Optimising quality of platelet transfusions
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Chapter 6

Comparison between a new PVC platelet storage container (UPX80) and a polyolefin container

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COMPARISON BETWEEN A NEW PVC PLATELET STORAGE CONTAINER (UPX80) AND A POLYOLEFIN CONTAINER

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Abstract

During storage of platelet concentrates (PCs), the quality of the platelets deteriorates gradually, which is partially dependent on gas exchange.

UPX80 (JMS, Japan) 1-L platelet storage PVC containers with increased gas transport capacity were compared with 1- and 1.5-L polyolefin (PO) containers (NPBI, The Netherlands) with filtered PC stored either in GAC (gluconate-acetate-citrate, < 10% plasma) or in plasma, for 8 days. In total 32 PCs, were made (260-330 x 10⁹ platelets per concentrate), and equally divided over different bags and storage media. During storage, gas exchange, metabolic, physical and activation parameters were measured.

No consistent differences for all parameters were observed between UPX80 and PO containers (1 L or 1.5 L). Blood gas parameters indicated better gas exchange for UPX80 containers compared with PO containers. Good morphology was observed in UPX80, and metabolic functions were not significantly different compared with PO containers. During prolonged storage (after day 6), some significant differences in CD62P and CD63 expression were found, indicating a higher degree of platelet activation in UPX80 containers, especially in GAC.

UPX80 PC containers are suitable for storage of PC. Although in UPX80 better gas exchange takes place, compared with PO containers, this does not improve the platelet quality during storage for 6 days, indicating that gas exchange above the level of PO containers has no effect on the switch to aerobic metabolism in platelets.
INTRODUCTION

Logistics of platelet transfusions greatly benefit from the possibility to store platelets for several days prior to transfusion. Although for storage longer than 5 days several bag types are available, the maximal storage time is restricted to 5 days due to concern about bacterial contamination [43;88]. During storage, the quality of the platelets needs to be preserved, which is greatly dependent on adequate exchange of gasses such as CO₂ and O₂ [119;120]. High O₂ permeability induces aerobic metabolism, producing less lactate compared with anaerobic conditions in which glycolysis takes place. Furthermore, CO₂ produced during metabolism of glucose or acetate can acidify the medium as well. Therefore, high CO₂ permeability is necessary. Theoretically, too high gas exchange can result in high pH values (> 7.4), with negative effects on platelet in vitro quality [121]. However, it was shown by Bannai [122], that the negative effect of high pH values was minimal when using gentle agitation, e.g. on flat bed devices.

New containers have been developed to improve the quality of platelets during storage [123]. In the present study, a platelet container, UPX80 from JMS (Hiroshima, Japan), made of PVC with a long-chain plasticizer (di-undecyl-phthalate) and with an increased surface area, was tested and compared with containers made of polyolefin (PO)(Compoflex; NPBI, Emmer Compascuum, the Netherlands). The plasticizer and the increased surface area of the container were expected to increase the gas exchange [124]. According to the manufacturer of UPX80, who compared its gas permeability with common PVC (gas permeability set at 100%), this material should increase the oxygen permeability to 360% and the carbon-dioxide permeability to approximately 400%. For the tested PO bags a similar comparison would lead to 260-310% for O₂ exchange, and 210-230% for CO₂ gas. Thus, UPX80 has a 1.16-1.38 times higher O₂ permeability and a 1.73-1.93 higher CO₂ permeability than PO. Containers made from PO are already being widely used for platelet storage with satisfying results [106]. The specifications of the manufacturer for the new 1000-ml UPX80 containers indicated a maximal filling of 200-400 ml of plasma containing 3.0-6.0 x 10¹¹ platelets. The manufacturer showed that the toxicity of UPX80 (di-undecyl-terephthalate) is lower than di-ethyl-hexyl phthalate (DEHP), the commonly used plasticizer in PVC containers. The LD50 of UPX80 in male rats is 835.875 mg/kg and in female rats 978.710 mg/kg compared with the LD50 of 250 mg/kg in case of DEHP (personal communication Dr. Suzuki, JMS, Hiroshima, Japan).

