SCIENTIFIC ARTICLES

EFFECTS OF INTERRUPTED POWER SUPPLY **on the viability of SAGM and CPDA1 stored erythrocytes**

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ABSTRACT

BACKGROUND
Benefits of stored red cell transfusion depend on minimal change of viability during storage. Viability depends mainly on storage medium and temperature.

MATERIALS AND METHODS
Two whole blood units collected into SAGM and CPDA1 blood bags from two volunteer donors were each aseptically divided into three transfer bags and one from each transfer bag was placed in each of three separate blood banks maintained at 4 °C. Controlled temperature fluctuations were produced by switching off electricity supply to blood banks 2 and 3 at scheduled intervals. Osmotic Fragility of red cells in each bag was determined weekly on days 1 to 35. Mean Cell Fragility (MCF) of each red cell lot was calculated from Osmotic Fragility Curves and analyzed.

RESULTS
Mean ±SD of MCF of red cells increased with days of storage and worsened with storage temperature fluctuations. Statistically significant differences existed in average MCF of red cells stored in SAGM or CPDA1 with fluctuations in storage temperatures but not in average MCF of red cells stored in SAGM and CPDA1 at same storage temperatures.

CONCLUSION
Storage temperature fluctuations affect viability of stored red cells. Viability in SAGM or CPDA 1 medium remained almost the same at the same storage temperatures.

INTRODUCTION

The beneficial effects of transfusion of stored red cells depend on the maintenance of normal viability of the cells during storage. Red blood cells (RBC) must be stored in an appropriate medium and at appropriate temperature within an acceptable shelf life to have a beneficial effect after transfusion. Different storage media or preservatives are available for storing blood and its components. For whole blood storage, ACD (Acid Citrate Dextrose), CPD (Citrate Phosphate Dextrose), CPDA (Citrate Phosphate Dextrose Adenine) have been used successfully with storage shelf life of 21, 28 and 35 days respectively at 2-6^{0}C. [1] For red blood cells storage, ACD, CPD and CPDA are still being commonly used in developing countries but superior additive solutions like SAGM (Saline Adenine Glucose Mannitol), ADSOL or NUTRICEL are currently used in the advanced transfusion services of developed nations to store plasma depleted red cells at 2-6 ^{0}C or 35-42 days.[2]

While the beneficial effects of RBC transfusions have been demonstrated universally, some recent clinical studies have indicated an inverse relationship between clinical outcomes and RBC transfusions.[3,4] Because of these adverse occurrences, much attention has been shifted on the quality and efficacy of stored blood used in transfusion medicine.[5] In fact, transfusion of damaged RBCs has been implicated as a cause of some observed complications of blood transfusions[4].

After two weeks of storage in low quality medium like Acid Citrate Dextrose (ACD) or Citrate Phosphate Dextrose (CPD) for example, a small percentage of red cells (<10%) are eliminated within the first 24 hours after transfusion while up to 25% are eliminated within the first few hours if cells had been stored for up to 4 weeks.[1]

Although biochemically, the rate of glycolysis in RBCs stored at 2-6^{0}C is greatly reduced, enough lactic acid is still produced to cause a progressive fall in pH which interferes with the functions of enzymes like hexokinase and phosphofructokinase. This in turn leads to further reduction in glycolysis which leads to depletion of ATP and slow but progressive loss of RBC viability. The fall in ATP and especially the total adenylates (i.e ATP+ADP+AMP) is associated with poor in-vivo survival of the transfused RBC.[6] Other biochemical changes such as reduction in 2,3diphosphoglycerate (2,3 DPG); and mechanical changes such as spherocyte formation also occur in stored RBCs that make them less functional in the immediate post transfusion period or susceptible to early destruction.

