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STUDIES ON THE STORAGE OF POOLED PLATELETS IN NON DOP PVC CONTAINERS

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ABSTRACT

There is an increasing demand for blood bag systems for the storage of pooled platelets from at least six units of blood for the treatment of bleeding cytopenia patients. PVC plasticized with DEHP, TEHTM, DNDP, BTHC and polyolefines are being used for the storage of platelets. However PVC containers of 1.0 to 1.2 litre capacity plasticised with BTHC have been found to be the best option to meet the stringent physical and chemical characteristics needed and hence such pooling bags are marketed by all reputed blood bag manufacturers. BTHC plasticiser is expensive, has a not too pleasant smell , has high leachability into blood plasma and there are problems relating to sterilization by steam. Blood bags made with this plasticiser have been reported to cause allergic reactions. In an effort to overcome these short comings, we have developed a new type of container made using PVC of Ultra High Molecular Weight, plasticised with the non DOP, non toxic plasticiser 1,2 - Cyclohexane dicarboxylic acid, di isononyl ester [DINCH] developed by M/s BASF.Blood of the same type collected from six voluntary blood donors in SAGM TAB bags made using PVC plasticized with the new plasticizer were separated by the buffy coat method and pooled using the train system to obtain pooled platelets which were leucodepleted by filtration and collected in containers of 1200ml capacity for test A standard platelet pooling bag of 1200ml capacity was used as control. Haematological, biochemical and aggregation characteristics of the stored platelet concentrates in these bags were studied. The results indicate that 1200ml containers made using DINCH plasticized Ultra High Molecular Weight PVC were well suited for the storage of at least six units of pooled platelets for up to seven days.The results of our studies are presented in this paper.

KEY WORDS : Platelets, Platelet rich plasma, DINCH Plasticiser, Platelet storage, Non DEHP plasticizer, Ultra High Molecular Weight PVC, Hexamoll [R] DINCH [R]

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INTRODUCTION

There is an increasing demand for the collection and storage of pooled platelet concentrates for the treatment of bleeding thrombocytopenia patients. The accepted practice is to increase the platelet content in the patient's blood by $50X10^9$/L by the administration of one unit of platelets per 10Kg body weight. Usually a platelet pool of 6 units is required, which may need to be increased to 10 units according to situations that arise. PVC plasticized with DEHP, TEHTM, DNDP, BTHC and polyolefines are being used for the storage of platelets. The requirements for the containers for storing of up to 10 units of platelet concentrates are very critical and demanding. The capacity of the containers vary from 600 ml to 1200ml, one liter being the generally preferred capacity keeping in view the essential requirement to store the platelet containing bags in platelet agitators at 20-24°C and ease of handling . The containers should also have optimum permeability to maintain the aerobic metabolism of the platelets and also to enable most of the carbondioxide generated to diffuse out, while maintaining carbondioxide partial pressure sufficient to build up an optimum level of bicarbonate buffer. PVC containers of 1.0 to 1.2 Liter capacity plasticised with BTHC are being used for pooling bags marketed by reputed blood bag manufacturers. BTHC plasticiser has disadvantages – it is expensive, has a not too pleasant smell, has high leachability into blood plasma and blood bags containing the plasticiser has been reported to cause allergic reactions. There are also difficulties regarding sterilization by steam.

Three methods are available for the collection of platelets-viz the PRP method, the buffy coat method and the apheresis method. The platelet concentrates prepared by the PRP method has a recovery of 60-75 percent where as by the buffy coat method it is slightly lower . However if buffy coats are prepared by pooling, the recovery increases to 60-75 percent. The WBC contamination of platelets in the PRP method is 1-2 per 1000 platelets where as by the buffy coat pooling method it is 2 per 100,000 platelets. The plasticizer of choice for PVC containers for the storage of platelet rich plasma is Tri-(2-ethyl hexyl), tri mellitate, even though other plasticizers have been studied in detail[1-17]. PVC containers plasticised with 1,2-cyclohexane di carboxylic acid, di isononyl ester -Hexamoll ^{R} DINCH^R - (hereinafter referred to as DINCH) have been found to be excellent for the storage of single unit platelet concentrates for over five days [18,19,20].This plasticizer has the advantage that it is free of DOP and thereby meets the requirements of the "REACH" regulation. The complete set of regulatory studies for safety and risk assessment regarding DINCH are available. The Scientific Committee for Emerging and Newly Identified Health Risks (SCENIHR) 2008, has identified DINCH as a possible alternative to DEHP for use in medical devices. The storage of multiple units of platelets in containers made using Ultra Higher Molecular Weight PVC of K value 77 to 90, plasticised with DINCH has been described in an Indian patent assigned to Terumo Penpol[21]. This paper presents the results of our studies on the storage of 6 units of platelet concentrates prepared by the buffy coat pooling method in 1200 ml capacity containers made using PVC of K-value 90, plasticized with DINCH. A well known platelet pooling bag of capacity 1200 ml plasticised with BTHC was used for comparison.

