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METHOD OF SEPARATING WHOLE BLOOD

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2 Claims

ABSTRACT OF THE DISCLOSURE

A solid granular material, e.g. polystyrene beads, having a coating of anti-coagulant, e.g., heparin, and having a specific gravity intermediate that of plasma and blood which can be used by adding to blood in vitro to separate plasma from the remainder of the blood. When the beads are added to whole blood, a separation occurs by which the plasma portion of the blood forms, by virtue of the presence of the anti-coagulant, in the resulting layer above the beads while the remaining portions of the blood form in a layer below the beads so that the plasma can easily be separated. In a preferred form, the material used to coat the beads also includes a wetting agent.

This invention relates to the separation of whole blood into plasma and red cell components. More particularly, this invention relates to such separation of blood where-in coagulation of blood by the blood clotting mechanisms is stopped so that whole plasma is recovered which contains an amount of prothrombin and/or clotting agents, in the same ratio as such clotting agents are present in the total plasma portion of the whole blood. The term "whole plasma," as used herein, refers to plasma which contains clotting agents in an amount proportional to the ratio of clotting agents:plasma in the whole blood, and the present invention assures separation of such plasma without clotting of the blood.

It has recently become a common technique to separate serum from other components of whole blood by using a particulate material, e.g. polystyrene particles or pellets, having a specific gravity intermediate the specific gravity of blood serum or plasma and the red blood cells. Accordingly, a sample of blood is permitted to clot in vitro. A quantity of the particulate material is introduced into the sample and the mixture is stratified by centrifuging or the like. The serum forms a top layer above the particulate material, and the blood cells form a bottom layer below the particulate material. The particulate material is used in an amount sufficient to form a tight layer between the two separate components. The serum may then be readily recovered as the top layer.

The above procedure is effective in obtaining serum from whole blood. However, where it is desired to obtain plasma, which differs from serum by the presence of clotting agents, the procedure is not sufficiently precise to assure that the clotting agents:plasma ratio of the separated plasma is the same as the clotting agent:plasma ratio of the whole blood. Assuring such ratio is important for a number of reasons. For example, it is often necessary to estimate the prothrombin content of the blood sample from a patient in order to determine and control dosages of anti-coagulant drugs, such dicumerol (bis-hydroxycoumarin). In such cases it is, of course, necessary to assure that the plasma is really whole plasma.

The above technique for obtaining serum has also previously been used in attempts to obtain plasma, except that an anti-coagulant is added to the blood sample and the blood is not permitted to stand. However, some

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clotting still takes place, thereby upsetting the ratio of clotting agents in the plasma recovered.

It is a general object of this invention to produce or separate whole plasma from blood and particularly from the red blood cells, in a new and improved manner assuring an absence of blood coagulation.

It is further an object of this invention to recover whole plasma from whole blood, wherein the whole plasma contains the same proportion of anti-clot agents to plasma as is present in the whole blood.

Another object of this invention is to provide a new and useful composition which can be used in combination with polystyrene particles or other particulate material having a specific gravity intermediate that of the red cells and plasma for assuring separation of whole plasma.

Yet another object of this invention is to provide, as a novel structure, a particulate material having a specific gravity intermediate that of plasma and red cells and having a dried coating thereon containing an anti-coagulant, which structure is useful in separating whole plasma in accord with any of the foregoing objects.

A still further object of this invention is to provide a new and useful blood component separation system and method in which an anti-coagulant can be carried throughout a blood sample in vitro by a solid particulate material having a specific gravity intermediate that of the plasma and red cells and having the anti-coagulant carried as a coating on the particulate material.

Other objects may be apparent to those in the art from the disclosure herein.

It has now been found that the whole plasma component of whole blood can be precisely recovered from whole blood in vitro to provide a plasma component which contains the same ratio of clotting agents:plasma or clotting agents:serum as was present in the whole blood starting material. The present invention provides particulate material having a specific gravity intermediate that of the plasma and red cells, and the surface of the particulate material is coated with and carries an anti-coagulant. In the most advantageous form, the particulate material surface is wetted with a water soluble non-ionic wetting agent to increase its capacity for absorption and carrying of the anti-coagulant. Preferably, the anti-coagulant is present on the particulate material structure in a known amount based on the weight of the particulate material.

The particulate material can be any material which is insoluble in an aqueous medium and which is non-reactive or inert with respect to the blood, wetting agent or anti-coagulant. For example, the polystyrene particles or pellets used in the separation of serum from whole blood can be used herein. Other suitable and usable plastic, and even non-plastic, materials which have the proper specific gravity intermediate that of the plasma and red cells will be evident. Where the material has a good surface absorption capacity, the wetting agent may prove wholly unnecessary. However, it is preferred to use the wetting agent with plastic particles since such particles are often not capable of absorbing and holding the anti-coagulant on their surfaces in the absence of the wetting agent.