SAGM, ADSOL or NUTRICEL have been developed to improve viability of plasma-depleted red cells on storage. By maintaining both ATP and 2,3-DPG levels, they provide good red cell storage conditions and are now the commonly used preservative solutions for red cells in most developed countries.[2]

The superiority of SAGM, ADSOL and NUTRICEL over CPDA as red cell storage media has been investigated and confirmed by several authors[7,8]. With storage in additive solutions, the concentration of ATP remains level early in storage, peaks at about 2 weeks, but subsequently falls to less than 50% by week 6 of storage.[7,8]

With higher storage temperatures above 6^{0}C, there is an exponential increase in the deleterious effects of biochemical changes occurring during storage in preservative media.[9] Higher storage temperature increases the metabolic rate of RBCs, and causes a faster consumption of nutrients with earlier depletion of ATP and more rapid loss of viability of the cells.

Electricity supply in Nigeria is very unstable. The total electricity generating capacity of the six major power stations in Nigeria is 3,450 megawatts for the teeming population of 160 million people[10]. Inevitably there are frequent power cuts from rationing of the supply; hence most blood banks in Nigeria depend on alternative energy supply to maintain constant temperature in their facilities. The cost of maintaining constant electricity supply to the blood banks through the use of electricity generating set is exorbitant. So, blood banks are often left without power for various periods with the hope that normal storage temperature of 2-6^{0}C will still be maintained.
Although factors causing loss of RBC viability are now much better understood, no method exists of predicting precisely from in-vitro tests how a given sample of RBC will survive in the circulation. Estimation of post-transfusion survival therefore continues to play an essential role in the development of improved methods of RBC preservation. Deformability has been found to be the only in-vitro change which best correlates with in-vivo survival of RBC.[6] It is an important determinant of RBC life span in-vivo[11,12] and is a function of the visco-elastic properties of the membrane, the viscosity of the intra cellular haemoglobin mileu and the surface area to volume relationship of RBC.[13] RBC deformability can be assessed in different ways like viscosity measurement,[14] passage of red cells through micropipette[15], rate of centrifugal packing[16], filtration through micropore filters of known porosity.[17] Adaptation of the filtration method has been used successfully at our centre to assess deformability of stored normal and sickle haemoglobin erythrocytes.[18] There have been few reports of studies conducted to quantify losses in stored RBC viability due to fluctuations in storage temperature.

The purpose of this study was to investigate and quantify, by osmotic fragility test, the effect of temperature fluctuations due to interrupted electricity supply on viability of stored red cells. The study was also intended to compare the extent of viability loss due to temperature fluctuations of RBC stored in CPDA1 and SAGM.

MATERIALS AND METHODS

Study Site

This study was conducted at the blood bank of University of Ilorin Teaching Hospital (UITH), a tertiary health institution that serves the people of North Central Zone of Nigeria with population of about 5-6 million. Approximately five thousand blood units are handled per annum. In the UITH, as in many other hospitals in the country, electricity supply to the entire hospital is from the main grid of the national supply. However, the blood bank has its own dedicated electricity generating sets for use when there is failure of supply from the national grid. Changing electricity supply to the dedicated electricity generator in case of failure from the national grid sometimes takes hours due to so many logistic reasons, ranging from non-availability of fuel for the generator, delayed attention by the technical personnel in charge, or complete breakdown of the generator.

Blood Collections and storage

Two volunteer donors who consented to participate in this study were bled. A quadruple SAGM blood collection pack was used for the first donor. The blood was taken into the main pack, which contained standard CPD preservative. After light centrifugation, plasma and buffy-coat were separated and transferred into empty satellite pack. The latter was detached and used for preparation of platelet concentrate. To the red cells left in the pack were added 100 mL of SAGM medium, contained in another satellite pack. From this pack, SAGM-suspended red cells were aseptically transferred into two empty satellite packs to obtain equal volume of SAGM suspended red cells in three small packs which were detached and labelled, AA, AA2 and AA4.

The second donor was bled by collecting 440 ml of blood into the main pack of triple blood bag system containing CPDA1. Blood was transferred into the other two bags to obtain three bags with equal volume. They were detached from each other and labelled BB, BB2 and BB4.