MATERIALS & METHODS

A. Platelet pooling bags evaluated.

a) Test bags: Platelet storage bags of capacity 1200ml made using PVC of K value 90 plasticised with the BASF plasticiser DINCH. (TPL compound 206)

b) Platelet storage bags used as control: - An internationally accepted platelet pooling bag of capacity 1200ml plasticised with n-butyryl, tri n-hexyl citrate.

The oxygen and carbondioxide permeability of the platelet bags used in the study are given in Table 1 below.

Table 1
Oxygen and carbondioxide permeability of the platelet bags used in the study.

Containers 1200 ml capacity	Permeability of the containers (ml/per container/24 hrs)	
	Oxygen	Carbondioxide
a. Test bag	130	291
b. Control bag	126	651

B. Collection of blood and preparation of platelet concentrate.

1. Blood collection

Blood donors were selected after taking their detailed medical history and after medical examination. Blood was taken from them only after the donors were found to fulfill all the eligibility criteria for a healthy donor. Written informed consent was also taken from them.

2. Buffy coat preparation

Blood collected from 12 donors of the same blood group was used for each test. Whole blood (450ml) was collected in the primary bag of QB SAGM TAB bags (all bags made of DINCH plasticised PVC) and kept at 20-22°C for two hours. The bags were centrifuged at 4500 rpm for 12 minutes at room temperature (22°C). The components RBC and plasma were separated using Terumo Automatic Component Extractor (TACE II). The separated plasma was stored at 2 - 6°C. The buffy coat with the platelets was retained in the primary bag and was stored at room temperature (20-24°C) for 24 hours.

3. Buffy coat pooling.

The six bags containing buffy coat were connected serially using Terumo Sterile Connecting Device (TSCD) to a transfer bag of 500ml to transfer the buffy coats. A transfer bag containing 300ml plasma from the plasma separated and stored earlier was connected to the top bag using TSCD. An equal volume of plasma was added in to the transfer bag by passing through all the six bags. On completion, the collection bag was sealed and separated.

4. PC preparation.

The bag containing pooled buffy was centrifuged at 1200 rpm for 5 minutes at 22°C to separate platelet rich plasma and a lower layer of RBC and leucocytes. The bag was connected through an Imugard III PL filter (Terumo) to the bags under test and the upper layer of platelet rich plasma expressed into the test bag using a component expressor. The same procedure was adopted for the preparation of Reference sample. A test sample was taken under sterile conditions and the platelet counts adjusted to almost the same level in the test and reference bags. The air inside the platelet storage bag was removed and the tube sealed off.

The following test samples were thus prepared.

i) Pooled platelet rich plasma from six donors of the same blood group collected in TPL-206 bag.
ii) Pooled platelet rich plasma from six donors as above of the same blood group collected in the Reference bag.

5. Storage

The pooled platelet rich plasma in test and reference bags were then positioned in a platelet agitator incubator with horizontal shaking and maintained at 22°C.

6. Periodicity of tests.

Tests were done after 1, 3, 5, 7 and 9 days.

C. Parameters studied.

The blood collection, separation processing and haematological studies were done at the R&D Centre of Terumo Penpol Ltd. The Biochemical and morphological studies were done at the Metabolic Disorders Research Centre, Trivandrum under the supervision of the Director, Dr. P.A.Kurup.

a) Haematological

1. pH, was measured using Systronics Digital pH Meter MK VI.

2. Morphology, platelet count and WBC count were measured using LABOMED microscope, Model - VISION 2000. Measurements were also done using automatic blood cell counter - Beckman LH 750- 5 part differential cell counter.3. p O_2, p CO_2 - These were measured using blood gas analyzer model - Bayer, Rapid Lab 248.
4. Bicarbonate (Spectrophotometric method)- was measured using test kit of M/s Erba Diagnostics, Mannheim, Germany.

