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(54) Title: PROCESS FOR PRODUCING A PLASMA PROTEIN-CONTAINING MEDICAMENT			
(54) Bezeichnung: VERFAHREN ZUR HERSTELLUNG EINES PLASMAPROTEIN-HÄLTIGEN ARZNEIMITTELS			
(57) Abstract			
<p>This invention describes a process for producing a plasma protein-containing medicament essentially free of undesired metals out of citrate plasma or a citrate-containing plasma fraction. This process consists of the following steps: replacement of the citrate and, optionally, the citrate-bound metals in a plasma protein-containing solution by a water soluble mono- or dicarboxylate or an organic mono- or dicarboxylic acid under non-precipitating conditions, production of the plasma protein or the plasma proteins, and production of the medicament.</p>			
(57) Zusammenfassung			
<p>Beschrieben wird ein Verfahren zur Herstellung eines Plasmaprotein-hältigen Arzneimittels, das im wesentlichen frei von unerwünschten Metallen ist, aus Zitratplasma oder einer zitrat-hältigen Plasmafraktion, welches die folgenden Schritte umfasst: Austauschen des Zitrats und gegebenenfalls zitratgebundener Metalle in einer Plasmaprotein-hältigen Lösung gegen ein wasserlösliches Mono- oder Dicarboxylat bzw. eine organische Mono- oder Dicarbonsäure unter nicht-präzipitierenden Bedingungen, Gewinnen des Plasmaproteins oder der Plasmaproteine und Fertigstellen des Arzneimittels.</p>			

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A b s t r a c t :

There is disclosed a method of preparing a plasma-protein-containing medicament from citrated plasma or from a citrate-containing plasma fraction, the medicament being substantially free from undesired metals, which method comprises the following steps:

- exchanging the citrate and optionally citrate-bound metals in a plasma-protein-containing solution for a water-soluble mono- or dicarboxylate or for an organic mono- or dicarboxylic acid under non-precipitating conditions,
- recovering the plasma protein or the plasma proteins, and
- finishing the medicament.



The invention relates to a method of preparing a plasma-protein-containing medicament from citrated plasma or from a citrate-containing plasma fraction, the medicament being substantially free from undesired metals.

With a concentration of 35-50 g/l, human albumin is the main component in plasma. Its therapeutic use has been known for a long time, an administration of albumin being indicated, e.g., in case of an acute loss of blood or plasma or in case of failure of the vasomotoric regulation. Since the osmotic pressure of a 20% (25%) albumin solution is approximately 4 times (5 times) that of normal human serum, the effect of albumin is mainly based on its ability to maintain the osmotic pressure.

Human albumin preparations are prepared from human plasma by multiple fractionation, e.g. by a fractionation according to Cohn, or they are prepared by means of recombinant methods. On account of various materials used during its preparation or when the albumin solution is stored in glass containers, aluminum gets into the albumin preparation so that the final content of aluminum in the respective preparations may be quite substantial.

Aluminum, which constitutes one of the most frequently occurring elements in nature, has recently been increasingly associated with various diseases of the human body, primarily with diseases of the nervous and bone systems. Although the lungs and the gastro-intestinal tract form an efficient barrier for the uptake of aluminum, this barrier is no longer effective in patients receiving intravenous preparations, and the aluminum possibly present in the administered preparations may be taken up without hindrance. Thus, e.g., D.S. Milliner et al. (N. Engl. J. Med. (1985), 312, pp. 165-167) report that some albumin products comprising large amounts of aluminum have lead to diseases of the bones or to encephalitis. Aluminum is also increasingly associated with Alzheimer's disease.

Therefore, efforts have been made to keep the aluminum content of, e.g., albumin preparations low. From U.S. Patent No. 5,372,997, e.g., a method of reducing the aluminum content of albumin preparations by using special glass containers on the one hand, and by treatment with an anion exchanger, on the other



hand, has been known. The aluminum is prevented from dissolving out of glass containers by using a special glass poor in aluminum and by dealkalizing the inner surfaces thereof with an ammonium sulfate solution or with sulphurous acid. Furthermore, a treatment with a cation exchanger is carried out. Finally, for heat-treating the albumin solution, the stabilizers commonly used therefor, such as sodium N-acetyl tryptophane or sodium caprylate, are admixed.

From EP-0 484 464-B1 a method of purifying an albumin from multivalent metal ions bound thereto by substituting them by monovalent metal ions, e.g. ammonium or alkaline metal ions, has been known.

