Storage of buffy-coat-derived platelets in additive solutions: 
in vitro effects on platelets stored in reformulated PAS 
supplied by a 20% plasma carry-over

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Background and Objectives Platelet additive solutions (PAS) have been shown to be suitable for extended platelet (PLT) storage. Depending on the PAS formulation, the percentage of plasma carry-over contributes to success. Improving PLT quality by optimizing the composition of PAS may allow a reduction to be made in the amount of plasma carried over to the final unit. Reducing the proportion of plasma carried over would probably decrease transfusion of unwanted antibodies and make greater amounts of plasma available for other needs.

Study Design and Methods Platelets from eight pools of 25 buffy coats were aliquoted and prepared for storage in plasma and different PAS units: InterSol and three alternate PAS named PSM1, PSM2 and PSM3. All PAS units were supplied with a 20% plasma carry-over and stored at room temperature with agitation for 9 days with in vitro testing for metabolic, cellular and activation parameters.

Results During storage, PLTs stored in InterSol displayed significantly lower glucose concentration (P < 0.01), lower adenosine triphosphate levels (P < 0.01), a higher mean PLT volume (P < 0.01), a lower response to hypotonic shock response activity (P < 0.01) and a higher CD62P expression (P < 0.01) when compared with PLTs stored in plasma and PSM1–3 solutions. pH was maintained at > 6.8 (day 9) and swirling remained at the highest level (score = 2) for all units throughout storage.

Conclusion Our results suggest that PLTs stored in PAS with addition of magnesium, potassium and glucose (PSM2 and PSM3) and 20% plasma carry-over maintained metabolic and cellular characteristics, equivalent to PLTs stored in 100% plasma during 9 days of storage. Our results also suggest that presence of potassium in addition to magnesium or alternatively the concentration of phosphate as well as the supply of additional glucose to normal plasma levels improve in vitro data of PLTs stored in PAS.

Key words: additive solutions, platelets, storage.
current commercially available PAS required the carry-over of substantial (30%) amounts of plasma for success. These data underline the need for improving the PLT quality by optimizing the composition of PAS, which may allow a supplementary reduction to be made in the amount of plasma carried over.

We investigated novel additive solutions (Reformulated PAS solution; Fenwal, La Châtre, France) containing the main components: citrate, acetate, phosphate as well as potassium, magnesium, dextrose monohydrate (as additional substrate for PLT metabolism) and calcium. Citrate included in the storage medium affects the rate of glucose consumption and lactate production and is useful as an anticoagulant for the PLTs. Acetate serves as a second metabolic fuel and has the added benefit of providing a buffer effect by generating bicarbonate (10). Phosphate has two possible roles in PLT metabolism: acting as a buffer to prevent a fall in pH and stimulating PLT glycolysis (11).

Potassium and magnesium have been shown to reduce the rate of metabolism and to reduce the storage-associated activation of PLTs (9,12). We investigated whether calcium chloride (1 mM) plays a role in PLT storage through evaluation of Reformulated PAS without calcium. In addition, a commercially available solution, also referred to as PAS III: InterSol (Fenwal, La Châtre, France) with or without addition of magnesium and dextrose monohydrate was investigated. Glucose (as a substrate for PLT metabolism) was supplied by a 20% plasma carryover in all InterSol and Reformulated PAS units and by the PAS formulation. The purpose of the present study was to determine the potential effects of reducing the amount of plasma carryover to 20% in combination with different PAS, as measured by in vitro parameters during a 9-day storage period.

Materials and methods

Preparation and storage of platelets

Platelets were collected from normal blood donors who met standard donation criteria and gave written, informed consent according to institutional guidelines. A total of 450 ml of whole blood (WB) was drawn into a citrate phosphate dextrose/saline adenine glucose manitol (SAG-M) quadruple-bag blood container system (Fenwal). After storage at room temperature for 2–6 h, WB units were centrifuged (2700 g) for 10 min at 22°C. Automatic equipment was used for the preparation of blood components (Optipress; Fenwal), including buffy coat (BC). Buffy coats were prepared with plasma inclusion of 15–20 ml to allow suspension of PLTs in InterSol (Fenwal) or Reformulated PAS (Fenwal) at 80%. Buffy coats (25 units) were stored overnight and combined in a large-volume container to create a primary pool (in total, eight primary pools from 200 BC units). The primary pools were split into five equivalent parts for the preparation of PLT units and storage in PLT storage bags (PL2410; Fenwal). All five BC units, derived from the primary pool, were prepared by the OrbiSac system (Caridian, Zaventem, Belgium) (13) to yield PLT units for matched studies stored in

