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(57) Abstract

The present invention relates to a biosynthetic cerebrospinal fluid control and method of use. Additionally, this invention relates to the isolation and purification of stable liquid human prealbumin, a component in the biosynthetic cerebrospinal fluid control.

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BIOSYNTHETIC CEREBROSPINAL FLUID CONTROL AND METHOD OF USE

Background of the Invention

Field of the Invention

This invention relates to a stable biosynthetic liquid cerebrospinal fluid control and method of use. Additionally, this invention relates to the isolation and purification of stable liquid human prealbumin, a component in the biosynthetic cerebrospinal fluid control.

Description of Related Art

Cerebrospinal fluid is formed by an ultrafiltration of the plasma. Normal values for cerebrospinal fluid analytes are not the same as plasma values. This difference is a result of the filtration process being selective and the fact that the chemical composition is adjusted by the blood-brain barrier. Analysis of this chemical composition is an important diagnostic procedure. Disease increases cerebrospinal fluid protein concentrations. Elevated cerebrospinal fluid total protein is an indicator of central nervous system pathology such as damage to the blood-brain barrier caused by meningitis or hemorrhage. IgG is the primary immunoglobulin of cerebrospinal fluid. It is increased in several neurological conditions such as multiple sclerosis and viral meningoencephalitis. Analysis of cerebrospinal fluid by serum protein electrophoresis is an important diagnostic test in the diagnosis of multiple sclerosis. Low glucose values signal infections such as bacterial, tuberculous or fungal meningitis. Low values are also seen as a result of infiltration of the meninges with malignant cells. High lactic acid levels in cerebrospinal fluid indicate bacterial or tuberculous infection and rule out viral meningitis. Low cerebrospinal fluid chloride levels can be used as an indicator of tuberculous meningitis.

Since the chemical composition of cerebrospinal fluid is similar to plasma comparable tests are performed. However, the levels of these constituents are not the same resulting in different normal values than those used for plasma. In order to assess the accuracy and precision of these diagnostic tests, a

control similar to cerebrospinal fluid must be run. In the case of serum protein electrophoresis, a known protein control is always run in a separate well. The protein fractions in cerebrospinal fluid are not always clearly detected. Therefore, a control in which all the serum protein fractions are clearly defined is important. Most cerebrospinal fluid controls are prepared from actual spinal fluid. There are no tests, however, to detect the presence of infectious diseases in spinal fluid. Additionally, the recovery of spinal fluid is difficult and expensive and the quality is varied. Other cerebrospinal fluid controls have been made from normal human blood serum diluted with a diluent containing glucose and chloride ions, and then lyopholized. Reconstitution of the control is then required before it can be used. See U.S. Patent No. 3,753,925.

Summary of the Invention

The present invention relates to biosynthetic cerebrospinal liquid controls based on human serum spiked with prealbumin. Two controls are disclosed: one simulating normal spinal fluid and the second simulating abnormal spinal fluid. The product is prepared from human serum and purified human prealbumin in a buffer matrix formulated to simulate human cerebrospinal fluid. In particular, this invention relates to a stable liquid human based cerebrospinal fluid control made by the process comprising: (a) combining a sufficient amount of lactic acid, chloride, glucose, serum, purified prealbumin, and potassium in a buffer to simulate normal human cerebrospinal fluid; (b) gassing said filtered fluid with oxygen to obtain normal electrophoretic pattern for human cerebrospinal fluid, and (c) filtering said fluid to remove all microbial contaminants.

The present invention also relates to high purity prealbumin and a process to make prealbumin. In particular, this invention relates to a purified prealbumin made by the process comprising: (a) diluting human serum with a first buffer; (b) extracting globulins, ceroplasm and albumin from normal serum diluted in a first buffer using ion exchange chromatography;

(c) isolating the prealbumin containing fractions eluded from Step (b) by immunodiffusion; (d) pooling, concentrating and buffer exchanging the prealbumin containing fractions of Step (c) with a second buffer; (e) removing albumin from the said prealbumin containing pooled fractions of Step (d) by affinity chromatography; (f) isolating the prealbumin containing fractions eluted from Step (e) by immunodiffusion; (g) pooling and concentrating and buffer exchanging said prealbumin containing fractions of Step (f) with a third buffer to increase the prealbumin concentration; (h) removing globulins from said pooled fractions of Step (g) by ion exchange chromatography; (i) pooling, concentrating and buffer exchanging with a fourth buffer to increase prealbumin concentrations; (j) purifying the prealbumin containing fractions of Step (i) by gel filtration to remove any residual proteins; (k) isolating purified prealbumin fractions from Step (j) by electrophoresis and immunodiffusion; and (l) pooling, concentrating and sterile filtering said purified prealbumin fractions of Step (k).

Brief Description of the Drawings

Fig. 1 shows protein electrophoresis of cerebrospinal fluid prepared according to this method.

Fig. 2 shows protein electrophoresis of cerebrospinal fluid prepared according to this method.

Fig. 3 shows protein electrophoresis of prealbumin prepared by the present method. Analysis by serum protein electrophoresis.

Fig. 4 shows an overlay of protein electrophoresis of prealbumin prepared by the present method, on to normal human serum pattern.

Fig. 5 shows protein electrophoresis of prealbumin prepared by the Raz method; analysis by serum protein electrophoresis.

Fig. 6 shows an overlay of protein electrophoresis of prealbumin prepared by the Raz method, onto normal human serum pattern.

