilization	60	10	coagulation factors	133
		Terminal heating			
in solution	sodium *N*-acetyl-DL-tryptophanate and/or sodium caprylate	60	10	albumin	(102,105)
in freeze-dried state	lyophilization with excipients[e]	80	72	coagulation factors	(134,135)

[a] For example, sucrose [57-50-1] with or without glycine [56-40-6].
[b] For example, sucrose and sodium sulfate [7757-82-6].
[c] For example, sodium citrate [994-36-5].
[d] Of the dried preparation.
[e] For example, sucrose.

readily and adsorb strongly to surfaces. These properties suggest that separations technologies used in plasma fractionation should be capable of removing prion agents to a high degree (148). This has been demonstrated in a number of experimental studies (146), but limitations in the sensitivity of methods of detection means that freedom from risk remains to be confirmed.

11. Process Rationale

The products of plasma fractionation must be both safe and efficaceous, having an active component, protein composition, formulation, stability, and dose form appropriate to the intended clinical application. Processing must address a number of specific issues for each product. Different manufacturers may choose a different set or combination of unit operations for this purpose.

Table 6. **Donation Method and Plasma Collection**[a]

	Source plasma		Recovered plasma	
Requirements	United States	Europe	United States	Europe
volume of plasma per donor, L/year	50–60	10–15	1.5	0.75
minimum number of donors for 1-t/year plasma	17–20	65–98	648	1296
donations required for 1-t/year plasma	1700	3333	4000	4000

[a] Figures are based on typical practice, described in a number of reports (149–151).

12. Plasma Collection

Human plasma is collected from donors either as a plasma donation, from which the red cells and other cellular components have been removed and returned to the donor by a process known as plasmapheresis, or in the form of a whole blood donation. These are referred to as source plasma and recovered plasma, respectively (Fig. 1). In both instances, the donation is collected into a solution of anticoagulant to prevent the donation from clotting and to maintain the stability of the various constituents. Regulations in place to safeguard the donor specify both the frequency of donation and the volume that can be taken on each occasion (149).

Procedures for the collection of whole blood are similar throughout the world. An interval from at least 8 weeks (United States) to 12 weeks (United Kingdom) is required between a donation of 450-mL blood, which yields ~250-mL plasma. In some countries, a smaller volume of blood is collected, eg, 350–400 mL in Italy, Greece, and Turkey and as little as 250 mL in some Asian countries (149). Regulations concerning plasmapheresis donations vary more widely across the world; eg, up to 300 mL of plasma can be taken in Europe in contrast to 1000 mL in the United States, both on a weekly basis. Consequently, both the mode of donation and the country in which it is given can have a profound effect on plasma collection (Table 6).

13. Initial Plasma Processing

Following donation, the separated plasma is frozen (152) and transported to a fractionation plant, where it is held in frozen storage before being released for processing. On entering processing, plasma is vulnerable to bacterial contamination and proteolytic degradation. The more labile constituents are particularly at risk. The early process steps aim for a degree of purification, the creation of a stable environment free from bacterial growth, and, where possible, a significant reduction in process volume. These objectives can be met by precipitation processes (153). Ideally, a range of intermediate products are produced at this stage that are held in storage pending release for further purification. The subdivision of processes in this manner carries a number of advantages including

Table 7. **Composition of Protein Precipitates**[a]

Cohn Fraction	Principal components	Initial plasma protein, %
I	fibrinogen; fibronectin; Factor VIII	5–10
II + III[b]	IgG; IgA; IgM; Factors II, V,[c] VII, IX, X; α- and β-globulins; fibrinogen; plasminogen; plasmin; β-lipoprotein	20–25
(III)	IgA; IgM; Factors II, V; β-lipoprotein; plasminogen; plasmin	6–7
(II-3)	IgG; fibrinogen; β-lipoprotein	4–5
(II-1,2)	IgG	3–4
IV_1	α-1-proteinase inhibitor; antithrombin III; IgM; ceruloplasmin[d]; α- and β-globulins; α-lipoprotein; albumin	5–10
IV_4	transferrin; haptoglobin; ceruloplasmin; α- and β-globulins; albumin	5–10
V	albumin; α-globulins	50–60

[a] Refs. 10 and 25.
[b] Subfractions given in parentheses.
[c] Factor V [9001-24-5].
[d] Ceruloplasmin [9031-37-2].

flexibility in scheduling and batch sizing, as well as in maximizing the utilization of limiting or expensive resources.