b) Biochemical

1. Lactate (Spectrophotometric method)- was measured using test kit, from Centrionic GmbH, Germany.
2. Glucose (Spectrophotometric method)- was measured using kit, from Aspen laboratories, Pvt. Ltd, Delhi
3. Pyruvate was determined by Spectrophotometric manual biochemical method[22].
4. ATP was measured by was determined by (Spectrophotometric method)- using test kit, of M/s Biovision Inc., California USA.
5. Acetyl Co Enzyme A was determined by Spectrophotometric manual biochemical method [23].
6. LDH was measured by Spectrophotometric manual biochemical method[22]
7. β-thromboglobulin was determined using ELISA kit, of M/s Uscn Life Science Inc, Wuhan, China.
8. Serotonin was determined using test kit, of M/s Demeditec Diagnostics, GmbH, Germany.
9. Cytochrome C was determined using test kit, of M/s ELISA Bender MedSystems, GmbH, Germany. UV/VIS Double beam spectrophotometer 2201 and Span ELISA Autoreader 4011 were used for the studies.
10. Osmotic fragility was determined by measuring the haemolysis colorimetrically due to the exposure of the cells to saline solution.

c) Aggregation studies.

1. Aggregation induced by collagen was measured using Whole blood Aggregometer, Chronolog Corporation.

RESULTS AND DISCUSSION

Two independent studies were made in which platelet concentrates were prepared from six blood donors of the same blood group in both the test and control bags. Each test was also done in duplicate. The results presented in the figures are average values with standard deviation. The results of haematological studies are given in Figures I to V. The results of biochemical studies are given in Figures VI to XVI.

Changes in pH values during the storage period.

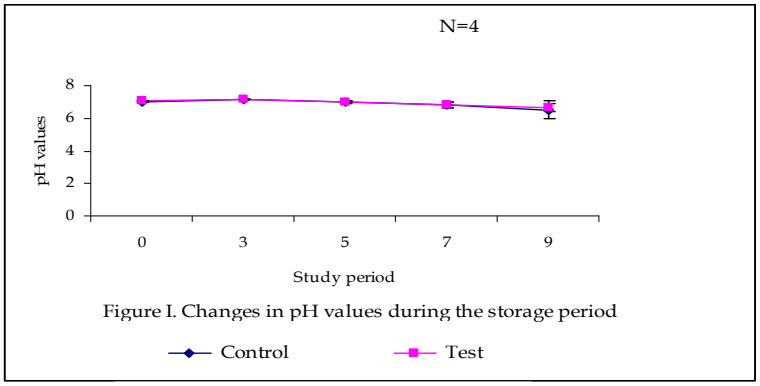
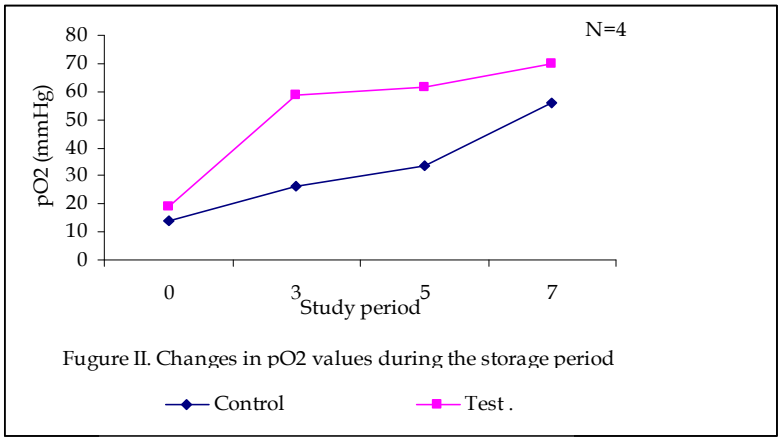


Figure I. Changes in pH values during the storage period

Changes in pO_2 values during the storage period.



Fugure II. Changes in pO2 values during the storage period

Changes in pCO_2 values during the storage period.

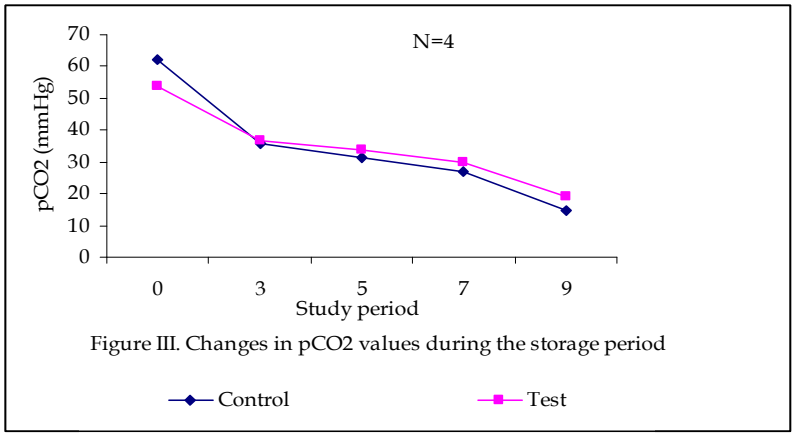


Figure III. Changes in pCO2 values during the storage period

Changes in platelet count during the storage period.

