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Determination of a Suitable Concentration of Disodium Hydrophosphate forStoring Red Blood Cell Mass

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ABSTRACT

Background: Donated blood and blood components are currently used in the treatment of patients to help replenish the blood cells and circulating blood volume lost due to blood loss. Therefore, there is a need for effective preservation solutions for the storage of blood components, especially red blood cells.
The aim of the work was to identify changes in red blood cell mass characteristics up to 42 days of age in disodium hydrophosphate solutions with different concentrations.
The relevance of the work for Vietnam is the need to develop its own solutions for the preservation of red blood cell mass instead of the total use of imported ones.
Materials and Methods. Six different types of solutions were studied, labelled from D1 to D6, which corresponded to increasing concentrations of disodium hydrophosphate from 0.1 to 0.6 g/100 mL of the preservative solution in 0.1 g increments. Donor blood from healthy volunteers was processed according to the developed study model. A haematology analyser, an osmometer and a pH meter were used to estimate the necessary values of the tested red blood cell mass.
Results. The effect of disodium hydrophosphate on erythrocyte mass during long-term storage up to 42 days was detected. The solution with the lowest concentration in this study, sufficient for the required level of erythrocyte preservation parameters, was determined.
Conclusions. Disodium hydrophosphate is a suitable solution for the storage of red blood cell mass. When the cells arestored for 42 days at concentrations between 0.2 and 0.6 g/100 mL, it ensures that the cell count, pH level and osmotic pressure are maintained at an adequate level. At the same time, the D2 solution is the formulation with the minimum concentration to maintain the required level of the target parameters. Further studies are needed to investigate the activity of disodium hydrophosphate in combination with other components.

Keywords
disodium hydrophosphate, red blood cell mass, red blood cell storage, haemotransfusion, blood transfusion

Introduction

In medical practice, there is often a need for transfusion of blood or blood components. These situations are often foreseen, but can also occur suddenly, as in the case of complications during labour. These situations frequently occur during childbirth in minors, because the expectant mother's body is not sufficiently functionally prepared for the birth process [1-2]. Whole blood transfusion has been used less and less frequently in recent years due to difficulties in its selection and the high risk of complications from haemotransfusion. Instead, the medical community considers it advisable to transfuse only blood components. This is safer, more effective in terms of clinical outcomes and cost-effective.
That is why preservative solutions are needed for the storage of individual blood components. The issue is particularly acute with regard to the preservation of red blood cells. Such attention to the problem of red blood cell preservation is due to the high susceptibility of this blood component to haemolysis. Occurring during the processing of components and storage of red blood cell units, it has serious clinical consequences for patients. Free haemoglobin from haemolysis can cause serious complications [3].

Studies have been carried out in the developed world to produce a universal preservative for the red blood cell mass. However, the results of the research have not been published and there is no pharmacopoeia of any country that has developed a complete guide to solutions for red blood cell storage. The novelty of the work is that it takes a step towards systematising and unifying the data regarding disodium hydrophosphate solution as a preservative for the red blood cell mass.
The relevance of the work lies in the fact that in Vietnam the preservation of red blood cells depends almost entirely on solutions purchased from foreign countries [4]. Thus, research on the production of red blood cell preservatives is an urgent need for the blood transfusion industry in Vietnam.
The aim of the study was to determine changes in the amount of red blood cells, pH and osmotic pressure when storing red blood cell mass up to 42 days in disodium hydrophosphate solutions withdifferent concentrations.
Research objectives:

1. to take blood from healthy volunteers for testing;
2. to anticoagulate it with citrate-phosphate-dextrose solution;
3. to obtain a red blood cell mass for examination;
4. to preserve red blood cells using disodium hydrophosphate solution with concentrationsranging from 0.1 to 0.6g/100mL;
5. to examine the preservatives on specific days using validated equipments;
6. to record the results.