Factor VIII, immunoglobulin, and albumin are all held as protein precipitates, the first as cryoprecipitate and the others as the Cohn fractions FI + II + III (or FII + III) and FIV_4 + V (or FV), respectively (Table 7; Fig. 2). Similarly, Fractions $FIV_1 + FIV_4$ can provide an intermediate product for the preparation of antithrombin III and α-1-proteinase inhibitor. This ability to reduce plasma to a number of compact, stable, intermediate products, together with the bactericidal properties of cold ethanol, are the principal reasons these methods are still used industrially.

14. Factor VIII Process

The Factor VIII molecule consists of multiple polypeptides having molecular weights of $\sim 80-210 \times 10^3$. The purified form consists of a light (mol wt = 80×10^3) and a heavy (mol wt = $90-210 \times 10^3$) chain (154), associated via a calcium linkage (155). The molecule is also bound noncovalenty to von Willebrand factor (mol wt = 220×10^3) forming complexes in the molecular weight range $1-10 \times 10^6$. Factor VIII is a particularly labile molecule vulnerable to degradation both by proteolysis (156,157) and by depletion of calcium ions (157,158). Factor VIII is contained in cryoprecipitate (159), the precipitate that forms when frozen plasma is thawed. This has enabled the molecule to be removed from the nonideal environment of the plasma stream at the very beginning of the manufacturing process (Fig. 2). Factor VIII is also vulnerable during cryoprecipitation because it is resolubilized as the temperature of the thawed

suspension rises. Consequently, processing must be carried out both rapidly and with a high degree of temperature control if loss of Factor VIII is to be minimized (160,161).

Fibrinogen and fibronectin are the other principal proteins in cryoprecipitate. Both are poorly soluble, adherent proteins that can limit the capacity of subsequent chromatographic and filtration operations. As for all protein precipitates, some supernatant remains trapped within the amorphous cryoprecipitate particles and is carried over with the mass of solids; other coagulation factors, which in their activated form can degrade Factor VIII, are of particular concern in this regard. The concentration of fibrinogen and fibronectin is normally reduced using further precipitation steps (129,134,162–168); residual coagulation factors of the prothrombin complex can be removed by adsorption to aluminium hydroxide (163). It is necessary to maintain a sufficient concentration of ionized calcium throughout the process to prevent the dissociation of Factor VIII (169,170).

At this point in the process, a virus inactivation step is normally included, eg, either incubation in the presence of a solvent–detergent mixture or heat treatment (Table 5). Further purification is subsequently required to remove chemicals, eg, tri-*n*-butyl phosphate [126-73-8] and polysorbate-80 [9005-65-6] used in the solvent–detergent treatment (132), or stabilizers used during pasteurization (129,130). This is achieved chromatographically using either ion-exchange adsorption (41), immunoadsorption (42,43), or size exclusion chromatography. Further protein purification is also obtained at this point, enabling the potency and solubility of the final product to be increased. Factor VIII is a trace substance in plasma and highly purified preparations contain very little protein. It is necessary to carefully formulate the solution to be compatible both with the final stages of manufacture, eg, sterile filtration, dispensing, and freeze drying, and with the product container. This can be achieved by increasing the solution ionic strength and by adding excipients, such as amino acids or human albumin, to prevent the adsorption of Factor VIII to surfaces and to provide a bulking agent for freeze-drying (81). Where a freeze dried Factor VIII is subjected to heat treatment to inactivate viruses, the formulation and physical nature of the frozen plug are important parameters which must be controlled in addition to the conditions under which the product is dried. In particular, it has been found that the frozen plug should consist of fine uniform crystals, a state that can be obtained at an industrial scale by a two-stage freezing process by which the solution is super-cooled prior to ice formation, which then takes place instantaneously (135,152).

