



Non-phthalate plasticizer DEHT preserves adequate blood component quality during storage in PVC blood bags

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Vox Sanguinis

Background and objectives Commercial blood bags are predominantly made of polyvinyl chloride (PVC) plasticized with di(2-ethylhexyl) phthalate (DEHP). DEHP is favourable for storage of red blood cells (RBC). Historically, removal of DEHP from blood bags has been linked to unacceptable haemolysis levels. Oncoming regulatory restrictions for DEHP due to toxicity concerns increase the urgency to replace DEHP without compromising RBC quality. Di(2-ethylhexyl) terephthalate (DEHT) is one suggested substitute. The aim of this study was to compare PVC-DEHT to PVC-DEHP blood bags using additive solutions saline-adenine-glucose-mannitol (SAGM) and phosphate-adenine-glucose-guanosine-saline-mannitol (PAGGSM), to determine whether DEHT can maintain acceptable component quality.

Materials and methods RBC concentrates (N = 64), platelet concentrates (N = 16) and fresh frozen plasma (N = 32) were produced from whole blood collected into either DEHT or DEHP plasticized systems. Using a pool-and-split study design, pairs of identical RBC content were created within each plasticizer arm and assigned either SAGM or PAGGSM. Storage effects were assessed weekly for 49 days (RBC), 7 days (platelets) and before/after freezing (plasma).

Results Though haemolysis was slightly higher in DEHT, all study arms remained below half of the European limit 0.8%. K⁺ was lower in DEHT than in DEHP independent of additive solution. The metabolic parameters were not influenced by choice of plasticizer. Platelet activation/metabolism and plasma content were similarly preserved.

Conclusion Our study demonstrates that the plasticizer DEHT provides adequate blood component quality. We propose DEHT as a strong future candidate for replacement of DEHP in blood bags.

Key words: DEHT, DEHP, phthalates, plasticizers, blood components, red blood cells.

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Introduction

Polyvinyl chloride (PVC) plasticized with di(2-ethylhexyl) phthalate (DEHP) has been the material of choice for

commercial blood containers since the mid-20th century. Plasticizers are essential for material flexibility; facilitating centrifugation, sealing, transport and general handling of blood bags without risk of breakage and product loss [1]. DEHP is a dipolar, lipophilic molecule non-covalently bound to the PVC polymer, that leaches from the plastics when in contact with the stored blood component. By incorporation into the red blood cell (RBC) bilayer membrane, DEHP helps preserve membrane integrity [2–4].

However, concerns have been raised about DEHP-linked endocrine-disrupting toxicity observed in animal models [5]. Though actual evidence of toxic impact in humans is ambiguous, it cannot be excluded that DEHP and/or its metabolites may be harmful during prolonged exposure. Therefore, replacement of DEHP in blood bags with a corresponding non-toxic plasticizer has long been desired, but not at the expense of blood component quality [6, 7]. The lack of an acceptable plasticizer substitute for RBCs has slowed down the conversion process considerably, but recently, the demand became urgently motivated due to the updated European Commission regulation (EC) 2017/745 allowing a maximum concentration of 0.1% weight/weight of DEHP in medical devices [8]. Though exceptions are possible, finding a substitute is nonetheless a high priority.

Several plasticizers have been suggested as substitutes to DEHP in blood bags. Di(2-ethylhexyl) terephthalate (DEHT) and di-isononyl cyclohexane-1,2-dicarboxylic acid (DINCH) have shown the greatest potential so far, despite providing inferior RBC quality in previous studies [9–13].

DEHT is a structural isomer of DEHP with no reported resemblance of the DEHP toxicity pattern [14, 15]. A recent study assessing RBCs stored in PVC-DEHT bags with phosphate–adenine–glucose–guanosine–saline–mannitol (PAGGSM) and AS-1 as additive solution (AS) showed promising results for RBC storage [9]. To our knowledge, there are presently no published studies where the collection bag is plasticized with DEHT, nor are there published assessments of the AS saline–adenine–glucose–mannitol (SAGM) paired with PVC-DEHT. As SAGM is presently the most widely used AS in Europe, this would be a valuable contribution to the collective gathering of DEHT *in vitro* data.