1. plasma (100%) (Plasma);
2. InterSol (80%) + plasma (20%) (InterSol);
3. InterSol + dextrose and magnesium (80%) + plasma (20%) (PSM1);
4. Reformulated PAS 0mM calcium (80%) + plasma (20%) (PSM2);
5. Reformulated PAS (with 1 mM calcium) (80%) + plasma (20%) (PSM3) Calcium Chloride dihydrate Injection USP 100 mg/ml was used (0.44 ml in 300 ml Platelet Concentrate). The addition of calcium was carried out by injection through the sampling site in a laminar flow hood.

The residual plasma has been estimated by calculation using the volume of the residual pooled BC, the haematocrit of the residual pooled BC and the volume of the PAS added. After the OrbiSac procedure, the PLTs were transferred and stored in commercially available PL2410 polyolefin bags (Fenwal) for 9 days. The air and foam were then excluded and the PLT units were stored on a flat bed agitator (60 cycles a minute, model LPR-3; Melco, Glendale, CA, USA) in a temperature-controlled cabinet at 22 ± 2°C. The samples were drawn aseptically on days 1 (PLT preparation day), 3, 5, 7 and 9. All sampling was carried out by sterile connection (TSCD-II, Terumo, Tokyo, Japan) of sampling bags to the respective containers. The composition of the different storage solutions used is shown in Table 1.

Analysis of metabolic and cellular parameters

Cellular and metabolic in vitro parameters were evaluated in a PLT storage study, including measurements of PLT

<table>
<thead>
<tr>
<th>Table 1 Composition of the different storage solutions</th>
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<tbody>
<tr>
<td>Name of PAS composition in mM</td>
</tr>
<tr>
<td>Sodium acetate</td>
</tr>
<tr>
<td>Sodium acid phosphate</td>
</tr>
<tr>
<td>Sodium phosphate</td>
</tr>
<tr>
<td>Sodium chloride</td>
</tr>
<tr>
<td>Potassium chloride</td>
</tr>
<tr>
<td>Dextrose monohydrate</td>
</tr>
<tr>
<td>Magnesium chloride</td>
</tr>
<tr>
<td>Calcium chloride</td>
</tr>
<tr>
<td>Osmolarity (mOsm/l)</td>
</tr>
</tbody>
</table>

PAS, platelet additive solutions.
counts (10⁹/L and 10⁹/unit) and mean PLT volume (MPV) using CA 620 Cellguard (Boule Medical, Stockholm, Sweden). The volume (ml) was calculated by weighing the contents of the storage bag, in grams, on a scale (Mettler PB 2000, Mettler-Toledo, Allbstadt, Switzerland) and the result, in grams, was divided either by 1.01 (1.01 g/ml is the density of the storage medium composed of approximately 80% InterSol or Reformulated PAS and 20% plasma) or 1.03 (the density of 100% plasma). By use of routine blood gas equipment (ABL 705, Radiometer, Copenhagen, Denmark) we also measured the pH, pCO₂ (kPa at 37°C), glucose (μmol/L), lactate (μmol/L) and calcium (μmol/L). Bicarbonate (μmol/L) was calculated based on the other measured variables.

The assessment of swirling was carried out by inspection and grading according to Bertolini (14). The white blood cell count on day 1 was determined with a Nageotte chamber and a microscope (Standard Zeiss, Chester, VA, USA) (15). Hypotonic shock response reactivity (HSR) measurements were performed using a dedicated microprocessor-based instrument (SPA 2000; Chronolog, Havertown, PA, USA) with the modifications of these tests described by VandenBroeke et al. (16). The total adenosine triphosphate (ATP) concentration, (μmol/10¹¹) was determined with a Luminometer (Orion Microplate Luminometer; Berthold Detection Systems GmbH, Pforzheim, Germany) on the basis of principles described by Lundin (17). Lactate dehydrogenase (LDH) activity, a marker for disintegration of PLTs, was measured with a spectrophotometric method (Sigma Aldrich kit 063K6003, Sigma-Aldrich, St Louis, MO, USA; Spectrophotometer DMS 100, Varian Techtron, Springvale, Australia) (18).