15. Factor IX Processes

Direct ion-exchange adsorption is used to recover Factor IX from the supernatant that remains following cryoprecipitation (38–40). Alternatively, Factor IX can be recovered from Cohn Fraction III (171,172). A number of coagulation factors tend to copurify with Factor IX, including Factor II [9001-26-7], Factor VII [9001-25-6], and Factor X [9001-29-0]. These form part of the coagulation cascade, which leads to blood clotting when activated (173,174). It is essential to avoid creating these activated states during processing, as they can lead to coagulation occur-

ring within the process solution. To avoid these difficulties, careful attention must be paid to process materials and reagents, and an adequate degree of anticoagulation must be available at all times.

Products prepared in this manner typically contain significant quantities of coagulation Factors II, VII, and X and other proteins as well as Factor IX (175). Although these preparations have been relatively well tolerated since their introduction in the early 1970s, some patients have experienced thrombotic reactions (176,177) primarily when relatively high doses have been used. The constituents responsible for these reactions have not been fully identified but candidate substances include phospholipid with activated coagulation factors (105,178); Factor IX degradation products (179) or the infusion of Factors II, VII, and X in patients deficient only in Factor IX (53). Further purification, introduced in an attempt to eliminate or reduce this problem (180) involves the use of a second anion-exchange adsorption/desorption step, followed by affinity chromatography using dextran sulfate [9011-18-1] (53,55) or heparin (54) bound to a solid-phase matrix. Immunoaffinity chromatography has also been used for this purpose (61). Methods for preparing both the highly purified Factor IX concentrate and the intermediate-purity Factor II, VII, IX, and X complex are illustrated in Fig. 2.

16. Immunoglobulin Processes

Virtually all immunoglobulin products consist of IgG, a globular protein composed of two heavy polypeptide chains that run the length of the molecule and two light-chains that are about half the length; together these form a Y-like topology. During processing, IgG is vulnerable to both proteolytic degradation, which results in fragmentation (105,181), and to self-association leading to aggregation of the molecules (182). Most IgG products are prepared using cold-ethanol fractionation, with the precipitate Cohn Fraction I + II + III, or Fraction II + III, being separated from the plasma stream and then processed to obtain Fraction II, which is predominantly IgG (Table 7). Specific conditions under which precipitates are formed for the principal fractionation schemes in use may be found in the literature (6,21–24). These conditions must be selected and controlled carefully to avoid copurification of proteolytic enzymes such as plasmin [9001-90-5], which can lead to fragmentation of the IgG molecule, with a concomitant loss of biological activity. Aggregation can be minimized by avoiding denaturing conditions and by ensuring that solutions are appropriately formulated at every step (183).

The resuspended and formulated Fraction II precipitate normally contains some aggregated IgG and trace substances that can cause hypotensive reactions in patients, such as the enzyme prekallikrein activator (184). These features restrict this type of product to intramuscular or subcutaneous administration. Further processing is required if products suitable for intravenous administration are required. Processes used for this purpose include treatment at pH 4 with the enzyme pepsin [9001-75-6] being added if necessary (131,182), or further purification by ion-exchange chromatography (44). These and other methods have been fully reviewed (45,183,185–188). Intravenous immunoglobulin products are supplied in the freeze-dried state and in the solution state (189).

17. Albumin Processes

The manufacture of albumin products, like the immunoglobulins, is usually based on cold-ethanol fractionation with the precipitate Fraction V being composed predominantly of albumin (Table 7). All albumin products are pasteurized at 60°C for 10 h to inactivate potential viral contaminants (Table 5); the purification process must remove components that would denature or aggregate on heating. This can be achieved with as little as 80% of the protein in the form of albumin, if the remaining proteins are α- and β-globulins, and not immunoglobulin or fibrinogen (190). This observation has led to the development of two types of product (105), ie, human albumin containing ≥ 96% albumin, and plasma protein fraction (PPF) containing ≥ 83% albumin. The first of these is prepared from Fraction V (6,22,23) and the latter from Fraction $IV_4 + V$ (24). During the 1970s, hypotensive reactions with plasma protein fraction were associated with the presence of prekallikrein activator (PKA) (191), a potent vasodilator generated from Factor XII [9001-30-3] (Hageman factor) by surface activation during processing. Operating conditions can be controlled to avoid PKA contamination, usually by ensuring that the Fraction IV cut, which precedes Fraction $IV_4 + V$ precipitation, is sufficiently large to remove PKA; rapid processing can also be used to minimize the degree of PKA contamination (31). The careful selection and control of precipitation parameters to avoid contamination with PKA applies to the more purified human albumin product as well as to the less purified plasma protein fraction. The presence of sodium acetate [127-09-3] in PPF has also been associated with adverse reactions (192). Acetate buffers have been used for pH control (6), and residual quantities may remain if the albumin precipitate is not washed and ethanol is removed by sublimation or evaporation. The use of diafiltration for the formulation of products (~1980s) means that salts, such as sodium acetate, and metal ions, such as aluminum, can be removed easily. Metal ions may be introduced with reagents such as sodium hydroxide [1310-73-2] or by leaching from depth filters (75,76) as well as from glass bottles into which products are dispensed.

