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"BLOOD COMPONENT PRESERVATION CONTAINER
AND MULTI-LINKAGE CONTAINER SYSTEM"

Background of the Invention

5 This invention relates to a blood component
preservation container which is suited for preserving
blood, a blood component, in particular an erythrocyte-
containing solution, and also to a multi-linkage
container.

10 In recent years, a blood component preservation
container of bag-like structure which is formed of
superimposed thermoplastic resin sheets whose
peripheries are fusion-bonded with each other has been
extensively utilized as a container for collecting,
separating or preserving blood.

15 This blood component preservation container (a
blood bag) is required to have various properties, such
as a sufficient transparency for enabling the condition
of blood or blood components contained therein to be
observed from outside; a sufficient flexibility
20 (pliability) for easily enabling various operations
such as blood collection, blood transfusion or
separation of blood components; a sufficient strength
for withstanding these operations; an excellent
sanitariness; and an excellent safety.

25 Since soft polyvinyl chloride meets most of these
requirements, it is predominantly utilized as a
material for the blood bag at present.

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Meanwhile, as far as the preservation of erythrocyte component is concerned, there has been an advancement in the development of a preservation solution for exclusive use therefor, i.e., a
5 preservation solution (such as M.A.P. solution) which is capable of effectively preserving erythrocyte component for 42 days has been developed and is actually put into use now.

However, in spite of the availability of such a
10 preservation solution to preserve erythrocyte component for a long period of time, there has been raised another problem that a big agglomerate or a so-called macroaggregate (hereinafter referred to as MA) is quite frequently generated during the storage of erythrocyte.
15 No measures have been developed as yet to prevent the generation of this MA.

It is also important to prevent the hemolysis (the destruction of blood cell) of erythrocyte being preserved. Namely, if the hemolysis degree of
20 erythrocyte is increased, the quality of erythrocyte preparation would be deteriorated.

Although various methods have been developed to prevent the hemolysis, all of these methods are only aimed at preventing the hemolysis, and a method for
25 suppressing not only the hemolysis but also the generation of MA has not been developed as yet.

Summary of the Invention

Accordingly, an object of the present invention is to provide a blood component preservation container which is excellent in blood component preservation property and capable of suppressing not only the
5 hemolysis of erythrocyte but also the generation of macroaggregate (MA).

Another object of the present invention is to provide a multi-linkage container system comprising
10 such a blood component preservation container as mentioned above.

These objects have been accomplished according to the present invention by a blood component preservation container for preserving a liquid containing at least a
15 blood component, which is formed of a soft polyvinyl chloride sheet containing a first plasticizer and a second plasticizer, wherein the first plasticizer is exudative to the blood component in such a degree that when it is singly mixed in an amount of 32% by
20 weight in a polyvinyl chloride sheet, and a predetermined amount of a blood component is preserved at a temperature of 4°C for 35 days in a blood component preservation container constituted by the resultant polyvinyl chloride sheet, 15 ppm by weight or more of
25 the added first plasticizer is eluted into the blood component, and the second plasticizer is capable of giving an oxygen gas permeability to a polyvinyl

chloride sheet in such a degree that when the second plasticizer is singly mixed in the polyvinyl chloride sheet at a ratio of 32% by weight, the oxygen gas permeability of the resulting polyvinyl chloride sheet is not less than $4.0 \text{ L/m}^2 \cdot \text{mm} \cdot \text{day} \cdot \text{atm}$ (30°C).

Brief Description of the Drawings

FIG. 1 is a plan view showing one example of a multi-linkage container system according to the present invention;

FIG. 2 is a graph showing the relationship between an amount of a plasticizer (DEHP) added and an amount of the plasticizer eluted; and

FIG. 3 is a graph showing the relationship between the amount of a plasticizer (DEHP) added and the rate of hemolysis.

Detailed Description of the Preferred Embodiment

A blood component preservation container and a multi-linkage container system according to the present invention will be explained in detail with reference to a preferred embodiment shown in the drawings.

FIG. 1 shows a plan view illustrating one example of a multi-linkage container system 1 according to the present invention. Referring to FIG. 1, this multi-linkage container system 1 is formed of quadruple containers including a blood collection bag (a first bag) 10, a blood plasma bag (a second bag) 20, a buffy coat bag (a third bag) 30 and a chemicals storage bag

(a fourth bag) 40, all of these bags being connected with each other via prescribed tubes.

Specifically, the blood collection bag 10 disposed leftmost side in FIG. 1 constitutes a blood component preservation container and includes a main bag portion 11 comprising two pliable polyvinyl chloride sheets which are superimposed one upon another and the peripheries of which are fusion-bonded (through heat bonding or high-frequency bonding) or adhered, thereby forming a sealed portion 12.