**Monoclonal antibody labelling**

Platelet concentrate samples, fixed by adding an equal volume of 1% paraformaldehyde (PFA), PFA–phosphate-buffered saline (PBS) (pH 7.2–7.4) at 22°C for 10 min were then stained for 20 min at the same temperature in the dark by incubating with 20 μl of fluorochrome-labelled monoclonal antibodies (mAb) per approximately 20 × 10⁶ PLTs. Phycoerythrin (PE)-conjugated IgG1 isotype CD62P (P-selectin/GMP-140/PADGEM; clone CLB Thromb/6) mAb was used for single colour staining purchased from Immunotech (Beckman Coulter, Marseilles, France). Control specimens were processed as above, but incubated with a PE-conjugated mAb (IgG1 isotype) with irrelevant specificity, purchased from Immunotech (Beckman Coulter). After incubation with fluorochrome-conjugated antibodies, the samples were washed twice by adding 20 ml filtered PBS–ethylenediamine tetra-acetic acid (0.33%, pH 6.9) with 0.1% Na-azide, and centrifuged at 2760 × g (Eppendorf 5810R, Eppendorf AG, Hamburg, Germany) for 10 min at +18°C.

**Flow cytometry analysis**

A total of 100000 PLT events were acquired on a FACS Calibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) equipped with 15 mW argon ion lasers and Cellquest pro software. Daily controls of optics and fluorescence intensity were performed using standardized beads (Calibrite, Becton Dickinson). The flow cytometer settings were optimized for the acquisition of PLTs by logarithmic signal amplification in all four detectors (forward and side scatter channels and fluorescence channels FL1 and FL2). For analysis, the gate was set around intact PLT population as defined by forward and side scatter characteristics. The percentage of positive PLTs of total PLT expressing activation markers CD62P above that of background (negative control) as well as the mean fluorescence intensity (MFI) were recorded.

**Bacterial cultures**

Bacterial cultures were performed at day 9 with the routine methods of the bacteriological laboratory at Karolinska University Hospital/Huddinge, including aerobic and anaerobic cultures performed on Columbia blood agar plates, with 48 h to final report.

**Statistical analyses**

The mean values and SD (n = 8) were usually given. Repeated measurement analysis of variance (ANOVA) was performed including ‘post hoc’ multiple comparison, Fisher’s LSD. Five different groups (storage solution) were studied over time (days). ‘Days’ were the repeated factor and ‘group’ a between factor. Results of Fisher’s LSD are presented in Tables 3 and 4 and Figures 1 and 2. The difference was considered statistically significant at P < 0.05. The analyses were carried out using the Statistica software, version 8.0, StatSoft, Inc 1984–2007 (SPSS, Chicago, IL, USA).

**Results**

In this study, PLTs stored in various storage solutions were studied. The mean plasma content in the various PAS units

**Table 2** Comparison of various storage solutions in platelet (PLT) concentrates on day 1 Results are expressed as mean ± SD, n = 8

<table>
<thead>
<tr>
<th>Storage solution</th>
<th>Volume (ml)</th>
<th>Platelets (10⁹/l)</th>
<th>Leucocytes (10⁹/units)</th>
<th>Ratio plasma (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>308 ± 5</td>
<td>930 ± 76</td>
<td>&lt;0.2</td>
<td>100</td>
</tr>
<tr>
<td>InterSol</td>
<td>294 ± 4</td>
<td>893 ± 76</td>
<td>&lt;0.2</td>
<td>19 ± 0.6</td>
</tr>
<tr>
<td>PSM1</td>
<td>308 ± 4</td>
<td>815 ± 42</td>
<td>&lt;0.2</td>
<td>19 ± 0.7</td>
</tr>
<tr>
<td>PSM2</td>
<td>300 ± 4</td>
<td>912 ± 67</td>
<td>&lt;0.2</td>
<td>20 ± 0.8</td>
</tr>
<tr>
<td>PSM3</td>
<td>300 ± 4</td>
<td>908 ± 62</td>
<td>&lt;0.2</td>
<td>20 ± 0.7</td>
</tr>
</tbody>
</table>
was calculated from 19% to 20% (Table 2). We found statistically significant differences in a variety of cellular, metabolic and flow cytometry parameters.

