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MONTMORILLONITE ADSORPTION OF FIBRIN FROM BOVINE BLOOD PLASMA

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The present invention relates to proteins. More in particular, the present invention relates to a method for producing stable proteins with a low tendency for coagulation, particularly, if subjected to heat.

It is known in the art to produce proteins, for example, from animal blood supplied by the slaughter houses by adding citrates, phosphates, and the like, stabilizing the same and separating the blood plasma from the blood corpuscles. A yellow-red colored liquid is thereby obtained consisting substantially of the dissolved proteins, red blood pigment and the dissolved salts.

This blood plasma is, however, not susceptible to useful application for pharmaceutical, cosmetic or alimentary products, for the blood plasma contains considerable impurities and, above all, has a percentage of fibrin. As a consequence, it still tends to coagulate thereby rendering a further use of the blood plasma rather difficult or even impossible.

While no satisfactory method has been found in the art to remove the impurities without damage to the blood plasma, an attempt has been made to provide a method for reducing the coagulability of the blood plasma for scientific purposes. However, these attempts have remained rather unsuccessful. It has been tried to treat the blood plasma with cation exchangers of the Na⁺ or H⁺ type. Where the blood plasma was treated with the Na cation exchangers a plasma was obtained containing only sodium and cations.

Where the blood plasma is treated with H cation exchangers a plasma was obtained in which the dissolved salts are converted and combined to the free acids in the plasma.

In both instances, a further treatment with anion exchangers has proved to be impossible as the proteins separated in the form of flakes immediately upon the anion exchangers coming into contact with the acid plasma. As a matter of fact, the proteins of the blood flocculate after a pH value of about 5 has been reached, and are not redissolved before the pH value has risen to 7 which value is necessarily reached.

Consequently, it was not possible to desalt the plasma, for example, by a subsequent treatment with cation and anion exchangers.

It is an object of the present invention to provide for a method of producing stable, non-gelatinizing proteins which makes it possible to remove the impurities therefrom.

It is another object of the present invention to provide for a method of producing stable proteins not coagulating under the influence of heat.

It is still another object of the present invention to provide a method for producing a protein which can safely be sterilized by heating.

It is a further object of the present invention to provide a method for producing a protein from animal blood which does not coagulate under the influence of heat and is tasteless, odorless, colorless and clear.

According to the present invention it has been unexpectedly found that the undesirable impurities can be removed from the plasma in a very simple manner by treating the same with montmorillonites. It has also been unexpectedly found that by such treatment, the proteins causing the coagulation can be removed.

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According to a preferred embodiment of the invention, the purifying effect can be further improved and the product is rendered tasteless and odorless by adding activated charcoal to the blood plasma after and/or before or simultaneously with adding the montmorillonites.

According to a further embodiment of the invention the plasma can be sterilized and its coagulability can be reduced by changing the salt equilibrium of the blood, thereby preventing coagulation of the latter when heated and sterilized.

According to the invention, the salt equilibrium is changed by slowly adding acidic or alkaline substances.

According to a preferred embodiment of the invention, the salt equilibrium of the plasma is changed by desalting the plasma by means of ion exchangers until the plasma has reached a small alkaline pH value or has reached the neutral point from alkaline pH values.

According to the invention, all undesirable impurities as well as the proteins causing coagulation are removed from the plasma by bringing the same into contact with the bleaching earth for a predetermined period, for example, 30 minutes, until the proteins causing coagulation are deposited on the bleaching earth, simultaneously taking along the impurities in the blood plasma. It has been found that the contacting period should be in a range of from 20 to 45 minutes. The amount of bleaching earth used depends on the composition of the blood which varies from case to case and must, therefore, be determined by a test. As a general rule, it will be sufficient to add about 5 percent by weight of bleaching earth to the plasma. The bleaching earth can be slightly acidic.

As a general rule, untreated, neutral, earth is best suited for the indicated purpose.

After the predetermined period, for example, 45 minutes, the bleaching earth together with the proteins deposited thereon and the impurities are separated from the plasma by filtering. As a result, a plasma is obtained which is a clear liquid, which has a slight viscosity, and which has lost its tendency to coagulate.