Literature Review

Hemotransfusion is one of the urgent operations for saving human life. It helps to stop or minimize blood or its component losing [5]. Red blood cells are the most sought-after blood transfusion product worldwide. According to statistics of 2016, approximately 85 million units of red blood cells are transfused annually [6]. Indications for transfusion include symptomatic anaemia, acute sickle cell crisis and acute blood loss exceeding 30% of blood volume in order to restore oxygen delivery to the tissues [7-9]. However, preserved red blood cells from donors are not a qualitatively equivalent product to autologous blood, and in many respects it causes concern in the practice of transfusiology [10].Storage time of red blood cell mass affects red blood cells on a qualitative level, as the latter get old in the storage bag and undergo haemolysis. Several studies have shown that cumulative damage results in increased clearance after blood transfusion, plasma transferrin saturation, nitric oxide uptake and/or immunomodulation with potential adverse clinical outcomes associated with blood transfusion [8, 9, 11]. Such outcomes include acute lung injury or a higher mortality rate. Therefore, it is so important to address the issue of quality and safe storage of red blood cell mass.
Units of red blood cells are obtained from whole blood by removing the plasma fraction after centrifugation. One unit of red blood cells should increase the recipient's haemoglobin level by 1g/dl and the haematocrit by 3%. Currently, red blood cells undergo leukofiltration before storage, which limits alloimmunisation associated with blood transfusion, and therefore they are considered safe with regard to cytomegalovirus transmission [12].
Preservative solutions are added to red blood cells to increase their shelf life and quality. In the early 1940s, the development of the first effective anticoagulant and preservative solution, acid citrate dextrose (ACD), made it possible to store red blood cells for up to 21 days. Over the following years, efforts were made to develop other solutions, such as citrate phosphate dextrose (CPD), which allows storage the mass for 21 days, and CPD-adenine, which allows storage for

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35 days. The current generation of supplemental solutions allows preservation of red blood cells for up to 42 days at 1-6°C [13]. In most European blood banks, the preservatives used for erythrocyte storage contain SAG-manitol (physiological solution-adenine-dextrose-manitol). The most common are AS-1 (Adsol®; Fenwal, Lake Zurich, Illinois, USA), AS-3 (Nutricel®, without mannitol; Haemonetics Corporation, Braintree, Massachusetts, USA) and AS-5 (Optisol®; Terumo Corporation, Elkton, MD, USA) [10]. However, as it was mentioned above, all these solutions are subject to importation for Vietnam, which requires in-house development of preservatives.

On this basis, a study was prepared to evaluate the effect of disodium hydrophosphate solution on red blood cell preservatives, and the optimal concentration was sought, based on data from the literature. It was hypothesised that all concentrations of the solution, starting from 0.2 g/10 mL, would be capable of maintaining the red blood cell mass in an accessible state for transfusion, preserving cell number, pH level and osmotic pressure.

Materials and Methods

The study was conducted at Bach Mai Hospital, which is one of the largest multidisciplinary medical facilities in Hanoi, Vietnam.

Materials

The objects of the study were 6 different types of solutions, labelled from D1 to D6 (Table 1). They corresponded to increasing concentrations of disodium hydrophosphate from 0.1 to 0.6 g/100mL of the preservative solution in 0.1 g increments.

Table 1. Variants of the tested solutions of the disodium hydrophosphate

Designation	Concentration, g/100 mL
D1	0.1
D2	0.2
D3	0.3
D4	0.4
D5	0.5
D6	0.6

The study materials were, firstly, whole blood taken from healthy volunteers and, secondly, an anticoagulant CPD solution from Terumo (Japan). The criteria for inclusion in the study were that the donor had been examined and had no laboratory detectable abnormalities. Selection was in accordance with the "Blood Transfusion Procedures" of the regional Department of Health, and the entire study was in accordance with the ethical standards of the responsible committee for human experimentation and the Declaration of Helsinki from 1975, revised in 2000.

The used instruments were:

- CellDyn 3700 laser automated haematology analyser;
- Roebling Osmometer;
- Metrohm 961 pH meter (Switzerland).