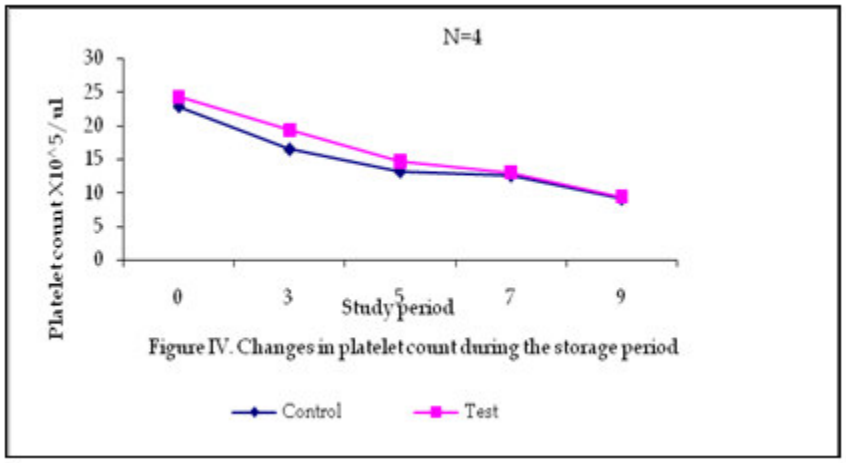


Figure IV. Changes in platelet count during the storage period

Changes in Bicarbonate during the storage period.

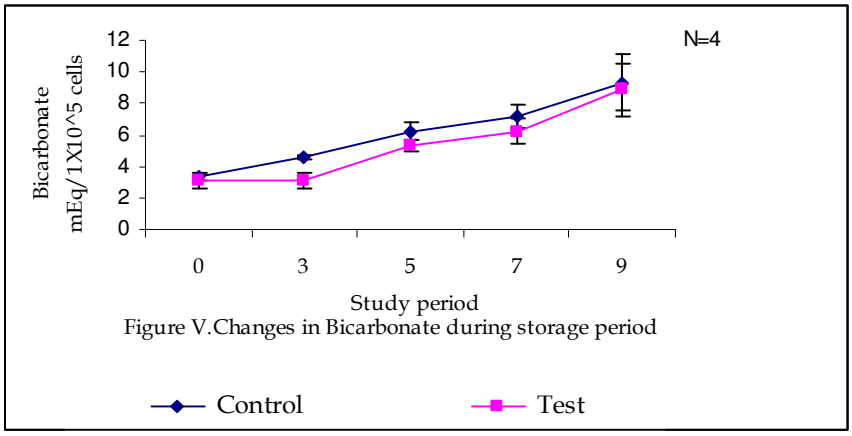


Figure V.Changes in Bicarbonate during storage period

Changes in Lactate level during the storage period.

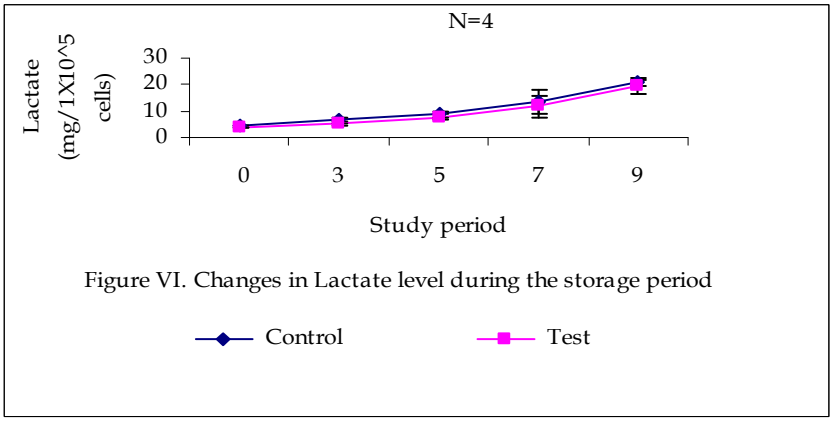


Figure VI. Changes in Lactate level during the storage period

Changes in glucose level during the storage period.