Detailed Description of the Invention

The disclosed invention involves diluting human serum with constituents adjusted within ranges for cerebrospinal fluid. Cerebrospinal fluid contains a very small amount of protein as compared to serum. The protein fractions are similar to those found in serum; however, for serum the quantity of prealbumin present is less than 1% whereas the quantity present in cerebrospinal fluid is 2 to 7% of the total proteins. In order to increase the level of this protein, a prealbumin spike was added. This protein was effectively isolated from human serum using column chromatography.

The product is formulated by the addition of the required constituents to a 50 to 80 mM HEPES buffer matrix. The pH of the buffered matrix is 7.3. Serum and prealbumin are added to the specifications required for each level. Glucose, lactic acid, chloride, sodium, potassium are added to obtain the desired concentrations as specified in Table I. The buffered solution is then gassed with 100% oxygen to remove a pre-albumin fraction that migrates faster than prealbumin and then sterile filtered.

The assayed constituents for this product are: protein, glucose, lactic acid, chloride, sodium, potassium, immunoglobulins and protein fractions by electrophoresis.

The Level I represents normal spinal fluid. Level II represents abnormal spinal fluid. The conditions observed in both levels of the control are most commonly seen in meningitis, multiple sclerosis, and brain trauma or injuries.

TABLE I

Constituent Targets:

PARAMETER	NORMAL	LEVEL I	LEVEL II	UNITS
Sodium	139-150	140-160	120-140	mmol/L
Potassium	2.7-3.9	2-4	3-6	mmol/L
Chloride	116-127	110-130	90-110	mmol/L
Lactic Acid	1.1-2.8	1-3	7-9	mmol/L
Glucose	45-80	45-80	25-40	mg/dL
Protein	15-45	15-45	50-80	mg/dL
ELECTROPHORETIC SEPARATION (% of Total Protein)				
PREALBUMIN	2-7	2-7	2-7	%
ALBUMIN	56-76	45-76	45-76	%
GLOBULINS:				
ALPHA 1	2-7	2-7	2-7	%
BETA	7-18	7-18	7-18	%
GAMMA	7-14	7-19	7-19	%
IMMUNOGLOBULINS (RID)				
IgA	0-0.2	trace	trace	mg/dL
IgG	10-40	0-15	5-40	mg/dL
IgM	0-0.6	trace	trace	mg/dL

Microbiology Specs: No growth to USP procedures.

Example 1 - Prealbumin Isolation from Serum

Units of normal human serum were pooled and the volume measured to be approximately two liters. The pooled serum was diluted 50% in 50mM potassium phosphate buffer, pH 7.5, 0.1% azide. The diluted serum was sterile filtered through a 0.22 micron filter into sterile containers. The prepared serum was then loaded on to an ion exchange column containing DEAE Sephacel™ or DEAE Sepharose™ (Pharmacia) that has been previously equilibrated with 50 mM potassium phosphate buffer, pH 7.5, 0.1% azide. After completion of the sample load, the column was washed with 50 mM potassium phosphate buffer, pH 7.5, 0.1% azide until an OD at 280nm is less than 0.2 as measured on an UV spectrophotometer. The bound proteins were eluted with a gradient from 0 to 1M NaCl in 0.5M potassium phosphate buffer, pH 7.5, 0.1% azide. 12 mL fractions were collected until the gradient was exhausted. This column removed ceruloplasmin, globulins and albumin from the sample.

The fractions are tested by immunodiffusion for the presence of prealbumin. When the fractions that contain prealbumin are identified, they are pooled, concentrated, and buffer exchanged with 20 mM potassium phosphate buffer, pH 7.1, 0.02% azide. The pooled fractions were concentrated to a total protein of approximately 4 to 5 g/dL. The fraction pool is then loaded on an affinity column containing Affi Gel Blue™ (BIORAD) or Blue Sepharose™ (Pharmacia) which has been equilibrated with 20 mM phosphate buffer, pH 7.1, 0.02% azide. This chromatography media contains Cibacron Blue Dye F3G-A which has an affinity for albumin. After the sample was loaded, the fraction collector was started and 6 mL fractions were collected as the column was washed with 20 mM phosphate buffer, pH 7.1, 0.02% azide. As the sample was loaded, albumin binds to the blue dye and the remaining proteins passed through the column. The prealbumin containing fractions were pooled, concentrated and buffer exchanged with 50 mM potassium phosphate buffer, pH 7.5, 0.1% azide. The fractions were concentrated to a total protein of approximately 3 to 4 g/dL. The concentrated fractions were then loaded onto an ion exchange column containing DEAE Sephacel™ or DEAE Sepharose™ (Pharmacia) which has been equilibrated with 50mM potassium phosphate buffer, pH 7.5, 0.1% azide. The proteins were eluted using a salt gradient of 0 to 1 M NaCl in 50mM potassium phosphate buffer, pH 7.5, 0.1% azide. Fractions of 3mL were collected until the gradient was exhausted. Fractions were tested for the presence of prealbumin using immunodiffusion.

When the prealbumin containing fractions have been identified these fractions were pooled, concentrated and buffer exchanged in 50 mM phosphate buffer with 170 mM sodium chloride, pH 7.5, 0.02% azide. The fraction pool was concentrated to a total protein of approximately 2-7 g/dL. This fraction pool was then loaded on a gel filtration column which contained ULTROGEL® AcA 54 (IBF Biotechnics) equilibrated with 50 mM phosphate buffer with 170 mM sodium chloride, pH 7.5, 0.02% azide. This column was then washed with 50 mM phosphate buffer with 170 mM sodium

chloride, pH 7.5, 0.02% azide. Fractions were collected of 3 mL each. Two protein peaks were collected. The prealbumin was mostly contained in the second peak. Fractions were tested for the presence of prealbumin using immunodiffusion. The fractions that contain prealbumin were then tested by serum protein electrophoresis for the presence of other serum proteins. The purified prealbumin fractions were selected, pooled and concentrated to a total protein of approximately 1 to 4 g/dL. These pooled fractions were sterile filtered and stored at 2-8°C.

