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(57) Abstract

A new citrate-based anticoagulant for donor whole blood provides good platelet yield and cell morphology at a significantly reduced risk of donor paresthesia during apheresis procedures. The primary citrate anticoagulant compositions include a citric acid to total citrate ration greater than about 30 %. The anticoagulants are mixed with whole blood to provide an anticoagulated blood mixture which contains a citric acid concentration of greater than about 5.0 mM, a total citrate concentration of less than about 20 mM, and an initial blood pH of less than about 6.75. The platelet rich products including PRP and PC prepared from blood collected in accordance with the invention exhibit better platelet yields and better platelet morphology on storage.

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CONTROLLING DONOR BLOOD CHARACTERISTICS

Background of the Invention

The present invention generally relates to blood collection procedures and blood component separation methods. More particularly, it relates to new and improved methods of collecting blood into anticoagulant formulations designed to promote increased platelet yield and improved overall platelet morphology in platelet collection procedures.

Today there exists a number of automated donor hemopheresis systems for separation of blood, including whole blood into components or fractions. The systems are designed to collect one or more components, such as plasma, white cells, platelets and red cells, for further use or for disposal; to return certain components to the donor, who may be a patient; and/or to treat a component, for subsequent return to a donor. One such system is the Autopheresis-C® systems sold by Baxter Healthcare Corporation of Deerfield, Illinois, a wholly-owned subsidiary of the assignee of the present invention. That system utilizes a microprocessor-controlled instrument including automated processing programs, in conjunction with a disposable set.

The Autopheresis-C® device may, when disposable plasmapheresis set is installed therein, be used to collect plasma from whole blood drawn from a donor. A rotating membrane in a separation chamber of the disposable may in fact be wetted by an anticoagulant priming operation before blood is withdrawn from the donor, as shown in U.S. Patent Application Serial No. 07/106,089, filed October 7, 1987, entitled "Method for Wetting a Plasmapheresis Filter with Anticoagulant" and the corresponding PCT International Application Publication No. WO89/03229.

For the collection of platelets and plasma, the Autopheresis-C® system uses a single, two-stage set as disclosed in U.S. Patent No. 4,851,126, entitled "Apparatus and Methods for Generating Platelet Concentrate". A set may include a rotating membrane separation chamber as set forth in U.S. Patent Application Serial No. 73,378, and in corresponding Canadian Patent No. 1,261,765, as well as a centrifuge separator as set forth in U.S. Patent Nos. 4,776,974 and 4,911,833, entitled "Closed Hemopheresis System and Method" and in International PCT Publication No. WO88/05332 entitled "Continuous Centrifugation System and Method for Directly Deriving Intermediate Density Material From a Suspension". If an anticoagulant source is pre-attached to the set, a biologically closed system, as medically defined, can be created.

A two-stage system enables the collection of blood from a donor for separation into platelet-rich plasma and packed red cells. The red cell suspension is returned to the donor by means of the same needle used to withdraw the whole blood. The platelet-rich plasma is collected in a container. The machine and set are disconnected from the donor. The collected platelet-rich plasma is then separated into plasma and platelet concentrate, utilizing a second stage of the biologically closed set.

Another automated closed system for separating blood fractions is the CS-3000® cell separator sold by Baxter Healthcare Corporation.

During withdrawal of blood and its subsequent treatment/separation, anticoagulant must be added in order to prevent clotting of the blood within the disposable tubing and separating set during the separation or collection of the blood. The conventional method of administering anticoagulant during automated apheresis procedures is to add anticoagulant during the step of withdrawal of the whole blood from the donor's vein.

Anticoagulant from an anticoagulant container is administered through tubing to a location just downstream from the phlebotomy needle at a tubing junction, where the anticoagulant tubing line merges with the nonanticoagulated whole blood tubing line adjacent the phlebotomy needle in the donor.

There are at least four separate reasons for the addition of anticoagulant to the donor's blood during extracorporeal blood procedures. The first reason is to prevent the blood from clotting as it travels through the various tubes to the blood separator of the disposable set. The second reason is to prevent the blood from clotting as it is being separated. All separators require some exposure of blood to fluid shear stresses and these shear stresses can induce coagulation or agglomeration. The third reason is to prevent the separated cells from coagulation as they are being pumped through reinfusion filters and back to the donor. The fourth reason is to provide enough nutrients and sufficient pH buffering to permit storage of the separated blood component for a required duration of time.

The demand for anticoagulant in each of the four general steps identified above depends on the particular automated apheresis procedure. Some systems may induce significantly more shear stress during blood separation than other systems and, therefore, the upper limit demand for anticoagulant would be set by the separation step. Also, the separation technology used may have different stages wherein each separation stage may have its own, different demand level for the amount of anticoagulant in the blood.

Generally, the prior art has dealt with the issue of anticoagulant demand in automated procedures by adding to the whole blood, almost immediately upon its withdrawal from the donor, enough anticoagulant to meet the highest

anticoagulant demand level during the entire withdrawal, separation, return and storage procedure. The anticoagulant is added adjacent the phlebotomy needle. The anticoagulant mixes with the whole blood upon being withdrawn from the donor. The prior art systems had been directed to adding as much anticoagulant as is necessary to prevent clotting with attention being paid to an upper limit dosage of anticoagulant, beyond which a so-called "citrate reaction" may occur in the donor upon return of an anticoagulated blood component to the donor.

Human blood collected by these apheresis procedures is anticoagulated in general practice by metering in a quantity of calcium ion chelating agent such as the sodium salts of citric acid. The ratio of human blood to citrate can be adjusted and controlled by various means and in general the ratio of blood to the citrate anticoagulant is made as large as possible to prevent the donor from experiencing any signs of paresthesia.

Reducing the quantity of anticoagulant administered to the patient on collection of whole blood is desirable for the patient donor but undesirable in terms of the ultimate storage stability and quantity of special blood components collected. More particularly to date, there is still a need to improve the total number of platelet cells recovered from these apheresis procedures and to improve the cell morphology on storage to provide better blood component products.

In the past, when using citrate-containing anticoagulants such as ACD-A and CPD-type anticoagulants, it has generally been believed that the ratio of whole blood to anticoagulant should be maintained in the range of 25:1 to 6:1, and the final blood pH should be maintained at about 6.8 to about 7.2. Despite these earlier efforts, it is still desired to improve upon the quantity and quality

of platelet concentrates by improving platelet collection conditions and procedures.

Summary of the Invention

Unexpectedly in view of the foregoing, it has now been discovered that platelet cells collected at low pH are morphologically better maintained than those collected at higher more physiological pH. In addition, better yields can be achieved if whole blood is collected into a lower pH. The unexpectedly good morphology results are contrary to previous literature on platelet cell storage which indicates that as the pH of the platelets falls during storage, the morphologies and other cell indexes of viability tend to decline.

In accordance with the present invention, a new citrate-based primary anticoagulant preparation for donor whole blood is provided for addition to a human blood sample being collected for separation into human blood platelet products. The primary anticoagulant preparation comprises a calcium ion chelating agent-type of anticoagulant including citric acid and trisodium citrate wherein the ratio of citric acid to total citrate present is greater than about 30% by equivalent weight. The primary anticoagulant is added to whole blood so that upon collection a resulting collected blood/anticoagulant mixture is provided which contains a citric acid concentration of greater than about 5.0 mM, preferably at least about 7.0 mM or more, a total citrate concentration of less than about 20 mM, preferably from about 7.0 mM to about 18 mM, and an initial pH of less than about 6.75. When citrate-based anticoagulant agents are formulated in accordance with these guidelines and platelet-rich plasma and platelet concentrates are derived therefrom, it has been discovered that an improvement in terms of total cell counts per milliliter, that is cell yields, and cell morphology and cell quality are significantly improved.

