The New Generation of Platelet Additive Solution for Storage at 22°C: Development and Current Experience

Juergen Ringwald, Robert Zimmermann, and Reinhold Eckstein

The storage of platelets (PLTs) in PLT additive solutions (PASs) might have several advantages. It can reduce allergic and febrile transfusion reactions, facilitate AB0-incompatible PLT transfusions, enable pathogen inactivation, and make more plasma available for other purposes (eg, for fractionation). For this reason, there has been considerable focus on the development of new PASs that assure maintenance of good PLT quality throughout storage. Several compounds in PASs such as citrate, acetate, phosphate, potassium, and magnesium have all turned out to be important, and the same applies to the necessary amount of glucose as determined by the plasma carryover. The latest generation of PASs, the modified PAS-III and Composol-PS, contains most or all of these compounds. Recently published data on the in vitro quality of either buffy coat– or apheresis-derived PLT concentrates stored in 70% or even 80% of PAS might encourage transfusion specialists to consider using these PASs in routine blood banking. However, because in vitro tests do not adequately predict clinical effectiveness of PLTs after transfusion, in vivo studies are still needed to assess the quality of PAS-stored PLTs.

CURRENT ATTEMPTS TO STORE PLATELETS IN PLATELET ADDITIVE SOLUTIONS

The idea of storing PLTs in plasma-free artificial media emerged in the 1950s in conjunction with early attempts in PLT preservation. Tullis' salt solution with acetate and gelatin or a phosphate-buffered salt solution containing glucose and some plasma were used in trials storing PLTs at 4°C.4,5 In 1985, Rock et al.6 studied the storage of PLTs concentrates derived from PLT-rich plasma in a modified Tyrode's medium at room temperature. For units containing approximately 15% residual plasma, the in vitro quality of PLTs after 72 hours of storage was similar to those with 100% plasma. One year later, Adams et al.7 published promising results of a trial investigating 5-day storage of PLTs in a medium containing citrate and dextrose in combination with Plasma-Lyte A (Table 1). Again, 1 year later, Holme et al.8 reported a significant improvement of in vitro characteristics and in vivo recovery and survival for PLTs stored in a different PAS containing glucose and bicarbonate. However, the presence of glucose in PAS is associated with a manufacturing problem. Caramelization of glucose occurs when heat sterilization is performed at neutral or alkaline pH. To overcome this problem, an acid pH of the glucose-containing solution is required. Unfortunately, acid pH is not conducive for PLT storage. Therefore, glucose-free solutions
were preferred by most investigators in various consecutive trials. In 1988, the results of a study by Adams and Rock⁹ indicated that only a very small amount of glucose from plasma carryover is sufficient to support PLT metabolism for up to 5 days. This finding was supported by Rock et al¹⁰ in 1991 using Plasma-Lyte A with a plasma carryover of only 11% to 17%. However, in the same year, Murphy et al¹¹ showed less encouraging results for a simple PAS containing only a few components.

Hence, Shimizu et al¹² decided to again use a glucose-containing PAS and tried to overcome the described manufacturing problems. To prevent the caramelization of glucose, they autoclaved their PAS, called Setosol (Table 1), in a nitrogen atmosphere at neutral pH. Although the quality of PLTs derived from PLT-rich plasma, stored for 5 days in Setosol with only 10% plasma carryover, was very promising, this approach has not been investigated further.

**PLATELET METABOLISM: WHICH FUEL IS NEEDED?**

As the lactate production caused by glycolysis was supposed to be the major cause for pH fall in stored PCs, the absolute need for glucose in a storage media was questioned. During storage at room temperature, PLTs use the anaerobic pathway of glycolysis for energy production only to a minor extent, up to approximately 15%, whereas the main part of the required energy (85%) is generated through the oxidative pathway of the tricarboxylic acid cycle.¹³ The major substrates for this pathway are free fatty acids representing the formerly described “unidentified endogenous fuel.”¹⁴ However, because of the intentionally low plasma carryover in PAS-containing PCs, one would expect only small amounts of free fatty acid to be available for PLT metabolism. Therefore, it was suspected that other substrates had to be fueling the oxidative pathway. The most important of these was found to be acetate (Fig 1).¹⁴ Whisson et al¹⁵ showed that washed PLTs can be stored for up to 72 hours in glucose- and plasma-free media containing acetate, which was the only available fuel for PLT metabolism. However, Holme¹⁶ found that acetate in PASs actually reduced the need for glucose during storage but was incapable of maintaining full PLT function and energy levels. Furthermore, Gulliksson¹,³ observed that the complete depletion of glucose is associated with PLT dysfunction despite a normal pH. Taken together, these findings led to the conclusion that for successful PLT storage, the presence of glucose seems to be necessary during the entire storage period.