Methods

Whole blood of each patient in a volume of 350 mL was treated with CPD solution. Then centrifugation was started at 4100 G programme for 3 minutes and 35 seconds.

In this case, $G = 1{,}118 \times 10^5 \times r \times N^2$,

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where “r” is a radius of the rotor in cm;
“N” is a centrifugation speed in revolutions/min.
Then, plasma with white blood cells and thrombocytes was decanted using separation forceps and red blood cell mass was obtained for further use in the study. To create D1-D6 solutions, 0.880 g of disodium chloride and 1.2 g of glucose and preservatives were added to disodium hydrophosphate.The total volume of the solutions corresponded to 100 mL.
The above listed operations were performed using a closed system and standard procedures at the Institute of Haematology and Blood Transfusion.
A research model was created specifically for the study, according to which the production of red blood cell mass is followed by its separation into six bags. 14 mL of one of the six red cell storage solutions is to be added to each of them. Storage takes place at a temperature of +4ºC. There are 7 checkpoints . They are 0, 7th, 14th, 21st, 35th and 42nd days with regard to the red blood cell amount, pH and osmotic pressure. After all the steps have been completed, the effect of disodium hydrophosphate on the storage quality of the red cell mass should be evaluated and theoptimum concentration of the solution identified.
Statistical processing of the results of the study was carried out according to SPSS-10.5, where "p" is the level of significance of the comparison of two mean values between the studied samples according to the T-criterion.

Results

Examination of the cell number in the red blood cell mass

The results of a study on the solutions' ability to retain the number of red blood cells in the mass were examined for 42 days (Table 2). However, the values differ for the different concentrations of disodium hydrophosphate (Fig. 1). The results are represented by the number of red blood cells per litre at each time of examination.

Table 2. Number of cells in the red blood cell mass preserved by solutions with different concentrations of disodium hydrophosphate

Solution / **Day**	Number of red blood cells (kl/l), **$x \pm SD$**, (n=10)					
	D_1 (0.1g/100 mL)	**D_2** (0.2g/100 mL)	**D_3** (0.3g/100 mL)	**D_4** (0.4g/100 mL)	**D_5** (0.5g/100 mL)	**D_6** (0.6g/100 mL)
0	6.68 ± 0.078	6.68 ± 0.076	6.67 ± 0.074	6.68 ± 0.077	6.68 ± 0.072	6.68 ± 0.084
7	6.58 ± 0.091	6.59 ± 0.081	6.59 ± 0.084	6.61 ± 0.087	6.61 ± 0.082	6.62 ± 0.097
14	6.40 ± 0.113	6.58 ± 0.081	6.58 ± 0.082	6.59 ± 0.096	6.60 ± 0.078	6.60 ± 0.094
21	6.20 ± 0.071	6.48 ± 0.043	6.47 ± 0.051	6.49 ± 0.049	6.49 ± 0.040	6.49 ± 0.058
28	5.68 ± 0.096	5.90 ± 0.082	5.91 ± 0.080	5.94 ± 0.084	5.96 ± 0.080	6.01 ± 0.089
35	2.46 ± 0.085	3.17 ± 0.133	3.17 ± 0.013	3.20 ± 0.131	3.24 ± 0.130	3.28 ± 0.136
42	1.77 ± 0.061	2.11 ± 0.140	2.10 ± 0.177	2.15 ± 0.143	2.16 ± 0.151	2.18 ± 0.171