We tested gas exchange parameters and an extended set of in vitro quality parameters that are sensitive indicators for platelet activation, and compared UPX80 of 1 L with two differently sized (1-L and 1.5-L nominal volume) PO containers. The UPX80 containers were tested in eight-fold with platelet concentrates (PCs) in plasma as well as in synthetic medium (GAC, gluconate-acetate citrate). Because of the well-known characteristics of PO [105;125], these
containers were tested in four-fold per size (1-L and 1.5-L). Per size we tested 4 PC stored in plasma and 4 stored in GAC.

GAC was tested because of its good storage properties [106] and to test the influence of various storage media on storage properties of the new UPX80 container.

MATERIALS AND METHODS

Preparation of PC

The study was performed in 4 series of experiments, each series consisting of 8 PCs. For each series, 40 whole-blood units (500 ± 50 ml, blood group A positive) were collected from normal blood donors in PVC-DEHP bottom-and-top containers with 70 ml of citrate-phosphate-dextrose (CPD) (T&B, Biopack, Compoflex NPBI, Emmer-Compascuüm, The Netherlands). After collection, the whole blood was stored overnight at 20°C on butane-diol cooling plates [30] until the next day (day 0 of storage, d0), at which PCs were prepared from pooled buffy coats (BCs). Whole blood units were centrifuged (8 minutes, 2817 x g, brake 3, start-up time 70 s, slow-down time about 270 s, 20°C; Hettich Roto Silenta R/P, Dépex, De Bilt, The Netherlands).

After centrifugation, BCs were collected by means of an automated device for component preparation, the Compomat; version G4) with Compomaster software (NPBI). Five BCs were pooled to prepare one PC. Plasma or GAC (gluconate, acetate, citrate: 90 mM NaCl, 23 mM sodium gluconate, 27 mM sodium acetate, 5 mM KCl, 3 mM MgCl₂, 0.32% (w/v) trisodium citrate, 294 mosm, pH 7.4) was added to the BC pool until a gross weight of 500 g was reached. One medium type was used for each series. BC pools in plasma were centrifuged at 704 x g for 5 minutes (brake 3, 20°C), BC pools in GAC were centrifuged at 313 x g for 5 minutes (brake 3, 20°C). The platelet-rich supernatants were transferred to a satellite container. After this, PC were filtered within 10 minutes after preparation over Sepacell PL-10A filters (Asahi, Japan) and stored either in UPX80 platelet storage containers (JMS1000, JMS, Hiroshima, Japan) (4 per run) or PO containers (Compoflex, NPBI), of either 1.5 or 1 L (nominal volume, 4 per series, one type per series). Volumes of the pooled PC were calculated by dividing the net weight of the PC by the specific gravity of plasma (1.026 g/cm³) or GAC with less than 10% remaining plasma (1.002 g/cm³).

PC storage and sampling

Concentrates were stored in a platelet incubator (22 ± 2°C) on a horizontal flatbed shaker (1 cycle/s)(Helmer labs Inc, Noblesville, IN, USA). Samples were taken aseptically via a sampling site coupler (NPBI) on day 0 (directly after
Comparison between UPX80 and PO containers

preparation and filtration, \textit{i.e.} about 16 hours after blood collection), day 1, 3, 6 and 8.

\textbf{Platelet and leukocyte counts}
Platelets and leukocytes in each PC were counted with an electronic counter (AcT10, Coulter Electronics, Mijdrecht, The Netherlands), after dilution (1:4) in PBS. After filtration, the filtrate was diluted (1:5) in PBS containing acridine orange (2.5 mg/l) and leukocytes were counted in a Nageotte bright-line counting chamber (Superior, Bad Mergentheim, Germany) by fluorescence microscopy (Leitz, Wetzlar, Germany).

\textbf{Blood gas parameters}
Within 5 minutes after sampling, PCs were used for blood gas measurements (pH, pCO$_2$, pO$_2$) in a blood gas analyzer (Ciba Corning 238 pH/Blood Gas Analyzer, Chiron Diagnostics NV, Houten, The Netherlands). Measurements were performed at 37°C.