Plasma, mostly prepared as fresh frozen plasma (FFP), is widely used, predominantly at emergency and critical care units as part of the treatment for critical bleeding [16]. Coagulation factors, except factor VIII, and to some extent factor V, are generally well preserved in FFP [17]. Although it is not expected that plasticizers influence the levels of coagulation factors, it is important to confirm this by comparing the concentrations of coagulation

factors/inhibitors preserved in FFP during PVC-DEHT and PVC-DEHP storage.

Platelets are generally stored with plasticizers that provide superior gas permeability to DEHP, for instance *n*-butyryl-tri-*n*-hexyl citrate (BTHC) [18] or tri-(2-ethylhexyl) trimellitate (TOTM) [19]. However, when prepared from whole blood (WB), platelets are in contact with the processing set plasticizer until preparation and may thus be affected.

In this study, we focused on assessing the quality of RBCs produced and stored in PVC-DEHT blood bag systems paired with SAGM or PAGGSM, during a 49-day storage period. The effects were compared to corresponding storage in PVC-DEHP. Secondary, we compared plasma and platelet components stored in DEHT or DEHP, to ensure their compatibility in a future WB set.

Materials and methods

Blood collection and component production

Prototype blood bags made entirely of PVC-DEHT or PVC-DEHP, respectively (including tubes, ports and filters), were manufactured for the study (Macopharma, Mouvoux, France), mimicking the commercially available quintuple bottom-and-top NPT reference (bag configuration: Supporting Information 1). Sixty-four WB units (450 ml \pm 10% in 63 ml citrate-phosphate-dextrose) from consenting donors were collected in the prototypes (N = 32; blood type A = 20, O = 12 of each plasticizer). Within each plasticizer arm, the WB units were pairwise ABO matched, pooled and split into new pairs of identical content. Each pair was assigned two additional sets of plasticizer-matched prototype bags for further WB processing into leucoreduced RBCs, leucoreduced plasma and buffy coat (BC). One of the sets contained 100 ml SAGM, the other 100 ml PAGGSM (Fig. 1). WB processing was performed according to Karolinska standard operating protocol (SOP): centrifugation at 3130 *g*, 11 minutes, MacoSpin, and separation by MacoPress Smart Revo (both MacoPharma).

Four study arms were generated for RBCs: DEHT/SAGM, DEHT/PAGGSM, DEHP/SAGM and DEHP/PAGGSM (N = 16 per arm). All units were leucoreduced by filtration and stored at 2–6°C within 8 hours of donation.

One plasma unit was randomly chosen from each processing pair, generating two plasma study arms: DEHT and DEHP (N = 16; blood type A = 10, O = 6 of each). They were leucoreduced by filtration, frozen in Lundair freeze LF Maxi (Ingenjörfirman P-O Persson AB, Helsingborg, Sweden) and stored in \leq –25°C within 18 hours of donation.

Double-dose BC platelet concentrates (PC) were prepared from pools of 8 same-plasticizer BCs and Platelet Additive Solution-E (PAS-E) using I-Platelet Pooling (IPP) set (Kansuk, Istanbul, Turkey), centrifugation (421 g, 9 minutes, Macospin) and extraction (Macopress Smart Revo), according to Karolinska SOP. Immediately after production, one of the units from each double dose was transferred into a prototype platelet storage bag plasticized with BTHC (ports of TOTM) and tubes of either DEHT or DEHP and then stored in platelet agitators at 18–22°C. Additional WB was collected until each of the two platelet study arms had N = 8.