Cellular assays
The PLT counts and contents on day 1 are given in Table 2. PLT counts and contents were significantly lower ($P < 0.01$) in PSM1 (only Day 1 data shown). No significant difference in PLT counts and contents between the other groups was detected throughout storage (data not shown). MPV increased in InterSol units during storage when compared with the other groups (Table 3). The LDH activity was higher in PLTs stored in plasma when compared with the other groups (Table 3). The percentage of PLTs expressing the activation marker CD62P (Fig. 2) as well as the mean fluorescence intensity, MFI (data not shown) increased during storage in all units. The mean expression in all preparations, with exception of InterSol units, was below 20% in fresh PLTs and below 35% in 9-day-old PLT units. An apparent decrease in CD62P expression was seen on Day 3 for PLTs stored in plasma and PSM1–3, while the activation level of PLTs stored in InterSol increased and became significantly higher ($P < 0.01$). Platelets stored in PSM1 solution differed significantly from PLTs stored in PSM2, PSM3 ($P < 0.01$) and plasma ($P < 0.05$). No significant difference in the expression of CD62P between PSM2 and PSM3 was detected but difference in the CD62P expression was found between plasma and PSM3 ($P < 0.05$). All bacterial cultures were negative.

Discussion
In this matched study, the potential *in vitro* effect associated with inclusion of potassium, calcium and glucose and the reduction of plasma carry-over to 20% in different PAS was evaluated and compared with data from buffy-coat derived PLTs stored in 100% plasma. The processing system for all PAS and plasma units was similar; and the plasma carry-over was calculated to be approximately 20% in all PAS units.

All PLT units had satisfactory PLT content (Table 1) according to current European standards (> 240 × 10⁹/ unit). However, we noticed that PLT yield was significantly lower in InterSol in combination with dextrose and magnesium (PSM 1). This observation may be associated with inclusion of potassium, calcium and glucose and the reduction of plasma carry-over to 20% in different PAS units. We found statistically significant differences in a variety of cellular, metabolic and flow cytometry parameters.

Metabolic assays
The glucose concentration, production of lactate as well as the bicarbonate concentration was higher in PLTs stored in plasma when compared with different PAS units (Table 3). The initial glucose concentration in InterSol-stored PLTs was approximately three times lower than in PLTs stored in plasma and PSM1–3 solutions (Table 3). A fall in ATP levels in InterSol-stored PLTs when compared with the other groups was detected (Table 3). The mean pH value in all preparations was above 6.8 on Day 1 and Day 9 respectively (Table 4). pH differed significantly between plasma and PAS units. With exception of the InterSol units, no significant difference of pH levels between the PAS (PSM1–3) units was detected (Table 4). Carbon dioxide ($pCO_2$) decreased in all units, but was higher in plasma when compared with all PAS units. $pCO_2$ was higher in PSM2 and PSM3 than in InterSol and PSM1 (Table 4). The extracellular calcium concentration remained unchanged during storage in all units but was higher in PSM3 units due to addition of this compound (Table 4).

HSR and swirling
The HSR reactivity of PLTs stored in plasma and PSM1–3 was significantly higher compared with PLTs stored in InterSol. HSR levels for PLTs stored in either plasma, PSM2 or PSM3 over 9 days were not significantly different. However, significant differences were found between plasma and PSM1 and between PSM3 and PSM1 (Fig. 1). Swirling remained at the highest level (score = 2) for all units throughout storage (Data not shown).

Flow cytometry analysis
The percentage of PLTs expressing the activation marker CD62P (Fig. 2) as well as the mean fluorescence intensity, pH, $pCO_2$ and $HCO_3^-$ values between plasma and PSM1–3 units might only be related to the amount of plasma in the units. Bicarbonate is the major buffer for plasma and contains initially roughly three times that in PSM1–3. Consequently, the buffering capacity of PAS seemed very limited compared with that of plasma (19).

To improve this situation, PSM1–3 and InterSol also contained phosphate (11) as well as acetate. Acetate served as a second metabolic fuel and gave an added benefit in providing a buffering effect by continuously generating bicarbonate (20) and thereby maintaining a more stable bicarbonate level during the entire storage period when compared with plasma. Moreover, a further benefit from acetate was derived from its oxidation and the use of a hydrogen ion during that oxidation, decreasing acidification of the storage environment (21).
In contrast to the plasma and PSM1–3 units, glucose in PLTs stored in InterSol was totally consumed on Day 5. For this reason, lactate production by anaerobic glycolysis also stopped at Day 5. Cessation in the production of lactate was associated with decrease in ATP levels and in the HSR reactivity and increase in pH and CD62P expression on the surface of the outer membrane of the PLTs.