\textbf{Swirling patterns and platelet morphology}
Swirling patterns were determined according to Fratantonì \textit{et al.} \cite{126}. This was done independently by two individuals. Swirling inhomogeneity visible throughout the whole bag, with contrast observable as fine detail received a score of 3. The same without fine detail received a score of 2 and some inhomogeneity visible in only a few places and with poor contrast obtained a score of 1. No inhomogeneity before and after squeezing the bag scored 0. For platelet morphology, 50 µl of PC was fixed in 250 µl of glutardialdehyde (0.5% in PBS, v/v) and stored at 4°C. Samples for morphology were stored for a maximum of 4 days and scored according to a slight modification of the method of Kunicki \textit{et al.}\cite{107} by light microscopy (Leitz, Wetzlar, Germany) with oil immersion (1000x). A total of 100 platelets was scored; the number of perfect discs and almost flat platelets (with dendrites) was multiplied by 4, filled discs with dendrites by 2 and spheres by 1.

\textbf{Nucleotide content and nucleobase release}
The nucleotide content of platelets was analysed with an anion-exchange HPLC method after perchloric acid extraction as described before \cite{127}. The neutralised samples were kept at -80°C until HPLC analysis. Columns were prepacked Partisphere Partisil-5 SAX cartridges (125 x 4.6 mm internal dimensions, Whatman, Clifton, NY, USA).
Chapter 6

The nucleobase release by platelets as a measure of degradation of nucleotides was determined by analysing the level of hypoxanthine, xanthine, uric acid, adenine and adenosine in perchloric acid extracts of supernatants after centrifugation (12,000 x g, 5 min) by reversed phase HPLC according to Van Gennip [128]. The C18 columns used were Partisphere 5 C18 (125 x 4.6 mm internal dimensions, Whatman).

The concentrations of the different substances were calculated by comparison with highly purified standards (Sigma Chem.Co., St. Louis, MO, USA).

Activation-dependent antigens

To measure activation of platelets, the following murine monoclonal antibodies (MoAbs) directed against human platelet antigens were used as described before [125;129]: MoAb CLB-Thromb/7 against GpIIb (CD41 antigen), MoAb Y2 against GpIIa (CD61 antigen), MoAb CLB-Thromb/6 against P-selectin (CD62P), MoAb CLB-gran/12 against a 53-kD protein (CD 63 antigen) and MoAb CLB-CD42 bb (all from CLB, Amsterdam, The Netherlands).

Glucose and lactate concentrations

Glucose and lactate concentrations were measured in PC supernatants (12,000 x g, 5 min) as described before [130].

β-thromboglobulin release and protein concentration

The supernatants of PC were used for protein assays and β-TG assays. PC were centrifuged for 5 minutes at 12,000 x g, supernatants were isolated and stored at -80°C until analysis. β-TG release was measured with a RIA (Kodak Clinical Diagnostics Ltd, Amersham, UK) and protein concentration was measured with a BCA Protein Assay (Pierce, Rockfield, IL, USA). β-TG concentrations were calculated per platelet. The protein concentrations were used for calculation of the plasma percentage in the PC stored in GAC.

Soluble P-selectin concentrations

Soluble P-selectin (sP-selectin) was measured in the PC supernatants (12,000 x g, 5 minutes) and quantified by a sandwich type ELISA [131]. Two non-competitive monoclonal antibodies were used; one of which was biotinylated. sP-selectin purified from plasma by immuno-absorption was used as a standard. sP-selectin concentrations were calculated per platelet.
Comparison between UPX80 and PO containers

Statistical analysis

Statistical analysis was performed with Student's two-tailed t-test for paired and unpaired observations, and differences were mentioned if significant (p < 0.05). When appropriate, significance levels were adjusted for multiple comparison according to the method of Bonferroni-Holm [132]. For significance, the total experiment-wise error was chosen to be lower than 0.05. Results are presented as the mean ± SD, except for the figures. In the figures we did not incorporate error bars, to avoid confusion with overlapping ranges. Instead, we included in the legends the range for the coefficient of variation (CV), to give an impression of the variation. Relevant statistical differences are mentioned in the text.