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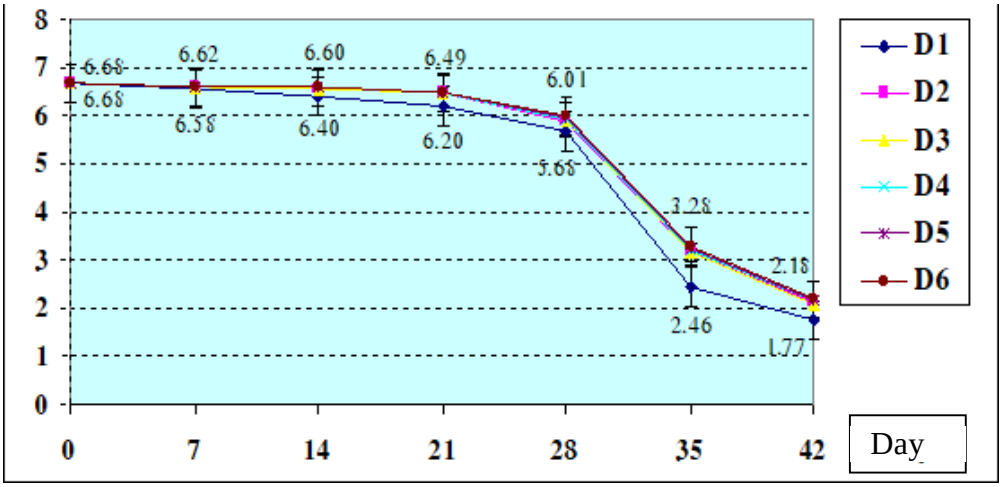

Figure 1. Diagram. Number of cells in a mass of red blood cells preserved by solutions with different concentrations of disodium hydrophosphate

According to Table 1 and Diagram 1 the number of erythrocytes in the mass did not change significantly in any of the solutions from day zero of the study to the 7 th day. From day 7 to 21st day the number of cells also remained fairly stable, although some loss started to occur. From day 21 until the end of the study the number of red blood cells in the D1 solution with the lowest concentration of disodium hydrophosphate decreased more rapidly. It was significantly different from the number of cells in the mixtures with higher preservative content. The differences were statistically significant and amounted to $p < 0.05$ and $p < 0.01$. Meanwhile, the number of red blood cells in D2, D3, D4, D5 and D6 solutions decreased more slowly from the 2nd to the 6th, but such a decrease did not have a statistically significant difference at a value of $p > 0.05$ during all study periods.

The study of the red blood cell mass pH

Table 3 illustrates the results of a study on the ability of the solutions to maintain the pH level in the mass for up to 42 days.

Table 3. pH level in the red blood cell mass preserved by solutions with different concentrations of disodium hydrophosphate

Solution / **Day**	**pH, $\bar{x} \pm$ SD), n=10**					
	D_1 (0.1g/100 mL)	**D_2** (0.2g/100 mL)	**D_3** (0.3g/100 mL)	**D_4** (0.4g/100 mL)	**D_5** (0.5g/100 mL)	**D_6** (0.6g/100 mL)
0	7.34 ± 0.014	7.34 ± 0.008	7.34 ± 0.004	7.34 ± 0.005	7.34 ± 0.009	7.34 ± 0.013
7	7.33 ± 0.014	7.33 ± 0.016	7.33 ± 0.008	7.33 ± 0.005	7.33 ± 0.010	7.33 ± 0.014
14	7.10 ± 0.016	7.31 ± 0.011	7.31 ± 0.016	7.31 ± 0.014	7.31 ± 0.010	7.31 ± 0.022
21	6.96 ± 0.014	7.27 ± 0.011	7.28 ± 0.013	7.29 ± 0.012	7.30 ± 0.012	7.30 ± 0.018
28	6.81 ± 0.016	7.24 ± 0.014	7.25 ± 0.007	7.26 ± 0.007	7.27 ± 0.016	7.28 ± 0.016
35	6.25 ± 0.016	7.00 ± 0.016	7.02 ± 0.010	7.03 ± 0.012	7.04 ± 0.016	7.05 ± 0.010
42	6.11 ± 0.024	6.81 ± 0.032	6.83 ± 0.020	6.83 ± 0.023	6.84 ± 0.014	6.85 ± 0.012

However, the values differ for different concentrations of disodium hydrophosphate (Fig. 2). The results are represented by the pH values at each moment of the study.