In accordance with this invention, a new and improved method of providing human blood platelet products exhibiting improved platelet storage properties comprises the steps of collecting whole blood and mixing it with a primary anticoagulant formulation so as to form an anticoagulated blood mixture. The anticoagulant preparation includes citric acid and trisodium citrate wherein the ratio of citric acid to total citrate is greater than about 30%. The anticoagulated blood mixture in accordance with the method of the invention has a citric acid concentration of greater than about 5.0 mM, a total citrate concentration of less than about 20 mM and an initial pH of less than about 6.75. Thereafter, the anticoagulated blood mixture is separated into a human blood platelet preparation selected from the group consisting of platelet-rich plasma and platelet concentrates.

In accordance with this invention, better quality, better yield blood component products may be produced in a given collection procedure without undesirably increasing the amount of anticoagulant. This in turn reduces the risk of adverse reaction to anticoagulants for the patient donor.

Other advantages of the present invention will become apparent from the following Detailed Description, Drawings and working Examples.

Brief Description of the Drawings

Fig. 1 is a graphical plot showing the blood citric acid concentration in millimoles as a function of total blood citrate concentration in millimoles resulting from the use of anticoagulant formulations comprising varying ratios of citric acid to trisodium citrate;

Fig. 2 is a graphical plot showing the blood pH achieved as a function of total blood citrate concentration

shown for various ratios of blood to anticoagulant created upon collection;

Fig. 3 is a contour plot graphically illustrating the effect of the ratio of blood citric acid concentration to blood total citrate concentration on the relative cell counts of platelets collected in a platelet-rich plasma product;

Fig. 4 is a contour plot graphically illustrating the effect of the ratio of blood citric acid concentration to blood total citrate concentration on the relative cell counts achieved in a platelet concentrate product;

Fig. 5 is a contour plot graphically illustrating the effect of the ratio of blood citric acid concentration to blood total citrate concentration on the morphology of platelet cells collected in a platelet-rich plasma fraction;

Fig. 6 is a contour plot graphically illustrating the effect of whole blood pH as a function of total blood citrate on relative cell counts collected in a platelet-rich plasma product;

Fig. 7 is a contour plot graphically illustrating the effect of whole blood pH as a function of total citrate concentration on the relative cell counts in a platelet concentrate product; and

Fig. 8 is a contour plot graphically illustrating the effect of whole blood pH as a function of total citrate concentration on cell morphology collected in a platelet product.

Detailed Description of the Presently Preferred Embodiments

In accordance with the present invention, improved human blood platelet products may be prepared from whole blood in a collection procedure wherein whole blood is mixed with an anticoagulant formulation carefully designed to provide a relatively low amount of total citrate concentration, a relatively low blood pH level, and which is also added at a low anticoagulant to blood ratio, to provide improved platelet-rich plasma and platelet concentrate blood products obtained in better yield and having better overall cell morphologies on storage.

In accordance with this invention, major indicators as to the success of an apheresis procedure such as total cell counts and morphology are improved by carefully selecting the total citrate concentration, the acid concentration present, the blood pH and the ratio of blood to anticoagulant.

In accordance with the present invention, improved anticoagulant formulations are provided including citric acid and sodium citrate wherein the ratio of citric acid to the total citrate present is greater than about 30% up to and including 100%. When calculated on the basis of blood dilution, citric acid concentrations of about 7.0 mM and higher provided good results.

In accordance with this invention, improved platelet products are produced using the anticoagulant formulations of this invention, when the anticoagulants are mixed with collected blood so that the mixture contains a citric acid concentration of greater than about 5.0 mM, a total citrate concentration of less than about 20 mM, and an initial pH of less than about 6.75. Typically sufficient anticoagulant in solution is mixed with blood being collected to chelate calcium ions in the blood and prevent activation of the clotting mechanism.

In accordance with the present invention, the level of anticoagulant to blood ratio may be from about 1:6 to about 1:14 with good results being obtained without the need to increase the anticoagulant ratio.

The present invention is based on the unexpected discovery that collecting platelets at lower pH using higher citric acid ratios and lower ratios of total citrate provides platelet concentrates having increased cell numbers and improved cell morphology.

Other advantages provided by the present invention will be apparent from the following Examples.

EXAMPLES 1-19

In the following examples, blood was collected into various formulations of anticoagulating agent. The anticoagulated whole blood mixtures were processed first to form a platelet-rich plasma product (PRP) and then a final platelet concentrate (PC) was prepared using conventional centrifugal techniques and equipment. Each of these platelet-rich products PRP and PCs were then examined to determine the optimum formulation for the anticoagulant in terms of cell counts per milliliter (yields) and cell morphology (quality). In addition, the adequacy of the anticoagulation was assessed by determination of plasma fibrino peptide-A (FPA) which is an early marker of coagulation activation. Moreover, a number of other chemical/physical indexes were measured to assist in the overall evaluation of the product characteristics.

In the following experiments, data were collected to evaluate the effect on platelet yield and cell morphology provided by deliberately changing the citric acid/trisodium citrate ratios of a citrate buffer solution used to anticoagulate whole human blood. Citrate buffer solutions are anticoagulants whose base neutralizing strength is directly proportional to the amount of citric "acid" these solutions contain. Whole human blood is the

"base" neutralized by the acidic citrate solution, thus the resultant blood pH falls in proportion to the amount of citric "acid" added. Total "citrate" (citric acid + trisodium citrate) is important for anticoagulant properties, but only the acid portion lowers blood pH.

The formulations tested and the results obtained are shown generally in Table 1 as follows:

TABLE 1

EXAMPLE	Citric Acid (mM)	Sodium Citrate (mM)	Total Citrate (mM)	Blood Citric Acid (mM)	Blood Sodium Citrate (mM)	Blood Total Citrate (mM)	Actual Whole Blood pH	NaCl (mg/dL)	Soln Osmolarity (OSM)
1	0.670	26.830	27.500	0.109	4.368	4.477	7.264	650.0	275
2	0.670	68.080	68.750	0.109	11.083	11.192	7.245	314.1	283
3	0.670	109.330	110.000	0.109	17.798	17.907	7.221	0.0	294
4	11.700	15.800	27.500	1.905	2.572	4.477	7.096	699.8	276
5	11.700	43.300	55.000	1.905	7.049	8.953	7.093	472.7	280
6	11.700	70.800	82.500	1.905	11.526	13.430	7.075	251.9	282
7	19.439	90.561	110.000	3.164	14.742	17.907	6.972	56.0	287
8	27.500	0.000	27.500	4.477	0.000	4.477	6.868	817.9	269
9	29.181	25.819	55.000	4.750	4.203	8.953	6.833	562.9	281
10	29.181	53.319	82.500	4.750	8.680	13.430	6.836	317.2	278
11	34.350	75.650	110.000	5.592	12.315	17.907	6.739	199.5	310
12	43.463	0.000	43.463	7.075	0.000	7.075	6.672	766.3	280
13	43.463	33.537	77.000	7.075	5.459	12.535	6.669	515.0	304
14	43.463	66.537	110.000	7.075	10.832	17.907	6.654	267.7	318
15	0.670	26.830	27.500	0.109	4.368	4.477	7.264	650.0	275

TABLE 1 - Continued

EXAMPLE	Solution pH	Actual Whole Blood pH	pCO_2	pO_2	Morph. Score (Morps cor)	Cell Count in PRP	Rel. Cell Count in PRP	Cell Count PC	Rel. Cell Count PC	HCT
1	6.61	7.268	51.7	26	0	2.2E+08	0.73	55230000	0.09	36
2	7.08	7.245	52.6	29	0	2.1E+08	0.72	1.8E+08	0.29	35
3	7.32	7.221	54.4	26	155	2.3E+08	0.78	4.7E+08	0.77	36
4	4.51	7.098	68.7	32	20	2.0E+08	0.69	51229000	0.08	36
5	5.41	7.093	69.5	29	185	2.4E+08	0.82	4.8E+08	0.80	36
6	5.74	7.075	70.9	32	135	2.1E+08	0.72	2.5E+08	0.41	37
7	5.63	6.970	85.2	31	185	2.3E+08	0.79	5.5E+08	0.90	36
8	2.29	6.868	100.2	42	0	2.3E+08	0.78	28825000	0.05	37
9	4.17	6.833	106.0	38	90	2.4E+08	0.82	4.7E+08	0.77	38
10	4.88	6.836	105.3	37	185	2.5E+08	0.84	3.5E+08	0.57	38
11	5.07	6.739	119.4	41	265	2.5E+08	0.85	5.7E+08	0.94	37
12	2.23	6.672	136.1	44	265	2.3E+08	0.79	4.9E+08	0.80	39
13	4.03	6.666	135.7	41	265	2.9E+08	0.97	4.5E+08	0.74	39
14	4.73	6.654	135.9	41	235	3.0E+08	1.00	5.3E+08	0.87	38
15	6.61	7.260	51.5	28	20	1.9E+08	0.65	88400000	0.15	36