The inner portion surrounded by the sealed portion 12 of the main bag portion 11 serves as a blood storage space 13 for storing a collected blood. Further, since the blood collection bag 10 can be utilized also as an erythrocyte bag for preserving erythrocyte therein, the blood storage space 13 may be used for ultimately storing and preserving a concentrated erythrocyte.

The main bag portion 11 is provided at its upper portion with a couple of sealed opening ports 14 each provided with a peeling tab for opening the opening ports 14. The main bag portion 11 is also provided, beside the opening ports 14, with an outlet port 18 for communicating the blood storage space 13 with other bags 30, 20 and 40. To this outlet port 18 is connected one end of a tube 61 via a connector member 17. Preferably, the connector member 17 is constructed in such a manner that the passage thereof is closed

before the connector member 17 is fractured, but can be opened once the connector member 17 is fractured.

To the other end of the tube 61 is connected a Y-shaped branch connector 64, to the branched ends of which each end of tubes 62 and 63 is connected respectively. The other end of the tube 62 is attached with a branch connector 19 of the same construction as that of the branch connector 64. The branched ends of the branch connector 19 are connected to each end of tubes 25 and 46 respectively.

A pliable tube 15 is attached via one end thereof to the upper portion of the main bag portion 11 so as to communicate with the interior of the blood storage space 13. The other end of the pliable tube 15 is attached via a hub 151 with a blood collecting needle 152. The hub 151 is adapted to be mounted with a cap 153 for encasing the blood collecting needle 152.

The blood plasma bag 20 includes a main bag portion 21 comprising two pliable soft resin sheets such as soft polyvinyl chloride sheets which are superimposed one upon another and the peripheries of which are fusion-bonded (through heat bonding or high-frequency bonding) or adhered thereby forming a sealed portion 22.

The inner portion surrounded by the sealed portion 22 of this main bag portion 21 is used as a plasma storage space 23 for storing plasma separated from the

blood in the blood collecting bag 10.

5 The main bag portion 21 is provided at its upper
portion with a couple of sealed outlet ports 24 for
blood transfusion each provided with a peeling tab for
opening the outlet ports 24. The main bag portion 21
is connected, beside the outlet ports 24, with the
other end portion of the tube 25 communicating with the
plasma storage space 23. Therefore, when the passage
of the connector member 17 is opened, the blood storage
10 space 13 of the blood collection bag 10 is allowed to
communicate, via the connector member 17, the tubes 61,
62 and 25 and the branch connectors 64 and 19, with
the blood plasma storage space 23 of the blood plasma
bag 20.

15 The buffy coat bag 30 includes a main bag
portion 31 comprising two pliable soft resin sheets
such as soft polyvinyl chloride sheets which are
superimposed one upon another and the peripheries of
which are fusion-bonded (through heat bonding or high-
20 frequency bonding) or adhered thereby forming a sealed
portion 32.

 The inner portion surrounded by the sealed portion
32 of this main bag portion 31 is used as a buffy coat
storage space 33 for storing buffy coat separated from
25 the blood in the blood collecting bag 10.

 The main bag portion 31 is connected via its upper
portion with the other end of the tube 63 communicating

with the buffy coat storage space 33.

The chemicals storage bag 40 includes a main bag portion 41 comprising two pliable soft resin sheets such as soft polyvinyl chloride sheets which are
5 superimposed one upon another and the peripheries of which are fusion-bonded (through heat bonding or high-frequency bonding) or adhered, thereby forming a sealed portion 42.

The inner portion surrounded by the sealed portion
10 42 of this main bag portion 41 is used as a chemicals storage space 43 for storing chemicals. In this embodiment, a predetermined volume of an erythrocyte preservation solution 47 is stored in the chemicals storage space 43. This erythrocyte preservation
15 solution may be a M.A.P. solution, a S.A.G.M. solution, an OPTISOL solution or an ADSOL solution.

The main bag portion 41 is provided at its upper portion with a couple of sealed outlet ports 44 each provided with a peeling tab for opening the outlet
20 ports 44. The main bag portion 41 is provided, beside the outlet ports 44, with a connector member 45 of the same construction as that of the connector member 17 for communicating with the chemicals storage space 43. To this connector member 45 is connected the other end
25 of tube 46. Therefore, when the passages of the connector members 17 and 45 are opened, all of the bags, i.e. the blood storage space 13 of the blood

collection bag 10, the chemicals storage space 43 of
the chemicals storage bag 40, the blood plasma storage
space 23 of the blood plasma bag 20 and the buffy coat
storage space 33 of the buffy coat bag 30 are allowed
5 to communicate with each other, via the connector
members 17 and 45, the tubes 61, 62, 63, 25 and 46 and
the branch connectors 64 and 19.

It is preferable to preliminarily introduce an
anti-coagulant in the blood collection bag 10. This
10 anti-coagulant is liquid in general and may be, for
example, an ACD-A solution, a CPD solution, a CPDA-1
solution or heparin sodium solution. The volume of
these anti-coagulants may be suitably determined in
advance depending on the volume of blood to be
15 collected.