The purified prealbumin was tested for total prealbumin content using serum protein electrophoresis, and radial immunodiffusion analysis for quantitative measurement of prealbumin. A single peak was observed and the prealbumin was found to be 90 to 100% pure by protein electrophoresis. See Fig. 3. When compared to the electrophoretic pattern of normal serum, the peak is observed in the prealbumin region and no other serum proteins are present. See Fig. 4. When spiked into normal serum, the resulting electrophoretic pattern showed a peak in the prealbumin region. See Fig. 1 and 2. SDS PAGE electrophoresis shows a single protein to be present. This protein is found in the correct molecular weight range for prealbumin (54,000) The quantity of prealbumin demonstrated yields of 80 to 100% depending on the purity of prealbumin required. A commercially available prealbumin prepared from human plasma using the method defined by Raz, A., et al., J. Biol. Chem., 244,12 (1969) was evaluated for purity. This prealbumin was found to be only 75% pure by protein electrophoresis. See Fig. 5. When compared to the electrophoretic pattern of normal serum the contaminating proteins are observed in the albumin, and alpha globulin regions. See Fig. 6.

The purified prealbumin has been monitored for stability while being stored refrigerated and frozen. The prealbumin has been tested for quantity by radial immunodiffusion and purity by

protein electrophoresis. After ten months storage at these conditions, the prealbumin has remained stable.

TABLE II

STABILITY OF PREALBUMIN

MONTHS	STORAGE AT 2-8°C
0	7395 mg of prealbumin/liter of solution
4	7020 mg of prealbumin/liter of solution
6	7879 mg of prealbumin/liter of solution
10	7005 mg of prealbumin/liter of solution
MONTHS	STORAGE AT -20°c
0	N/A
4	N/A
6	7724 mg of prealbumin/liter of solution
10	7275 mg of prealbumin/liter of solution

Example 2 - Preparation of Cerebrospinal Fluid Control

A clean container with a stirring device is prepared. 800mL of distilled is placed into the container. While mixing, the following chemicals are added:

Constituents	Level I	Level II
HEPES (N-2-hydroxyethyl piperazine-N_2'-2-ethane sulfonic acid)	12.3 gm	9.2 gm
Sodium HEPES	9.4 gm	7.0 gm
Sodium Chloride	6.6 gm	5.3 gm
Potassium Chloride	0.19 gm	0.3 gm
Glucose	0.57 gm	0.33 gm
Sodium Lactate	0.38 gm	1.5 gm
Human Serum	0.29 gm	0.63 gm
Prealbumin	10.0 mg	15.0 mg
Sodium Azide 25%	0.8 mL	0.8 mL

After all chemicals are dissolved, the total volume of the solution is brought to one liter with distilled water. All constituents are analyzed and adjusted within the above described specifications. A gas cylinder of oxygen is connected to a two stage regulator. Rubber tubing or equivalent is connected to the regulator and to the batching container. The first stage of the regulator is opened. The second stage is slowly opened until the

gas flow through the solution is approximately 0.4 SCFH (square cubic feet per hour). While mixing, the pool is flushed in this manner at room temperature.

After flushing, a sample of the solution is removed and concentrated approximately 60 times. This concentrated sample is then evaluated by serum protein electrophoresis. If the electrophoretic pattern does not show a single peak in the prealbumin region, reflushing is necessary.

After a normal electrophoretic pattern is recovered, the solution is sterile filtered through 0.22 micron membranes into sterilized containers. The sterile solution is then filled into sterilized vials at three mL each.

These cerebrospinal fluid controls were evaluated for stability according to a protocol for the evaluation of the stability of diagnostics products. This protocol states guidelines for accelerated stability studies. According to this protocol, a product that is stored at 37°C for one week is stable for one year at 2-8°C. Accelerated stability studies were used to determine the performance characteristics of the product under storage conditions which stress the product in comparison to those recommended for use and handling of the product. The cerebrospinal fluid controls were analyzed after storage at 25°C for three months and 37°C for four weeks. Results from these analyses show the product to be stable and therefore have a predicted shelf life of greater than three years. The product has been monitored at 2-8°C for greater than one year. See Table III.

TABLE III

STABILITY OF CEREBROSPINAL FLUID CONTROL

LEVEL I

CONSTITUENTS	UNITS	2-8°C Storage	25°C Storage	37°C Storage
PROTEIN	mg/dL	28	30	28
LACTIC ACID	mM	1.2	1.1	1.2
GLUCOSE	mg/dL	56	56	56
CHLORIDE	mM	120	127	122
SODIUM	mM	149	150	149
POTASSIUM	mM	2.6	2.6	2.6
IgA	mg/dL	1.2	1.1	1.3
IgG	mg/dL	4.6	5.0	4.3
IgM	mg/dL	1.2	1.6	1.4
ELECTROPHORESIS:				
PREALBUMIN	% OF TOTAL	6.2	6.2	5.2
ALBUMIN	% OF TOTAL	66	65	66
ALPHA 1	% OF TOTAL	3.2	3.0	3.7
ALPHA 2	% OF TOTAL	6.3	6.3	6.2
BETA	% OF TOTAL	7.7	8.2	8.2
GAMMA	% OF TOTAL	10.5	11.2	11.6