**THE BUFFERING CAPACITY OF PLATELET ADDITIVE SOLUTIONS**

The glycolytic breakdown of glucose results in the production of lactate and hydrogen ions and may lead to an acidification of the PCs with a negative impact on PLT quality. This occurs when the production of hydrogen ions exceeds the buffering capacity of the medium. PAS-containing PCs have a rather low buffering capacity due to their low plasma content and the subsequently associated low concentration of bicarbonate,

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**Table 1. Composition of Different PASs**

<table>
<thead>
<tr>
<th></th>
<th>Plasma-Lyte A</th>
<th>PAS (1)</th>
<th>PAS</th>
<th>PSM1 pH</th>
<th>Setosol</th>
<th>Composol</th>
<th>PAS-II</th>
<th>PAS-III</th>
<th>PAS-IIIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>90.0</td>
<td>70.0</td>
<td>110.0</td>
<td>98.0</td>
<td>90.0</td>
<td>90.0</td>
<td>115.5</td>
<td>77.3</td>
<td>69.3</td>
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<tr>
<td>KCl</td>
<td>5.0</td>
<td>10.0</td>
<td>5.1</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
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<td>–</td>
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<tr>
<td>CaCl₂</td>
<td>–</td>
<td>–</td>
<td>1.7</td>
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<td>–</td>
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<tr>
<td>MgCl₂</td>
<td>3.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3.0</td>
<td>1.5</td>
<td>–</td>
<td>–</td>
<td>1.5</td>
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<tr>
<td>MgSO₄</td>
<td>–</td>
<td>0.8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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</tr>
<tr>
<td>Na₃ citrate</td>
<td>–</td>
<td>30.0</td>
<td>15.2</td>
<td>23.0</td>
<td>17.0</td>
<td>11.0</td>
<td>10.0</td>
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<td>Citric acid</td>
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<td>NaHCO₃</td>
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<td>2.7</td>
<td>25.0</td>
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<td>–</td>
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<tr>
<td>Na phosphate</td>
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<td>5.0</td>
<td>2.7</td>
<td>25.0</td>
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<td>–</td>
<td>–</td>
<td>28.2</td>
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<td>27.0</td>
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<td>–</td>
<td>–</td>
<td>23.0</td>
<td>27.0</td>
<td>30.0</td>
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<tr>
<td>Na gluconate</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>23.0</td>
<td>–</td>
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<tr>
<td>Glucose</td>
<td>–</td>
<td>35.5</td>
<td>–</td>
<td>–</td>
<td>23.5</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Maltose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>28.8</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Mannitol</td>
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<td>–</td>
<td>–</td>
<td>–</td>
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**NOTE.** All values are expressed as mmol/L.

Abbreviation: PSM1, platelet storage medium 1.
the most important buffer system in human plasma. Therefore, it is of special importance for PAS-containing PCs that the glycolytic activity should be reduced to a minimum during storage. Attempts to increase the buffering capacity of PASs by adding bicarbonate were not successful. As PLTs are stored at nearly neutral pH, this brings the bicarbonate into equilibrium with CO₂, and this, in turn, is capable of easily escaping the gas permeable bags. An alkaline pH would be necessary to prevent this. Holme et al.17 tried to solve this problem by producing 2 separately stored parts of a PAS, one with an acid pH-containing glucose and one with an alkaline pH-containing bicarbonate. Those 2 parts were mixed immediately before usage for PLT storage. Although they obtained good PLT quality during subsequent storage, their approach has not been further investigated. This was most likely because of the need for the rather complicat-ed preparation of the PCs using this technique.