Also from U.S. Patent No. 5,250,663 a method has been known according to which an albumin substantially free from aluminum can be obtained. This method starts with an albumin-containing fraction, a Cohn fraction, e.g., and at first various precipitations are carried out. I. a., a heat shock treatment is carried out in the presence of sodium caprylate as stabilizer and 10 to 20% ethanol. Finally, this solution is subjected to an ultrafiltration and a diafiltration. After the diafiltration, aluminum and other impurities have been removed, weak salt solutions, such as 3% NaCl, sodium acetate or, in some instances, sodium caprylate solutions being used for this diafiltration. With this use of caprylate, no exchange of salts will occur, since caprylate has already been admixed as a stabilizer for the heat shock treatment prior to diafiltration; thus, substantial amounts of citrate ions will still be present in the preparation.

As has, e.g., been described by J.C. May et al. (1992) (Vox Sang., 62, pp. 65-69), the presence of citrate ions, which have a high affinity to aluminum, plays an essential role in the uptake of aluminum (for this, cf. e.g. also R.B. Martin (1986), J. Inorgan. Biochem., 28, pp. 181-187), and thus the mere presence of citrate ions constitutes a general risk of metal ion contaminations for all the preparations.

In EP-0 696 595-A1, the citrate ions are removed in the course of a DEAE Sephadex chromatography in a method in which caprylate is used for the fractionation of albumin. Yet there is no simple exchange of citrate ions for other ions, but - on



account of the high costs of the DEAE Sephadex anion exchangers - an expensive separation of citrate takes place.

The present invention relates to a

method of avoiding or reducing, respectively, undesired metals in plasma-protein-containing medicaments, in which both, the undesired metals are removed in the course of the preparation method and a contamination of the finished preparations during storage in metal-containing containers is prevented or reduced, respectively.

In a first aspect, the invention provides a method of preparing a plasma-protein-containing medicament from citrated plasma or from a citrate-containing plasma fraction, which medicament is substantially free from undesired metals and also does not take up any metals when stored in metal-containing containers, which method comprises the following steps:

- exchanging the citrate and optionally citrate-bound metals in a plasma-protein-containing solution for a water-soluble mono- or dicarboxylate or for an organic mono- or dicarboxylic acid under non-precipitating conditions,
- recovering the plasma protein or the plasma proteins, and
- finishing the medicament.

Surprisingly, it has been found that it is just the anions contained in the plasma-protein-containing solution which decisively contribute to the removal of the metal cations.

Contrary to the method described in U.S. Patent No. 5,250,663, it is not only sodium caprylate which is added to a citrate-containing solution or to a citrate-containing precipitate, but the citrate which, on the one hand may carry bound metal ions in complexed form and, on the other hand, is responsible for the dissolving out of undesired metals within the course of the preparation method or during the storage of the finished medicament, is replaced by a water-soluble mono- or dicarboxylate.

For it has been shown that if citrate ion-containing preparations are precipitated and subsequently taken up in a citrate ion free buffer, the new solution may still contain considerable portions of citrate ions. Since most of the fractionation methods in the recovery of pharmaceutical preparations from plasma - to which nearly always citrate is added during the extraction - comprise one or several precipitation steps, the inventive exchange method for citrate ions thus constitutes an interesting possibility for a simple,



low-cost and efficient removal of citrate ions which can easily be incorporated into already established procedures.

As the plasma fraction, e.g. a fraction obtained according to the Cohn fractionation, will serve. For the preparation of albumin, e.g., an albumin-containing precipitate from the Cohn fractionation is used.

To allow for as complete an exchange of the citrate as possible, the exchange step thus must be carried out under non-precipitating conditions, since otherwise - as has been mentioned - there will be a risk that the citrate can be removed only insufficiently because of its high affinity to the precipitated protein. Preferably, the exchange of the citrate will take place at an early time in the preparation method.

According to a preferred embodiment

as the plasma-protein-containing medicament a medicament comprising one or several factors of coagulation and fibrinolysis, immunoglobulins, glycoproteins and/or albumin, is prepared. There, in particular fibrinogen, prothrombin, the factors V, VII, VIII, IX, X, XI, XII and XIII, optionally in their activated form, von Willebrand factor, but also anticoagulants, such as heparin, heparinoids or cumarin derivatives, or fibrinolytic agents, such as streptokinase, urokinase, pro-urokinase, t-PA or plasmin, can be considered as the coagulation and fibrinolysis factors. As the immunoglobulins, various preparations comprising immunoglobulins of the classes IgG, IgA, IgM and mixtures thereof, optionally in high titers, may be prepared.