TABLE 1 - Continued

EXAMPLE	Citric Acid (mM)	Sodium Citrate (mM)	Total Citrate (mM)	Blood Citric Acid (mM)	Blood Sodium Citrate (mM)	Blood Total Citrate (mM)	Actual Whole Blood pH	NaCl (mg/dL)	Soln. Osmol. (OSM)
16	0.670	68.080	68.750	0.109	11.083	11.192	7.245	314.1	283
17	11.700	15.800	27.500	1.905	2.572	4.477	7.096	699.8	276
18	19.439	90.561	110.000	3.164	14.742	17.907	6.972	56.0	287
19	43.463	33.537	77.3000	7.075	5.459	12.535	6.669	515.0	304
ACD 6.7	38.073	74.812	112.886	5.711	11.222	16.933	6.767		
8				4.759	9.352	14.111	6.847*		
9				4.230	8.312	12.543	6.892*		
10				3.807	7.481	11.289	6.928*		
11				3.461	6.801	10.262	6.957*		
12				3.173	6.234	9.407	6.982*		
13				2.929	5.755	8.684	7.003*		
14				2.720	5.344	8.063	7.020*		
CPD 7.1	15.563	69.434	104.997	2.179	12.521	14.700	7.066*		
8				1.945	11.179	13.125	7.086*		
9				1.729	9.937	11.666	7.104*		
10				1.556	8.943	10.500	7.119*		
11				1.415	8.130	9.545	7.131*		
12				1.297	7.453	8.750	7.141*		
13				1.197	6.880	8.077	7.149*		
14				1.112	6.388	7.500	7.157*		

* = Predicted pH

TABLE 1 - Continued

EXAMPLE	Solution pH	Actual Whole Blood pH	pCO_2	pO_2	Morph. Score (Morps cor)	Cell Count in PRP	Rel. Cell Count in PRP	Cell Count PC	Rel. Cell Count PC	HCT
16	7.08	7.244	52.9	27	50	2.1E+08	0.70	2.5E+08	0.41	35
17	4.51	7.093	69.3	33	10	1.9E+08	0.65	68289000	0.11	37
18	5.63	6.973	85.0	28	195	2.3E+08	0.78	6.1E+08	1.00	36
19	4.03	6.672	137.7	40	235	2.4E+08	0.80	4.9E+08	0.80	39

Regression Output:

Constant:	7.250857
Std. Err of Y Est.	0.017085
R Squared	0.994573
No. of Observations	19
Degrees of Freedom	17
X Coefficient(s)	-0.08476
Std. Err of Coef.	0.001518

As shown in Table 1, the experimental solutions contain a widely varied amount of "acid" concentrations of from about 0.67 mM to 43.46 mM combined with various concentrations of trisodium citrate from about 26.8 mM to 109.3 mM, providing total citrate concentrations of from about 27.5 mM to 110.0 mM. A total of 19 sample runs were made using the 14 distinct anticoagulant compositions of citric acids/trisodium citrate ratios with five trial replicates. Table 1 also contains information on the amount of sodium chloride (NaCl) added to each solution to insure isotonicity, as well as calculated resultant blood concentration of the citrate species using the tested ratio of blood/experimental solution of 6/1. Because of the neutralizing aspect of the addition, no actual citric acid exists in the final collected blood. However, the pH of the collected blood is reduced from a normal level of about 7.4 to the lower level shown in Table 1 under the heading actual WB pH. The right-hand portion of Table 1 provides data on each sample including specific values of resultant experimental solution osmolarity (OSM) and pH and then gives the resultant whole blood pH, pCO_2, pO_2, hematocrit (HCT).

In addition, isolated platelet characteristics including morphology (Morpscor 0-400) and platelet cell counts in the platelet-rich plasma (PRP) and platelet concentrate (PC) are also reported in Table 1. A "relative" cell count in both PRP and PC was calculated by dividing each specific result by the maximum results, giving the proportion for each result when compared to the maximum or best result. This was done because the total amount of whole blood used in each sample was only a fraction of a total normal blood unit and thus could not be processed in a standard fashion, so the proportion process standardizes the data.

Fig. 1 provides a graphic display of the data presented in Table 1 showing the blood citric acid concentration on the y-axis and the blood total citrate concentration on the x-axis. Superimposed on this graph are calculated concentrations of the above x and y values for standard ACD (open circles) and CPD (asterisks) standard anticoagulants at blood/anticoagulant ratios of approximately 7/1 to 14/1. This was done to illustrate how the new and improved anticoagulants of the present invention, at the 6/1 ratio used, compare to various standard ratios currently used in the apheresis field for ACD anticoagulants. CPD values are also shown for comparative purposes and provide a good illustration of anticoagulants containing less citric acid as compared to ACD. As is shown in Table 1 and Fig. 1, the new and improved anticoagulant composition of this invention encompass and extend potential usable anticoagulant products over standard ACD types.

Fig. 2 provides a graphical illustration of the pH of the resultant anticoagulated whole blood on the y-axis with the total blood citrate concentration shown on the x-axis. Again, the straight lines describing the result obtained with ACD and CPD standard anticoagulants are shown for comparison purposes. As shown in Fig. 2, blood pH of the experimental systems varied over a wide range of from about 6.0 to about 7.25, encompassing the standard anticoagulants under normal use.

An important and substantive point revealed by these data is that standard ACD anticoagulants employed under apheresis conditions are pushed to a ratio of 14:1 whole blood:anticoagulant levels to prevent donor paresthesia. In accordance with the present invention, the new and improved anticoagulant composition when used at a 6:1 whole blood:anticoagulant ratio and when citrate concentration was reduced provided equivalent and less

citrate than standard anticoagulants delivered at much lower ratios, even at a 14:1 whole blood:ACD ratio.

The surprising results of these experiments are further illustrated in the contour plot shown in Figs. 6-8. The surfaces described in these plots were obtained by a multivariate regression analysis using an ECHIP computer program on the data of Table 1. Fig. 6 shows the relative count of platelet cells obtained in the PRP. Fig. 7 shows the relative count of platelet cells in the PC. Fig. 8 shows the morphology of the collected platelet cells employing the Morpscor of 0 to 400, wherein 400 equals perfect discoid cells. The higher score, the better the result in each of these indices. The surface graphs are displayed using alpha characters, A-D, which describe the numerical trend going from low (A) to high (D).

Figs. 6-7 describe "best yield" conditions. These occur when citrate levels are high and pH is low. This result was expected and it is likely due to a pH-associated deactivation of deleterious platelet activity. The unexpected result of improved morphology at low pH is illustrated by the contour plot shown in Fig. 8. As shown therein, good morphological characteristics were obtained at low pH over most of the evaluated citrate concentration range of from about 5.0 mM to 20 mM of citrate, with preferred C's occurring from about 7.0 mM to 18 mM citrate.

As indicated in Table 1, major indices such as cell count and morphology appear to change as a function of the specific anticoagulant formulation. A statistical analysis (multivariate regression of the data on blood citric acid concentration and total blood citrate concentration) was performed and the graphical representation of the resulting regression analysis for cell counts in PRP or PC and cell morphology are depicted in Figs. 3, 4 and 5, respectively.