According to another, preferred embodiment of the invention, the purification of the plasma can be even further improved by treating the same with activated charcoal. This renders the product tasteless and odorless, which makes it particularly suitable for pharmaceutical and alimentary purposes. The charcoal can be added before or after the treatment with bleaching earth or simultaneously therewith.

If a particularly great degree of purity is called for, the plasma can be processed as follows:

After the aforescribed treatment with bleaching earth has been completed, a second treatment with bleaching earth is effected. For that purpose, acid earth has been particularly found of advantage. This earth is added, together with a predetermined amount of activated charcoal, and the entire mass is stirred for a predetermined period of time, for example, 15 minutes. Thereafter, the plasma is filtered so as to separate the bleaching earth as well as the charcoal and the impurities from the plasma. As a result, a clear plasma free from impurities is obtained which does not gelatinize.

The amount of charcoal and bleaching earth are determined by the degree of the desired purification but, as a general rule, will, together not exceed 5% by weight, of the plasma.

According to a further embodiment of the invention, a coagulation of the blood is prevented when heated, and a sterilization of the blood plasma by heating is thus made possible by changing the salt equilibrium of the blood plasma. This can be done by slowly adding acid, for example, hydrochloric acid, sulfuric acid, or citric acid. The acids are to be added very slowly and carefully, preferably drop by drop. For example, 50 cm.³ of

raw plasma having a pH value of 6.82 require the slow and drop by drop addition of 14.25 cm.³ n/2 HCl, until the plasma has reached a pH of 2.25, whereupon it can be safely boiled without flocculation of the protein.

According to a still further embodiment of the invention, the blood plasma is desalted with the aid of ion exchangers so that the plasma has a slight alkaline pH value.

According to the invention, the plasma can be desalted by first treating the same with anion exchangers. Thereby, a plasma with a strong alkaline reaction is obtained which plasma is then contacted with a cation exchanger binding the free cations, thereby producing a plasma which is substantially neutral and which does not have any tendency to flocculate.

However, a still better product is obtained if first the cations are removed from the plasma prior to treating the same with the anion exchangers, because in this case an acid plasma is obtained which is protected against decomposition, particularly, by bacteria, and can be preserved for several months.

According to the last-mentioned embodiment the purified blood plasma is treated with a strongly acid cation exchanger of the H⁺ type. Thereby the dissolved cations (Na, Ca, Li, Fe) are removed from the plasma and the equivalent amounts of the respective free acids are formed. Thus, the reaction of the plasma is a strongly acid one.

After this treatment with the cation exchanger the plasma has become largely insensitive to flocculation and can be heated to temperatures up to 100° C.

A small sample of this plasma must then be subjected to titration in order to ascertain how much of, for example, NaOH is sufficient in order to raise the pH value, which is in the order to 7.0, to the point where the proteins will be clearly dissolved. It has been found that this point is in a range of from 8.0 to 10.5 pH.

Thereafter, the acid blood plasma is supplied with the requisite amount of a soluble alkali salt or a base, so that the produced alkali hydroxide amount will bring the pH value of the plasma to the requisite value after removal of the anions. The alkali salt can be added as a pure salt, as a solution or as non-de-salted or only partly de-salted plasma.

It has, for example, been found of advantage to add to the acid plasma NaCl, HCl, or H₂SO₄, or sodium citrate.

The amount to be added is governed by the following: 50 cm.³ of plasma call for 1.94 cm.² of n/2 NaOH from pH 6.8-9.0. This amount corresponds to a supply for each liter of plasma of 1.13 grams NaCl or 0.78 gram NaOH or 140 cm.³ raw plasma with 0.32% Na.

Thereafter, the plasma is treated with a strongly basic anion exchanger of the OH⁻ type. The exchanger is agitated in order to prevent its obstruction by the proteins which first flocculate. After the above-mentioned pH values have been reached, the protein flakes are completely dissolved. The agitation can be effected by a stirrer or by the introduction of air. The plasma obtained from the exchanger is completely clear and contains only small traces of free alkali. For most purposes it can be readily used. However, even these small alkali traces can be removed where necessary by adding a cation exchanger, without causing flocculation of the protein. A plasma thus produced does not contain any dissolved salts and is simultaneously stable with respect to coagulation. It is colorless, clear, odorless and tasteless. For some purposes it is necessary to have a completely colorless plasma. This can be done, for example, by adding 0.05-1% H₂O₂ and heating the mixture until no further decoloring effect is observed. This decoloring is effected either after the treatment with an anion exchanger or after the treatment with a cation exchanger and before the anion treatment. The latter is

preferable, since H₂O₂ has a better reaction in an acid environment.