LEVEL II

CONSTITUENTS	UNITS	2-8°C Storage	25°C Storage	37°C Storage
PROTEIN	mg/dL	61	66	64
LACTIC ACID	mM	7.6	7.7	7.6
GLUCOSE	mg/dL	31	34	33
CHLORIDE	mM	102	106	102
SODIUM	mM	127	127	127
POTASSIUM	mM	4.1	4.2	4.1
IgA	mg/dL	2.5	2.5	3.0
IgG	mg/dL	10.2	10.4	10.3
IgM	mg/dL	1.9	2.4	1.9
ELECTROPHORESIS:				
PREALBUMIN	% OF TOTAL	5.4	4.9	4.6
ALBUMIN	% OF TOTAL	63	63	62
ALPHA 1	% OF TOTAL	2.8	2.4	2.7
ALPHA 2	% OF TOTAL	7.4	7.6	7.8
BETA	% OF TOTAL	8.6	9.6	9.6
GAMMA	% OF TOTAL	12.5	12.6	13.5

The cerebrospinal fluid controls were also evaluated for open vial stability. Vials were tested after being open for two weeks. Analyses of the opened vials showed no change when compared to vials that were freshly sampled. See TABLE IV.

TABLE IV

CONSTITUENT	UNITS	FRESH VIAL	OPEN 14 DAYS
OPEN VIAL STABILITY LEVEL I			
Protein	mg/dL	25.5	25.7
Glucose	mg/dL	60.0	60.2
Sodium	mM	158	158
Chloride	mM	113	112
IgG	mg/dL	4.98	4.99
IgA	mg/dL	1.18	1.16
IgM	mg/dL	<0.69	<0.69
ELECTROPHORESIS:			
Prealbumin	% of Total	3.6	4.4
Albumin	% of Total	65	64
Alpha 1	% of Total	3.0	3.6
Alpha 2	% of Total	6.8	7.1
Beta	% of Total	9.2	9.2
Gamma	% of Total	11.9	12.1
OPEN VIAL STABILITY LEVEL II			
Protein	mg/dL	59.5	59.1
Glucose	mg/dL	33.3	33.2
Sodium	mM	127	127
Chloride	mM	96	97
IgG	mg/dL	11	11
IgA	mg/dL	2.59	2.58
IgM	mg/dL	0.89	0.91
ELECTROPHORESIS:			
Prealbumin	% of Total	2.5	2.6
Albumin	% of Total	61	62
Alpha 1	% of Total	4.0	4.1
Alpha 2	% of Total	8.5	7.8
Beta	% of Total	10.0	9.5
Gamma	% of Total	13.5	13.8

Example 3

The cerebrospinal fluid control prepared in Example 2 was used as a control in several diagnostic tests. The results of these assays are reported in TABLE V.

TABLE V
METHODS COMPARISON

CONSTITUENT	UNITS	METHOD	LEVEL I	LEVEL II
Protein	mg/dL	DuPont aca 4	28.5	59.8
	mg/dL	DuPont aca 3	22	57
	mg/dL	Kodak Ektachem	25.6	75.6
	mg/dL	Abbott Spectrum	28.6	60.2
Lactic Acid	mM	DuPont aca 3	1.2	7.0
	mM	Baxter Paramax	1.4	7.1
Glucose	mg/dL	DuPont aca 4	57.2	33.5
	mg/dL	DuPont aca 3	57	33.8
	mg/dL	DuPont Dimension	56.6	33.4
	mg/dL	Kodak Ektachem	60.1	35.9
	mg/dL	Abbott Spectrum	58.5	35.4
	mg/dL	Baxter Paramax	60.0	36.0
Chloride	mM	DuPont aca 3	122	104
	mM	Kodak Ektachem	112	93
	mM	Abbott Spectrum	119	101
	mM	DuPont Dimension	114	97
	mM	NOVA Biomedical	119	101
	mM	Baxter Paramax	113	95
Sodium	mM	Abbott Spectrum	152	130
	mM	DuPont Dimension	153	128
	mM	NOVA Biomedical	150	126
Potassium	mM	NOVA Biomedical	2.7	4.1
IgG	mg/dL	RID	8.0	16
	mg/dL	Beckman Array	5.0	11
IgA	mg/dL	RID	1.4	3.0
	mg/dL	Beckman Array	1.2	2.6
IgM	mg/dL	RID	1.4	1.9
	mg/dL	Beckman Array	<0.69	0.91

TABLE V (Continued)

ELECTROPHORESIS:	% OF TOTAL	
	HELENA	
Prealbumin	7.0	5.5
Albumin	63	61
Alpha 1	3.8	3.9
Alpha 2	6.4	7.4
Beta	7.3	8.3
Gamma	13.0	14.0
	BECKMAN PARAGON	
Prealbumin	5.5	3.5
Albumin	66	67
Alpha 1	3.7	3.7
Alpha 2	6.6	7.1
Beta	7.6	7.6
Gamma	10.0	11.0

WE CLAIM

1. A stable liquid human based cerebrospinal fluid control made by the process comprising:
   (a) combining a sufficient amount of lactic acid, chloride, glucose, serum, purified prealbumin, and potassium in a buffer to simulate normal human cerebrospinal fluid;
   (b) gassing said filtered fluid with oxygen to obtain normal electrophoretic pattern for human cerebrospinal fluid; and
   (c) filtering said fluids to remove all microbial contaminants.
2. The control of Claim 1 further characterized by stability of up to about 24 months when stored at between about 2-8°C.
3. The control of Claim 1 further characterized by open vial stability of about seven days when stored at between about 2-8°C.
4. An electrophoretic method to assess human cerebrospinal fluid comprising: comparing the controls of Claim 1 with the electrophoretic pattern of sample human cerebrospinal fluid.