Lipoproteins may denature on heating and if present during pasteurization can result in the formation of haze or turbidity in the final product. This material was removed traditionally by filtration through asbestos (qv) sheets (6); however, health hazards associated with asbestos have led to its replacement by alternative filter materials (23,37,193). These media have been less effective than asbestos and further measures have been required to ensure the visual clarity of albumin products, eg, further filtration developments for lipid removal (194), preferential denaturation of contaminants using in-process heat treatment, and anion-exchange chromatography (49).

18. Other Processes

A number of other plasma products are entering into clinical use; growth is expected in at least some of these areas. Fibrinogen, previously withdrawn because of the hepatitis risk (112), can now be supplied in a virally inactivated

form suitable either for infusion or as part of a fibrin [9001-31-4] sealant kit used for wound healing (195). Fibrinogen can be recovered from cryoprecipitate, Cohn Fraction I (Table 7), or from side fractions of Factor VIII processing. Low solubility, characteristic of fibrinogen, means that precipitation technology is particularly suitable for its preparation. However, ion-exchange chromatography may be required to remove chemicals used in virucidal treatments.

Another by-product of Factor VIII processing having clinical value is von Willebrand factor. It has been recovered from side fractions using ion-exchange and affinity chromatography (196).

Alpha-1-proteinase inhibitor and antithrombin III are used to treat people with hereditary deficiencies of these proteins. Both can be recovered from Cohn Fraction IV (Table 7) using ion-exchange chromatography (52) and affinity chromatography (197), respectively. Some manufacturers recover antithrombin III directly from the plasma stream by affinity adsorption (56,198,199).

19. Economic Aspects

Estimates for a number of economic aspects of plasma fractionation can be made (200–206). The world capacity for plasma fractionation exceeded 30,000 t of plasma in 1999 and has increased by ~150% since 1980 (Fig. 4). The quantity of plasma processed in 1999 was ~26,000 t/year; the commercial sector accounts for ~70% of this, with 15,000 t/year in the form of source plasma from paid donors (Fig. 5). Plant capacities and throughput are usually quoted in terms of principal products, such as intravenous immunoglobulin, albumin and Factor VIII. These figures may not encompass manufacture of other products.

Worldwide the clinical use of albumin is almost 400 t/year and IgG iv about 46 t/year, however, the level of use varies widely between regions (Table 8) with commercial products often being imported to meet demand. In 2000, the total world sales of plasma derivatives exceeded $5,000,000,000 (Fig. 6) with 35% of sales being in North America, 29% in Europe and 26% in Asia/Pacific. In