RESULTS

Characteristics of PC before and after filtration

Platelet recovery after filtration of GAC PC was significantly lower than plasma PC whereas the initial concentration and volume was similar. Concentrates stored in GAC had a very low plasma concentration (table 6.1) due to the use of a Compomat for preparation of BC with maximal plasma removal. During storage for 8 days, the number of platelets decreased in all PC to a similar degree. The platelet and leukocyte concentrations between different series were comparable. The number of leukocytes after preparation ranged from 23 to 221 x 10⁶ leukocytes per unit, which were effectively removed by filtration until less than 1% of the original concentration (table 6.1).
Table 6.1: Properties of platelet concentrates (PCs) before and after filtration and during storage. Except when stated otherwise, data for PO containers are not split per container volume because this is not relevant for the given parameters. Mean values ± SD are shown.

<table>
<thead>
<tr>
<th>PC, bag type and medium</th>
<th>Plt. (^b) x 10^9/ml (day 0)</th>
<th>% Plt. decr. due to filtr. (day 0)</th>
<th>Total plt. content(^a) x 10^9</th>
<th>Volume(^a) (ml)</th>
<th>% Plt. decrease day 0-8</th>
<th>Total leuk.(^b) x 10^6</th>
<th>Total leuk.(^a) x 10^3</th>
<th>% Plasma in stor. medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO, plasma (n=8)</td>
<td>0.998 ± 0.129</td>
<td>7 ± 4.3</td>
<td>332 ± 32</td>
<td>359 ± 28</td>
<td>5 ± 3.7</td>
<td>59 ± 5.3</td>
<td>&lt;48 ± 5.3</td>
<td>100 ± 5.3</td>
</tr>
<tr>
<td>UPX80, plasma (n=8)</td>
<td>1.002 ± 0.151</td>
<td>10 ± 4</td>
<td>311 ± 48</td>
<td>346 ± 30</td>
<td>9 ± 3.6</td>
<td>40 ± 12</td>
<td>40 ± 23.1</td>
<td>100 ± 5.3</td>
</tr>
<tr>
<td>PO, GAC (n=8)</td>
<td>0.963 ± 0.244</td>
<td>16 ± 8.8</td>
<td>266 ± 80</td>
<td>329 ± 28</td>
<td>5 ± 5.2</td>
<td>39 ± 12</td>
<td>139 ± 8.5</td>
<td>5±0.5(^{(1L)})</td>
</tr>
<tr>
<td>UPX80, GAC (n=8)</td>
<td>1.054 ± 0.153</td>
<td>20 ± 17.6</td>
<td>294 ± 98</td>
<td>342 ± 35</td>
<td>7 ± 4.4</td>
<td>62 ± 65</td>
<td>75 ± 40.6</td>
<td>7 ± 1.2</td>
</tr>
</tbody>
</table>

Footnotes table 6.1:
- Plt. = platelet number
- \(^a\) = after filtration
- \(^b\) = before filtration
- PO = polyolefin container
- UPX80 = new JMS container
- GAC = platelet storage solution
- Leuk. = leukocyte number per PC

**Physical parameters of the PC**

Platelets stored in plasma in 1-L PO containers acidified faster than platelets stored in 1.5-L PO containers (fig. 6.1). Differences already became significant (p < 0.05) at day 1 of storage. The pH in (1-L) UPX80 containers was significantly higher than in 1-L PO (day 1, 3 and 6) and 1.5-L PO containers (day 1 and 3).

In GAC, the changes in pH showed an opposite pattern to that seen with platelets stored in plasma. A marked increase in pH was seen after day 6. From day 0 until day 8, no significant differences in pH between UPX80 and PO (1-L and 1.5-L) were observed. At day 6 and 8, the pH was higher (p < 0.05) in UPX80 compared with 1.5-L PO and at day 8 compared with 1-L PO. In all PCs stored in plasma as well as in GAC, the pH remained in the range 7.3-6.8 during storage for 8 days.
Comparison between UPX80 and PO containers

Figures 6.1-6.7: Changes in platelet parameters during storage in plasma or GAC in UPX80 and PO containers. PCs stored in plasma (solid symbols) and GAC (open symbols); either in PO 1.5-L (circle), PO 1-L (square) or UPX80 (triangle) containers. As mentioned in Materials and Methods, no error bars are shown in the figures (to avoid crowding); instead, the ranges for the coefficients of variation (CVs) are given between brackets.