As the glycoprotein, orosomucoid may, e.g., be used.

Preferably, a salt of an organic carboxylic acid having 2 to 20 carbon atoms is used for the exchange of the citrate, a caprylate or a tartrate or mixtures thereof being particularly preferred.

For the purposes of the present invention, also an organic mono- or dicarboxylic acid is to be understood as a mono- or dicarboxylate, since in any case the exchange of the citrate in solution will always be for the anion of the acid. Preferably, an organic mono- or dicarboxylic acid having 2 to 4 carbon atoms is used for exchanging the citrate.

The method according to the invention has proved



particularly suitable for the preparation of plasma-protein-containing medicaments that exhibit excellent properties particularly in respect of their aluminum contamination by being substantially free from any detectable aluminum.

As has been mentioned, exchanging of the citrate must be effected under non-precipitating conditions. Preferably, the exchange step is effected during a diafiltration, ultrafiltration or during a chromatographic process, since these steps have proved particularly suitable for a simple, low-cost and efficient exchange.

The conditions during the exchange step depend on the method used and in particular will be chosen such that the exchange of the citrate and optionally also of citrate-bound metals will be as complete as possible. Therefore, the respective parameter which determine the method, in particular the temperature, the duration of the exchange step and the concentration of the respective mono- or dicarboxylate or mono- or dicarboxylic acid, respectively, have to be optimized.

During the exchange, the temperature will preferably be in a range of between 0 and 50°C, more preferred in a range of between 10 and 30°C, most preferred approximately at room temperature. The respective period of time for the exchange is particularly dependent on the ratio of the volume to be exchanged to the membrane surface and on the temperature and preferably is at least 30 minutes, in particular the period of time will be in a range of between 30 minutes and several hours.

In general, a parameter like the period of time will just as well depend on the respective exchange volume of the material in question. Preferably, the exchange volume will be at least 5 times, most preferred at least 30 times that of the starting solution, and the period of time for the exchange will be chosen accordingly.

The concentration of mono- or dicarboxylate or of mono- or dicarboxylic acid preferably is in a range of between 0.001 and 10 mol/l, most preferred in a range of between 0.001 and 1 mol/l.

Sodium caprylate, e.g., is added at a concentration of between 1.0 mmol/l and 1.5 mol/l, preferably in a range of between 1.0 mmol/l and 25 mmol/l.



Sodium acetate, e.g., is added at a concentration of between 1 mmol/l and 5.5 mol/l, preferably between 50 mmol/l and 1.0 mol/l.

The sodium salt of the hexanoic acid, e.g., is added at a concentration of between 1.0 mmol/l and 1.0 mol/l, preferably in a range of between 5.0 mmol/l and 0.1 mol/l.

Sodium tartrate may be added at a concentration of between 1.0 mmol/l and 1.2 mol/l, preferably between 10.0 mmol/l and 0.2 mol/l.

For salts of higher acids, thus the efficient amounts can already be found in a range of from 0.001 to 0.1 mol/l, while salts of lower acids preferably are added at somewhat higher concentrations.

A further parameter which is decisive for the method is the pH of the solution. Preferably, it is at pH 6 to 8, most preferred at pH 6.5 to 7.5.

Beside carboxylate or carboxylic acid, respectively, also inorganic salts, such as, e.g., sodium or potassium salts, may be contained in the solution for increasing the ionic strength thereof. For instance, an at least 4% sodium chloride solution is contained. Furthermore, various buffer salts may be contained.

As the materials for the respective exchange method, in particular commercially available materials, such as, e.g., diafiltration membranes, ultrafiltration units, various chromatographic gels, molecular sieves and others may be used. All these materials may be based on organic or inorganic materials; they may be of synthetic or biological origin.

It has been shown that the method according to the invention will be particularly advantageous if the plasma-protein-containing solution is purified and/or concentrated before the exchange.

As with all the medicaments based on plasma as raw material, one or several steps for inactivating possibly present viruses should also be provided within the scope of the preparation method according to the invention.

A preferred embodiment of the method according to the invention thus relates to a method in which the plasma-protein-containing solution is treated, preferably heat-treated, before

and/or after the exchange so as to inactivate possibly present viruses. Common virus inactivation treatments which may be used within the scope of the present method have been described in EP-0 159 311, EP-0 519 901 or in EP-0 674 531.