The buffy coat bag 30 may be the one which
contains in advance a platelet preservation solution.
Examples of such a platelet preservation solution are a
physiological saline solution, a GAC solution, a PAS
20 solution, a PSM-1 solution and a synthetic storage
medium solution.

Next, the conditions for the materials that can
be employed for the bags 10, 20, 30 and 40 will be
explained.

25 (1) The blood collection bag 10:

The blood collection bag 10 constituting a blood
component preservation container of the invention is

formed of a soft polyvinyl chloride sheet containing a first plasticizer and a second plasticizer, wherein the hemolysis depression effect (an effect to minimize the amount of hemoglobin to be eluted into a liquid to be preserved) is attained by the first plasticizer which is eluted from the soft polyvinyl chloride sheet into a liquid to be preserved (hereinafter referred to as a preservation liquid or solution) and the second plasticizer which is eluted from the soft polyvinyl chloride sheet into a preservation liquid or solution in a very small amount. In addition, the MA generation suppression effect is attained by a high gas permeability of the soft polyvinyl chloride sheet that is provided by the second plasticizer. Due to a balanced combination of these hemolysis depression effect and MA generation suppression effect, it is possible to obtain a blood collection bag, which is excellent in blood cell preservation property.

The resin constituting the soft polyvinyl chloride sheet may be selected from polyvinyl chloride and polymeric materials mainly comprising polyvinyl chloride (such as a copolymer, a polymer blend and a polymer alloy, each containing a small amount of other polymer materials).

The first plasticizer should be exudative to the blood component in such a degree that when it is singly mixed in an amount of 32% by weight in a polyvinyl

chloride sheet, and a predetermined amount of a blood component is preserved at a temperature of 4°C for 35 days in a blood component preservation container constituted by the resultant polyvinyl chloride sheet, 5 15 ppm by weight or more of the added first plasticizer is eluted into the blood component.

This limitation of the elution level on the first plasticizer is based on the results obtained from the relationship between an amount of plasticizer added to 10 the sheet and the amount of the plasticizer eluted (FIG. 2), and on the results obtained from the relationship between the amount of the plasticizer eluted and the rate of hemolysis (FIG. 3), with di-2-ethylhexyl phthalate (DEHP) used as the plasticizer.

15 The first plasticizer should preferably be an aliphatic or aromatic acid ester containing at least two ester linkages and containing an aliphatic hydrocarbon group having 4 to 12 carbon atoms in its hydrocarbon chain. Among them, dialkyl phthalate and 20 dialkyl adipate are preferable in particular.

Specific examples of the first plasticizer are dioctyl phthalate, dioctyl adipate, di-2-ethylhexyl phthalate, diisononyl phthalate, diisodecyl phthalate, di-2-ethylhexyl maleate, dibutyl phthalate and dihexyl 25 phthalate. These esters may be employed singly or in combination of two or more of these. Among them, di-2-ethylhexyl phthalate (DEHP) or a composition mainly

comprising DEHP is most preferable, because of the stability in the amount of elution and an excellent hemolysis depression effect that may be obtained therefrom.

5 The content of the first plasticizer in the polyvinyl chloride sheet should preferably be 5 to 40% by weight, more preferably 10 to 30% by weight, and most preferably 10 to 25% by weight. If the content of the first plasticizer is less than 5% by weight, the
10 amount of elution of the first plasticizer becomes too small to obtain a sufficient hemolysis depression effect. On the other hand, if the content of the first plasticizer exceeds 40% by weight, the amount of elution of the first plasticizer becomes excessive so
15 that the physiological activity of blood cell may be adversely affected by the first plasticizer, and at the same time, the inclusion of such a large amount of the first plasticizer may deteriorate the mechanical strength of the bag so that the bag may be fractured
20 during the centrifugation, for instance.

 The second plasticizer is capable of giving an oxygen gas permeability to a polyvinyl chloride sheet in such a degree that when the second plasticizer is singly mixed in the polyvinyl chloride sheet in an
25 amount of 32% by weight, the oxygen gas permeability of the resultant polyvinyl chloride sheet becomes not less than $4.0 \text{ L/m}^2 \cdot \text{mm} \cdot \text{day} \cdot \text{atm}$ (30°C), more preferably not

less than $4.2 \text{ L/m}^2 \cdot \text{mm} \cdot \text{day} \cdot \text{atm}$ (30°C), and preferably also the carbon dioxide gas permeability of the resultant polyvinyl chloride sheet becomes not less than $23.0 \text{ L/m}^2 \cdot \text{mm} \cdot \text{day} \cdot \text{atm}$ (30°C). In particular, the second plasticizer should preferably be the one, which is exudative to the blood component in such a degree that when it is singly mixed in an amount of 32% by weight in a polyvinyl chloride sheet, and a predetermined amount of a blood component is preserved at a temperature of 4°C for 35 days in a blood component preservation container formed of the resultant polyvinyl chloride, 2 ppm by weight or more of the added second plasticizer is eluted into the blood component, but the elution amount of the second plasticizer is smaller than that of the first plasticizer. Namely, the second plasticizer should be slightly exudative to the blood component.