All RBC and plasma components followed assigned plasticizer (DEHT or DEHP) from collection throughout the entire process including sampling. Similar for PCs, except for briefly during production, as the IPP set is plasticized with TOTM with a hard housing filter of polycarbonate, but contains DEHP in tubes and forks. Potential cross-contamination was avoided through separated storage and handling, both during bag manufacturing and of the collected and produced components. Measurements of raw material, semi-finished bags and sterilized finished products confirmed DEHP content <0.1% weight/weight in the DEHT material at all measuring points.

TSCDII (TerumoBCT, Lakewood, CO, USA) was used for all sterile docking. Seals were made with Qseal-free, Qseal-opti and Qseal-air (Conroy Medical AB, Upplands Väsby, Sweden).

Sampling

Prototype sampling bags (nominal volume 40 ml) were used. The RBCs were sampled every 7 days from day (d) 1 to 49. Plasma was sampled on the day of collection (d0 pre-freezing). After thawing (Barkey plasmatherm, Barkey GmbH & Co. KG Leopoldshöhe, Germany), the bags were kept at 2–6°C and sampled on d0 (within 30 minutes of thawing), d7 and d14 (factor VIII only) post-thawing. Aliquots were refrozen at ≤−65°C until analysis. PCs were sampled on d2, d5 and d7.

Analysis of storage effects

Production process

To verify conformity of the production process, residual white blood cells (WBC) were counted on the first day of sampling in all components, by use of cell counter ADAM-rWBC (NanoEnTek, Seoul, South Korea) (RBCs and plasma) or Nageotte/microscopy (PCs). RBC concentrates (RCC) were analysed for haematocrit (Hct) and Hb_{RCC} (g/unit and g/l) using Swelab Alfa Plus Basic hematology analyzer (Boule Diagnostics AB, Spånga, Sweden). Residual RBCs in plasma were counted by flow cytometry (CytoFLEX, Beckman Coulter, Brea, CA, USA). Platelet count (/l and /unit) was obtained by CA 620 Cellguard (Boule Medical, Stockholm, Sweden). The PCs were visually checked for swirling and aggregates.

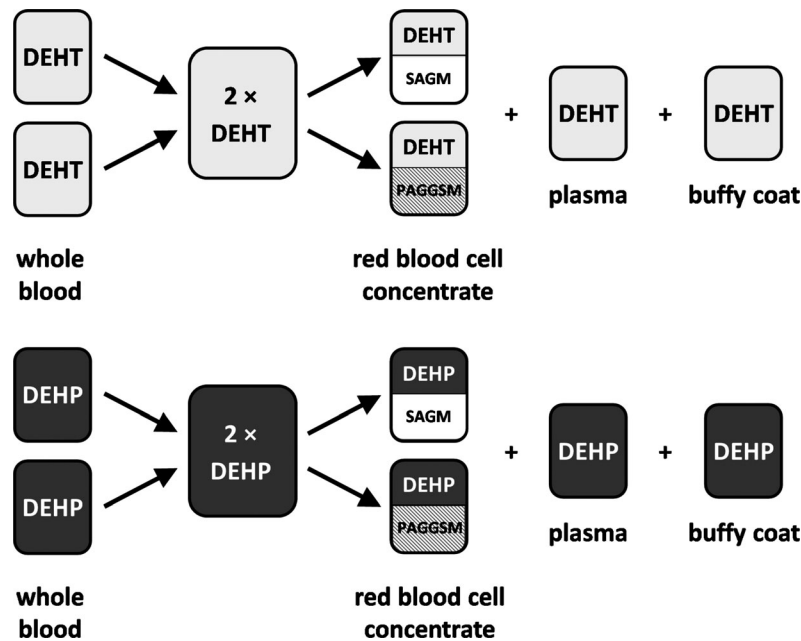


Figure 1 Pool-and-split study approach for PVC bags plasticized with DEHT (N = 32) or DEHP (N = 32), with additive solutions SAGM and PAGGSM for red blood cell storage.

Serology tests were performed for all donors at donation. All units were subjected to bacterial screening after completed storage (Karolinska University Laboratory, Clinical chemistry and Clinical microbiology departments).