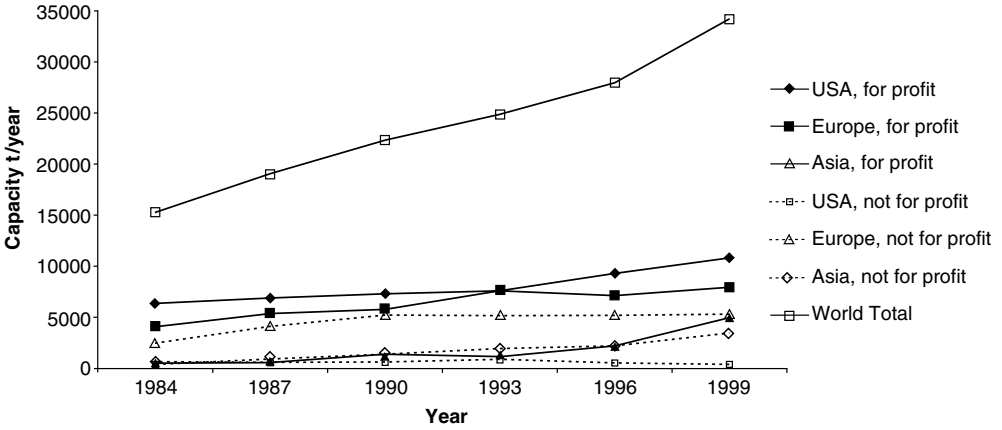

Fig. 4. Capacity of plasma fractionation plants.

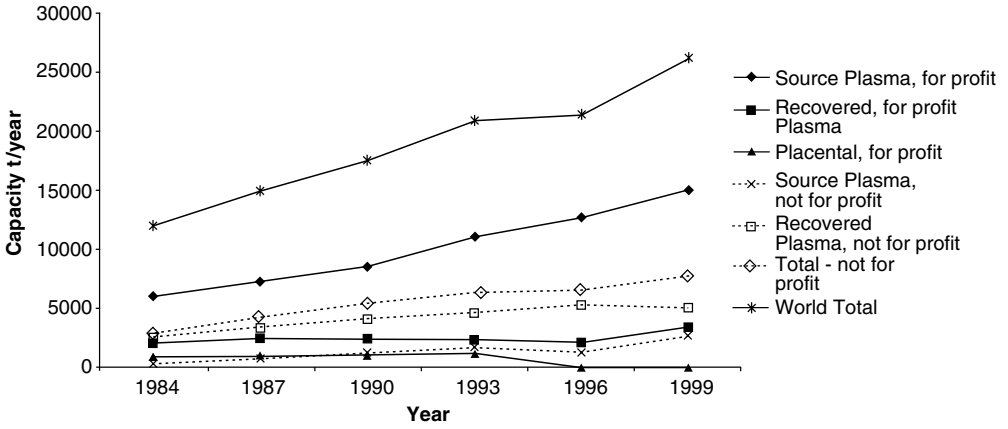

Fig. 5. Processed plasma, by origin, 1984–1999.

North America, sales increased from $818,000,000 in 1990 to $1,533,000,000 in 2000 with sales of IgG iv increasing by 360%, but with sales of albumin and Factor VIII falling by 8% and by 53%, respectively, over the same period. The reduction in the market for plasma derived Factor VIII resulted from development of genetically engineered Factor VIII prepared by recombinant technology (recombinant Factor VIII) and its introduction into the North American market in 1996. By 2000, 70% of the Factor VIII used in North America and 37% in Europe was provided from recombinant sources. Between 1984 and 2000, the worldwide demand for Factor VIII increased from 1300 million IU to almost 4000 million IU, with ~50% of clinical usage in 2000 being met by plasma derived Factor VIII. Recombinant Factor IX was introduced in 1998, accounting for 80% of the Factor IX used in North America and 25% in Europe by 2000.

Many countries aspire to supply their requirement for plasma products from their own plasma resource, a position supported by the World Health Organization (WHO) (207). Where multiple products are prepared from a common feedstock, the product in shortest supply dictates the scale of the manufacturing operation. The driving force for plasma fractionation was originally provided by albumin; this was replaced by Factor VIII in the 1970s and by IgG, iv in the

Table 8. **World-wide Distribution of Plasma Derivatives, 2000**

Region	Population, million	Quantity of plasma derivative distributed		
		Albumin, t	IgG iv, t	Factor VIII[a], IU × 10^6
Asia	3680	176.2	9.4	225
Africa	796	7.6	0.6	47
Europe	728	100.2	14.6	978
South America	520	22.8	0.8	328
North America	316	79.3	19.7	403
Oceania	31	4.6	1.0	48
World Total	*6071*	*390.7*	*46.1*	*2029*

[a] excluding recombinant Factor VIII.