Preferable examples of the second plasticizer are phthalic diester, dialkyl phthalate, adipic ester and dialkyl adipate, each having two or more aliphatic hydrocarbon groups each containing 4 to 12 carbon atoms. In this case, the phthalic diester should preferably be di-n-decyl phthalate, and the dialkyl adipate should preferably be di-octyl adipate.

In particular, a diester which can be derived from phthalic acid and an alcohol or aliphatic hydrocarbon group having 8 to 12 carbon atoms is preferable. Among

them, di-n-decyl phthalate (DnDP) or a composition mainly comprising DnDP is more preferable. Of the dioctyl adipate, di-2-ethylhexyl adipate (DEHA) is more preferable.

5 The content of the second plasticizer in the polyvinyl chloride sheet should preferably be 5 to 45% by weight, more preferably 10 to 30% by weight, and most preferably 12 to 30% by weight. If the content of the second plasticizer is less than 5% by weight, the
10 gas permeability of the resultant polyvinyl chloride sheet becomes too low and the MA generation suppressing effect of the sheet would be deteriorated. On the other hand, if the content of the second plasticizer exceeds 45% by weight, the content of the first
15 plasticizer would inevitably become very little due to the limitation on the total amount of the first and second plasticizers (the total amount of the first and second plasticizers is confined to a certain level with a view to maintain the mechanical strength of the
20 resultant sheet), so that the hemolysis depression effect to be derived from the first plasticizer would be deteriorated.

 Since the second plasticizer of the present invention is slightly exudative to blood plasma, the
25 following advantages will be obtained.

 If the second plasticizer not exudative at all, only the first plasticizer would elute, so that

a fairly large amount of the first plasticizer is required to be added in order to sufficiently suppress the hemolysis. However, since there is a limitation (for example, 50% by weight) on the total amount of the first and second plasticizers in order to maintain the mechanical strength of the resultant sheet as explained below, each amount of the first plasticizer and the second plasticizer is also restricted to a certain level.

By contrast, when the second plasticizer is slightly exudative, both first plasticizer and second plasticizer would elute, so that a sufficient hemolysis depression effect can be attained with a relatively smaller amount of the first plasticizer as compared with the case where the second plasticizer is not exudative at all. As a result, the amount of the second plasticizer to be added can be correspondingly increased, thus making it possible to obtain a polyvinyl chloride sheet excellent in gas permeability. Namely, it is possible according to the present invention to obtain a polyvinyl chloride sheet improved in both plasticizer elution property and gas permeability, thereby achieving an effective and balanced suppression of the hemolysis and the MA generation, while maintaining a predetermined mechanical strength of the polyvinyl chloride sheet.

The total content of the first plasticizer and the

second plasticizer should preferably be in the range of 20 to 50% by weight, more preferably 25 to 40% by weight. If this total content is less than 25% by weight, at least one of the hemolysis depression effect and the MA generation-suppressing effect may become insufficient. On the other hand, if this total content exceeds 50% by weight, the mechanical strength (such as tensile strength) of the resultant polyvinyl chloride sheet would be deteriorated, and hence a bag formed of such a polyvinyl chloride sheet may be fractured if it is exposed to a severe condition such as a centrifugation.

The polyvinyl chloride sheet may contain, if desired, various additives such as a stabilizer, in addition to the first and second plasticizers. As the stabilizer, various kinds of epoxy compounds, compounds of zinc, calcium, barium or magnesium may be added in the polyvinyl chloride sheet at a ratio of 0.1 to 15% by weight, for instance.

The thickness of the polyvinyl chloride sheet may be suitably determined taking into consideration the balance between the mechanical strength and the gas permeability required for the polyvinyl chloride sheet. However, in general, the thickness of the polyvinyl chloride sheet should preferably be in the range of 0.2 to 0.5 mm, more preferably 0.25 to 0.4 mm.

The polyvinyl chloride sheet having the

aforementioned features may be manufactured, for instance, by the following method.

5 A soft polyvinyl chloride, the first plasticizer and the second plasticizer are sufficiently kneaded by a kneader to obtain a kneaded product, which is then extruded via a T-die or a circular die to obtain a flat sheet. The sheet is then subjected to a sequence of manufacturing steps including a thermoforming step, a blowing step, a stretching step, a cutting step and a
10 step of sealing (fusion bonding) the end portion to obtain a product of predetermined shape and structure.

The surface of the sheet (raw material) may be roughened (or embossed), or coated or applied with an anti-blocking agent or a slip agent for preventing the
15 blocking of the interface between the sheets.