AA and BB, AA2 and BB2, AA4 and BB4 were stored in three different blood banks 1, 2 and 3 respectively. Constant electricity supply was maintained in the three blood banks throughout the study period, with a dedicated generating set which was automatically switched on immediately in the event of power cut. Blood bank 1 was maintained at a temperature of 2 - 6 °C (average 4.8 °C) throughout the study period. Electricity supply to Blood bank 2 was switched off for two hours at midday everyday while that to blood bank 3 was switched off for four hours at midday everyday throughout the study period. Average temperature in blood bank 2 at the end of two hours switch off was 7.2 °C, while it was 9.8 °C for blood bank 3 at the end of each four hours switch off. The temperature in both banks 2 and 3 returned to the normal basal temperature of 2 - 6 °C within three hours of switching back at the end of the power interruption.

Osmotic Fragility Test (OFT)

In this study, osmotic fragility test was used to assess the red cell viability changes. OFT was performed and recorded on red cells in each bag on days 1, 8, 15, 22, 29 and 35.

On the day of OFT, small aliquot of red cells was expressed through the pilot tube of each bag into empty sterile specimen bottles and labelled appropriately and the bags immediately returned to their respective blood banks.

Eleven saline solutions (9g/L, 7.5 g/L, 6.5 g/L, 6.0 g/L, 5.5 g/L, 5.0 g/L, 4.0 g/L, 3.5 g/L, 3.0 g/L and 2.0 g/L) were prepared. Six rows of twelve tubes, one row for each sample were set up. 5 ml each of the eleven saline solutions was delivered into eleven tubes of each row and 5 ml of water was dispensed into tube 12.50µl of blood from each sample was added to the twelve tubes and mixed immediately by inverting the tubes several times and allowed to incubate at room temperature (average 25^0C) for 30 minutes. The contents were mixed again and lightly centrifuged for 5 min at 1200g and the supernatant was transferred into other sets of sterile bottles and properly labelled. Optical density (OD) in each tube was determined using spectrophotometer at wavelength of 540nm. Percentages or degrees of haemolysis in each tube was calculated by assigning 100% haemolysis to OD of the tube containing distilled water and red cell concentrate. Osmotic Fragility Test curves were plotted and Mean Corpuscular Fragility (MCF) or P50 in g/L (the concentration at 50% lysis) was derived from the OFT graph for red cells in AA, AA2, AA4, BB, BB2 and BB4 on days 1, 8, 15, 22, 29 and 35.

Interpretation of OFT Results

Normal RBCs can withstand considerable hypotonicity because of the biconcave shape which allows the cell to increase its volume by about 70% before the surface membrane is stretched and the limit reached to cause lysis.

MCF above the normal range of 4.0-4.45 g/L indicates a greater concentration of normal saline at lysis, a reduced ability to withstand hypotonicity and a greater osmotic fragility of RBC. MCF changes are presumed to be due to RBCs membrane alterations during storage[6,13] and are determined by storage medium characteristics and storage temperature.

DATA ANALYSIS: The analysis of MCF data in Table 1 was performed using R 3.1.0 (www.cran.org) and IBM SPSS 20 statistical packages. Mean and standard deviations of MCF were calculated for Pair-wise. Comparisons of the average MCF within each group (SAGM or CPDA1) and between the two groups (SAGM and CPDA1) were performed using the paired t-test and two independent samples t-test respectively. Under the two methods, significant difference in the average MCF compared was determined at 5% level.

RESULTS

TABLE 1: Mean Corpuscular Fragility, MCF g/L (P_{50}) of red cells stored in SAGM and CPDA1 without and with electricity interruptions.

Storage Media	Blood Bag	Mean Cell Fragility (P50) in g/L						Mean ± SD MCF g/L
		Day 1	Day 8	Day 15	Day 22	Day 29	Day 35	
SAGM	AA	4.1	4.1	4.3	4.2	4.4	4.8	4.32 ± 0.26
	AA2	4.2	4.1	4.5	4.6	4.8	5.1	4.55 ± 0.37
	AA4	4.1	4.4	4.8	4.9	5.0	5.3	4.75 ± 0.43
CPDA	BB	4.0	4.3	4.3	4.5	4.6	4.8	4.42 ± 0.28
	BB2	4.1	4.4	4.5	4.7	4.9	5.4	4.67 ± 0.45
	BB4	4.1	4.6	4.8	4.9	5.1	5.6	4.85 ± 0.50