THE MOST IMPORTANT COMPOUNDS IN PLATELET ADDITIVE SOLUTIONS

Gulliksson and colleagues18,19 studied the impact of citrate, acetate, and phosphate on PLT metabolism and on the buffering capacity of PAS-containing PCs. They were able to show that PLTs stored in a sodium chloride solution with 8 mmol/L of citrate produced only half the quantity of lactate compared with PLTs in a similar solution with approximately 20 mmol/L of citrate.18 Nonetheless, lowering of the citrate concentration to levels below 8 mmol/L should be avoided as this might give rise to clotting problems. Acetate, however, was found to be able to reduce the high lactate production at a citrate concentration of 20 mmol/L to the same rate found at a citrate concentration of 8 mmol/L. Beside the ability to reduce lactate formation, acetate has a further alkalinizing effect in that it acts as a buffer substance. A hydrogen ion is required for the oxidation of acetate before it is metabolized as acetyl-Co-A in the tricarboxylic acid cycle (Fig 1).20 Against this background, a PAS containing 10 mmol/L citrate and 30 mmol/L acetate, called PAS-II, was composed. Using this solution for PLT storage, Gulliksson et al.21 made another important observation. Specifically, they found significantly lower values for adenine nucleotide levels in apheresis-derived PCs using phosphate-free acid citrate dextrose as anticoagu-lant compared with those in buffy coat–derived PLTs stored in phosphate-containing citrate.
phosphate dextrose. In a subsequent study, they investigated apheresis-derived PLTs stored either in PAS-II or in PAS-II with additional phosphate (PAS-III) and were able to show that higher adenine nucleotide levels were indeed associated with the presence of phosphate in the PCs. Consequently, some authors concluded that the presence of phosphate in PAS might not be desirable. However, the higher ATP levels in PAS-III containing units in combination with the fact that higher ATP levels are associated with a higher PLT viability led Gulliksson et al to the conclusion that phosphate is an important ingredient in PAS and should therefore be regarded as one cornerstone of modern PASs. Nowadays, phosphate-containing PAS-III is mainly used in the context of the photochemical treatment process of pathogen inactivation of PCs with a plasma carryover of approximately 35%.

THE ADDITION OF MAGNESIUM AND POTASSIUM TO PAS-III

A few years ago, the impaired in vitro function of PLTs stored in 65% to 70% PAS-III compared with PLTs stored in 100% of plasma was discussed among the members of the Biomedical Excellence for Safer transfusion (BEST) group of the International Society of Blood Transfusion. They suggested that the inclusion of magnesium and potassium chloride in PAS-III might help further improve the quality of the stored PLTs. This assumption was based on several reasons. First, trials with PLTs stored in the very early PASs, containing those 2 electrolytes by accident, had previously shown good PLT quality results already. Second, several authors had described an inhibiting effect of magnesium and/or potassium on PLT activation and aggregation in the past. Furthermore, for PAS-II with additional potassium and magnesium, a positive effect had also been demonstrated before. In addition, the members of the BEST group hypothesized that such an alteration of PAS-III might allow storage of PLTs at a plasma carryover as low as 20%. Consequently, the new PAS-III with additional magnesium and potassium, called modified PAS-III (PAS-IIIM), was investigated for the first time in a pilot study comparing the in vitro quality of PLTs stored in 70% or 80% PAS-III, 70% or 80% PAS-IIIM, or 100% plasma. This small study consisting of 6 buffy coat- and 5 apheresis-derived PCs had primarily 2 important outcomes. The in vitro quality of the PLTs stored in PAS-IIIM was significantly improved compared with the quality of PLTs stored in PAS-III. Furthermore, the results were similar for PLTs stored in 70% or 80% of PAS-IIIM, whereas for PAS-III–stored PLTs, the in vitro quality was significantly more impaired when using 80% instead of 70% of PAS-III. This indicated that a plasma carryover of only 20% might be sufficient to maintain PLT in vitro quality during a 7-day storage when PAS-IIIM is used.

FURTHER EVALUATION OF PAS-IIIM

In the next step, the BEST group wanted to confirm the promising findings of the pilot study in a large international multicenter study. However, because of a failure in the production of a batch of PAS-IIIM, all but 2 centers (Baden-Baden and Erlangen, Germany) received a PAS-IIIM with a wrong acetate concentration (6.12 g/L instead of 4.42 g/L). Therefore, the published results of the BEST multicenter study and the 2 descendants could not be used for an exact assessment of the originally described PAS-IIIM, which is now commercially available in Europe (SSP+, MacoPharma International, Langen, Germany). Just recently, 3 studies have been published using the original PAS-IIIM for 7-day storage of PLTs. As we had received the correct PAS-IIIM when participating in the multicenter study of the BEST group, we were able to confirm the findings of the pilot study for our apheresis derived PCs completely. This was slightly different for Van der Meer et al comparing metabolic parameters of buffy coat–derived PCs stored in 70% or 80% PAS-IIIM, 70 or 80% Composol-PS, or 70% PAS-III. Although they also found superior results for PLTs stored in PAS-IIIM compared with PLTs stored in PAS-III, they obtained inferior results for PLTs stored with a plasma carryover of only 20%. This finding was similar for PAS-IIIM as well as for Composol-PS–stored PLTs. Therefore, further studies are needed before a definite conclusion can be drawn on whether 7-day storage of PLTs in...
those 2 PASs is feasible with plasma carryovers of only 20%.