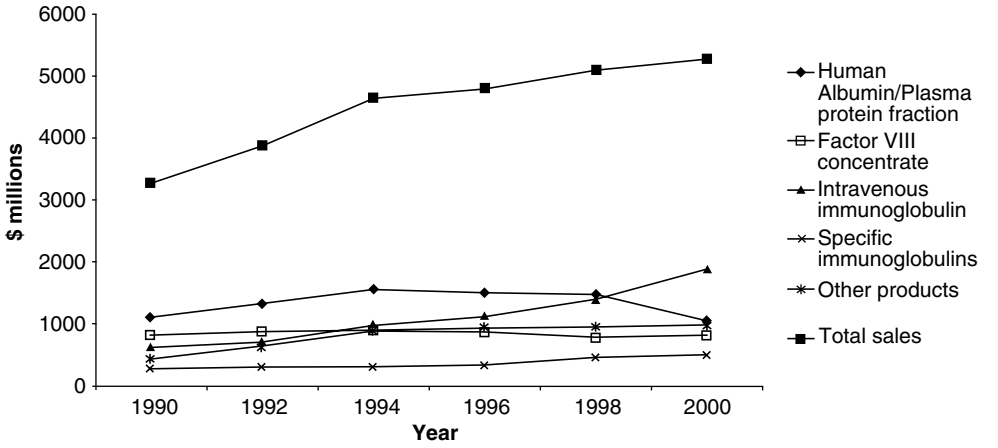

Fig. 6. Worldwide sales of plasma derivatives, 1992–2000.

1990s. The quantity of plasma required is determined by clinical demand and by the yield of the product from the manufacturing process. Clinical demand can vary from country to country (Table 9). Yield of IgG iv is typically 3–4-g/L plasma. For Factor VIII, the quantity of plasma required increases sharply at lower yields, a trend that increases with increasing demand. The yield of high purity Factor VIII is ~150-IU/L plasma (206). This emphasizes the critical importance of Factor VIII yield for those countries concerned with national self-sufficiency. The extent to which the demand for plasma products can be met from national supplies of plasma is determined by the volume of plasma collected as well as by-product yields. The relatively large volume of plasma taken

Table 9. **Distribution of Intravenous Immunoglobulin and Factor VIII per 10^6 Population**[a]

Country	IgG iv, kg	Factor VIII[b], IU × 10^6
Australia	51.4	3.0
Canada	99.8	4.0
France	40.6	2.9
Germany	42.1	5.0
Hong Kong	8.1	1.1
Italy	30.3	2.5
Japan	28.5	2.0
New Zealand	40.0	2.8
Spain	32.7	2.7
Sweden	29.2	6.8
Switzerland	29.1	2.8
Taiwan	3.7	1.2
United Kingdom	26.2	3.4
United States	76.1	3.9

[a] Estimates for 2000–2001.
[b] Inclusive of recombinant Factor VIII.

from each paid U.S. donor (Table 6) has led to ~50% of the total world supply of plasma being provided from the United States. In 2001, 6.5 million L of plasma were exported from the United States for fractionation elsewhere, with 1.5 million L being imported into Spain, 1.4 million L into Switzerland, 1.3 million L into Austria, 0.6 million L into Germany, and 0.6 million L into the United Kingdom.

20. Regulation and Control

The preparation of clinical products from human plasma is regulated as a pharmaceutical manufacturing operation by national authorities who are responsible for giving authorization to distribute a product in their country. To obtain authorization, manufacturers must obtain approval for their manufacturing operation and for each of their products. In most countries, this is granted by awarding a manufacturing license for the overall operation, and individual product licenses for each of the products involved. This is done by the FDA in the United States and by the Medicines & Health Related Products Agency (MHRA) in the United Kingdom. In Europe, a centralized approval procedure is operated by the European Agency for the Evaluation of Medicinal Products (EMEA).

To obtain a manufacturing license it is necessary to comply with current good manufacturing practice (cGMP). In the United States the cGMP guidelines, published in the *Code of Federal Regulations* (208) encompass organization and personnel; buildings and facilities; equipment; control of components, containers, and closures; production and process controls; packaging and labeling; holding and distribution; laboratory controls; records and reports; and return of products. Manufacturing guidelines have also been published by WHO (149), as well as by other national or regional authorities (151,208–211) There is interest in harmonizing regulatory standards between different countries and to assist with this the EMEA has produced a number of guidelines on the manufacture of plasma derivatives, summaries of product characteristics and guidelines for the clinical investigation of individual products.