Results in this study generally showed that the Mean Cell Fragility increased progressively with duration of storage in all the samples (Table 1). The mean ±SD of MCF g/L of red cells stored in SAGM with uninterrupted power supplies (AA), two hour interrupted power supplies per day (AA2), and four hour interrupted power supplies per day (AA4) were 4.32±0.26 g/L, 4.55±0.37 g/L and 4.75±0.43 g/L respectively, while for red cells stored in CPDA1, the mean ± SD of MCF g/L were 4.42±0.28 g/L, 4.67±0.45 g/L and 4.85±0.47 g/L with uninterrupted power supplies (BB), two hour interrupted power supplies per day (BB2) and four hour interrupted power supplies per day (BB4) respectively. The increments in MCF with storage days during electricity interruptions appeared to be more pronounced, but not statistically significantly so, in red cells stored in CPDA1 (see Table 2).

TABLE 2: Paired Samples Test of differences in average MFC within and between groups of stored red cells

Paired Samples Test of Average MCF Differences

		Paired Differences					T	d.f.	P-value
		Estimated Means' Difference	Std. Deviation of Means' Difference	Std. Error of Means' Difference	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	AA/ AA2	-.23333	.16330	.06667	-.40471	-.06196	-3.500	5	0.017
Pair 2	AA/ AA4	-.43333	.25033	.10220	-.69604	-.17062	-4.240	5	0.008
Pair 3	BB/ BB2	-.25000	.18708	.07638	-.44633	-.05367	-3.273	5	0.022
Pair 4	BB/ BB4	-.43333	.23381	.09545	-.67870	-.18797	-4.540	5	0.006
Two Independent Samples Test of Average MCF Differences									
Pair 5	AA/ BB	-0.1000	0.1549	0.0632	-0..4492	0.2492	-0.638	10	0.538
Pair 6	AA2/ BB2	-0.1167	0.5844	0.2386	-0.6484	0.4150	-0.489	10	0.635
Pair 7	AA4/ BB4	-0.1000	0.1265	0.2702	-0.7020	0.5020	-0.370	10	0.719

Results from paired t-tests for two related samples in Table 2 showed significant differences in average viabilities (MCF) among red cells stored in the same medium at different degrees of power interruption compared with viabilities (MCF) at no power interruption, (AA/AA2, AA/AA4, BB/BB2, BB/BB4) with p-value =0.017 for AA/AA2, p-value=0.008 for AA/AA4, p-value=0.022 for BB/BB2 and p-value=0.006 for BB/BB4 comparisons. The differences in average MCF of RBCs stored in the same medium at varying temperatures are clearly shown by the boxplots of MCF in Figure 1.

DISCUSSION OF RESULTS

Figure 1 shows the boxplots of MCF g/L for the six Blood Bags over 35 days of storage. There are significant differences in the average MCF (black horizontal line in each boxplot) in AA/AA2, AA/AA4, BB/BB2, and BB/BB4. No significant difference was observed in the average MCF in AA/BB, AA2/BB2 and AA4/BB4 as shown by the respective plots. There were statistically significant differences in the average MCF g/L of blood bag within each group as revealed by Figures 2 a, b, c and d and there were no statistically significant differences between the average MCF g/L of blood bags between the two groups of storage medium (SGM and CPDA) as shown by Figures 3 a, b, and c. Figures 4, 5 and 6, show Osmotic Fragility Curves of AA and BB at day1; AA, AA2 and AA4 at day 35 and BB, BB2 and BB4 at day 35 respectively. A progressive shift to the right (increase in MCF) in the Osmotic Fragility Curves was noticed from day 1 to day 35 for red cells stored in both SAGM and CPDA1.

FIGURE 1: Boxplots of MCF g/L for the six Blood Bags over thirty five days of storage showed remarkable differences in the median MCF (black horizontal line in each box plot) in AA/AA2, AA/AA4, BB/BB2, and BB/BB4. No significant difference in the mean MCF in AA/BB, AA2/BB2 and AA4/BB4 comparisons is observed as shown by the respective plots.