5. A purified prealbumin made by the process comprising:

(a) diluting human serum with a first buffer;

(b) extracting globulins, ceroplasm and albumin from said diluted serum using ion exchange chromatography;

(c) isolating the prealbumin containing fractions eluded from Step (b) by immunodiffusion;

(d) pooling, concentrating and buffer exchanging the prealbumin containing fraction of Step (c) with a second buffer;

(e) removing albumin from the said prealbumin containing pooled fractions of Step (d) by affinity chromatography;

(f) isolating the prealbumin containing fractions eluded from Step (e) by immunodiffusion;

(g) pooling and concentrating and buffer exchanging said prealbumin containing fractions of Step (f) with a third buffer to increase the prealbumin concentration;

(h) removing globulins from said pooled fractions of Step (g) by ion exchange chromatography;

(i) pooling, concentrating and buffer exchanging the prealbumin containing fraction of Step (h) with a fourth buffer to increase the prealbumin concentration;

(j) purifying said prealbumin containing fraction of Step (i) by gel filtration to remove any residual proteins;

(k) isolating purified prealbumin fractions from Step (j); and

(l) pooling, concentrating and sterile filtering said purified prealbumin fractions of Step (k).

6. The method of Claim 5 wherein the affinity chromatography media is Cibacron Blue Dye.

7. The method of Claim 5 wherein said purified prealbumin fractions of Step (k) are isolated by electrophoresis and immunodiffusion.

8. The method of Claim 5 wherein the ion exchange media is selected from the class consisting of DEAE Sephacel™ or DEAE Sepharose™ (Pharmacia).

9. The method of Claim 5 wherein the gel filtration media is ULTROGEL® AcA 54 (IBF Biotechnics).

FRACTION	%
Prealbumin	4.6
Albumin	58.3
Alpha 1	3.6
Alpha 2	7.1
Beta	11.0
Gamma	15.3

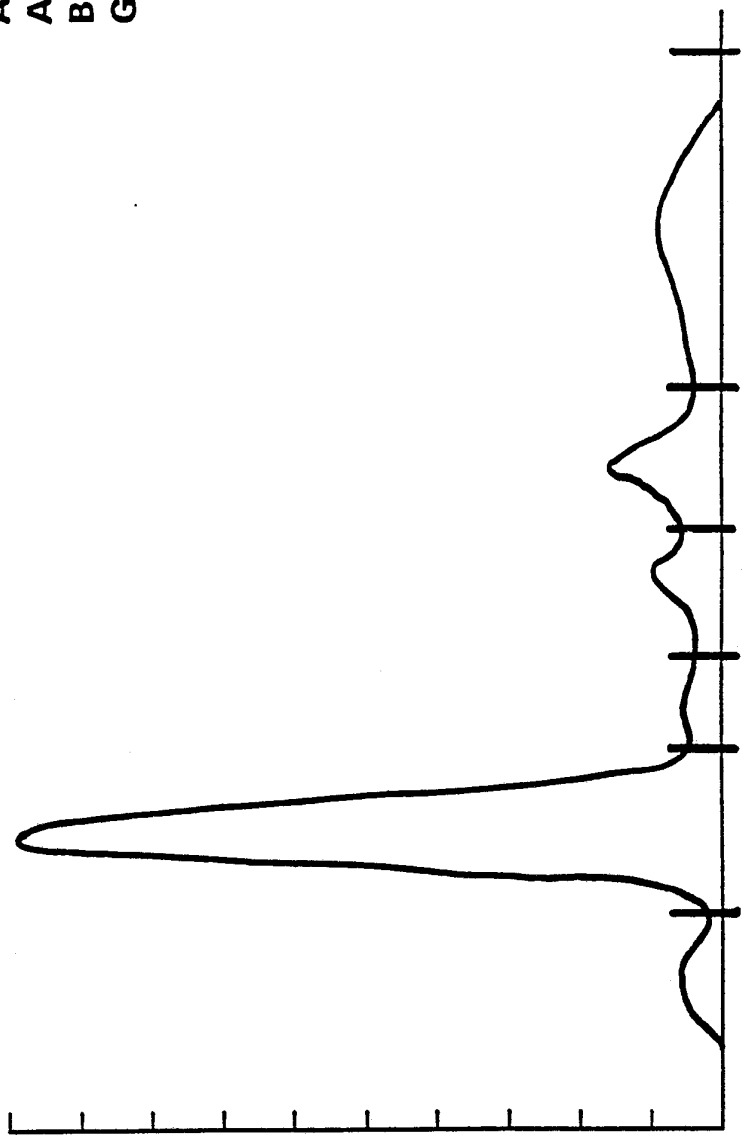

Fig. 1

FRACTION	%
Prealbumin	3.9
Albumin	59.8
Alpha 1	3.0
Alpha 2	7.9
Beta	11.9
Gamma	13.6