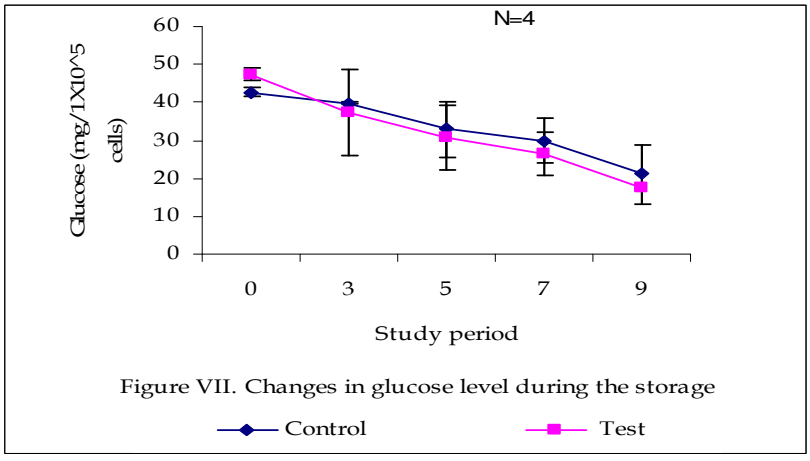


Figure VII. Changes in glucose level during the storage

Changes in HSR values during the storage period.

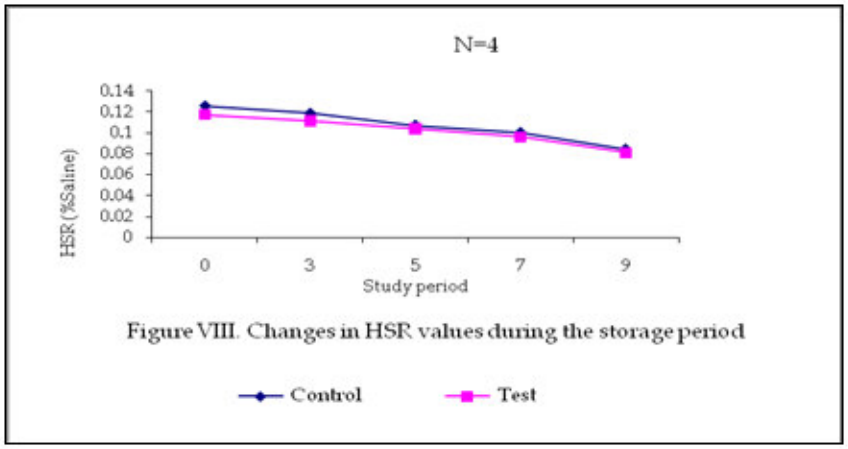


Figure VIII. Changes in HSR values during the storage period

Changes in pyruvate values during the storage period.

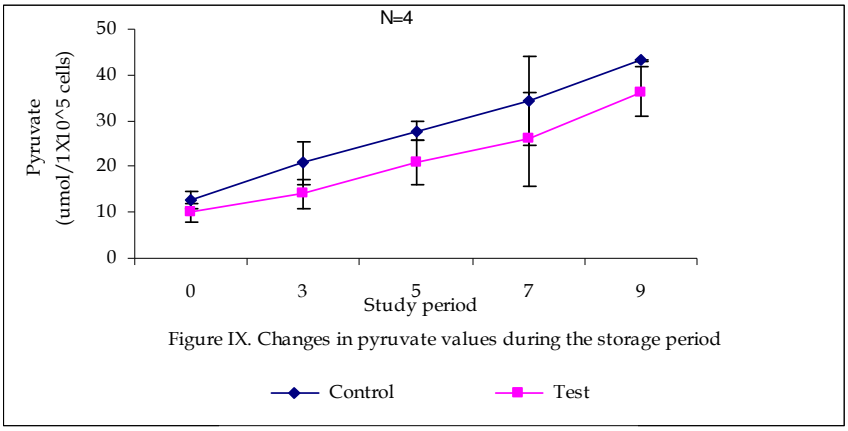


Figure IX. Changes in pyruvate values during the storage period

Changes in ATP values during the storage period.

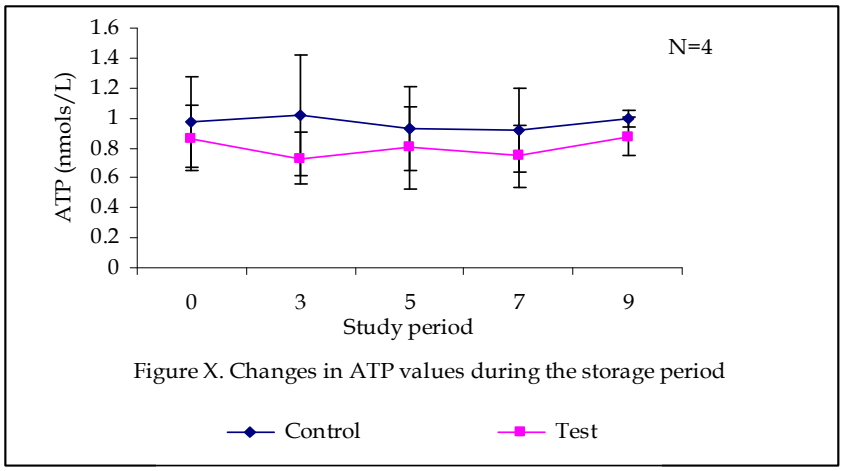


Figure X. Changes in ATP values during the storage period

Changes in Acetyl Co A during the storage period.