(2) The platelet bag:

The chemicals storage bag 40 may be utilized as a platelet bag for preserving platelet after the erythrocyte preservation solution 47 is transferred
20 to the blood storage space 13 of the blood collection bag 10.

There is not any particular limitation regarding the composition and property of the sheet material for constituting the chemicals storage bag 40. For
25 example, the same materials as employed for the blood collection bag 10 may be employed. If the same materials as employed for the blood collection bag 10

are employed in the manufacture of the chemicals storage bag 40, the manufacture of the bag may be simplified because of the decrease in kinds of sheet materials to be prepared, and at the same time, the preservation of platelet will be improved due to the elution of the plasticizers and also to the excellent gas permeability of the bag (for example, the effective preservation period will be extended to 3 to 5 days).

(3) The blood plasma bag 20:

There is not any particular limitation regarding the composition and property of the sheet material for constituting the plasma bag 20. For example, the same materials as employed for the blood collection bag 10 may be employed. If the same materials as employed for the blood collection bag 10 are employed in the manufacture of this plasma bag 20, the manufacture of the bag may be simplified because of the decrease in kinds of sheet materials to be prepared.

(4) The tubes 15, 61, 62, 63, 25 and 46:

As for the materials for the tubes 15, 61, 62, 63, 25 and 46, polyvinyl chloride, polyethylene, polypropylene, polyester (such as PET, PBT), ethylene-vinyl acetate copolymer, polyurethane, a thermoplastic elastomer (such as polyester elastomer, styrene-butadiene-styrene copolymer) or a material mainly comprised of any of these polymers may be employed. Among them, polyvinyl chloride and a material mainly

comprising polyvinyl chloride are preferable for use.

5 If each of these tubes is formed of polyvinyl chloride, they may be excellent in pliability and flexibility, thereby making it easy to handle them, and at the same time they may be easily closed with a clamp and the like. Moreover, since a polyvinyl chloride tube is excellent in compatibility with the sheet material forming the bags and made of the same kind of material as that of the tube, a bonding or adhesion 10 which is high in bonding strength and excellent in gas-tightness will be effected between the tubes and the bags, which is preferable in view of assuring the invulnerability to the centrifugation and retaining the sterilized condition of the bags. There is not any particular limitation with respect to the kind and 15 content of plasticizer to be employed for these tubes.

As for the multi-linkage container system of the present invention, it is not limited to the construction shown in the drawing, but may be of any 20 construction as far as it includes a blood component preservation container for preserving a liquid containing a blood component, i.e., a blood cell preservation bag such as a blood collection bag or an erythrocyte preservation bag as explained above. For 25 example, the multi-linkage container system of the present invention may be a double bag comprising a blood collection bag and a platelet bag;

a triple-linkage bag comprising a blood collection bag,
a blood plasma bag and a platelet bag; a quadruple-
linkage bag comprising a blood collection bag, a
platelet bag, a blood plasma bag and an erythrocyte bag
5 for exclusive use therefor; a quadruple-linkage bag
comprising a blood collection bag which may be used
also as an erythrocyte bag, a platelet bag, a plasma
bag and a buffy coat bag (a small bag for relay); or
any other multi-linkage bags where any additional bag
10 for additional end-use is attached to any of the
aforementioned bags.

The container to be connected with the blood
component preservation container may be formed of a bag
made of the same material as used for forming the tubes
15 or formed of a container of any other shape and
construction.

This invention will be further explained with
reference to the following specific examples.

Examples 1 - 2, and Comparative Examples 1 to 4

20 A. Method of experiment:

(1) Manufacture of a multi-linkage bag system

Various kinds of sheets having compositions and
properties as shown in Table 1 below were employed and
formed into a 2-ply structure, which was then cut and
25 the periphery thereof was heat-sealed thereby to form
a blood collection bag. Likewise, a platelet bag
(chemicals storage bag), a blood plasma bag and a buffy

coat bag were prepared.

95 ml of an erythrocyte preservation liquid (an aqueous solution containing M.A.P. solution/sodium chloride: 5.52 g/l; adenine: 0.16 g/l; glucose: 8.01 g/l; mannitol: 16.19 g/l; citric acid: 0.22 g/l; sodium citrate: 1.67 g/l; sodium phosphate: 1.04 g/l) was introduced via the tube into the platelet bag (chemicals storage bag). Then, all of the bags were connected via pliable tubes, and at the same time, a blood collecting tube was connected to the blood collection bag. Thereafter, 60 ml of an anticoagulant (an aqueous solution containing ACD-A solution/sodium citrate: 22.0 g/l; citric acid: 8.0 g/l; and glucose: 22.0 g/l) was introduced via the tube into the blood collection bag, thereby preparing a quadruple-linkage bag system comprising four bags connected with each other.

This quadruple-linkage bag system was then subjected to a high pressure steam sterilization (121°C, 30 minutes). The construction of each bag in this quadruple-linkage bag system is shown in Table 2 below.