According to still another embodiment of the invention the protein is rendered stable and does not flocculate when exposed to heat sterilization simply by treatment in a cation exchanger and without anion exchanger treatment. For this purpose the cation exchanger is first charged with H⁻ ions. If the protein solution is highly alkaline it can be neutralized by adding an organic acid such as citric acid. The protein solution is then treated in the cation exchanger as afore-described. In view of the fact that the treatment in the hydrogen ion exchanger may lead to an acid reaction of the protein solution, it will be indicated in some instances to neutralize the solution. This is particularly indicated if the proteins are to be used for pharmaceutical or alimentary purposes. The neutralization can be effected, for example, with sodium hydroxide, bi-carbonate or calcium carbonate. This can be done either before or after the heat sterilization.

The heat sterilization can, for example, be effected by passing the protein solution through a pipe system heating the same to, for example, 85° C. and filling the same into airtight containers which are then sealed and are again heated up to the sterilization temperature.

Example I

100 kg. of blood plasma are obtained from the blood of cows after stabilization with sodium citrate by centrifuging. The blood plasma is stirred and during the stirring 2.5 kg. of slightly acidic montmorillonite are added, as well as 0.5 kg. of activated charcoal. The mixture is then stirred for about 30 minutes and thereafter the bleaching earth with the protein and the impurities deposited thereon are separated from the plasma by filtering. A clear, non-gelatinizing impurity-free plasma is obtained.

Example II

100 kg. of blood plasma are processed as in Example I with the exception that 5.2 kg. of montmorillonite and 0.8 kg. of activated charcoal are added and the mixture is stirred for 45 minutes.

Example III

100 kg. of blood plasma are processed as in Example I with the exception that no charcoal is added and 5% of untreated neutral montmorillonite are added and the mixture is stirred for 20 minutes.

Example IV

100 kg. of blood plasma are processed as in Example I with the exception that 3% of untreated neutral montmorillonite and 1% of active coal are added and the mixture is stirred for 25 minutes.

Example V

100 liters of blood plasma are obtained from the blood of cows after stabilization with sodium citrate by centrifuging. The blood plasma is stirred and during the stirring 5.2 kilograms of an untreated neutral montmorillonite are added. After 45 minutes the bleaching earth with the protein and the impurities deposited thereon are separated from the plasma by filtering. The filtered plasma is then supplied with 1.9 kilograms of an acid montmorillonite and 0.9 kilogram of activated charcoal. After stirring for about 15 minutes the plasma is filtered and a clear, non-gelatinizing plasma, free from impurities, is obtained.

25 liters of this purified plasma having a pH value of 7.9 are then fed into a strongly acid cation exchanger of the H⁺ type. The column of the cation exchanger has a diameter of 68 millimeters and a height of 660 millimeters; its volume is 2 liters, the speed of flow is 1.38 meters per hour. After having passed through the exchanger the plasma has a pH value of 1.75.

To 4 liters of this plasma there are added 8 grams

of NaCl and are passed through the column of a strongly basic anion exchanger of the OH⁻ type having the same dimensions as the cation exchanger.

The mixture is passed with the same speed of flow as in the cation exchanger through a grid stirrer revolving at a speed of about 60 revolutions per minute. In the lower part of the exchanger column a white deposit is formed, whereas the portion thereabove becomes increasingly clear. This clear plasma is drawn off. It has a pH value of 8.45 and contains about 0.03% Na by weight. This plasma is mixed with 21 cm.³ of a strongly acid cation exchanger of the H⁺ type and is separated therefrom after 5 minutes. The plasma then has a pH value of 8.2. Thereafter, the plasma is heated for 1 hour to a temperature of 95° C. and is thereby sterilized. The plasma can then be further processed in a manner known per se; for example, it can be concentrated by evaporation, or it is dried by spraying or it is deep frozen.