Products licensed for use in the United States are listed in Table 1. Product licenses cover method of preparation and product characterization, including safety and toxicity, as well as the intended clinical applications. Where monoclonal antibodies are used as process reagents, these must comply with regulatory guidelines for the preparation of clinical products (212–214). National control authorities are responsible for ensuring that products continue to meet the relevant standards; in some countries independent testing is carried out on a batch-by-batch basis. This is done in the United States by the Food and Drug Administration (FDA), and in the United Kingdom by the National Institute for Biological Standards and Control.

21. Specifications and Analytical Methods

Fundamental product specifications are published, with those for other drug substances, in national or regional pharmacopoeias. Plasma products described

in the *U.S. Pharmacopeia* (USP) (215) include human albumin, plasma protein fraction, antihemophilic factor, Factor IX complex, normal immune globulin, and a variety of specific immune globulins such as Rh_o (D), hepatitis B, tetanus, and varicella zoster. The *European Pharmacopoeia* (PhEur) (216) lists human albumin solution, antithrombin III, Factor VII, Factor VIII, Factor IX, prothrombin complex, fibrinogen, normal imunoglobulin, normal imunoglobulin for intravenous administration (IgG iv), and a variety of specific immunoglobulins. Principal characteristics of human albumin, plasma protein fraction, normal immune globulin, IgG iv, and Factor VIII and Factor IX concentrates are summarized in Tables 10–12. Draft versions of new monographs are published for comment in the journals *Pharmacopoeial Forum* and *Pharmeuropa* for the United States and European *Pharmacopoeias,* respectively.

Pharmacopoeial monographs describe products and methods that are well established and may not necessarily reflect state-of-the-art manufacturing or clinical practices. Issues concerning current practice may be dealt with by regulatory authorities (eg, FDA, EMEA) or learned societies, eg, the International Society of Thrombosis and Haemostasis (ISTH) provides international coordination in the treatment of coagulation disorders. Specialist ISTH subcommittees hold open meetings biannually and their recommendations are published in the journal *Thrombosis and Haemostasis.* The International Society of Blood Transfusion (ISBT) has a similar role with recommendations being published in the jounal *Vox Sanguinis.* Patients interests are represented by bodies such as the World Federation of Hemophilia (WFH) and the Primary Immunodeficiency Association (PiA), whereas manufacturers are represented by the Plasma Protein Theraputics Association (PPTA) and by the European Plasma Fractionation Association (EPFA) for the commercial and not-for-profit sectors,

Table 10. **Properties of Human Albumin and Plasma Protein Fraction (PPF)**

	Human Albumin		PPF
Characteristic	USP XXVII	PhEur 4th ed.	USP XXVII
form	solution	aqueous solution[a]	solution
protein concentration, g/L	50 250	40–50 150–250	50
albumin content, %	≥96	≥ 95	≥ 83
α- and β-globulins, %			≤17
γ-globulins, %			≤1
polymers and aggregates, %		≤ 10	
prekallikrein activator, IU/mL		≤ 35	
haem, OD at A_{403}[b]	≤0.25	≤0.15	
aluminium, μg/L		≤ 200	≤2.0[c]
potassium, mmol/g		≤0.05	
sodium, mmol/g	130–160	≤160	130–160
pH[d]		6.7–7.3	6.7–7.3

[a] Specified as a clear, slightly viscous liquid; almost colorless, yellow, amber, or green Ref. 216.
[b] OD at A_{403} = optical density at absorbance at 403 nm.
[c] mEq/L. Ref. 215.
[d] Measured after dilution to 10-g/L protein with a 9-g/L solution of sodium chloride.