In forming the coated particles of this invention, the particulate material or particles are coated with the anti-coagulant, e.g. in admixture with a wetting agent or after coating of the particles with a wetting agent, and the coated structure is then dried to provide a dry coating. The amount or concentration of wetting agent and amount or concentration of anti-coagulant used during the coating step will depend to some extent on the surface area and absorption characteristics of the particle being coated, as is well known in the absorption of liquids on solid

surfaces. For a particular size and nature of solid particle, the amount absorbed or absorbable can be readily determined by simple experimentation, i.e. by performing a test run of the coating method to determine how much liquid is actually absorbed per unit of particulate material.

The present method may advantageously be carried out by supporting the particles on a foraminous structure such as a screen and pouring the wetting agent and anti-coagulant solution or solutions over the particles. Preferably, in runs standardizing the method, the screen should be pre-treated with additional coating materials so that the amount absorbed by the screen does not affect the calculation of the amount absorbed by the particles and the amount, e.g. by weight, of particulate material used should be predetermined. Where the amount and concentration of solution poured over the particles is known and the solution draining from the screen is collected, the amount absorbed can readily be determined. Since the amount absorbed by the particles is known, the amount per unit weight of particles can be readily calculated. The particles are dried, e.g. by placing in a vacuum oven, after coating, so that the anti-coagulant does not flow from the particle surface. The particles can then be packaged and labeled with their concentration. If necessary or desired, prior to packaging, the particles from each run can be diluted with uncoated particles or particles carrying lesser amounts of anti-coagulant or can be concentrated with particles carrying greater amounts of anti-coagulant to provide a standardized mixture of particles in which the amount of anti-coagulant per unit weight of particle is precisely known.

The water soluble non-ionic wetting agents are preferably used in an aqueous solution containing a small amount, e.g. from .02 up to 20% and preferably .2 to 2% of the wetting agent. The wetting agents often can cause rupture of red blood cells, in whole blood or otherwise adversely affect the red blood cells, and therefore it is preferred that the amount of wetting agent be sufficient to wet the surface of the particulate material with only little excess, if any. Thus, where the surface of the particulate material is more highly absorptive or has a greater surface area, weaker solutions of wetting agent will be used while the stronger solutions will be used where surface area or absorptivity is low.

The anti-coagulant is used in a concentration sufficient to provide the proper amount of anti-coagulant on the particulate material surface for preventing coagulation of the blood. Thus, the amount of anti-coagulant per gram of particulate material will depend upon the amount of blood to which a given weight of particulate material is intended to be added. Anti-coagulant materials such as heparin are very expensive, so for sake of economy it is preferred that they not be used in excess above the amount necessary, although no excess appears to adversely affect the blood. Proper amounts of anti-coagulant for addition to blood are well known and sufficient amounts of anti-coagulant should be used to coat the particles to provide the proper amount in the blood during separation of plasma. The amount of anti-coagulant is usually sufficient to provide about 10 U.S.P. units of heparin per ml. of blood, although lesser amounts, e.g. down to 1 U.S.P. unit, can be used where lesser amounts of anti-coagulant activity are desired. Of course, excesses up to 100 U.S.P. units and higher can be used, but at a cost disadvantage. Usually from about one to about 1000 U.S.P. units of coagulant per gram of particulate material will be sufficient for any application.

Anti-coagulants are obtainable in prepared solutions, or concentrates of anti-coagulant can be diluted with water to prepare a solution of a known strength, e.g. usually in the range of 100-2000 U.S.P. units per ml. Stronger or weaker solutions can be prepared, and it may be desirable to do so in the present invention, especially with respect to stronger solutions, since many of the more

readily available usable particulate materials have relatively low surface areas per unit of weight, due to a large particle size.

The following specific examples are given in illustration of the present invention and are not intended to be limiting on the invention.

Example I

500 grams of polystyrene pellets having an average diameter of $\frac{1}{15}$ inch and an average length of $\frac{1}{16}$ inch are supported on a retaining screen which has been previously dipped in a 2% solution of liquid water soluble polyethylene glycol (averaging tetraethylene glycol) and thereafter dipped in a heparin solution containing 1000 U.S.P. units of heparin per ml. and dried. 250 mls. of the polyethylene glycol solution are mixed with 250 mls. of the heparin solution, and the resulting mixture is then poured over the polystyrene pellets. About 80% of the combined solution is recovered beneath the screen, indicating absorption of about 20%, or about 100 mls., by the pellets. The coated pellets are then quickly dried in a vacuum oven until the water has been evaporated therefrom, leaving a dry coating of the anti-coagulant.