The individual effect of total citrate concentration and acid concentration were statistically significant on cell counts in both the PRP (citrate, p is less than 0.05; acid, p is less than 0.001) and PC (citrate, p is less than 0.001; acid, p is less than 0.05) and on cell morphology (citrate, p is less than 0.01; acid, p is less than 0.001).

As the contour plots shown in Figs. 3, 4 and 5 indicate, cell counts are higher in both PRP or PC and morphologies of the PC cells are better as the citrate concentration increases or the acid component increases. Since the final blood pH is inversely correlated with the citric acid component of the anticoagulant ($R^2 = 0.995$, p is less than 0.0001), when cell counts (PRP or PC) and morphology are regressed on blood pH in total citrate, the contour plots depicted by Figs. 6, 7 and 8 result. Statistical significance remains the same as above.

A graphical example of the observed blood pH at various ratios of blood to ACD-A is shown in the attached Fig. 2. Also shown are comparable changes using "CPD" anticoagulant. Experimental anticoagulants (Exp.-Anti) formulated in accordance with this invention with a variation to add fourteen different citric acid/trisodium citrate combination levels were evaluated and these are illustrated in Fig. 1. The appropriate combination for both ACD-A or CPD-type anticoagulants is also shown in the same figure.

Fig. 2 shows the resultant blood pH achieved when the specific combination of each of the above experimental anticoagulants is added to blood at a blood to experimental anticoagulant ratio of approximately 6:1. As indicated by Fig. 2, precise blood pH can be achieved at various total citrate concentrations by simply increasing the amount of citric acid (and reducing trisodium citric as shown in Fig. 1).

The unexpected conclusion from this study is that platelet cells collected at low pH are morphologically better maintained than those collected at higher, more physiological pH. In addition, better yields (higher cell counts) can be achieved at lower pH. The above was also true of total citrate concentration and this result was expected.

The unexpectedly good morphology results are contrary to the literature on platelet cell storage. That literature indicates that as the pH falls during storage, the morphologies and other cell indexes of viability tend to decline. This study contradicts that finding in that the lower the pH on collection, the better the overall morphology of the platelets.

These data support the general use of a new anticoagulant compositions based on reduced citrate concentrations containing relatively high concentrations of the "acid" component. Specifically, Examples 11-14 produced results leading to high morphology scores coupled with good yields. These Examples included a range of total blood citrate of 7 mM to 18 mM and each used citric acid concentrations of 7 mM when calculated on the basis of blood dilution. At the lowest citrate concentration of 7 mM this is equivalent to standard ACD delivered at high (greater than 14/1) blood/anticoagulant ratios. Accordingly, improved anticoagulant formulations are provided herein as compared to standard ACD and CPD types.

In accordance with this invention, any and all means available to those skilled in the art of formulating specific anticoagulant formulations may be used to enhance the combined effects of total citrate concentration and citric acid concentration to maintain a low blood pH while reducing (or raising) the total citrate concentration. Various mechanical means known to those skilled in the art may be used to separately meter in the appropriate

quantities of the acid or salt of the citrate to formulate a final mixed anticoagulated blood product having the appropriate pH and ratios of anticoagulant to provide the improved platelet storage described herein.

Although the present invention has been described with reference to certain preferred embodiments, modifications or changes may be made therein by those skilled in this art without departing from the scope or spirit of the invention, as defined in the appended claims.

WE CLAIM:

1. A primary anticoagulant preparation for addition to a human blood sample unit intended for separation into a human blood platelet preparation comprising: a calcium ion chelating agent-type anticoagulant including citric acid and sodium citrate wherein the ratio of citric acid to total citrate is greater than about 30% by equivalent weight, said primary anticoagulant being added to a blood donation unit such that upon collection of the unit of blood, a resulting collected blood/anticoagulant mixture contains a citric acid concentration of greater than about 5.0 mM, a total citrate concentration of less than about 20 mM, and an initial pH of less than about 6.75.

2. A primary anticoagulant preparation as in Claim 1 wherein the calcium ion chelating agent-type anticoagulant is ACD-A.

3. A primary anticoagulant preparation as in Claim 1 wherein the calcium ion chelating agent-type anticoagulant is CPD.

4. A primary anticoagulant preparation as in Claim 1 wherein the calcium ion chelating agent-type anticoagulant includes citric acid and trisodium citrate.

5. A primary anticoagulant preparation as in Claim 1 wherein the total citrate concentration is between about 5.0 mM to about 20 mM of citrate.

6. A primary anticoagulant preparation as in Claim 1 wherein the total citrate concentration is between from about 7.0 mM to about 18 mM citrate.

7. A primary anticoagulant preparation as in Claim 1 wherein the citric acid concentration is greater than or equal to about 7.0 mM.

8. A primary anticoagulant preparation as in Claim 1 wherein the level of anticoagulant to whole blood in the resulting mixture is from about 1:6 to about 1:14 by volume.

9. An intermediate blood product useful for making improved platelet preparations on further processing, the intermediate blood product comprising:

an admixture of freshly collected human blood and an anticoagulant composition including citric acid and sodium-citrate, the admixture having a citric acid concentration of greater than about 5.0 mM, a total citrate concentration of less than about 20.0 mM and an pH of less than about 6.75.

10. A method of collecting human blood for preparing improved platelet preparations therefrom comprising the steps of:

introducing an anticoagulant preparation to a human blood sample being collected in a collection set adjacent a phlebotomy needle, the anticoagulant preparation being a solution including citric acid and sodium citrate wherein the ratio of citric acid to total citrate is greater than about 30% by equivalent weight, the anticoagulant being introduced at rate sufficient to provide a resulting collected blood anticoagulant mixture containing a citric acid concentration of greater than about 50 mM, a total citrate concentration of less than about 20.0 mM and an initial pH of less than about 6.75.

11. A method as in Claim 10 wherein the anticoagulant is introduced at a rate sufficient to provide a resulting collected blood/anticoagulant mixture having a citric acid concentration of greater than about 5.0 mM, a total citrate concentration of less than about 20.0 mM and an initial pH of less than about 6.75.

12. A method as in Claim 10 wherein the anticoagulant is introduced at a rate sufficient to provide a resulting collected blood/anticoagulant concentration of greater than or equal to about 7.0 mM and a total citrate concentration of about 7.0 mM to about 18.0 mM.

13. A method as defined in Claim 10 wherein the anticoagulant is introduced at a rate of whole blood:anticoagulant preparation of from about 6:1 to about 14:1.

14. A method as in Claim 10 wherein the anticoagulant preparation is introduced through a tubing junction provided in the collection set.

15. A method as in Claim 10 wherein the anticoagulant preparation is introduced by flowing it into collection tubing provided in the collection set.

16. A method as in Claim 10 wherein the anticoagulant preparation is introduced by pumping it into collection tubing provided in the collection set.

17. A method as in Claim 10 further comprising the step of: subjecting the resulting collected blood/anticoagulant mixture to blood separation procedures to provide a platelet product selected from the group consisting of platelet-rich plasma products and platelet-concentrate products.

18. A method as in Claim 10 further comprising the step of subjecting the resulting collected blood/anticoagulant mixture to automated blood separation procedures to provide a platelet concentrate product.

19. A method as in Claim 10 further comprising the step of subjecting the resulting collected blood/anticoagulant mixture to centrifugation separation processing to provide a platelet product selected from platelet-rich plasma and platelet-concentrate products.

20. A method of human blood platelet preparation to improve platelet storage comprising:

collecting whole blood into a primary anticoagulant formulation to form an anticoagulated blood mixture, the anticoagulant preparation including citric acid and trisodium citrate and the ratio of citric acid to total citrate being greater than about 30%, the anticoagulated blood mixture having a citric acid concentration of greater than about 5.0 mM, a total citrate concentration of less than about 20 mM, and an initial pH of less than about 6.75.