Fig. 6.1 - Changes in pH (range of CVs: 0.141-1.88%).

The pCO₂ of platelets stored in plasma started to become lower in UPX80 containers compared to 1-L PO containers at day 0 (p < 0.05) and 1.5-L PO containers at day 1 (p < 0.01) (fig. 6.2). The pCO₂ of GAC-stored PC was lower (p < 0.05) in UPX80 compared to 1-L PO containers at day 6 and 8 and to 1.5-L PO at day 8.

Fig. 6.2 - Changes in pCO₂ (range of CVs: 5.54-26.8%).
pO$_2$ in plasma-stored PC was higher (p < 0.05) in UPX80 containers than in 1-L PO containers at day 1, 6 and 8 (fig. 6.3). In GAC-stored PC, no differences in pO$_2$ were observed between the different storage containers.

![Graph showing changes in pO$_2$](image)

**Fig. 6.3** - Changes in pO$_2$ (range of CVs: 9.31-39.2%).

Swirling changed during the eight days of storage as described previously [133] and ranged from a score of 3 at the first day of storage till 1 at day 8. Differences between storage containers were not observed. At day 8, GAC-stored PCs showed a higher swirling score than plasma-stored PCs.

When platelets were stored in plasma, all containers gave a satisfactory maintenance of platelet morphology that slightly decreased during storage and started for 1-L containers (PO and UPX80) at quite low values (fig. 6.4). A significant difference between UPX80 containers and 1.5-L or 1-L PO containers was not observed except for day 0 when 1.5-L PO containers had a much higher morphology score than UPX80 containers (p < 0.001) and 1-L PO containers (p < 0.01).

In GAC, there was an increase in morphology score between day 0 (directly after preparation and filtration) and day 1. At day 0, 1.5-L PO gave a lower (p < 0.05) score than 1-L PO.

Low scores of 1.5-L PO at day 0 in plasma as well as in GAC were not caused by bag size, because morphology was measured directly after preparation. Platelets stored in 1-L PO or 1.5-L PO were prepared on different days, and possible day-to-day variances are the only explanation, because the experimental conditions were the same on all preparation days. A consistent difference in morphology score between UPX80 and PO containers during the whole storage period of 8 days was not demonstrated in GAC or in plasma.
Comparison between UPX80 and PO containers

**Fig. 6.4** - Changes in morphology score according to Kunicki (range of CVs 0.273-35.9%).

**Table 6.2**: Metabolic parameters in 1- and 1.5-L (1-L and 1.5-L) polyolefin (PO) and UPX80 containers. Mean values ± SD are shown