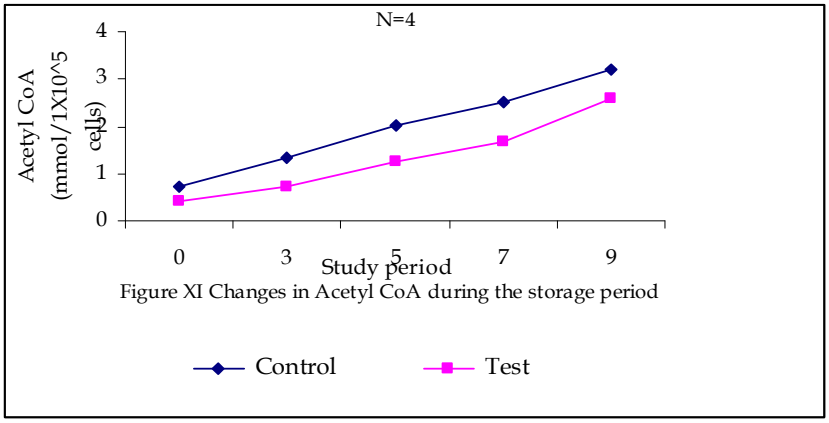


Figure XI Changes in Acetyl CoA during the storage period

Changes in LDH release during the storage period.

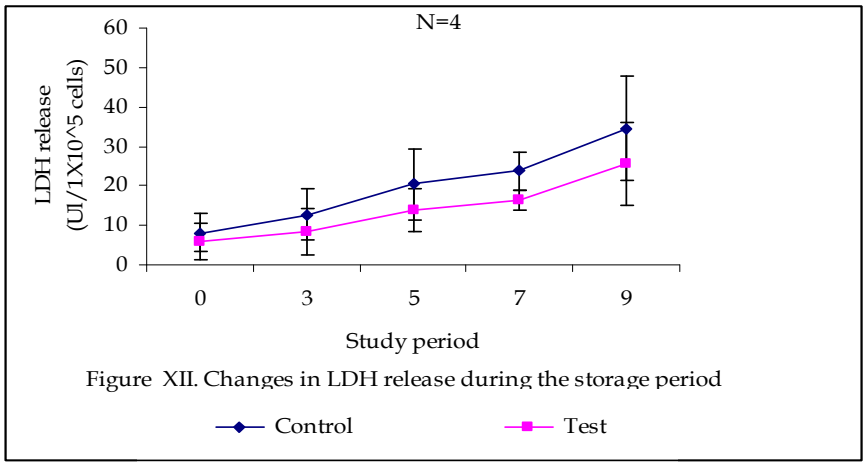


Figure XII. Changes in LDH release during the storage period

Changes in B thromboglobulin release during the storage period.

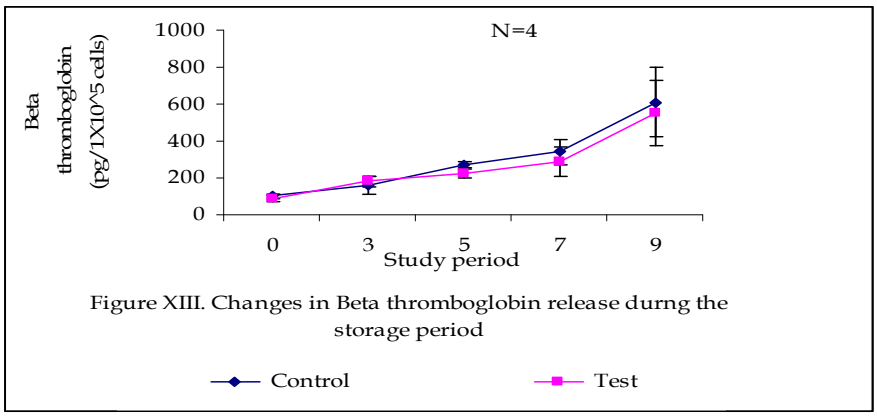


Figure XIII. Changes in Beta thromboglobin release durng the storage period

Changes in Serotonin release during the storage period.