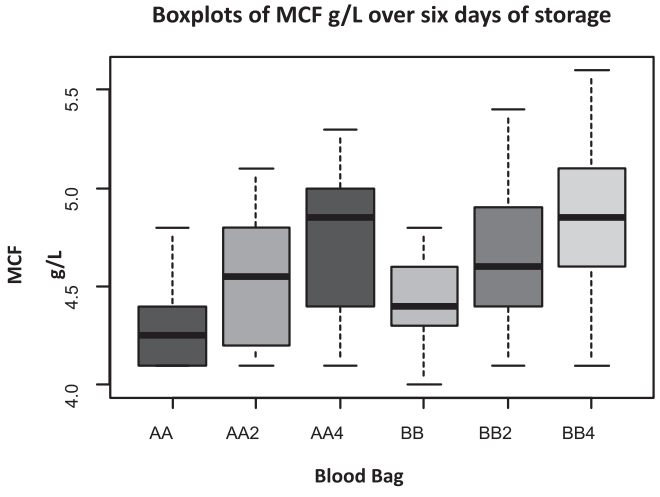


FIGURE 2: Line graphs of average MCF g/L over the storage days for SAGM and CPDA Blood bags. The graphs showed remarkable differences in the average MCF g/L of blood bags within SAGM (Figure 2a and b) and CPDA (Figure 2 c. and d.) blood bags.

However, the results of the two independent samples t-tests, as in Table 2, showed no statistically significant differences in the average MCF of red cells stored in different medium at the same degree of power interruption (p-value = 0.538 for AA/BB, p-value = 0.635 for AA2/BB2, p-value = 0.719 for AA4/BB4). These results are clearly shown by the line graphs of average MCF g/L of each pair of blood bags over storage period as presented by Figure 3.