<table>
<thead>
<tr>
<th></th>
<th>Day</th>
<th>PO plasma 1.5-L (n=4)</th>
<th>PO plasma 1-L (n=4)</th>
<th>UPX80 plasma 1-L (n=8)</th>
<th>PO GAC 1.5-L (n=4)</th>
<th>PO GAC 1-L (n=4)</th>
<th>UPX80 GAC 1-L (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lactate</strong></td>
<td>1</td>
<td>6.8±1.08</td>
<td>6.8±0.72</td>
<td>7.0±1.21</td>
<td>2.9±0.50</td>
<td>3.8±0.98</td>
<td>3.9±1.68</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8.7±1.26</td>
<td>9.4±0.66</td>
<td>9.8±1.64</td>
<td>5.5±1.05</td>
<td>6.3±1.27</td>
<td>6.3±1.73</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>12.8±1.87</td>
<td>14.0±1.22</td>
<td>15.6±2.96</td>
<td>9.4±2.00</td>
<td>9.5±2.01</td>
<td>10.1±3.06</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>15.6±2.39</td>
<td>16.8±1.70</td>
<td>19.1±4.16</td>
<td>10.7±2.63</td>
<td>11.0±3.86</td>
<td>10.5±3.92</td>
</tr>
<tr>
<td><strong>Adenylic acid</strong></td>
<td>1</td>
<td>74.5±6.7</td>
<td>76.5±9.9</td>
<td>80.3±4.6</td>
<td>81.0±7.7</td>
<td>74.3±10.7</td>
<td>80.5±4.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>79.3±4.5</td>
<td>80.8±6.6</td>
<td>73.8±5.2</td>
<td>70.5±3.1</td>
<td>71.0±12.2</td>
<td>75.5±6.8</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>67.8±2.2</td>
<td>66.0±5.6</td>
<td>65.4±5.1</td>
<td>70.0±5.9</td>
<td>61.8±8.1</td>
<td>64.0±5.1</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>63.5±2.9</td>
<td>59.5±3.3</td>
<td>58.1±6.1</td>
<td>75.5±18.0</td>
<td>53.0±6.3</td>
<td>66.9±15.5</td>
</tr>
<tr>
<td><strong>ATP</strong></td>
<td>8</td>
<td>92±3.5</td>
<td>85±12.0</td>
<td>79±8.0</td>
<td>72±4.1</td>
<td>70±2.9</td>
<td>68±10.2</td>
</tr>
<tr>
<td><strong>ATP/ADP</strong></td>
<td>1</td>
<td>1.8±0.13</td>
<td>1.9±0.06</td>
<td>1.8±0.08</td>
<td>1.7±0.14</td>
<td>1.9±0.10</td>
<td>1.8±0.16</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.0±0.08</td>
<td>1.9±0.10</td>
<td>1.9±0.08</td>
<td>1.8±0.21</td>
<td>1.9±0.10</td>
<td>1.9±0.14</td>
</tr>
<tr>
<td><strong>ATP ratio</strong></td>
<td>6</td>
<td>2.1±0.08</td>
<td>2.0±0.15</td>
<td>2.1±0.10</td>
<td>1.8±0.17</td>
<td>1.9±0.05</td>
<td>2.1±0.20</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>2.2±0.18</td>
<td>2.2±0.05</td>
<td>2.3±0.08</td>
<td>1.8±0.14</td>
<td>2.0±0.25</td>
<td>2.0±0.11</td>
</tr>
</tbody>
</table>

Footnotes table 6.2:

*a* cumulative production in pmol per platelet

*b* (AMP+ADP+ATP) pmol per 10⁶ platelets

*c* percentage of the value on day 1
**Metabolic parameters**

The lactate concentration in the supernatant was related to the platelet count at day 1. No differences were found between the various containers (table 6.2). Lactate production divided by the glucose consumption ranged from about 1.2 to 1.9 over the whole storage period (data not shown), indicating at least partial aerobic glycolysis.

Adenyllic acid was expressed as the sum of AMP, ADP and ATP amount per $10^6$ platelets. At the various storage days no differences were observed between the different container types and between the two storage media. Over the whole storage period, the decrease in adenyllic acid was $20 \pm 16.4\%$ in all storage containers (table 6.2).

The ATP content at day 8 as percentage of the ATP present on day 1 is also shown in table 6.2. The ATP concentration in plasma PC in PO containers on day 3 (1-L: $107 \pm 8.4\%$; 1.5-L: $110 \pm 4.7\%$) was increased compared to the first day of storage. This was different from the $94 \pm 6.4\%$ observed on the same day for UPX80 containers, indicating a good metabolic condition of the platelets and a net production of ATP due to aerobic phosphorylation. Compared to GAC-stored platelets, a significantly higher plasma ATP concentration was found at day 8 in 1.5-L PO containers and in UPX80 containers. The ATP/ADP ratio was always between 1.8-2.3 and did not differ between the GAC- and the plasma-stored PC (table 6.2). The rise in the ATP/ADP ratio as observed during storage in plasma indicates some decrease of the storage pool content (dense granules; [134]), which contain ATP and ADP in a much lower ratio than does the metabolic pool.

The increase in hypoxanthine concentrations was in conformity with the observed decrease in adenyllic acid. Hypoxanthine concentrations were higher in GAC than in plasma-stored PC on day 1, 3 and 6 (data not shown).

**β-thromboglobulin and P-selectin release**

β-TG and soluble P-selectin amounts (sP-selectin) were related to the platelet count and expressed as ng per $10^9$ platelets. In plasma, on day 6 and 8, the supernatants of PC in PO containers (1-L and 1.5-L) showed a lower ($p < 0.05$) β-TG concentration than those from PC in UPX80 containers (fig 6.5). β-TG concentrations in GAC in (1- and 1.5-L) PO containers were comparable with those in UPX80 containers at all time points.