**PAS-IIIM AND COMPOSOL-PS: THE LATEST GENERATION OF PLATELET ADDITIVE SOLUTIONS**

Given the recently published promising data on the in vitro quality of PLTs stored in PAS-IIIM or Composol-PS, these PASs may be of special interest for blood bankers already producing PAS-stored PCs or planning to produce such PCs in the near future. Moreover, an analysis of the currently very scarce data on comparisons of PLT storage in the above-mentioned PASs could be of particular interest, as both PASs differ qualitatively in only 2 compounds (Table 1). Composol-PS is a phosphate-free PAS containing gluconate, whereas PAS-IIIM contains phosphate but no gluconate. Although counteracting influences on PLT metabolism are known for phosphate,19 this remains open for gluconate. Some authors have shown that gluconate does not offer any benefits for PLT metabolism, and moreover, it shows insufficient buffering capacity.12,34

Van der Meer et al22 showed similar glycolytic activity for buffy coat–derived PLTs stored in either PAS. They found lower pH values in Composol-PS–containing units, which was primarily found on the lower basic pH of Composol-PS (7.0) compared with PAS-IIIM (7.2). However, this difference was of minor relevance as the pH values in Composol-PS containing units remained within an acceptable range throughout the storage period. Just recently, we obtained similar results for changes in pH values when comparing the in vitro quality of washed apheresis PCs with 48 hours postwash storage in Composol-PS or PAS-IIIM.35 The results for the other in vitro quality parameters investigated in this study were similar for the PLTs washed and stored in the 2 PASs except for a higher PLT activation rate for PLTs stored in Composol-PS. However, the PLT activation remained within an acceptable range, and therefore, we concluded that both PASs could be used for successful PLT washing.

Probably, the most interesting data on the comparison of 7 days PLT storage in 70% PAS-IIIM or Composol-PS were recently published by the BEST group.33 They investigated the effect of the interruption of agitation on PLTs’ in vitro quality parameters. Overall, they had shown that PLTs stored in 70% PAS-IIIM with a PLT concentration of approximately $1 \times 10^9$/mL can sustain 4 days without agitation. Compared with PLTs in PAS-IIIM, PLTs stored in Composol-PS showed lower pH values and impaired results for hypotonic shock response (HSR) when the agitation was interrupted for 2 or 4 days. Without the interruption of the agitation however, the results for HSR were similar in both PASs. Thus, the authors concluded that PLTs stored in Composol-PS show a poorer capacity to withstand a period without agitation than PLTs stored in PAS-IIIM. Furthermore, they stated that the beneficial effects of phosphate might outweigh the disadvantage of this compound of inducing glycolysis especially under stressful conditions such as the interruption of agitation. In summary, the data of the BEST group could be taken as an argument in favor of PAS-IIIM as the currently available PAS of choice. However, when considering this, the quandary of the in vitro testing in general has to be taken into account at this stage. It is quite difficult to define good and acceptable results of in vitro tests, given the uncertainty surrounding the correlation of these parameters with in vivo viability and hemostatic effectiveness of the PLTs. Therefore, only in vivo studies can give definite information on the definite quality of the PLTs after ex vivo storage in PAS-IIIM or in Composol-PS. Such studies are under preparation at present and might be available in the future.

**CONCLUSIONS AND OUTLOOK**

Ex vivo storage of PLTs in PASs with a low plasma carryover offers several advantages compared with storage of PLTs in 100% plasma. Especially for the latest generation of PASs, promising in vitro results for PLT quality were recently obtained. Nonetheless, whether PLT storage in these PASs is indeed equal to or even better than PLT storage in plasma still remains uncertain and under discussion. The comparison of functional in vitro quality parameters between PLTs stored in 70% of PAS-IIIM or more, and PLTs stored in 100% of plasma revealed still better results for the latter.2,29,35 However, the differences were rather small, and results for PLTs stored in PAS-IIIM were in acceptable ranges. Therefore, only comparative in vivo studies of PLTs stored either in 100% plasma or one of the latest PASs might help to find a definite answer to the question whether the slightly impaired in vitro
results for PAS-stored PLTs are indeed of clinical relevance. As long as these studies are outstanding, each blood banker considering PLT storage in PASs has to decide individually whether the known advantages of this method might outweigh the slightly impaired in vitro quality of PAS-stored PLTs. However, the improved in vitro quality of PLTs stored in PAS-IIIM or Composol-PS should at least encourage blood bankers already storing PLTs in PASs to substitute their currently used PAS by one of those PASs of the latest generation.

REFERENCES

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