The tubes and the blood collecting tube employed herein were made of a soft polyvinyl chloride containing 36.7% by weight of di-2-ethylhexyl phthalate.

Table 1

Sheet No.	Materials	Plasticizers	Content (wt%)	O ₂ gas permeability	CO ₂ gas permeability	Thickness of sheet (mm)
				(L/m ² · mm · day · atm (30°C))		
Sheet A	PVC	Di-2-ethylhexyl phthalate	17.1	4.5	25.0	0.36
		Di-n-decyl phthalate	17.1			
Sheet B	PVC	Di-2-ethylhexyl phthalate	17.1	3.9	21.5	0.36
		Di-2-ethylhexyl adipate	17.1			
Sheet C	PVC	Di-2-ethylhexyl phthalate	31.7	3.1	15.8	0.36
Sheet D	PVC	Di-n-decyl phthalate	31.7	5.3	31.8	0.36
Sheet E	PVC	Di-2-ethylhexyl adipate	31.7	4.0	24.0	0.36
Sheet F	PO	None	-	7.5	30.0	0.25

PVC: Polyvinyl chloride

PO : A mixture of poly (ethylenebutylene) polystyrene block copolymer, polypropylene and polyethylene vinyl acetate

An apparatus for measuring O₂ gas permeability: L-100, Dr. Lyssy, Zollikon, Switzerland

Table 2

	Blood collection bag	Buffy coat bag	Platelet bag	plasma bag
Purpose/blood component	Blood collection/erythrocyte	Buffy coat	Concentrated platelet	Platelet-poor plasma
Volume (ml)	500	150	400	400
Inner surface area (cm ²)	380	200	400	340
Present invention 1	Sheet A	Sheet A	Sheet A	Sheet A
Present invention 2	Sheet B	Sheet B	Sheet B	Sheet B
Comparative Example 1	Sheet D	Sheet D	Sheet D	Sheet D
Comparative Example 2	Sheet C	Sheet C	Sheet C	Cheet C
Comparative Example 3	Sheet E	Sheet E	Sheet E	Sheet E
Comparative Example 4	Sheet F	Sheet F	Sheet F	Sheet F

(2) Heavy metals and eluate test

Tests on heavy metals and eluate were performed according the specification prescribed in Japanese Pharmaceutical Codex, General Testing Method, RPlastic container for Transfusion Testing MethodS.

(3) Separation of blood components

About 400 ml of whole blood was introduced into the blood collection bag, and then the multi-linkage bag system was subjected to a centrifugation by a centrifugal separator, thereby separating the whole blood into three phases, i.e., a platelet-poor plasma, a buffy coat and a concentrated erythrocyte.

Then, the platelet-poor plasma in the blood collection bag was transferred via the tube to the blood plasma bag. Thereafter, the buffy coat was transferred via the tube to the buffy coat bag, leaving the concentrated erythrocyte in the blood collection bag.

Then, the erythrocyte preservation solution which had been charged in advance in the platelet bag was transferred via the tube to the blood collection bag, and after a predetermined portions of the tubes were heat-sealed, the tubes were cut off to separate the multi-linkage bag system into a blood collection bag (erythrocyte bag) filled with the concentrated erythrocyte mixed with an erythrocyte preservation solution, a platelet bag filled with platelet poor

plasma, and a double-linkage bag system consisting of the buffy coat bag filled with buffy coat and the platelet bag.

5 The double-linkage bag system was then subjected to a slight centrifugation to separate the buffy coat in the buffy coat bag into two layers, i.e., an upper layer comprising a concentrated platelet and a lower layer comprising leukocyte. Then, the concentrated platelet was transferred via the tube to the platelet
10 bag.

Finally, the tube linking the platelet bag and the buffy coat bag was cut off to separate these bags from each other.

(4) Confirmation of MA generation

15 The blood collection bag which was obtained in the aforementioned procedure (3) and filled with the concentrated erythrocyte mixed with a erythrocyte preservation solution was preserved for 35 days at a temperature of 4°C, and then the generation of MA was
20 observed for each of the samples according to Example 1 and Comparative Examples 1 to 3. The results are shown in Table 3 below.

(5) Confirmation of hemolysis level

25 The blood collection bag which was obtained in the aforementioned procedure (3) and filled with the concentrated erythrocyte mixed with a erythrocyte preservation solution was preserved for 35 days at

a temperature of 4°C, and then 1 ml of sample solution after the preservation was aseptically taken out from each of the bags according to Example 1 and Comparative Examples 1 to 3 to measure the hemoglobin concentration and the hematocrit value by using an automatic blood cell counter (Sysmex CC-180 available from Toa Iyo Densi Co. Ltd., Japan).

Furthermore, a portion of the sample solution taken out was subjected to a centrifugation for 15 minutes under the condition of 1,500 G by using a centrifugal separator (KR-702 available from Kubota Seisakusho Co. Ltd., Japan) to take out the upper layer constituted by blood plasma. Thereafter, the hemoglobin concentration in the blood plasma was measured by means of Leuco-crystal violet method (LCV method).