FIG. 1

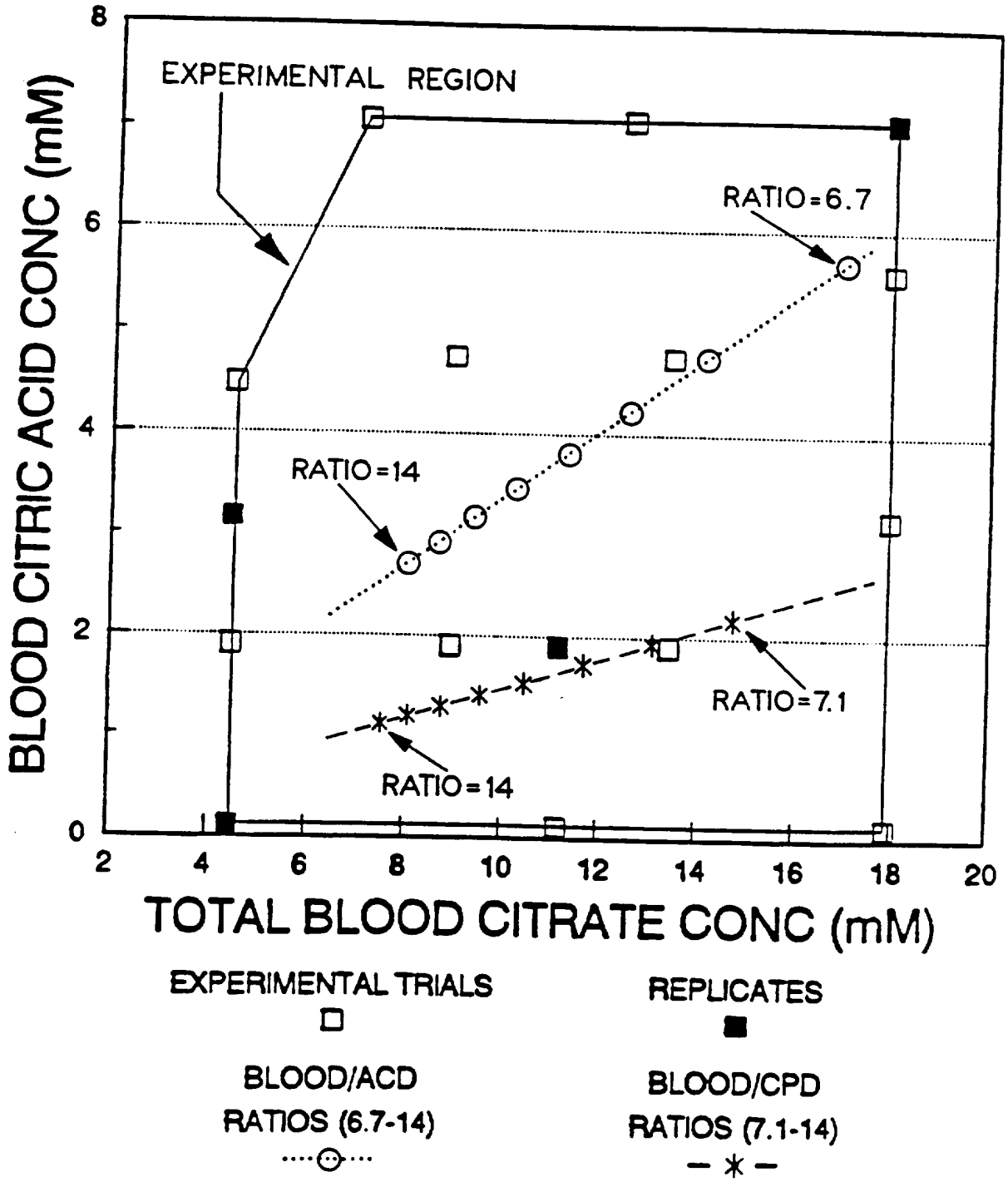

BLOOD CITRIC ACID CONC (mM)
TOTAL BLOOD CITRATE CONC (mM)
EXPERIMENTAL REGION
RATIO=6.7
RATIO=14
RATIO=7.1
RATIO=14
0
2
4
6
8
2
4
6
8
10
12
14
16
18
20
EXPERIMENTAL TRIALS
REPLICATES
BLOOD/ACD
RATIOS (6.7-14)
BLOOD/CPD
RATIOS (7.1-14)

FIG. 2

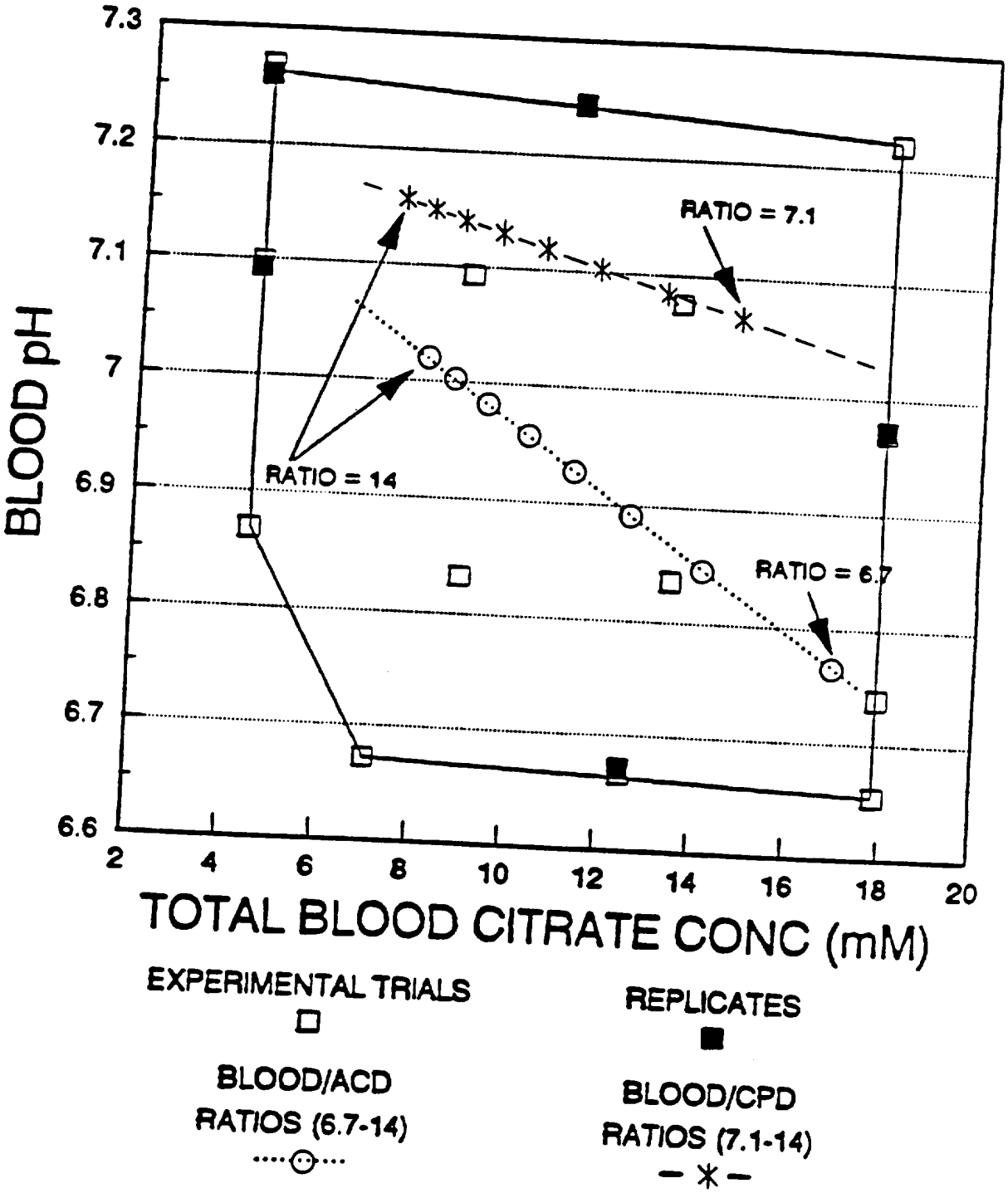

7.3
7.2
7.1
7
6.9
6.8
6.7
6.6
BLOOD pH
RATIO = 7.1
RATIO = 14
RATIO = 6.7
2
4
6
8
10
12
14
16
18
20
TOTAL BLOOD CITRATE CONC (mM)
EXPERIMENTAL TRIALS
REPLICATES
BLOOD/ACD
RATIOS (6.7-14)
BLOOD/CPD
RATIOS (7.1-14)