Red blood cells

The storage effects on RBCs were assessed by analysis of haemolysis, mean corpuscular volume (MCV), RBC microvesicle (RMV) count, RMV phosphatidylserine translocation, pH and concentrations of potassium ions (K^+), adenosine triphosphate (ATP), glucose, lactate and 2,3-diphosphoglycerate (2,3-DPG).

Haemolysis analysis was performed using HemoCue plasma/low haemoglobin photometer (Radiometer Medical ApS) following previous protocol [20]. Haemolysis (%) = $(100 - Hct) \times Hb_{supernatant} (g/l) / Hb_{RCC} (g/l)$. MCV (fL) was determined by Swelab Alfa. ABL 800 Flex blood gas analyzer (Radiometer Medical ApS, Brønshøj, Denmark) assessed extracellular pH (37°C) and concentrations (mmol/l) of K^+ , glucose and lactate. Count and phosphatidylserine externalization (annexin V) of RMV were investigated by flow cytometry (CytoFLEX): PE-Cy7 and BV421 mouse anti-human CD235a (BD Biosciences, San Jose, CA, USA) were used for glycophorin A (marker for RBC origin) detection; aside from this, protocol and reagents have been previously described [20]. The results were analysed with FlowJo v.10.6 (Ashland, OR, US). Concentration of ATP ($\mu\text{mol/g Hb}$) was assessed by luminometry (Orion Microplate Luminometer, Berthold, Pforzheim, Germany) using ATP Kit SL (BioThema, Handen, Sweden), while 2,3-DPG ($\mu\text{mol/g Hb}$) was analysed through spectrophotometry (Jenway 6500 Spectrophotometer, Barloworld Scientific Ltd., Dunmow, Essex, UK) with 2,3-DPG test kit 10148334001 (Roche Diagnostics, Mannheim, Germany).

Plasma

Plasma was analysed for coagulation factors V, VII, VIII, X, XI (coagulation, turbidimetry), XIII, protein C (enzymatic activity, chromogenic), protein S-free (immunological, turbidimetry) and von Willebrand factor (photometry) (all IU/100 ml) on haemostasis analyser BCS-XP (Siemens, Munich, Germany). Triglycerides (enzymatic photometry, mmol/l) and α -1-antitrypsin (immunological, turbidimetry, g/l) were assessed using the Cobas 8000 platform (Roche Diagnostic, Basel, Switzerland). Fibrinogen (Clauss coagulation photometry, g/l) was measured with Sysmex CS 5100 (Kobe, Japan).

Platelets

Mean platelet volume (MPV, fL) was measured using CA 620 Cellguard. Cell disintegration was determined

spectrophotometrically (Jenway 6500, test kit 063K6003 Sigma-Aldrich, St Louis, MO, USA) through determination of extracellular lactate dehydrogenase (LDH, %). Parameters of extracellular metabolism, pH (37°C, corrected to 22°C through Rosenthal's factor), glucose (mmol/l) and lactate (mmol/l), were measured, and bicarbonate (mmol/l) calculated, by ABL 800. Intracellular ATP content ($\mu\text{mol}/10^{11}$ platelets) was assessed through luminometry (same equipment/reagents as RBCs). Mitochondrial membrane potential (determined through JC-1, %), activation level (CD62P, %), expression of surface glycoprotein CD42b (MFI), expression of platelet endothelial cell adhesion molecule PECAM-1 (MFI), and response capacity to agonist stimulation through PAC-1 with collagen, ADP and thrombin (%) were all analysed as previously described [21, 22].

Statistical analysis

Gaussian distribution was verified (D'Agostino-Pearson normality test), and mean \pm standard deviation was calculated. Two-way ANOVA was chosen for statistical significance testing of RBCs, and unpaired t-test for plasma and platelets (RBCs: $N = 16$, plasma: $N = 16$, platelets: $N = 8$ per study arm), with Holm-Sidak's correction for multiple comparisons. For contingency test of 2,3-DPG at d21, Fisher's exact test was used to compare AS and plasticizers, respectively.