The values obtained in this manner were put into the following equation to calculate the degree of hemolysis, the results being shown in Table 3.

$$\text{Hemolysis degree (\%)} = \left\{ \frac{[\text{hemoglobin concentration (mg/dl) in plasma} \times (1 - \text{hematocrit}/100)]}{[\text{hemoglobin concentration (g/dl) in M.A.P.-added concentrated erythrocyte} \times 1,000]} \right\} \times 100$$

(6) Confirmation of preservation state of platelet

The platelet bag which was obtained in the aforementioned procedure (3) and filled with a concentrated platelet was preserved with stirring at

a temperature of 22°C, and then 1 ml of sample solution was aseptically taken out from the bag on 0th day, 3rd day and 5th day to measure the number of platelets in the sample solution by using the aforementioned
5 automatic blood cell counter, the results being shown in Table 4.

Furthermore, the pH value (at 37°C) of the sample solution taken out as mentioned above was measured by using a blood gas/pH measuring apparatus (IL 1304
10 available from Instrumentation Lab.), the results being shown in Table 5.

(7) Centrifugation

The blood collection bag filled with a predetermined volume of distilled water was subjected
15 to a centrifugation under the following conditions.

Centrifugal separator: Himac CR7B3, Hitachi
Seisakusho, Japan

Conditions : 1.80×10^8 gasec., 4,000 rpm
(4,670 G), 22°C.

20 After the centrifugation, the blood collection bag was taken out of the auxiliary cup and examined to see if any damage was caused thereto, the results being shown in Table 6.

B. Results of the experiment

25 (1) Preparation of sheet and bag

The preparation of sheets could be smoothly performed, obtaining sheets excellent in uniformity.

The preparation of each bag was also smoothly performed.

(2) Results of the heavy metals and eluate test

It was confirmed that all of the samples satisfied, in terms of heavy metals and eluate, the conditions prescribed in the specification of Japanese Pharmaceutical Codex, General Testing Method, RPlastic container for Transfusion Testing MethodS.

(3) Evaluation on the preservation of erythrocyte

Table 3 shows findings on the generation of macroaggregate (MA) and hemolysis level. As seen from Table 3, the blood collection bags according to Examples 1 and 2 indicated no generation of MA, and at the same time it was confirmed that the level of hemolysis in the blood collection bags according to Examples 1 and 2 was very low.

Table 3

	Number of MA generated	Hemolysis degree (%) average value $\pm$ SD
Present invention 1	0	0.40 ± 0.03
Present invention 2	0	0.41 ± 0.03
Comparative example 1	0	0.82 ± 0.05
Comparative example 2	10	0.40 ± 0.03
Comparative example 3	0	0.51 ± 0.04
Comparative example 4	0	0.80 ± 0.06

(n=10)

(4) Evaluation on the preservation of platelet

Table 4 shows the change with time in the number of platelets, and Table 5 shows the change with time of pH value. As shown in Table 4, any substantial change in the number of platelets was recognized in any of the samples. As shown in Table 5, Comparative Example 2 indicated the lowering of pH value or the deterioration in quality of platelets under the aforementioned condition that the number of platelets was almost the same in all of the samples, which was assumed to be caused by the low gas permeability of sample according to Comparative Example 2. However, with respect to other samples, it was confirmed that they could be preserved in a stable condition up to 5th days of storage.

Table 4

No.	Number of platelets ($\times 10^7/\text{ml}$) average value $\pm$ SD		
	Initial moment	After 3 days	After 5 days
Present invention 1	201 $\pm$ 13	202 $\pm$ 14	201 $\pm$ 14
Present invention 2	200 $\pm$ 14	201 $\pm$ 14	200 $\pm$ 14
Comparative example 1	201 $\pm$ 13	201 $\pm$ 14	201 $\pm$ 14
Comparative example 2	199 $\pm$ 13	198 $\pm$ 14	200 $\pm$ 14
Comparative example 3	200 $\pm$ 16	199 $\pm$ 15	201 $\pm$ 16
Comparative example 4	198 $\pm$ 15	197 $\pm$ 14	199 $\pm$ 16

(n=10)

Table 5

	pH vale	average value $\pm$ SD	
	Initial moment	After 3 days	After 5 days
Present invention 1	6.99 ± 0.06	6.98 ± 0.07	6.98 ± 0.05
Present invention 2	7.00 ± 0.06	6.99 ± 0.07	6.99 ± 0.05
Comparative example 1	6.97 ± 0.04	7.03 ± 0.06	7.05 ± 0.05
Comparative example 2	7.00 ± 0.05	6.60 ± 0.06	6.07 ± 0.04
Comparative example 3	7.00 ± 0.05	7.02 ± 0.06	7.03 ± 0.06
Comparative example 4	6.99 ± 0.06	7.11 ± 0.07	7.18 ± 0.07

(5) Evaluation on the strength of bag by making use of centrifugation test

Table 6 shows the number of fractured blood collection bags which were subjected to a centrifugation test. As seen from Table 6, the blood collection bags according to Examples 1 and 2 were not fractured at all, indicating a sufficient degree of strength of the bag to withstand the operation of centrifugation.