FIG. 3

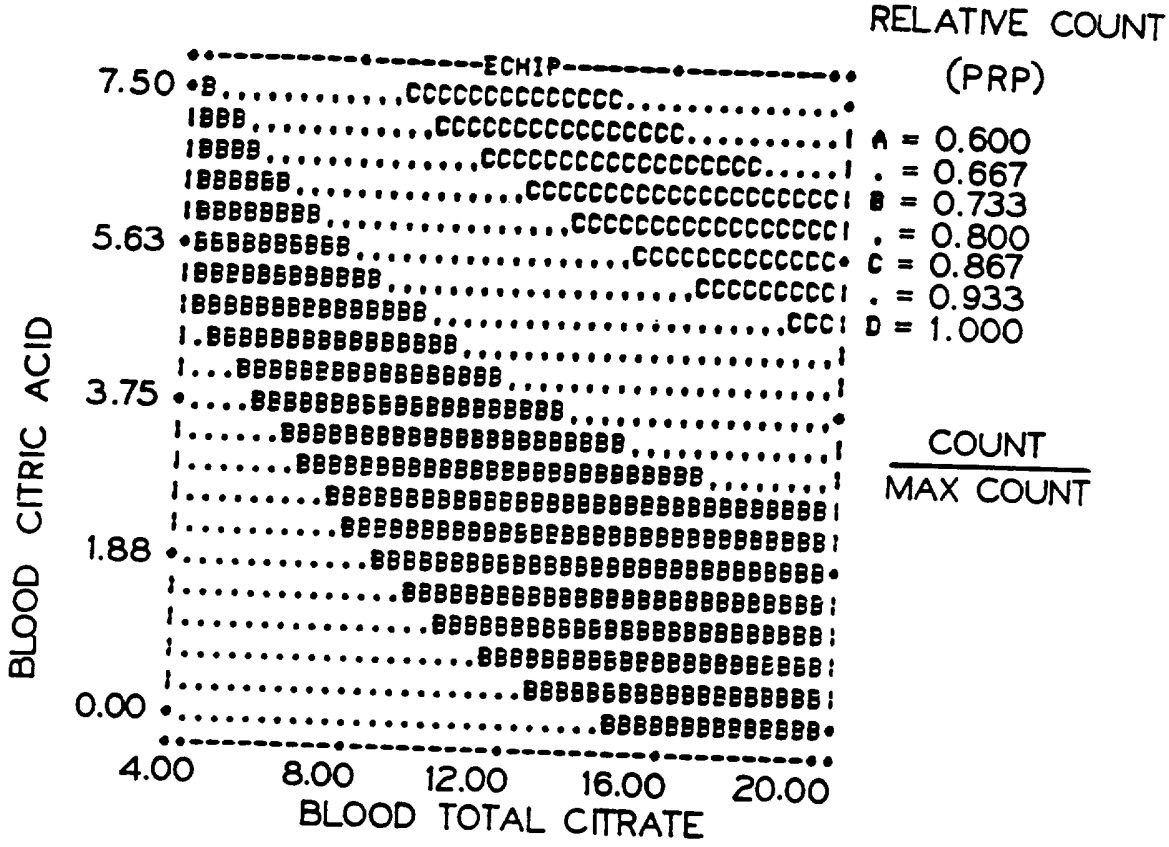

RELATIVE COUNT
(PRP)
ECHIP
7.50
5.63
3.75
1.88
0.00
A = 0.600
. = 0.667
B = 0.733
. = 0.800
C = 0.867
. = 0.933
D = 1.000
COUNT
MAX COUNT
BLOOD CITRIC ACID
4.00
8.00
12.00
16.00
20.00
BLOOD TOTAL CITRATE

FIG. 4

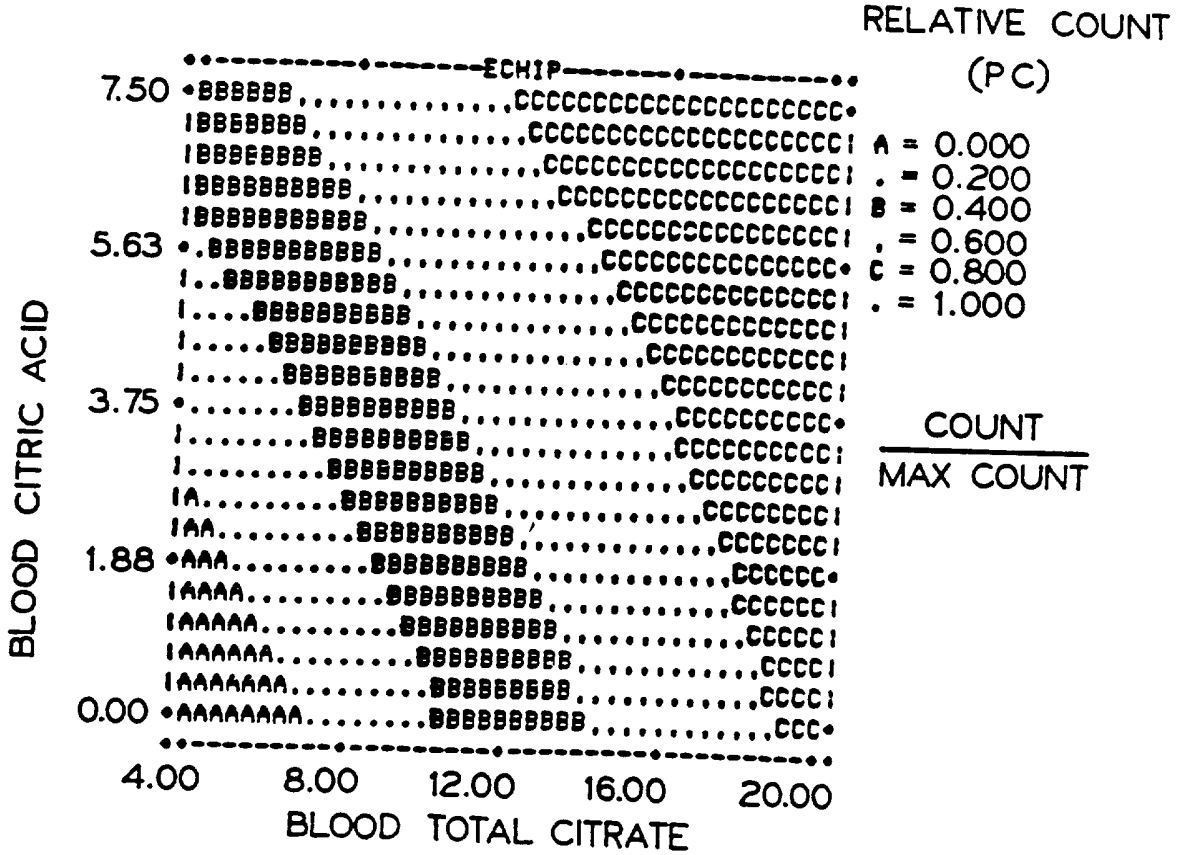

RELATIVE COUNT
(PC)
ECHIP
7.50
5.63
3.75
1.88
0.00
A = 0.000
. = 0.200
B = 0.400
. = 0.600
C = 0.800
. = 1.000
COUNT
MAX COUNT
BLOOD CITRIC ACID
4.00
8.00
12.00
16.00
20.00
BLOOD TOTAL CITRATE