GraphPad Prism v.8.2 for Windows (GraphPad Software Inc., La Jolla, CA, USA) was used for computation of all statistics.

Ethical approval

Ethical approval was applied for but considered not applicable according to the Stockholm Regional Ethical Review Board.

Results

Component acceptance criteria

After production, all components fulfilled European [23] and local quality criteria without non-conformities (Supporting Information 2). In addition, screening for infectious markers [23] was negative for all donors.

There were no visual or physical differences between the plasticizers in processing functionality or handling. There were no leakages during transport, processing, storage or freezing/thawing of plasma. 100% of the tube seals and sterile dockings held adequate quality and sterile connections were easily opened.

Minor effects on RBC membrane and metabolism

Haemolysis, RMV and K^+ were analysed to assess the effects of plasticizer and AS on cell membrane degradation.

Haemolysis was higher in DEHT than in DEHP from d14 onwards ($P < 0.001$). From day 35, AS also affected haemolysis, with lower haemolysis levels in PAGGSM for both DEHT and DEHP ($P < 0.05$ within plasticizers, $P < 0.001$ for all other combinations). However, at d49, DEHT/SAGM, highest of the study arms (0.39%), still barely reached half of the European limit 0.8% [23] (Fig. 2a and b, Table 1, Supporting Information 3).

RMV count is an indicator of membrane integrity loss. Reflecting the haemolysis results, the count was higher in DEHT than in DEHP and in SAGM than in PAGGSM (Fig. 2c, Table 1). We also noticed a similar highest-to-lowest order of glycophorin A expression decrease throughout storage (data not shown).

Externalization of RMV phosphatidylserine, apoptosis marker, was also elevated in DEHT/SAGM storage. At d49, DEHT/SAGM was higher than both DEHP arms ($P < 0.05$), whereas DEHT stored with PAGGSM remained below or did not differ from the DEHP arms throughout storage (Fig. 2d, Table 1).

Elevated extracellular K^+ indicates membrane damage or inhibited metabolism. The extracellular K^+ levels were

lower ($P < 0.001$) in DEHT from d28 onwards, independent of AS (Fig. 3a). End K^+ concentration in DEHT finished 5–10 % lower than in DEHP (Table 1).

2,3-DPG preservation, associated with oxygen unloading ability, was notably higher ($P < 0.05$ or higher significance level) in DEHT/SAGM at d14. At d21, a significantly higher percentage of DEHT-stored RCCs had some 2,3-DPG left (DEHT 66%, DEHP 38%, $P < 0.05$), while there was no difference when comparing AS (SAGM 47%, PAGGSM 56%, ns)(Fig. 3b).

The RBC metabolism was further studied through analysis of pH (Fig. 4a), glucose, lactate and ATP (Fig. 4b, all Table 1). For units with the same AS, there were no significant differences (ns), regardless of the plasticizer used. An overall increased metabolism rate in SAGM, predominantly visualized by higher lactate generation ($P < 0.01$ or higher significance level at d7, significance level decreasing over time), resulted in better ATP preservation in PAGGSM storage from d28 onwards ($P < 0.05$ or higher significance level).

Mean corpuscular volume, indicator of cell swelling, was not affected by the plasticizer (Fig. 4c, Table 1), but showed markedly higher values for SAGM storage from d14 onwards ($P < 0.05$ or higher significance level).

Complementary details for RBCs are available in Supporting Information 3.