Table 11. **Properties of Normal Immunoglobulin and Normal Immunoglobulin for Intravenous Administration**

	Immunoglobulin		Immunoglobulin Intravenous
Characteristic	USP[a] XXVII	PhEur[b] 4th ed.	PhEur[c] 4th ed.
form	solution	liquid or freeze-dried	liquid or freeze-dried
number of donors for plasma pool	$\geq$ 1000	$\geq$ 1000	$\geq$ 1000
protein concentration, g/L	150–180	100–180	$\geq$30
immunoglobulin G, %	$\geq$ 90	$\geq$ 90	$\geq$ 95[d]
polymer and aggregate, %		$\leq$10	$\leq$3
pH		5.0–7.2	4.0–7.4

[a] Antidiptheria activity, $\geq$ 2I U/mL; positive antimeasles and antipolio activity. Ref. 215.
[b] Clear and pale-yellow to light brown liquid or white or slightly yellow powder; antibody to hepatitis B surface antigen of $\geq$ 0.5 IU/g; antibody to hepatitis A virus of $\geq$ 100 IU/mL if intended for use in prophylaxis of hepatitis A.
[c] Clear or slightly opalescent and colorless or pale yellow liquid or white or slightly yellow powder; $\geq$ 80% Fc function (portion of fully functional immunoglobulin molecule); prekallikrein activator, $\leq$ 35 IU/mL; HBsAs antibody, $\geq$ 1 IU/g; normal subclass distribution; osmolality, $\geq$ 240 mosmol/kg. Ref. 216.
[d] Excluding albumin stabilizer.

respectively. Recommendations may also be made by national groups of clinicians (217). For products other than coagulation factors, international consensus meetings may be held under the auspices of bodies such as the National Institute for Health (218,219) and the American Association of Blood Banks (220) in the United States.

A wide range of analytical methods are employed in characterizing plasma products, and details of reference methods can be found in *European Pharmacopoeia* monographs (216) and the U.S. Code of Federal Regulations (208).

Table 12. **Properties of Factor VIII (Antihemophilic Factor) and Factor IX Concentrates**

	Factor VIII (AHF)		Factor IX	
Characteristic	USP XXVII	PhEur[a] 4th ed.	USP[b] XXVII	PhEur[c] 4th ed.
form	freeze-dried powder	powder or friable solid	freeze-dried powder	powder or friable solid
colour		white or pale yellow		white or pale yellow
potency IU/mL		$\geq$ 20		$\geq$ 20
% of label	80–120	80–120	80–120	80–125
specific activity IU/g	$\geq$ 100	$\geq$ 1000		$\geq$ 50,000
osmolality, mosm/kg		$\geq$240		$\geq$240
solubility, min		$\leq$ 10		$\leq$ 10
pH		6.5–7.5		6.5 – 7.5

[a] No anti-A and anti-B haemagglutination at 1/64 dilution Ref. 216.
[b] Factor IX Complex. Ref. 215.
[c] Factor IX concentrate, Heparin, $\leq$0.5 IU/IU Factor IX; nonactivated partial thromboplastin time, $\geq$ 150 s; Factors II,VII and X $\leq$ 5% of Factor IX activity.

22. Health, Safety, and Environmental Factors

The possibility that infectious donations of plasma may enter the fractionation process places staff at risk; the transmission of hepatitis B to fractionation workers had been reported (221) before the screening of plasma for hepatitis B infection was introduced. The extensive testing of donations that takes place (~1993) (121) reduces this risk substantially. Nevertheless, it is assumed that plasma for fractionation may be contaminated with viruses such as hepatitis B, hepatitis C, and HIV, and appropriate precautions should be taken. For example, automation should be used where possible, all equipment should be decontaminated for maintenance and repair, and all staff should wear appropriate protective clothing. These precautions apply to laboratory facilities as well as to the manufacturing plant. Materials that are potentially contaminated, such as used filter pads and waste fractions, should be sterilized prior to disposal. Fractionation staff now receive hepatitis B vaccination routinely and normal immunoglobulin may be administered following accidental exposure to potentially infectious materials (222). The need to validate methods of virus inactivation (223) has led some manufacturers to establish containment laboratories for handling HIV and other viruses; these facilities must comply with national regulations concerning the handling of dangerous pathogens.

Ethanol (qv), the principal bulk reagent in plasma fractionation, is categorized as a highly flammable material with vapor concentration of 3–19% ethanol being explosive (224) at temperatures above the flash point of 13°C (225). These properties must be considered in the design and specification of equipment and facilities involved in ethanol fractionation. Once the fractionation process has been completed, there are waste solutions containing up to 40% ethanol that require disposal. Some manufacturers recycle this material using distillation (qv), a procedure that must be regulated and controlled to the satisfaction of local environmental authorities.

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