FIG. 5

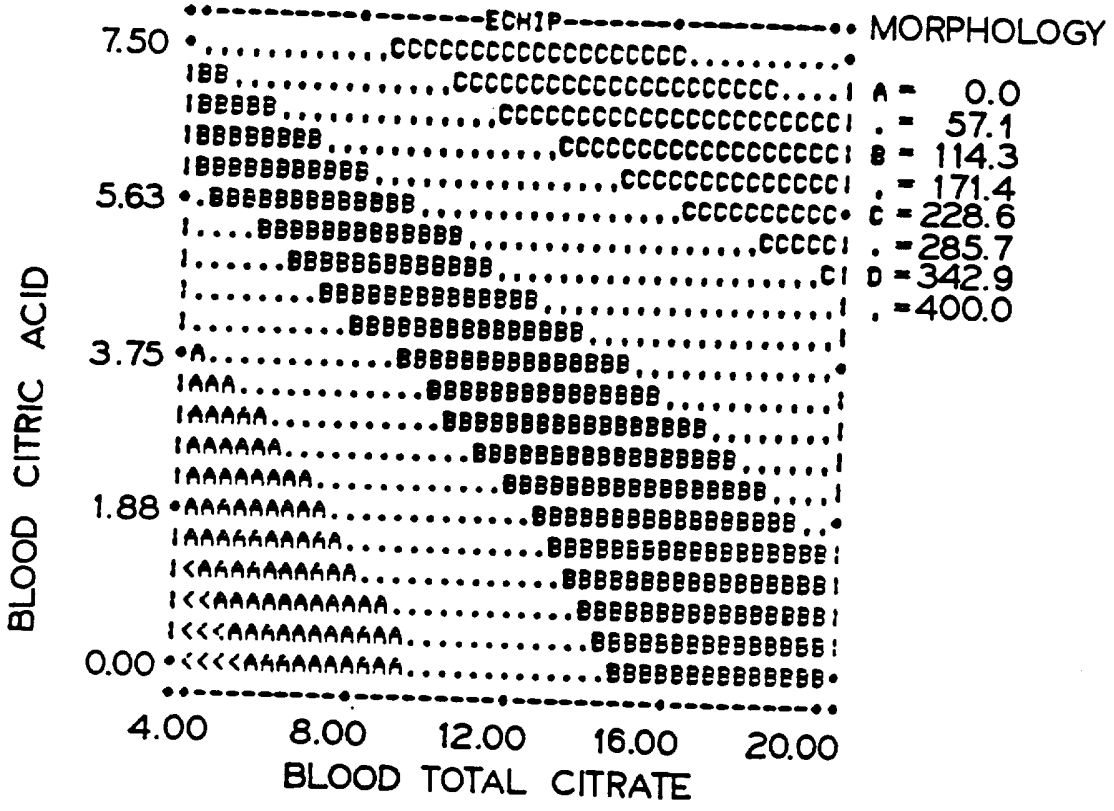

ECHIP
MORPHOLOGY
A = 0.0
. = 57.1
B = 114.3
. = 171.4
C = 228.6
. = 285.7
D = 342.9
. = 400.0
7.50
5.63
3.75
1.88
0.00
BLOOD CITRIC ACID
4.00
8.00
12.00
16.00
20.00
BLOOD TOTAL CITRATE

FIG. 6

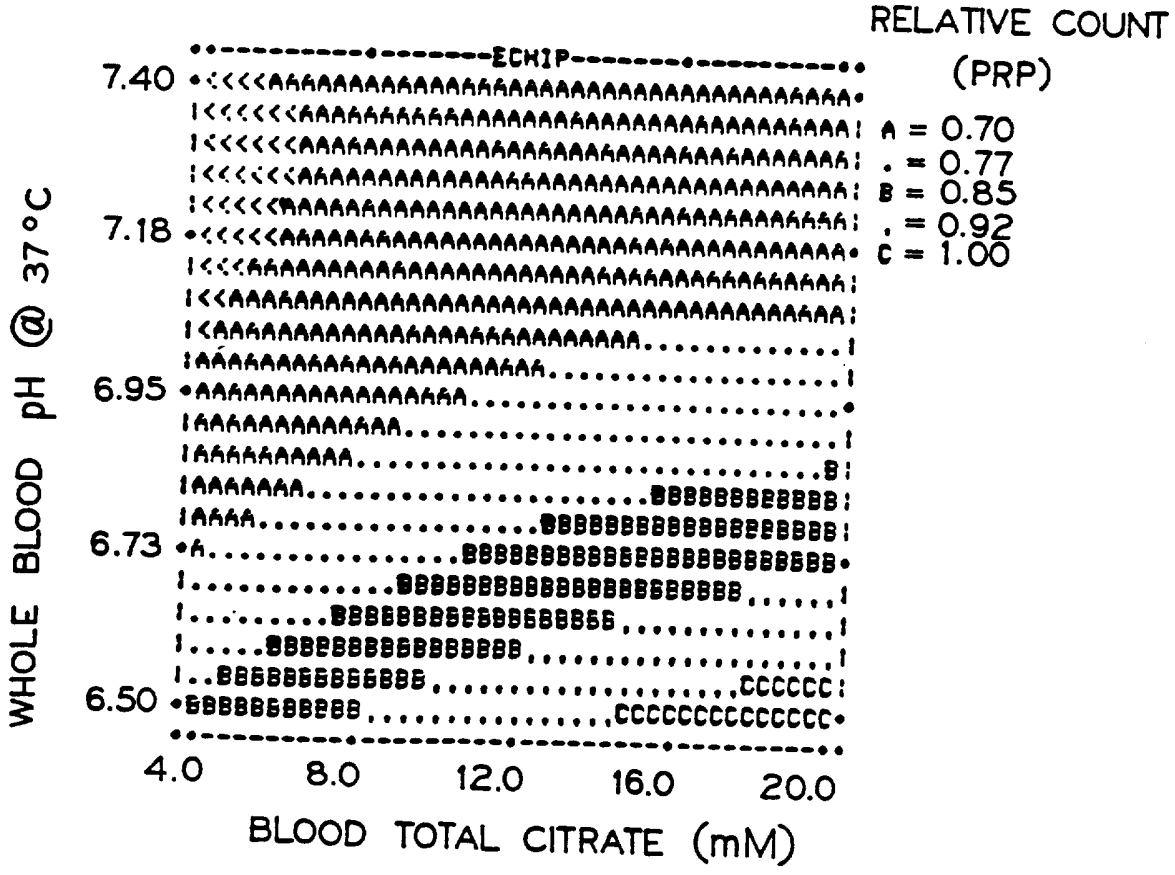

ECHIP
RELATIVE COUNT
(PRP)
A = 0.70
. = 0.77
B = 0.85
. = 0.92
C = 1.00
7.40
7.18
6.95
6.73
6.50
WHOLE BLOOD pH @ 37 °C
4.0
8.0
12.0
16.0
20.0
BLOOD TOTAL CITRATE (mM)