Example VI

10 grams of crystalline egg-white of fowls' eggs are dissolved in 90 cm.³ of water. They are then passed through a cation exchanger of the H⁺ type with sulfurized polystyrol. Thereafter, it is drawn off the cation exchanger and is sterilized by being heated to 88° C.

Example VII

Fresh egg-white from fowls' eggs are mixed with 15% of water and treated as described in Example VI. Again, the protein can be safely sterilized.

Example VIII

100 liters of blood plasma are obtained and processed as described in Example I. Thereafter, 50 liters of raw plasma having a pH value of 6.82 are supplied with 14.25 liters of n/2 HCl, the latter being slowly and carefully added, in very small quantities, over a period of about 1 hour, until the whole has been added and mixed with the raw plasma. Thereafter, a plasma is obtained which has a pH value of about 2.25 in which state it is heat-resistant up to and including the boiling temperature of the plasma.

It is a particular advantage of the method of the invention that the sterilized protein solutions can be very practically stored and conveyed, as it is no longer necessary to keep them in a deeply cooled or dried state. Rather, they can be stored in cans which are airtightly sealed.

A further advantage of the invention resides in the fact that the temperature stability can be adjusted by varying the contacting period of the plasma with the exchange substances. For example, in the case of protein obtained from the egg-white of fowls' eggs it is entirely sufficient to have a temperature stability not exceeding 70° C.

Furthermore, it is possible to obtain proteins having the desired temperature stability while having an acidic pH-value; if the pH-value is in the order of 2, the eggs are resistant to decomposition for several months, the acids having a germ-killing effect.

It will be understood that this invention is susceptible to further modification and, accordingly, it is desired to comprehend such modifications within this invention as may fall within the scope of the appended claims.

What we claim is:

1. A process for upgrading impure, fibrin-containing, slaughterhouse blood plasma, which process comprises the steps of contacting said blood plasma with about 2.5-

5.2% by weight of a montmorillonite for 20-45 minutes, thereby adsorbing the fibrin and impurities on said montmorillonite, and then removing from the blood plasma the montmorillonite containing the deposited fibrin and impurities, whereby the resultant purified blood plasma is rendered heat stable.

2. The process of claim 1, further comprising the step of sterilizing said purified blood plasma at about 85-100° C.

3. The process of claim 1, wherein the blood plasma is bovine blood plasma.

4. The process of claim 1, wherein the montmorillonite is an untreated, neutral montmorillonite.

5. The process of claim 1, wherein the montmorillonite is a slightly acidic montmorillonite being present in a quantity of 5% by weight based on the blood plasma.

6. The process of claim 1, further comprising adding activated charcoal to the blood plasma thereby adsorbing odor-forming and taste-forming impurities from said blood plasma, and removing from the plasma said activated charcoal with said impurities, whereby the resultant purified blood plasma is tasteless and odorless.

7. The process of claim 1, further comprising the steps of passing the fibrin-free blood plasma through a cation exchanger, and then through an anion exchanger so as to reach a pH value of about 8-10.5.

8. The process of claim 1, further comprising the steps of passing the fibrin-free blood plasma through a cation exchanger, and then through an anion exchanger so as to reach a neutral pH.

9. The process of claim 1, further comprising the steps of adding to the fibrin-free blood plasma an alkali salt, and then substantially desalting the plasma by passing the plasma first through a cation exchanger, and then through an anion exchanger, the plasma having thereafter a pH of about 8-10.5.

10. The process of claim 1, further comprising the step of adding to the fibrin-free blood plasma a sufficient amount of hydrogen peroxide to completely decolorize the plasma.

11. The process of claim 10, further comprising the step of adding to the plasma after contact with the cation exchanger, a sufficient amount of hydrogen peroxide to completely decolorize the plasma.

12. The process of claim 1, further comprising the step of adding to the fibrin-free blood plasma, a base, and substantially desalting the plasma by passing said plasma through a cation exchanger and then through an anion exchanger, the plasma having thereafter a pH of about 8-10.5 due to the presence of the base, and removing the cations of the base by a cation exchanger.

13. The process of claim 1, further comprising the steps of adding an acid to the fibrin-free plasma so as to lower the pH of the blood plasma to about 2.25 in which state it is heat-resistant up to and including the boiling temperature of the plasma.

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