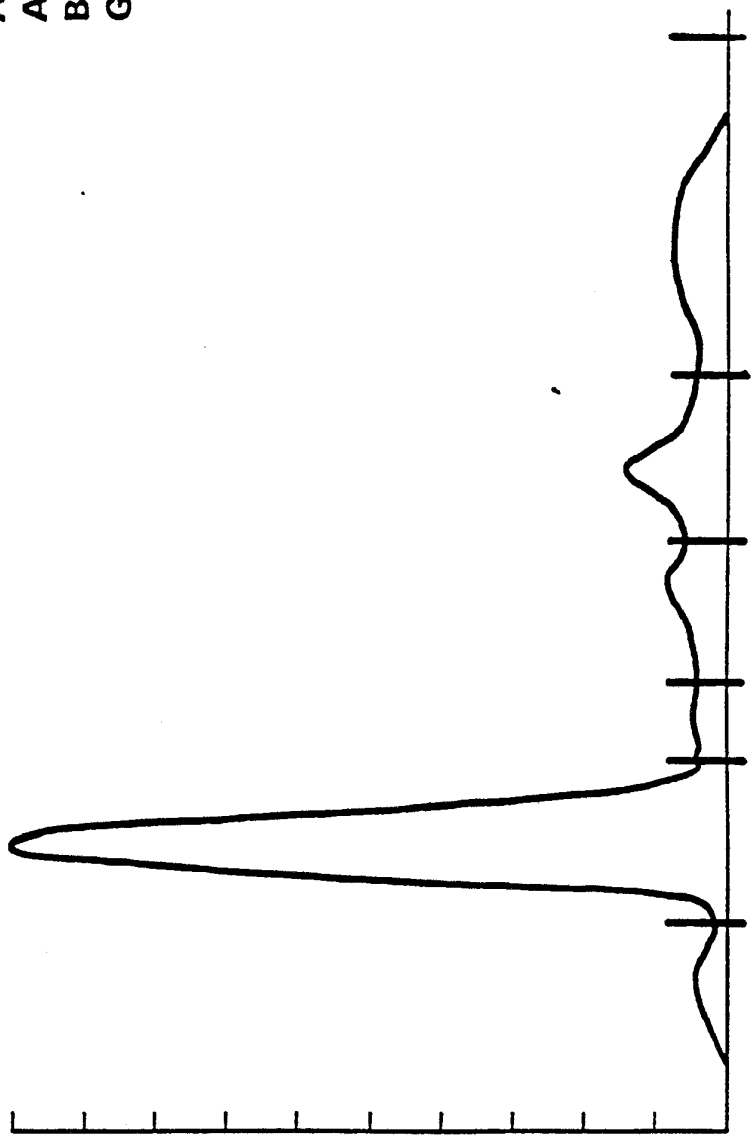

Fig. 2

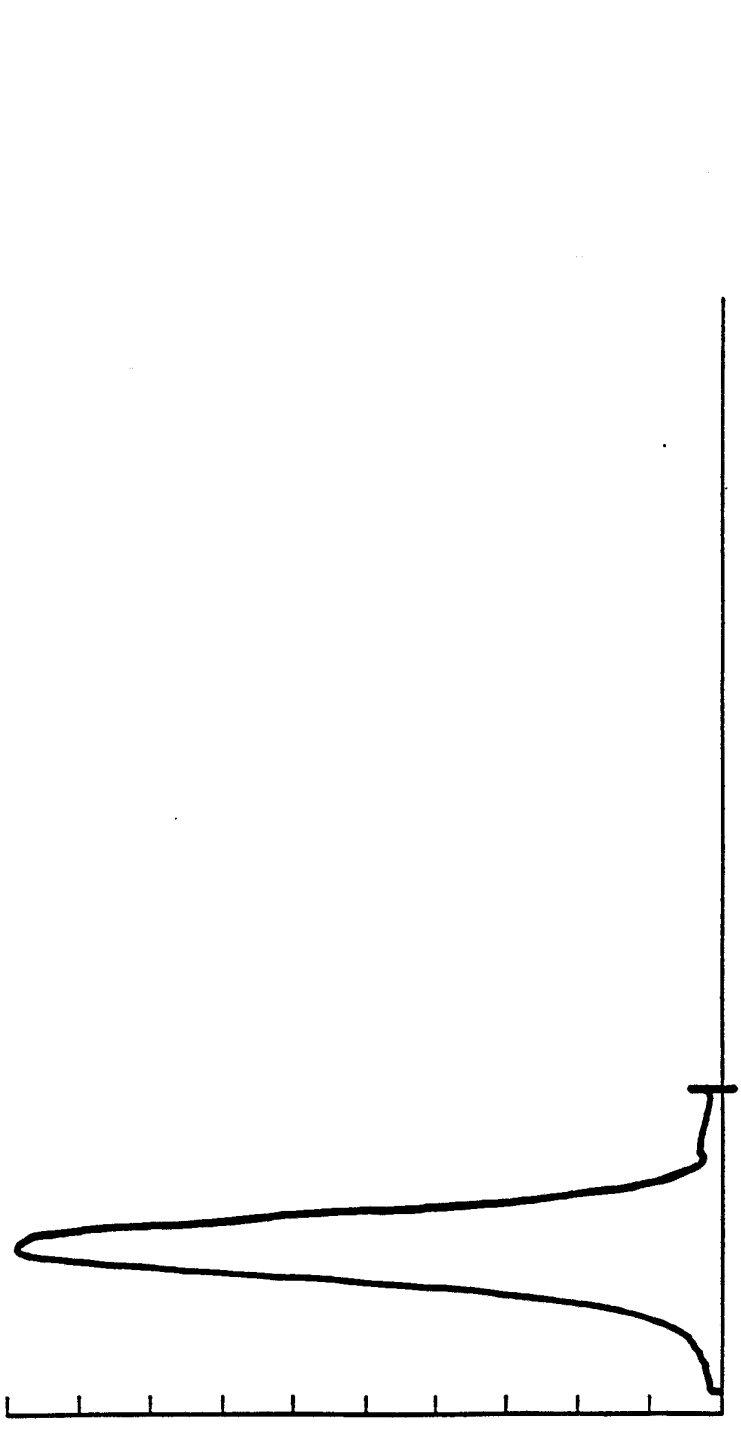

Fig. 3

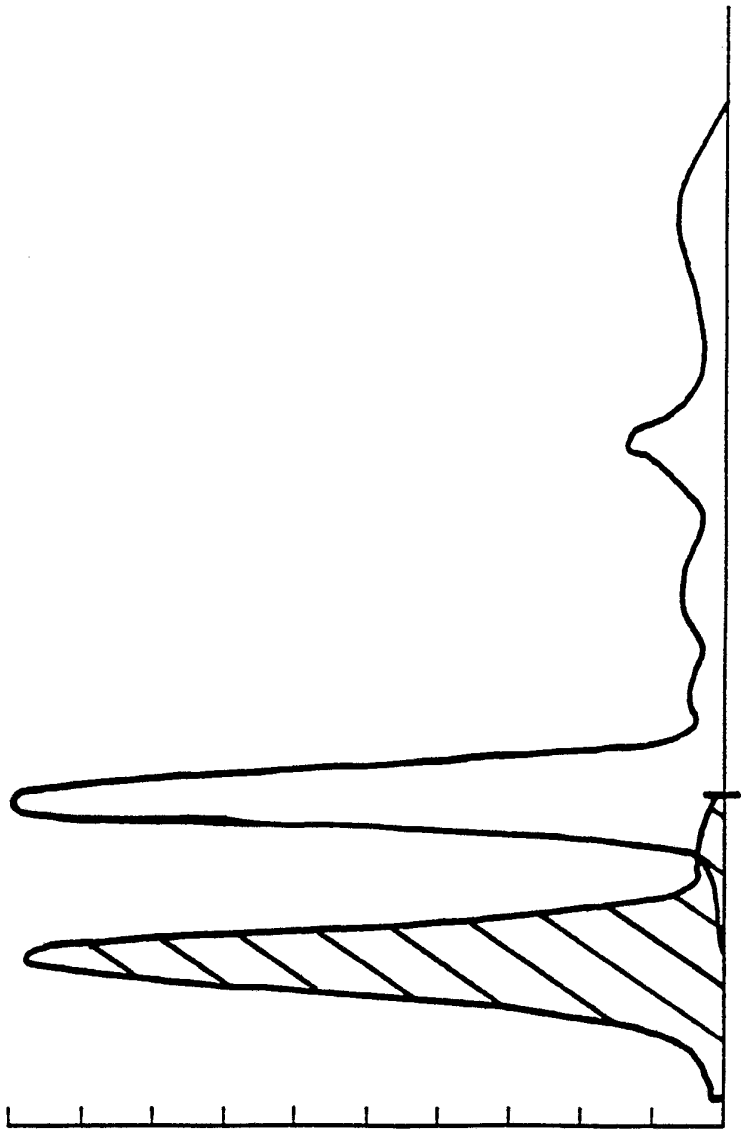

Fig. 4

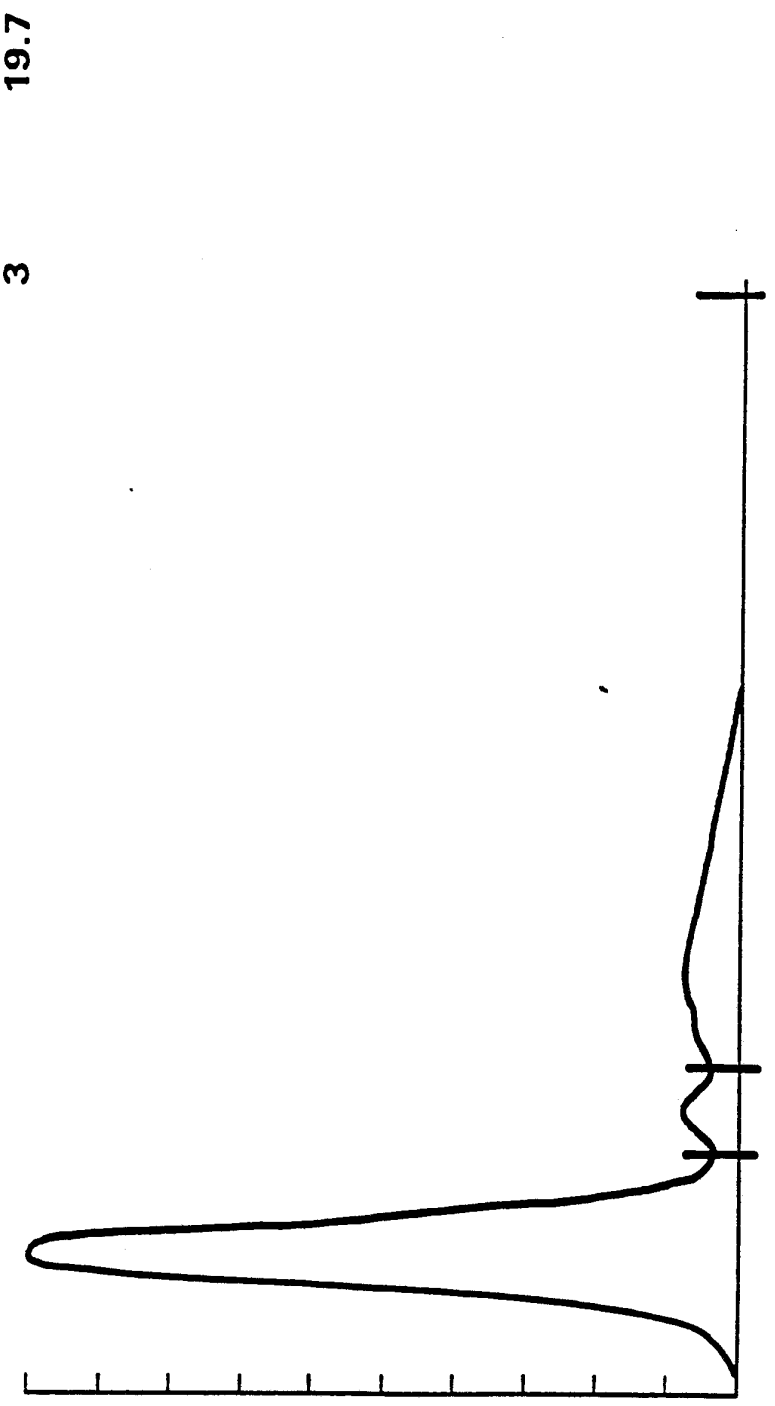

Fig. 5

FRACTION	%
1	75.3
2	5.0
3	19.7

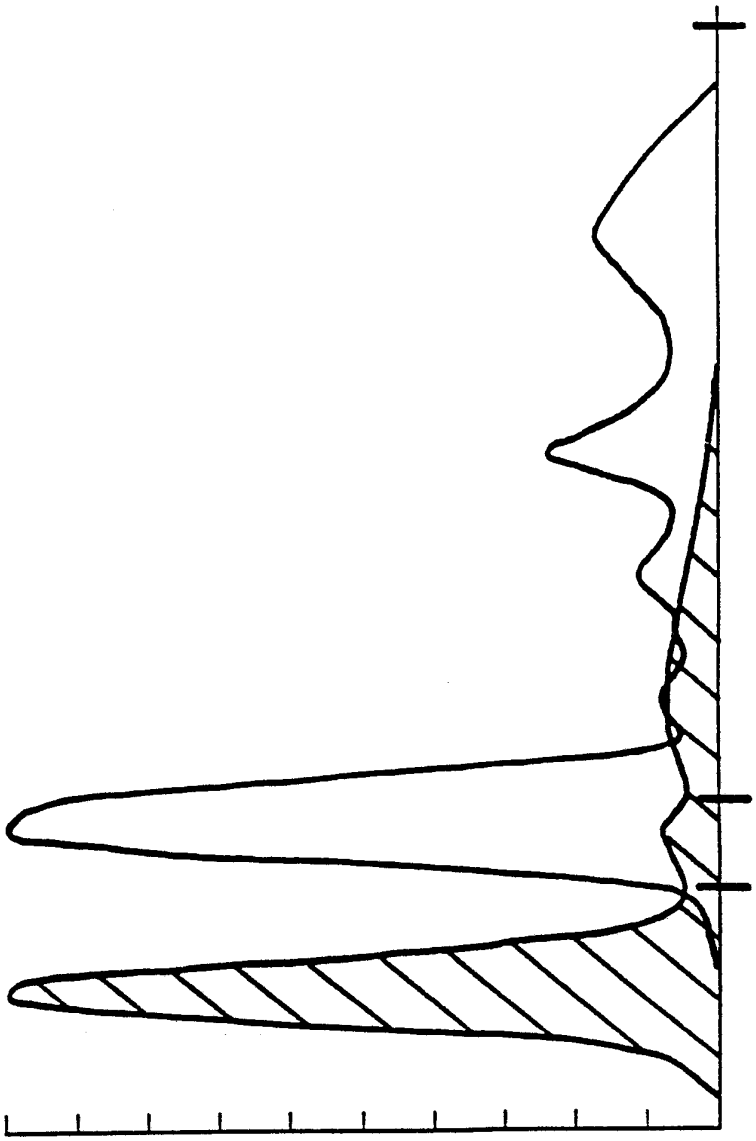

Fig. 6

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 92/01555

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 G01N33/96; C07K13/00; C07K3/12

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	G01N ; C07K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	US,A,3 753 925 (A.L.LOUDERBACK ET AL.) 21 August 1973 cited in the application see the whole document	1,4