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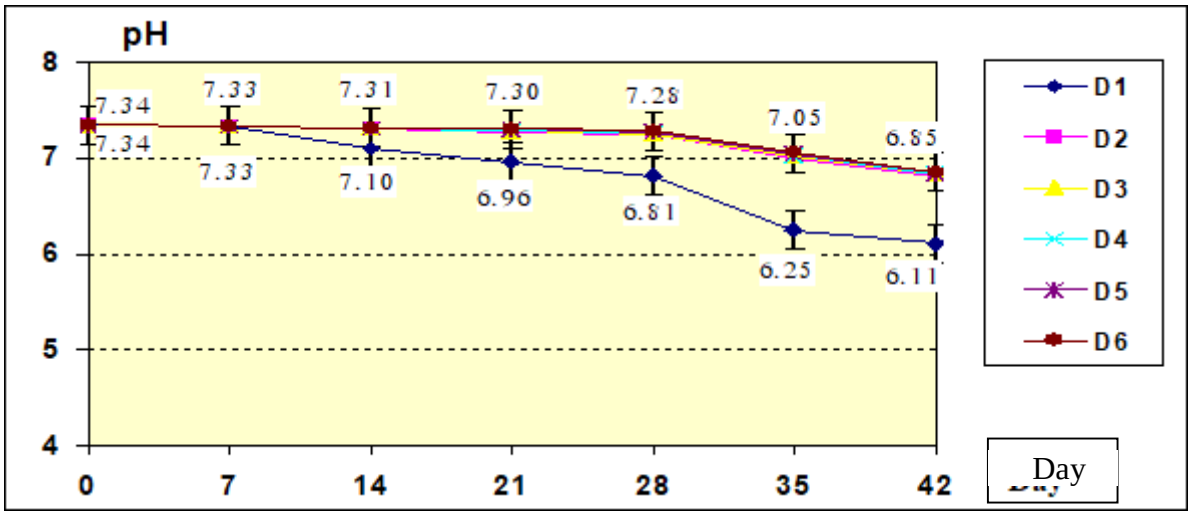

Figure 2. Diagram. pH level in the red blood cell mass preserved by solutions with different concentrations of disodium hydrophosphate

The results of the study of pH of the solutions practically coincide with the data obtained from the analysis of the cells number. At the same time, the pH values did not differ significantly from day 0 to day 7, although they decreased from day 0 to day 7. These changes were not statistically significant, the p-value was higher than 0.05. From day 14 until the end of the study the pH values began to decrease. Attention was drawn to the fact that this decrease was much faster in the D1 solution than in the others. The differences were considered statistically significant with a level of $p < 0.01$. The pH value of solutions D2 to D6 decreased, but slowly, and such a decrease was not statistically significant.

Study of the osmotic pressure of the red blood cell mass

The results of a study on the ability of the solutions to maintain the osmotic pressure level in the red blood cell mass up to 42 days are shown in Table 4.

Table 4. Level of osmotic pressure in the mass of red blood cells preserved by solutions with different concentrations of disodium hydrophosphate

Solution / **Day**	**Osmotic pressure (mosm/l), $x \pm SD$,** (n =10)					
	D_1 (0,1g/100 mL)	**D_2** (0,2g/100 mL)	**D_3** (0,3g/100 mL)	**D_4** (0,4g/100 mL)	**D_5** (0,5g/100 mL)	**D_6** (0,6g/100 mL)
0	314,6 ± 1,82	315,0 ± 0,71	315,2 ± 1,64	315,8 ± 1,30	316,0 ± 1,58	316,4 ± 2,41
7	315,8 ± 1,79	316,2 ± 1,48	317,4 ± 0,54	317,8 ± 1,09	317,8 ± 1,30	317,2 ± 2,05
14	318,8 ± 1,79	318,2 ± 1,79	319,2 ± 1,79	319,2 ± 1,30	319,0 ± 1,41	319,0 ± 0,71
21	325,2 ± 2,59	320,2 ± 1,79	320,4 ± 1,52	321,4 ± 1,14	321,4 ± 1,14	321,0 ± 0,71
28	338,2 ± 2,39	325,2 ± 1,64	325,0 ± 1,00	325,8 ± 1,30	325,0 ± 1,00	325,6 ±1,82
35	346,0 ± 1,87	330,8 ± 2,17	331,0 ± 1,87	330,8 ± 2,17	331,6 ± 1,82	331,6 ± 1,52
42	354,4 ± 2,41	345,0 ± 1,92	344,6 ± 1,22	343,9 ± 1,30	343,6 ± 0,71	342,9 ± 1,67