These findings have also been described in previous reports (22,23). This observation emphasizes the importance of glucose being present in the storage medium during the entire storage period as it may also be associated with decreased post-transfusion recovery and survival (24).

There was a statistically significant fall in HSR in PLTs stored in InterSol compared with the other groups. This may be explained by morphological transformations as
The HSR reactivity during 9 days storage of platelets (PLTs) stored with lack of magnesium and potassium in InterSol, and depletion. The initially lower response to HSR in InterSol expression as well as a loss of functionality due to glucose evidenced by an increase in PLT size (MPV) and CD62P expression as well as a loss of functionality due to glucose depletion. The initially lower response to HSR in InterSol units when compared with PSM1-3 units may be associated with lack of magnesium and potassium in InterSol, and/or higher glucose content, resulting in better maintenance of PLT integrity. Although HSR values decreased in all units, all HSR scores of PSM1-3 on day 9 were above the level that indicated poor in vivo viability as predicted to occur for PLTs stored for 7 days (1).

In contrast to an earlier report (9) when PLTs were stored in PAS IIIM, we experienced HSR reactivity similar to that of plasma when PLTs were stored in PSM2 with ingredients similar to those of PAS IIIM but also supplemented with glucose and with a different concentration of phosphate. This effect may be associated with the higher levels of

Data are reported as mean ± SD (n = 8); the P value represents the LSD test group–group significance.

<table>
<thead>
<tr>
<th>pH (37°C)</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>6.99 ± 0.03</td>
<td>7.22 ± 0.04</td>
<td>7.21 ± 0.04</td>
<td>7.13 ± 0.05</td>
<td>6.98 ± 0.06bde</td>
</tr>
<tr>
<td>InterSol</td>
<td>6.91 ± 0.02</td>
<td>6.93 ± 0.02</td>
<td>6.88 ± 0.02</td>
<td>6.96 ± 0.03</td>
<td>7.04 ± 0.04a</td>
</tr>
<tr>
<td>PSM1</td>
<td>6.91 ± 0.01</td>
<td>7.00 ± 0.02</td>
<td>7.02 ± 0.03</td>
<td>6.99 ± 0.04</td>
<td>6.92 ± 0.04a</td>
</tr>
<tr>
<td>PSM2</td>
<td>6.85 ± 0.02</td>
<td>7.00 ± 0.03</td>
<td>7.03 ± 0.03</td>
<td>6.99 ± 0.05</td>
<td>6.89 ± 0.08a</td>
</tr>
<tr>
<td>PSM3</td>
<td>6.83 ± 0.02</td>
<td>7.01 ± 0.03</td>
<td>7.04 ± 0.04</td>
<td>7.01 ± 0.06</td>
<td>6.90 ± 0.08a</td>
</tr>
<tr>
<td>pCO₂ (37°C)</td>
<td>8.98 ± 0.57</td>
<td>4.12 ± 0.24</td>
<td>3.46 ± 0.17</td>
<td>3.22 ± 0.14</td>
<td>2.92 ± 0.20bde</td>
</tr>
<tr>
<td>Plasma</td>
<td>3.66 ± 0.23</td>
<td>2.74 ± 0.17</td>
<td>2.19 ± 0.13</td>
<td>1.74 ± 0.16</td>
<td>1.46 ± 0.14a</td>
</tr>
<tr>
<td>InterSol</td>
<td>3.63 ± 0.14</td>
<td>2.44 ± 0.10</td>
<td>2.07 ± 0.11</td>
<td>1.96 ± 0.13</td>
<td>1.83 ± 0.13a</td>
</tr>
<tr>
<td>PSM1</td>
<td>4.16 ± 0.16</td>
<td>2.54 ± 0.14</td>
<td>2.27 ± 0.17</td>
<td>2.17 ± 0.15</td>
<td>2.08 ± 0.15c</td>
</tr>
<tr>
<td>PSM2</td>
<td>4.22 ± 0.25</td>
<td>2.50 ± 0.11</td>
<td>2.21 ± 0.15</td>
<td>2.14 ± 0.12</td>
<td>2.04 ± 0.18abc</td>
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<tr>
<td>Calcium (µM)</td>
<td>0.05 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.05 ± 0.00bde</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.02 ± 0.00</td>
<td>0.02 ± 0.00</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01ac</td>
</tr>
<tr>
<td>InterSol</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01ac</td>
</tr>
<tr>
<td>PSM1</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.00</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01ac</td>
</tr>
<tr>
<td>PSM3</td>
<td>0.07 ± 0.00</td>
<td>0.08 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.08 ± 0.01abcd</td>
</tr>
</tbody>
</table>