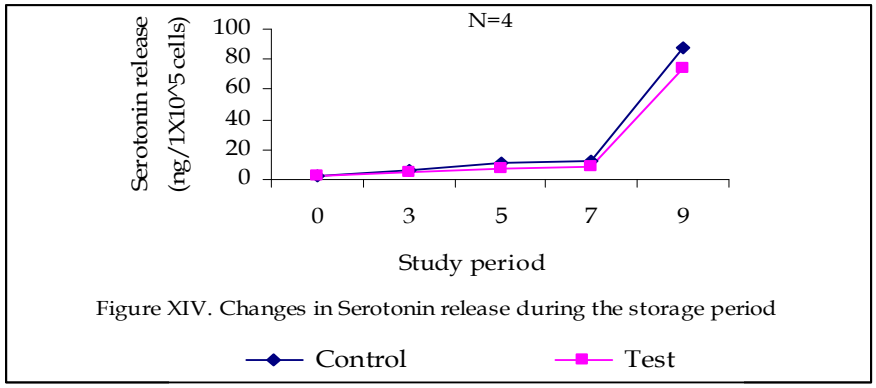


Figure XIV. Changes in Serotonin release during the storage period

Changes in aggregation during the storage period.

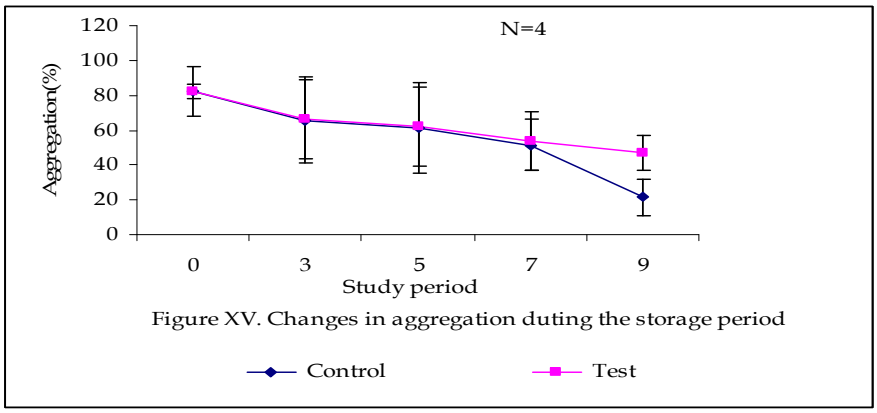


Figure XV. Changes in aggregation duting the storage period

Changes in morphology during the storage period.

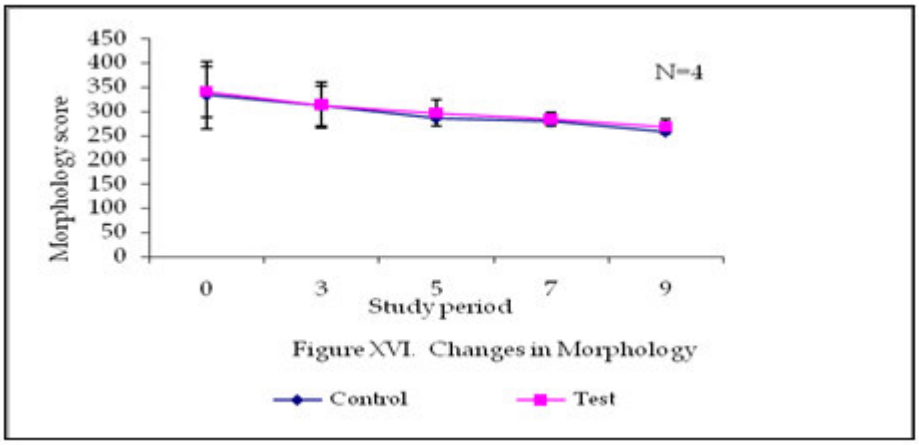


Figure XVI. Changes in Morphology

The platelet count in the test and control bags showed a decrease with storage time and the decrease was lower in the case of the test bags. The partial pressure of oxygen maintained a steady increase throughout the period of study indicating that the containers ensured adequate oxygenation of the platelet concentrates. The bicarbonate level in the plasma varied from 3 – 7 mEq x 10^5 which indicates good buffering potential in both test and control bags even on

the seventh day of storage. pH is an indicator of the overall balance between aerobic and anaerobic glycolysis, production of bicarbonate buffer, and escape of CO_2 from the system and has to be maintained above 6.4. In these studies, the pH was maintained at 6.7 – 6.9 on the 7^{th} day of storage in both test and control bags. The trend in glucose consumption in the test and control bags indicates good oxidative activity of the platelets. The Pyruvate level had a slow increase with days of storage, which was however less in the case of the test bags – indicating the maintenance of a healthy level of the oxidative process. This observation is supported by the gradual increase of the acetyl coenzyme A. The ATP levels remained almost steady in both test and control bags indicating good balance between the aerobic and anaerobic pathways.