Figure 3 illustrates that the values of osmotic pressure in the mass vary for different concentrations of disodium hydrophosphate. The results are represented by the osmotic pressure values at each moment of the study and are recorded in milliosmoles per liter.

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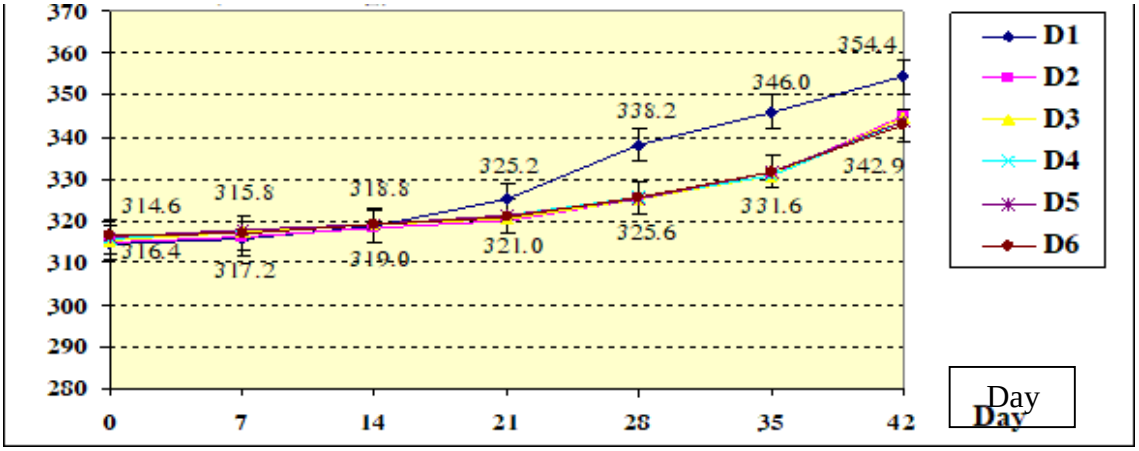

Figure 3. Diagram. Level of osmotic pressure in the mass of red blood cells preserved by solutions with different concentrations of disodium hydrophosphate

The data in Table 3 and Figure 3 illustrate that from the beginning of the study to day 21 the osmotic pressure in the red blood cell mass remained practically unchanged. From day 21 to the end of the study the osmotic pressure in the solution with the lowest concentration of disodium hydrophosphate D1 increased faster. It began to differ from the osmotic pressure of mixtures with higher concentrations of disodium hydrophosphate, such as D2, D3, D4, D5 and D6.

Discussion

In Vietnam there have been no previous studies on the effect of disodium hydrophosphate on cell number, pH and osmotic pressure in the red blood cell mass during storage. The presence of this component in the erythrocyte storage solution at the appropriate concentration has not been studied.

The results show that disodium hydrophosphate has a favourable effect on red blood cell storage by stabilising the pH. Up to day 21, solutions from D1 to D6 were equally active, allowing the preservation of the main studied parameters in the red blood cell mass. However, then the values of the target parameters began to change. At the same time, solution D1 showed statistically significant deterioration of the parameters, which leads to the conclusion that the concentration of disodium hydrophosphate 0.1g/100 mL does not meet the requirements and is not a sufficient preservative for red blood cells storage. At concentrations from 0.2 g to 0.6 g per 100 mL of solution, the preservative preserves cell quantity and osmotic pressure for 42 days of storage, and changes of these parameters are statistically insignificant during this period.