In plasma as well as in GAC there was no difference in sP-selectin concentration related to the platelet count when UPX80 containers were compared with PO containers (fig 6.6).
Comparison between UPX80 and PO containers

**Fig. 6.5** - Changes in β-thromboglobulin concentrations related to platelet numbers (range of CVs: 14.0-44.7%).

**Fig. 6.6** - Changes in soluble P-selectin (sP-selectin) concentrations related to platelet numbers (range of CVs: 9.65-73.1%, high CVs are caused by large range of values for healthy individuals).

*Activation-dependent antigens*

No differences were observed between the mean fluorescence intensity of platelets labelled with anti-CD42b (GpIb) in plasma and in GAC or between UPX80 containers and 1.5-L PO containers. Moreover, platelets stored in 1- and
1.5-L PO containers showed no significant differences in CD42b expression either (data not shown).

![Graph showing changes in CD62P expression](image)

**Fig. 6.7** - Changes in CD62P expression (P-selectin) on the platelet membrane (range of CVs: 9.35-40.0%).

The CD61 (GpIIIa) expression on platelets stored in plasma or in GAC showed no differences between the three container types. CD62P expression in plasma was higher (p < 0.01) at day 6 when UPX80 were compared with 1.5-L PO containers and at day 8 when compared with both PO containers (fig 6.7). In GAC, differences (p < 0.05) in expression were found between 1- and 1.5-L PO containers at day 8. CD62P expression in UPX80 was higher than in PO containers at day 6 and 8 (p < 0.01).

The CD63 expression increased during storage of PC in plasma. The CD63 expression was higher in UPX80 containers at day 6 compared with 1.5-L PO containers and at day 8 compared with 1- and 1.5-L PO containers (p < 0.01). In GAC, UPX80 gave higher CD63 expression at day 8 compared to 1-L PO containers (p < 0.05) (data not shown).

**DISCUSSION**

Filtration of PC prepared in GAC/plasma medium resulted in a higher platelet loss than filtration of PC prepared in plasma. Similar results were described previously by Shimizu et al. [135]. Low initial morphology scores of GAC PC indicate a high activation degree directly after preparation and filtration and might be an explanation for the high platelet loss during filtration. However, platelets stored in GAC showed a recovery of morphology score between day 0 (directly after preparation and filtration) and day 1 in PO and UPX80 containers.
Comparison between UPX80 and PO containers

UPX80 containers, developed for increased gas exchange showed better blood gas values compared to PO containers. In plasma, UPX80 yields lower pCO₂ and higher pO₂ values than PO on different storage days, starting at day 1. In contrast to plasma, in GAC there is only a lower pCO₂ in UPX80 compared to PO at day 6 and 8 of storage, whereas pO₂ was similar in both container types. The pH maintenance in plasma and GAC in UPX80 containers is better than in PO containers, due to the increased CO₂ exchange because the production of lactate is similar.

The increase in pH between day 6 and 8 in GAC can be explained by the nearly complete absence of bicarbonate and carbonic anhydrase, which are both present in plasma. These systems compete for CO₂ with the diffusion of CO₂ through the storage bag. CO₂, easily escapes in absence of both systems (i.e. in GAC), resulting in an increase in pH.

During normal shelf life, UPX80 compared to PO containers do not demonstrate differences in activation degree for the various activation parameters CD62P, CD63, soluble P-selectin and β-TG. However, during prolonged storage (day 6 and 8), the CD63 and CD62 expression and β-TG release are significantly higher in UPX80 compared to PO, indicating more activation in UPX80.

We conclude that containers made from UPX80 show good gas exchange, good morphology of the platelets and a platelet metabolism that is not different from PO containers [105]. The degree of activation is similar during the normal shelf life of PC (5 days) for platelets stored in UPX80, compared to PO containers. After storage longer than 5 days, studied to simulate bad conditions and to show possible differences more easily, the degree of activation in UPX80 became significantly higher, but this was not consistent for all measured activation markers.

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