Thromboglobulin release was low in these studies for both test bags and controls for up to seven days. Thromboglobulin is present in the cytoplasm of platelet cells and its presence in the plasma at high levels is indicative of cell damage. No cytochrome C release observed in the test and control bags indicating no damage to the mitochondria of platelets. The serotonin and LDH release were low in these studies indicating that the platelet cell membranes maintained their viability during the period of study. Distinct increase in these parameters was observed after 7 days storage. The change in hypotonic shock response, an indicator of the ability of platelets to recover from the shock of exposure to hypotonic medium was very similar in the test and control bags, The morphology scores relating to the change of shape of platelets from disc to spheres on storage was similar in test and control bags indicating favourable storage environment. Platelet aggregation is a key indicator of the ability of platelets to aggregate under critical conditions. In these studies the platelet aggregation was maintained well for up to seven days of storage after which a distinct fall was observed in the control bags. The energy requirements of platelets involve both oxidative metabolism and anaerobic glycolysis depending on the availability of oxygen. Since the anaerobic pathway is less efficient for ATP generation, adequate aerobic metabolism is very crucial for optimum maintenance of platelet functions. In these studies the test bags were made of a special formulation in which ultra high molecular weight PVC of K value 90 was plasticised with the nonphthalate plasticizer - 1,2 cyclohexane dicarboxylic acid di isononyl ester(DINCH), stabilizers and lubricants (TPL compound 206). The results presented in these studies indicate that the containers had adequate permeability to oxygen to maintain optimum levels of oxygenation of the platelets and also ensured adequate carbondioxide partial pressure within the container for creating appropriate levels of the bicarbonate buffer to maintain the viability of platelets for up to seven days. Statistical analysis of the experimental data for both test and control were done by the paired T test method. The following parameters are found to be statistically significant between control and test bag - Lactate, Glucose, Pyruvate, Bicarbonate, Acetyl CoA, ATP, LDH release. In all these studies the actual values for the test bag is less compared to the control bag, which means that test bag is better than control bag. Other study parameters were not significantly different. This study highlights the effectiveness of DINCH plasticized UHMW PVC bags of capacity 1200ml for the storage of six units of platelets. The level of platelets used in this study varied between 4.7 – 5.7 X 10^{11} per bag of 1200 ml capacity. The only reference to the use of Ultra High Molecular Weight PVC for platelet storage is US patent (to Carmen etal) in which the PVC plasticized with TEHTM was used for the storage of platelet concentrates[24].

CONCLUSION

The present studies show that the Platelet storage bags of capacity 1200ml made of PVC plasticised with the non phthalate, non aromatic non toxic plasticiser DINCH was suitable for the storage of platelet concentrates collected from six units of blood for up to 7 days. The performance of the test bags was superior to that of the control bag studied which was made of PVC plasticised with n-butyl, tri, n- hexyl

citrate. The test bags have the additional advantages that they have very low odour, are non allergenic, and have low leachability into blood plasma. In the studies reported herein pooled platelets from six units of blood with an average count of 8-9X10 ^{10} platelets were used in the test and control. The upper limit for the storage of platelets have to be determined.

SUMMARY

This paper presents the results of our studies on the storage of six units of platelet concentrates prepared by the buffy coat method in 1200 ml capacity containers made using PVC of K-value 90 plasticised with 1,2 Cyclohexane di carboxilic acid, di isononyl ester. A well known platelet pooling bag of capacity 1200 ml, plasticised with BTHC was used for comparison. In each study, blood was collected from six donors of the same group and separated into RBC, plasma and buffy coat. The buffy coats were pooled and the platelet rich plasma separated by centrifugation, filtered to remove leukocytes and collected in the bags under test and stored in a platelet agitator maintained at 22°C. 16 nos of heamatological and biochemical parameters were studied at 1,3,5,7 and 9 day intervals. The results showed that the containers ensured sufficient oxygen permeability to maintain an oxidative metabolism for the platelets. The stored platelets retained their viability for seven days and there was no indication of morphological or functional deterioration of the platelets on storage. The studies clearly show that containers of capacity 1200ml made using higher molecular weight PVC, plasticised with the non DOP plasticiser DINCH was well suited for the storage of at least six units of pooled platelets for seven days.

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