Thus, disodium hydrophosphate at a concentration of 0.2 g or more per 100 mL solution has similar red cell preservation values to solution D6, and formula D2 represents the solution with the lowest concentration of disodium hydrophosphate in the present study, demonstrating the ability to maintain maximum red cell number, stabilize pH and osmotic pressure well.

However, considering that the parameters did undergo changes, it must be concluded that the ability of disodium hydrophosphate to preserve red blood cells should be studied in combination with other agents, such as, for example, adenine or mannitol, in multi-component synthetic formulations.

The disodium hydrophosphate results in this study are consistent with the very important role of pHstabilisation in red blood cells storage. Even in the state of preservation, red blood cells still obtain energy to sustain life and their function through anaerobic metabolism of glucose. The end product of this process is lactic acid, which lowers the pH and has many negative effects on the quality of stored red blood cells and blood transfusion safety.For example, red blood cell enzymes decrease their activity at low pH, and a decrease in ATP leads to a reduction in cell life span. 2.3-Bisphosphoglyceric acid, or bisphosphoglycerate (BPG) is an isomer of 1.3-bisphosphoglyceric acid. It is formed as a by-product of glycolysis and present in red blood cells at a concentration of approximately 5 mmol/l. It is used in blood diagnostics and serves as an indicator of haemoglobin oxygen saturation. The 2.3-BFG cycle causes a change in the dissociation efficiency of oxygen from haemoglobin, leading to a decrease in red blood cells function [14].The pentose monophosphate pathway reduces red blood cells activity at low pH, provoking NADPH deficiency and causing the inability of red blood cells to maintain glutathione in a restored state. It leads to an inability to maintain intracellular antioxidants and results in globin degeneration and red blood cells precipitation. NADPH deficiency also increases the oxidation of haemoglobin to methemoglobin and choleglobin [15]. Choleglobin gets recrystallised, causing mechanical rupture of red blood cells [16].Furthermore, when there is a deficiency in NADPH, red blood cells have to use NADPH instead. It induces a lack of energy for red blood cells function and Na+-ATPase pumping. Therefore, Na+ remains in them, drawing in water and causing cells destruction.

Under normal conditions, there are 6 buffers in the blood that regulate and maintain a relatively constant pH between 7.4 and 0.05 [17]. The buffer system of haemoglobin is the most important for red blood cells. The buffer systems of protein, bicarbonate and phosphate play an important role in plasma [18]. These buffers were removed with the plasma in the storage medium, therefore the pH of the red blood cells mass was rapidly decreasing over time.

In the presence of disodium hydrophosphate preservative solution (which is a weak dibasic alkali in fact) converts lactic acid to neutral disodium lactate and disodium hydrophosphate to dihydrophosphate (which is a weaker acid than lactic acid) [19]. Through this mechanism, disodium hydrophosphate better maintains the pH of the red blood cells mass and helps to maintain the necessary metabolic processes in the red blood cells [20-21]. All these factors are of crucial importance to ensure the quality of the red blood cells and the safety of haemotransfusions.

Conclusions

So, our study gives us possible to conclude.

1. Disodium hydrophosphate is a suitable solution for the storage of red blood cell mass. It ensures that the cell number, pH and osmotic pressure are maintained at a sufficient level when the cells are stored for 42 days at concentrations ranging from 0.2 to 0.6 g/100 mL;
2. D2, i.e. a solution with a concentration of 0.2g/100mL is the formula with the lowest concentration of disodium hydrophosphate in the present study to maintain the test values at the proper level;
3. After 42 days at all studied concentrations, disodium hydrophosphate allowed to maintain only 32% of the cells volume, a stable pH of 6.81 and an osmotic pressure of 345 mosmol/l.

Prospects for further research on this topic are to study the ability of disodium hydrophosphate to preserve red blood cells when they are preserved in combination with other agents such as

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adenine, mannitol or as part of multicomponent solutions.

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