Table 4 In vitro effects of platelets (PLTs) stored in various storage solutions for 9 days

<Fig. 1 The HSR reactivity during 9 days storage of platelets (PLTs) stored in plasma, InterSol, and PSM units (bc); PSM1, Δ (abc); PSM2, × (b); and PSM3, ○ (bc). Values given as mean ± SD, n = 8. aP < 0.01 vs. Plasma; bP < 0.01 vs. InterSol; cP < 0.01 vs. PSM1; dP < 0.01 vs. PSM2; eP < 0.01 vs. PSM3; fP < 0.05 vs. Plasma; gP < 0.05 vs. InterSol; hP < 0.05 vs. PSM1; iP < 0.05 vs. PSM2; jP < 0.05 vs. PSM3.>

<Fig. 2 Changes in platelet (PLT) expression of CD62P during 9 days storage of PLTs stored in plasma, InterSol, and PSM units (bc); PSM1, Δ (abc); PSM2, × (b); and PSM3, ○ (bc). Values given as mean ± SD, n = 8. aP < 0.01 vs. Plasma; bP < 0.01 vs. InterSol; cP < 0.01 vs. PSM1; dP < 0.01 vs. PSM2; eP < 0.01 vs. PSM3; fP < 0.05 vs. Plasma; gP < 0.05 vs. InterSol; hP < 0.05 vs. PSM1; iP < 0.05 vs. PSM2; jP < 0.05 vs. PSM3.>
glucose available for PLT metabolism in PSM2 compared with PAS III M. Alternatively, the difference in phosphate concentration may explain this effect. There is also a significant difference between plasma 100% and PSM1, and between PSM1 and PSM3, suggesting that lack of potassium in PAS may have a negative effect on HSR levels. Alternatively, the lower levels of phosphate in PSM2 and PSM3 may explain these differences.

Calcium ions (Ca$^{2+}$) actively participate in several control functions in PLTs (25), as well as imply membrane-modifying effects (26). As a net influx of Ca$^{2+}$ into the PLTs will probably cause PLT activation (27), our results may suggest that calcium (1 m) included in the PSM3 units either was actively removed from the cytosol or sequestered within an internal site by the action of different Ca$^{2+}$-ATPases and exchangers or alternatively the calcium ions may be sequestered by the citrate as well as the phosphate in the PAS solution. As we did not notice any significant differences between PSM2 and PSM3, the role of calcium during storage of PLTs will need to be further explored.

Our data showed slight differences in the expression of the activation marker CD62P between groups, with exception of PLTs stored in InterSol. In these units, we found an increase in CD62P from Day 5. CD62P is a constituent of alpha granules in resting PLTs that can be detected on the surface of activated PLTs after alpha granule secretion (24). The increase in CD62P from Day 5 may be associated with metabolic changes due to the glucose depletion described earlier.

The initial higher levels of CD62P on Day 1 in all units are probably attributed to PLT stress due to the preparation process. After Day 1 we found, more or less, an apparent recovery from activation in all storage solutions with exception of InterSol. In PSM1-3, this phenomenon probably is associated with inclusion of magnesium (9,12). The significantly lower levels in PSM2 and PSM3 when compared with PSM1 may again be explained by the lack of potassium in PSM1 or alternatively the difference in phosphate concentrations. However, the surface expression of CD62P in all plasma and PSM1-3 stored PLTs were within the range recently described by Curvers et al. (28) for fresh and 8-day-old PLTs.

To summarize, the results of the present study suggest that PLTs stored in Reformulated PAS (PSM2 and 3) with 20% plasma carry-over maintained metabolic and cellular characteristics, equivalent to PLTs in 100% plasma during 9 days of storage. Our results suggest that presence of potassium in addition to magnesium or alternatively the concentration of phosphate as well as supply of additional glucose to normal plasma level improves in vitro data of PLTs stored in PAS. In addition, the introduction of such solutions may allow reduction in plasma carryover, thus potentially further reducing the risk of transfusion reactions and allowing increased recovery of plasma for other purposes such as fractionation.

However, the impact of these findings needs to be examined performing in vitro studies of recovery and survival, as well as/or increments in thrombocytopenic patients to confirm the haemostatic effect of PLTs stored in solutions with 20% plasma carry-over and extended storage.

References
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