It is particularly suitable if prior to a virus inactivation treatment, the plasma protein recovered is further subjected to a dialyses against a medium with a low salt content, e.g. water. This may provide an additional stabilizing effect for the plasma protein because of the presence of the mono- or dicarboxylate. The recovery of the plasma protein or plasma proteins and the finishing of the medicament preferably should be effected exclusively with citrate-free components so as to avoid the renewed contamination of the preparation with citrate ions which are responsible for a renewed contamination with metal ions in case of longer storage.

It is true that the method according to the invention has proved quite particularly suitable for removing aluminum ions or for preventing a renewed contamination with aluminum ions, respectively, during storage of the medicament. Yet also other metal ions from which it is known that they may contaminate plasma-protein-containing medicaments, such as aluminum-like metals, cadmium, zinc, lead, iron and others, may efficiently be reduced.

Thus, in a second aspect, the present invention provides a plasma-protein containing medicament when produced by a method according to the first aspect of the invention, having a content of undesired metals, in particular aluminum, of less than 100 µg/l, preferably less than 10 µg/l, in particular less than 200 ng/l. The content of undesired metal can be determined, e.g., by atomic absorption spectroscopy, this maximum content not being exceeded even after extended storage, even when stored for more than 5 years.

The plasma-protein-containing medicament of the invention may be stored in the most varying containers of the prior art. Such containers may consist of glass, synthetic material, metals or combinations thereof. The containers may also be specially pre-treated; thus, the surface may have been siliconized, e.g.. As the glasses, both hard glasses and soft glasses (cf. e.g. glasses of the classification USP 23, p. 1781) may be used.

In particular, the plasma-protein-containing medicament of



the invention has a low content of undesired metals when stored in hard glasses; particularly preferred the latter is less than 100 $\mu\text{g/l}$, more preferred less than 10 $\mu\text{g/l}$, most preferred less than 200 ng/l .

The present invention will be explained in more detail by way of the following examples to which, however, it shall not be restricted.

Example 1:

A precipitate of Cohn fractionation comprising albumin in a purity of >95% is dissolved 1+2 (w/v; 1kg in 2 l) in 50 g/l NaCl solution at a neutral pH. The solution is continuously diafiltered with a regenerated cellulose membrane at +4°C against water. At this, 0.1 mmol caprylate is added per g of protein.

In the following Table 1 a), the aluminum decrease and the citrate decrease after diafiltration without addition of a carboxylate, such as, e.g., caprylate, tartrate, salt of hexanoic acid or an acetate, are illustrated.

TABLE 1:

a) without addition of a carboxylate:

Sample	Aluminum content		Citrate content	
	$\mu\text{g/g}$ Protein	in %	$\mu\text{mol/g}$ Protein	in %
Before diafiltration	2.802	100.0	166	100.0
Diaconcentrate	0.704	25.1	8.6	5.2

The following Table 1 b) shows the aluminum decrease and the citrate decrease after diafiltration with the addition of 0.1 mmol caprylate.



1 b) with the addition of caprylate

Sample	Aluminum content		Citrate content	
	$\mu\text{g/g}$ Protein	in %	$\mu\text{mol/g}$ Protein	in %
Before diafiltration	2.999	100.0	139.5	100.0
Diaconcentrate	0.096	3.2	1.1	0.8

A comparison of this table with Table 1 a), i.e. with the corresponding decreases without the addition of a caprylate, clearly shows that by an 0.1 mmol caprylate addition per g of protein a clearly higher decrease of citrate and aluminum can be attained than is the case without the addition of a carboxylate, such as, e.g., caprylate.

Example 2:

An albumin-containing precipitate is dissolved as described in Example 1 and subsequently diafiltered. There, 0.5 mmol of a sodium salt of the hexanoic acid were added per g of protein. After termination of the diafiltration, there result the following aluminum and citrate decreases (Table 2).

TABLE 2:

Sample	Aluminum content		Citrate content	
	$\mu\text{g/g}$ Protein	in %	$\mu\text{mol/g}$ Protein	in %
Before diafiltration	3.368	100.0	135	100.0
Diaconcentrate	0.127	3.8	1.8	1.3

As appears clearly from this Table as compared to Table 1 a), by an addition of 0.5 mmol of a sodium salt of hexanoic acid per g of protein, a clearly higher decrease of citrate and



aluminum can be attained than is the case without the addition of the hexanoic acid salt.

Example 3:

A precipitate of Cohn fractionation comprising albumin in a purity of >95% is dissolved 1+2 (w/v; 1kg in 2 l) in 50 g/l NaCl solution at a neutral pH. The solution is continuously diafiltered with a regenerated cellulose membrane at +4°C against water. At this, 5 mmol acetate is added per g of protein.