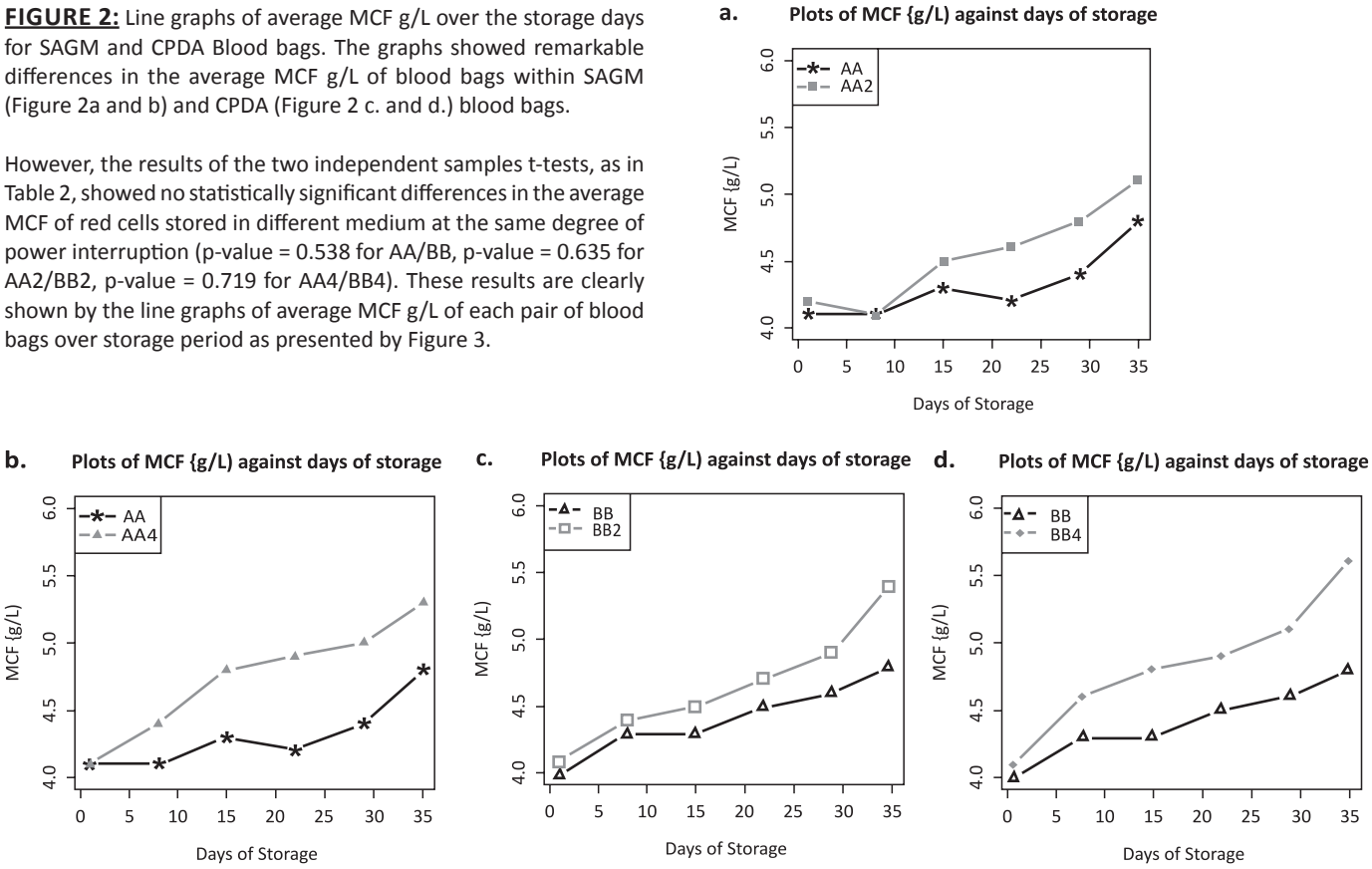
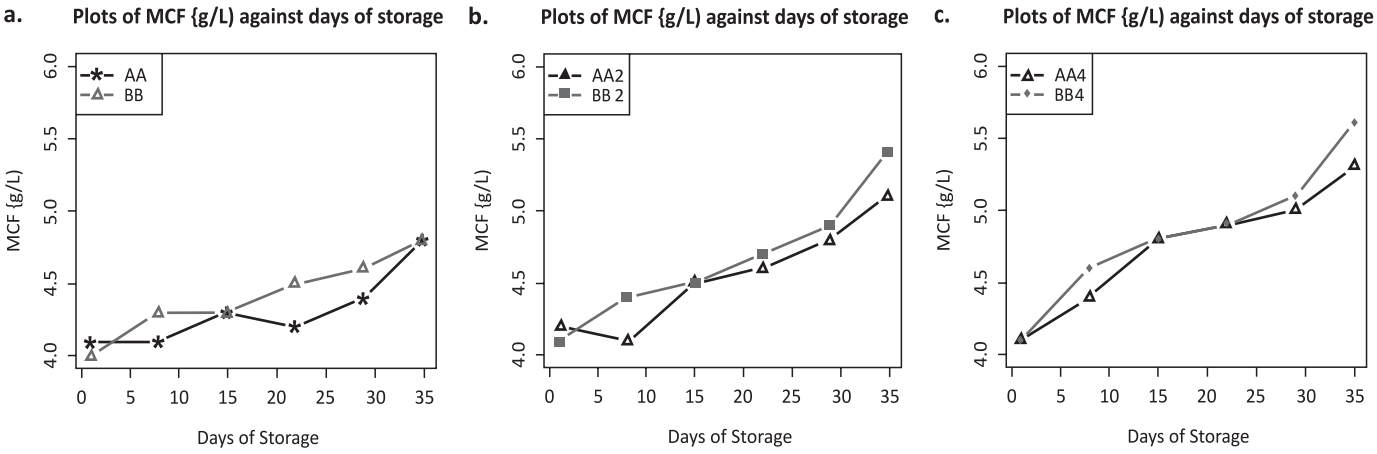


FIGURE 3: Line graphs of average MCF g/L over the storage days for SAGM and CPDA Blood bags. The graphs showed no remarkable difference in the patterns of the average MCF g/L of blood bags for each pair AA/BB, AA2/BB2 and AA4/BB4 over the thirty five days storage periods.