A	THE JOURNAL OF BIOLOGICAL CHEMISTRY vol. 244, no. 12, 25 June 1980, pages 3230 - 3237; A-RAZ ET AL.: 'The Interaction of Thyroxine with Human Plasma Prealbumin and with the Prealbumin-Retinol-binding Protein Complex.' cited in the application see page 3230 - page 3233	5,7,8

-/--

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
25 JUNE 1992	08. 07. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	HITCHEN C.E.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	CHEMICAL ABSTRACTS, vol. 107, no. 13, 28 September 1987, Columbus, Ohio, US; abstract no. 112019X, M.M.BASHOR ET AL.: 'Purification of prealbumin from human serum.' page 311 ;column 1 ; see abstract & Prep. Biochem. 1987, 17(3), 209-227. ---	5,8
A	CHEMICAL ABSTRACTS, vol. 102, no. 15, 15 April 1985, Columbus, Ohio, US; abstract no. 128116J, A.PHILIPPE ET AL.: 'Combined pseudo-ligand affinity chromatography as a general method for plasma protein purification.' page 291 ;column 2 ; see abstract & Protides Biol. Fluids 1984, 32, 1125-1128. ---	5,6

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9201555 SA 58238

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 25/06/92

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		CA-A- 985996	23-03-76
		DE-A,C 2314263	04-10-73
		FR-A- 2185248	28-12-73
		GB-A- 1372812	06-11-74
		JP-A- 49031390	20-03-74