FIG. 7

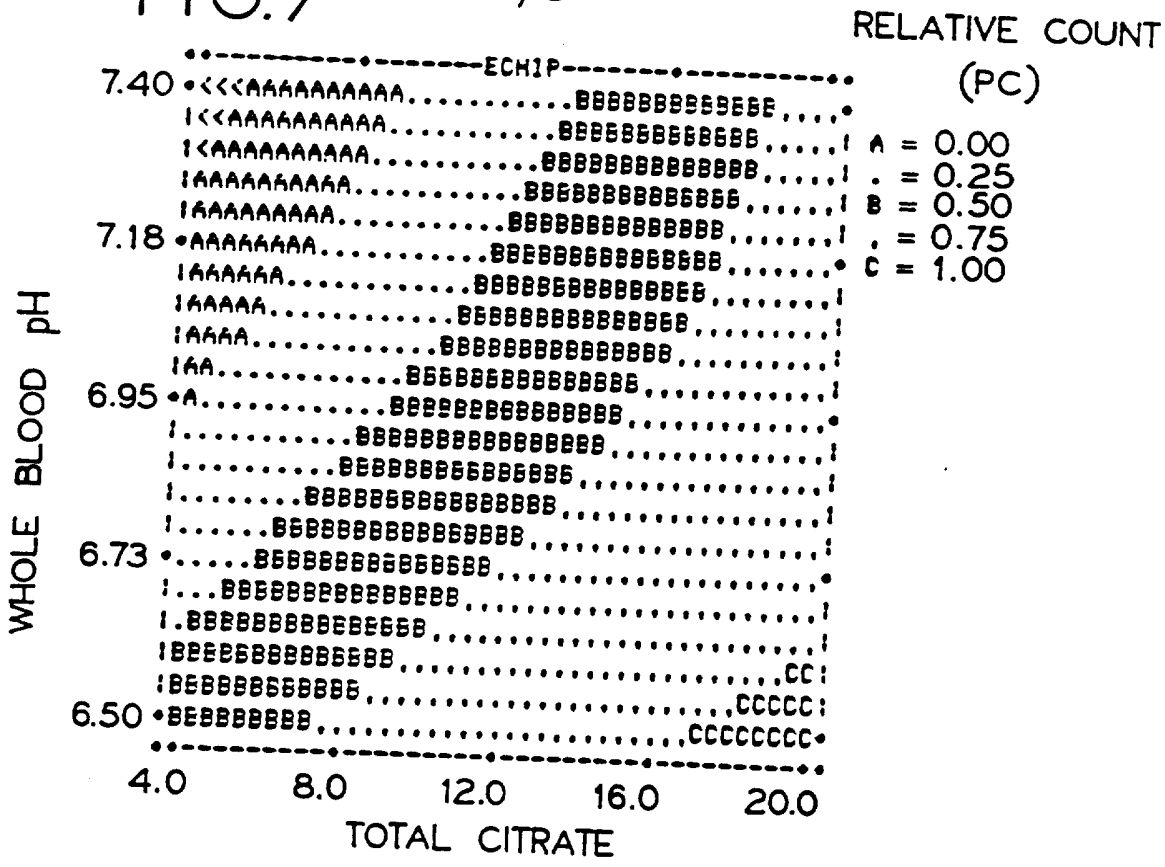
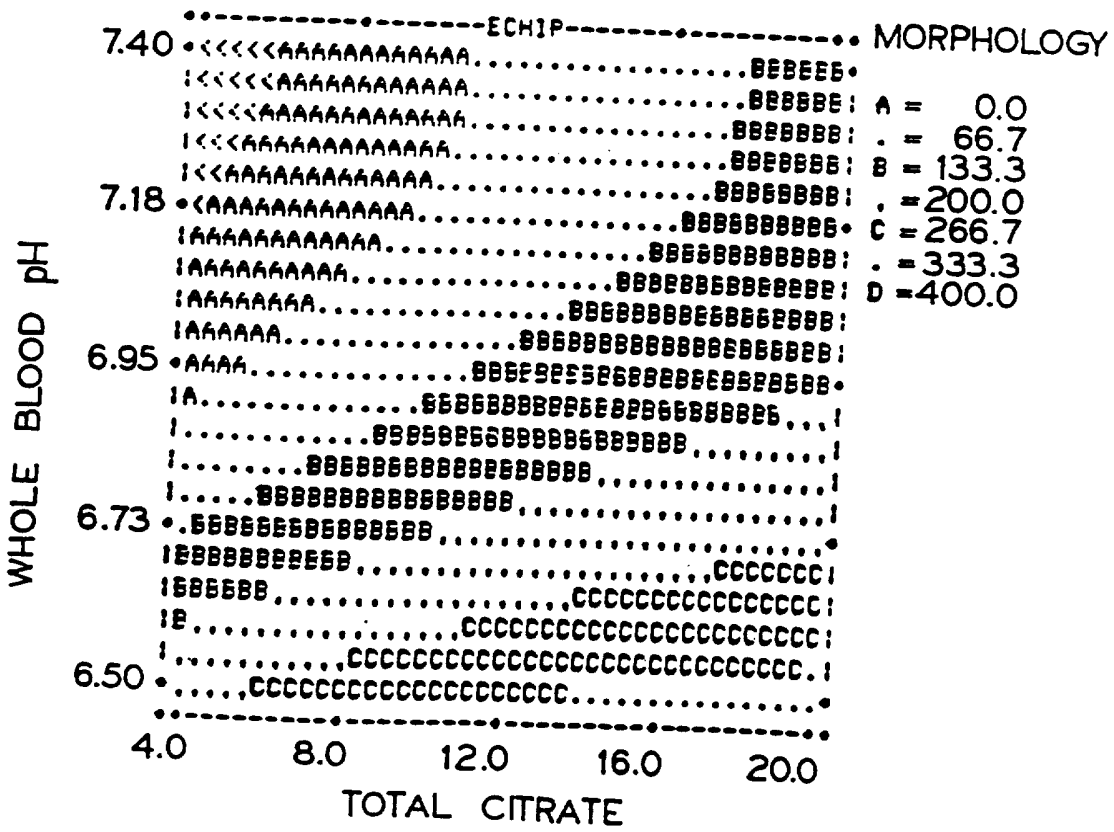

RELATIVE COUNT
(PC)
A = 0.00
. = 0.25
B = 0.50
, = 0.75
C = 1.00
WHOLE BLOOD pH
7.40
7.18
6.95
6.73
6.50
4.0
8.0
12.0
16.0
20.0
TOTAL CITRATE

FIG. 8


MORPHOLOGY
A = 0.0
. = 66.7
B = 133.3
, = 200.0
C = 266.7
. = 333.3
D = 400.0
WHOLE BLOOD pH
7.40
7.18
6.95
6.73
6.50
4.0
8.0
12.0
16.0
20.0
TOTAL CITRATE

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/10705

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A01N 1/02; A61K 35/14
US CL :435/2; 424/529
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/2; 424/529

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, CASPlus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 4,961,928 (HOLME ET AL.) 09 October 1990, see abstract.	1-8
X	US, A, 4,992,363 (MURPHY) 12 February 1991, see column 9, line 64.	1-8
X	Sigma Catalogue, published 1992, page 263, see entry 85-4.	1-9
X	Vox Sang, Volume 52, issued 1987, Prowse et al., "Studies on the Procurement of Blood Coagulation Factor VIII in vitro Studies on Blood Components Prepared in Half-Strength Citrate Anticoagulant", pages 257-264, see table 1.	1-20

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"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
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"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
"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)	"&"	document member of the same patent family
"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		

Date of the actual completion of the international search 29 OCTOBER 1995	Date of mailing of the international search report 27 DEC 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Sandra Saucier Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/10705

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Vox Sang, Volume 55, issued 1988, Griffin et al., "Studies on the Procurement of Blood Coagulation Factor VIII", pages 9-13.	1-20
A	Transfusion, Volume 22, Number 3, issued 1982, Valeri et al., "Viability and function of red blood cell concentrates stored at 4°C for 35 days in CPDA-1, CPDA-2 or CPDA-3", pages 210-216.	1-20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/10705

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/10705

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-8 and 10-20, drawn to a first anticoagulant composition and a method of using the composition.
Group II, claim 9, drawn to a second composition comprising an anticoagulant composition and blood.

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The claims of these two groups are drawn to distinct inventions which are not linked so as to form a single inventive concept because only one product is permitted in a category. PCT Rules 13.1 and Rule 13.2 do not permit multiple products within the definition of a single inventive concept. Accordingly, the claims are not so linked by a special technical feature with the meaning of PCT Rule 13.2 so as to form a single inventive concept.