The results of this study confirm the earlier knowledge of progressive loss of red cell viability with increasing days of storage[1]. What the study also now confirms is the significant influence of storage temperature fluctuations on rate of loss of viability of stored red cells. Loss of red cell viability is common but its severity or degree is said to differ in different red cells storage media.[19,20] In our study there was progressive loss of red cell viability in both SAGM and CPDA 1 which was only slightly more pronounced in CPDA 1 at normal (4.8 °C) and interrupted (7.2 °C and 9.8 °C) storage temperatures. Thus, the superiority of SAGM over CPDA as previously reported[7,8] was not conclusively confirmed in this study.

As there are few reports of studies of the effects of storage temperature fluctuations on stored red cell viability, our findings may need to be confirmed by further studies possibly using other measures of red cell viability.

FIGURE 4: Osmotic Fragility Curve of AA and BB at day 1.

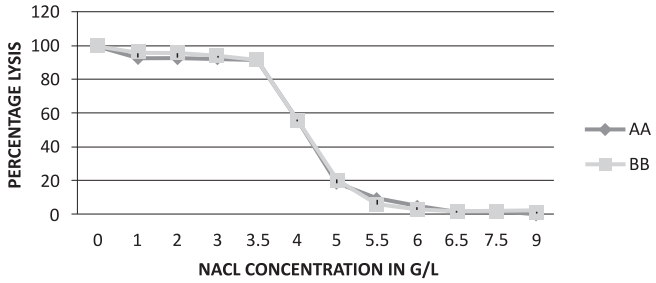


FIGURE 5: Osmotic Fragility Curve of AA, AA2 and AA4 at day 35.

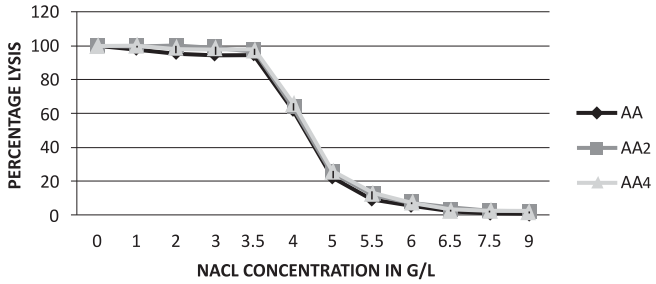
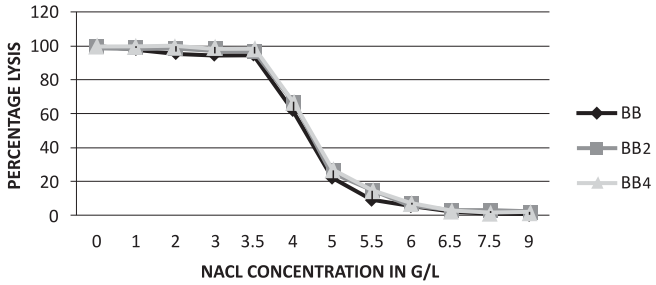


FIGURE 6: Osmotic Fragility Curve of BB, BB2 and BB4 at day 35.



CONCLUSIONS

From the results of this study, storage temperature fluctuations do significantly affect the viability of stored red cells. Optimal or expected benefits can therefore not be obtained when stored red cells that have suffered temperature fluctuations during storage are transfused to patients. Differences between the loss of viability of red cells stored either in SAGM or CPDA 1 are not pronounced, provided the temperature of storage is kept constant.

RECOMMENDATIONS

In spite of the unstable public power supply in Nigeria and possibly in other developing countries, every effort must be made to prevent power supply fluctuations in our blood banks. Only blood banks fitted with temperature fluctuation alarms should be used. Stand-by generators should always be in working condition, and be adequately fuelled. Frequent opening and closing of blood banks or cold rooms should be avoided during power cuts. However, where power fluctuations persist, it may be necessary to study, and set the expiry date of our units of stored red cells at a level shorter than is recommended for the respective storage media.

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A Shittu was the principal investigator of this study. He carried out the laboratory work as well as drawing of osmotic fragility curves and calculation of MCF. H.O.O was also involved in the laboratory works and in the reading of Optical Density with the spectrophotometer and calculation of percentage lysis from osmotic fragility curves. W.B.Y analysed the data, while J.O.A. designed the study and supervised the procedures.

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