In the following Table 3, the aluminum decrease and the citrate decrease after diafiltration with addition of 5 mmol acetate per g of protein are illustrated.

TABLE 3:

Sample	Aluminum content		Citrate content	
	$\mu\text{g/g}$ Protein	in %	$\mu\text{mol/g}$ Protein	in %
Before diafiltration	3.95	100.0	155	100.0
Diaconcentrate	0.3	7.6	2.6	1.7

As appears clearly from this Table as compared to Table 1 a), by an addition of 5 mmol acetate per g of protein, a clearly higher decrease of citrate and aluminum can be attained than is the case without the addition of the acetate.

Example 4:

An albumin-containing precipitate is dissolved as described in Example 1 and subsequently diafiltered. There, 1.0 mmol tartrate per g of protein is added. After termination of the diafiltration, there result the following aluminum and citrate decreases (Table 4).



TABLE 4:

Sample	Aluminum content		Citrate content	
	$\mu\text{g/g}$ Protein	in %	$\mu\text{mol/g}$ Protein	in %
Before diafiltration	6.29	100.0	129	100.0
Diaconcentrate	0.62	9.9	1.0	0.8

As appears clearly from this Table as compared to Table 1 a), by an addition of 1.0 mmol tartrate per g of protein, a clearly higher decrease of citrate and aluminum can be attained than is the case without the addition of the tartrate.

In the claims which follow and in the preceding summary of the invention, except where the context requires otherwise due to express language or necessary implication, the word "comprising" is used in the sense of "including", i.e. the features specified may be associated with further features in various embodiments of the invention.



Claims:

1. A method of preparing a plasma-protein-containing medicament from citrated plasma or from a citrate-containing plasma fraction, which medicament is substantially free from undesired metals and also does not take up any metals when stored in metal-containing containers, which method comprises the following steps:
 - exchanging the citrate and optionally citrate-bound metals in a plasma-protein-containing solution for a water-soluble mono- or dicarboxylate or for an organic mono- or dicarboxylic acid under non-precipitating conditions,
 - recovering the plasma protein or the plasma proteins, and
 - finishing the medicament.
2. A method according to claim 1, characterized in that as the plasma-protein-containing medicament, a medicament comprising one or several plasma proteins selected from the group consisting of the factors of coagulation and fibrinolysis, immunoglobulins, glycoproteins and albumin is prepared.
3. A method according to claim 1 or 2, characterized in that a salt of an organic carboxylic acid having 2 to 20 carbon atoms is used for exchanging the citrate.
4. A method according to any one of claims 1 to 3, characterized in that a caprylate and/or a tartrate is used for exchanging the citrate.
5. A method according to any one of claims 1 to 3, characterized in that an organic mono- or dicarboxylic acid having 2 to 4 carbon atoms is used for exchanging the citrate.
6. A method according to any one of claims 1 to 5, characterized in that a plasma-protein-containing medicament which is substantially free from aluminum is prepared.



7. A method according to any one of claims 1 to 6, characterized in that exchanging of the citrate is effected

during a diafiltration, ultrafiltration, a gel permeation chromatography or a chromatographic separation method, respectively, which enable the separation of the protein from salts.

8. A method according to any one of claims 1 to 7, characterized in that before the exchange, the plasma-protein-containing solution is purified and/or concentrated.

9. A method according to any one of claims 1 to 8, characterized in that the plasma-protein-containing solution is treated, preferably heat-treated, before and/or after the exchange, so as to inactivate possibly present viruses.

10. A method according to any one of claims 1 to 9, characterized in that the virus inactivation treatment is effected immediately after the recovery of the plasma protein in the presence of the mono- or dicarboxylate.

11. A method according to any one of claims 1 to 10, characterized in that finishing of the medicament is effected exclusively with citrate-free components.

12. A method according to any one of claims 1 to 11, characterized in that the exchange of the citrate is effected in the presence of sodium chloride, preferably with an at least 4 % by weight sodium chloride solution.

13. A plasma-protein-containing medicament when produced by a method according to any one of claims 1 to 12, having a content of undesired metals, in particular aluminum, of less than 100 $\mu\text{g/l}$, preferably less than 10 $\mu\text{g/l}$, in particular less than 200 ng/l.



14. A method of preparing a plasma-protein-containing medicament the method being substantially as described herein with reference to any of Examples 2 to 4.

- 5 15. A plasma-protein-containing medicament when produced by the method of claim 14.

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