Example II

The screen is prepared as above, and 250 mls. of the polyethylene glycol solution are poured over a fresh 500 grams of polystyrene pellets supported by the screen. The coated pellets are then vacuum oven-dried until water is evaporated having a waterless or dry coating of the polyethylene glycol. 250 mls. of the heparin solution are then poured over the pellets and the coated pellets are again dried by evaporation of water in the vacuum oven. The total amount of solution collected indicated that approximately the same amount of wetting agent and heparin was absorbed on the surfaces of the pellets.

In the above two examples, pellets were prepared having dried coatings containing 100 U.S.P. units of heparin per gram of pellets.

Example III

An anti-coagulant solution of 10% neutral sodium oxalate salt is prepared. 2% polyethylene glycol is added to the solution, and the procedure of Example I is repeated using the resulting solution. The resulting coated polystyrene pellet product has a dry coating which contains approximately 200 U.S.P. units of sodium oxalate per gram of material.

Example IV

2 grams of pellets prepared as in Example I with the dry coating containing 100 U.S.P. units of heparin per gram were added to 10 mls. of fresh whole blood in a test tube. The test tube was closed and shaken vigorously for a few seconds. The tube was then spun in a centrifuge until the red cells had settled to the bottom of the tube. The materials in the tube appeared in three strata, bottom red cell stratum, an intermediate stratum of the pellets and an upper stratum of plasma. The anti-coagulant had been desorbed from the pellets. The upper plasma layer was poured off from above the layer of pellets, the pellets forming a firmly packed layer and retaining the red blood cells within the test tube. When compared with an analysis of the blood, it was found that the plasma contained the same ratio of clotting agents, i.e. prothrombin and fibrinogen, per unit of plasma, as was present in the original whole blood per unit of plasma.

Although heparin and sodium oxalate have been used in the above examples as anti-coagulant materials, it is to be understood that any water soluble salt of oxalic acid or citric acid, including, but not limited to, the sodium, potassium and ammonium salts, may be used in lieu thereof. Sodium or other citrate or oxalate functions as an anti-coagulant by preventing the prothrombin from being converted to thrombin. Other useful anti-coagulants include hirudin and the chondroitin sulfuric acid anti-coagulants which contain chondroitin sulfuric acids or esters

thereof as active ingredients thereof. Among the latter anti-coagulants is heparin, which functions as an anti-coagulant by blocking the reaction of thrombin with fibrinogen, a reaction which would otherwise form fibrin to clot the blood.

The water soluble non-ionic wetting agents are well known, and any such wetting agents can be used. Particularly preferred are the water soluble polyhydroxy hydrocarbon and polyhydroxy ethers such as alkylene and polyalkylene glycols, e.g. ethylene glycol, propylene glycol, diethylene glycol, triethylene glycol, tetraethylene glycol, and other polyethylene glycols and polypropylene glycols, pentaerythritol, etc. Other such wetting agents include, but are not limited to water soluble alcohols such as methanol, ethanol and propanol, amides such as formamide and butyramide, ketones and aldehydes such as acetone, methylethylketone, acetaldehyde and butyraldehyde, ethers such as ethyl ether and methylethyl ether, and other water soluble organic compounds having at least one non-ionic polar group, and mixtures of such compounds. Where compounds herein are described as "water soluble," it is intended that the compounds be soluble in the amounts and under the conditions used.

The coated particles can be supplied in a glass container approximately of test tube dimensions such as the well known and available tapered bottom test tubes conventionally used in gravitational separations of blood samples. The container can have a sealed or stoppered top for preventing contamination. When it is desired to conduct a blood separation, the seal or stopper can be removed and the blood can be introduced and agitated in the presence of the coated particles and the mixture can be permitted or caused to separate according to specific gravities. Thus, although the present invention is described with respect to addition of the coated particles to the blood, it is fully intended that such addition includes the introduction of blood into a container which already contains the coated particles.

The foregoing detailed description is given for clear-

ness of understanding only and no unnecessary limitations are to be understood therefrom, as some modifications will be obvious to those skilled in the art.

I claim:

1. The method of separating plasma from whole blood which comprises bringing into contact with the blood a separating amount of inert particulate material having a specific gravity intermediate that of plasma and red cells and having a dry coating of an anti-coagulant amount of anti-coagulant on the surface thereof in an amount sufficient to form a barrier layer between the plasma and red cells, agitating the particles and whole blood, centrifuging the resulting mixture and recovering the plasma from above the particulate material.
2. The method of separating plasma from whole blood, which method comprises bringing into contact with the blood a separating amount of inert particulate material having a specific gravity intermediate that of plasma and red cells, said particulate material having a dry coating comprising a surface wetting amount of wetting agent and an anti-coagulant amount of an anti-coagulant, agitating the resulting mixture, centrifuging the resulting mixture, and recovering the stratified layer above said particulate material as whole plasma containing the blood clotting agents in an amount proportional to the ratio of clotting agents to the whole plasma in the whole blood.

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