Table 6

	Number of feature generated
Present invention 1	0
Present invention 2	0
Comparative example 1	0
Comparative example 2	0
Comparative example 3	0
Comparative example 4	15

As explained above, it is possible, according to the blood component preservation container and the multi-linkage container system of the present invention, to attain a suitable degree of elution of plasticizer and a high gas permeability so as to concurrently achieve an excellent hemolysis depression effect and an excellent macroaggregate (MA) suppression effect as a blood component is preserved therein.

These effects will be further promoted, while assuring a sufficient mechanical strength of the blood component preservation container, in particular a mechanical strength sufficient to withstand the operation of centrifugation, if a plasticizer of slightly exudative is employed as the second

plasticizer; if a preferable composition is employed
for the first plasticizer and for the second
plasticizer; if each content of the first plasticizer
and the second plasticizer are suitably selected and at
5 the same time the total content of the first and second
plasticizers is selected within the aforementioned
predetermined range.

Due to the aforementioned features of the blood
component preservation container and the multi-linkage
10 container of the present invention, it is possible to
preserve erythrocyte and other blood components for a
long period of time, while maintaining the quality of
the erythrocyte, etc., in an excellent condition.

Furthermore, the blood component preservation
15 container and the multi-linkage container system of the
present invention are easy in handling and manufacture,
and excellent in safety.



Claims:

1. A blood component preservation container for preserving a liquid containing at least a blood component, which is formed of a soft polyvinyl chloride sheet containing a first plasticizer and a second plasticizer,

wherein said first plasticizer is exudative to the blood component in such a degree that when it is singly mixed in an amount of 32% by weight in a polyvinyl chloride sheet, and a predetermined amount of a blood component is preserved at a temperature of 4°C for 35 days in a blood component preservation container constituted by the resultant polyvinyl chloride sheet, 15 ppm by weight or more of the added first plasticizer is eluted into the blood component, and

said second plasticizer is capable of giving an oxygen gas permeability to a polyvinyl chloride sheet in such a degree that when said second plasticizer is singly mixed in said polyvinyl chloride sheet in an amount of 32% by weight, the oxygen gas permeability of the resultant polyvinyl chloride sheet becomes not less than $4.0 \text{ L/m}^2 \cdot \text{mm} \cdot \text{day} \cdot \text{atm}$ (30°C).

2. The container according to claim 1, wherein said second plasticizer is exudative to the blood component in such a degree that when it is singly mixed in an amount of 32% by weight in a polyvinyl chloride sheet, and a predetermined amount of a blood component



is preserved at a temperature of 4°C for 35 days in a blood component preservation container formed of the resultant polyvinyl chloride, 2 ppm by weight or more of the added second plasticizer is eluted into the blood component, but the elution amount of the second plasticizer is smaller than that of the first plasticizer.

3. The container according to claim 1 or 2, wherein said second plasticizer is an aliphatic or aromatic ester having at least two ester linkages and containing an aliphatic hydrocarbon group having at least four carbon atoms in its hydrocarbon chain.

4. The container according to claim 3, wherein said second plasticizer is selected from the group consisting of phthalic diester, dialkyl phthalate, adipic ester and dialkyl adipate, each having two or more aliphatic hydrocarbon groups each containing 4 to 12 carbon atoms.

5. The container according to claim 4, wherein said phthalic diester is di-n-decyl phthalate, and said dialkyl adipate is di-octyl adipate.

6. The container according to any one of claims 1 to 5, wherein said first plasticizer is an aliphatic or aromatic ester having at least two ester linkages and containing an aliphatic hydrocarbon group having 4 to 12 carbon atoms in its hydrocarbon chain.

7. The container according to claim 6, wherein



said first plasticizer is dialkyl phthalate or dialkyl adipate.

8. The container according to any one of claims 1 to 5, wherein said first plasticizer is dioctyl phthalate.

9. The container according to any one of claim 1 to 8, wherein said first plasticizer is contained in an amount of 5 to 40% by weight.

10. The container according to any one of claims 1 to 9, wherein said second plasticizer is contained in an amount of 5 to 45% by weight.

11. The container according to any one of claims 1 to 10, wherein the total content of said first plasticizer and said second plasticizer is 20 to 50% by weight.

12. A multi-linkage container system comprising a plurality of containers connected together via tubes, said containers including at least one blood component preservation container according to any one of claims 1 to 11.

13. A blood component preservation container and multi-linkage container system, substantially as hereinbefore described with reference to the accompanying drawings.