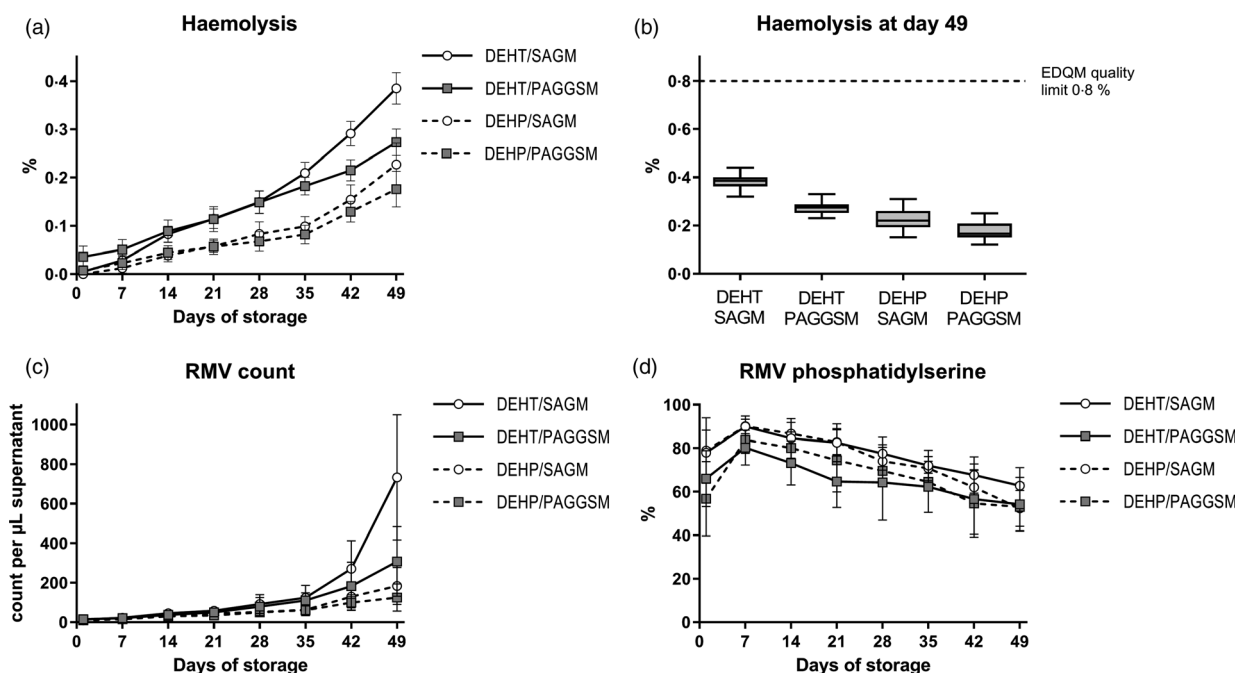


Figure 2 The RBC membrane was acceptably preserved in DEHT plasticized bags, visualized through (a) haemolysis over 49 days, (b) day 49 haemolysis in relation to European limit (0.8%, dashed line), (c) RBC microvesicle (RMV) count and (d) percentage of RMV phosphatidylserine externalization, all stored in DEHT or DEHP with additive solution SAGM or PAGGSM. Values are displayed as (a, c–d) mean \pm standard deviation and (b) boxplot with Tukey whiskers. Significance was tested using two-way ANOVA with Holm–Sidak’s correction for multiple comparisons (Table 1, Supporting Information 3).

Table 1 Storage analysis results for red blood cells (N = 16 of each study arm) on day 1 and 49 (day 42: available in Supporting Information 3)

Analysis parameter, day 1	DEHT/SAGM Mean ± SD	DEHT/PAGGSM Mean ± SD	DEHP/SAGM Mean ± SD	DEHP/PAGGSM Mean ± SD
Haemolysis (%)	0.01 ± 0.01	0.04 ± 0.02	below detection limit	0.01 ± 0.01
	Significant differences: ***, ###, □□□			
Red blood cell microvesicles, count (× 10 ³ /μl)	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0
	Significant differences: ns			
Red blood cell microvesicles, phosphatidylserine positive (%)	77.9 ± 10.4	66.0 ± 12.9	78.9 ± 15.1	56.8 ± 17.1
	Significant differences: **, †††, ##, □, §			
Extracellular K ⁺ (mmol/l)	2.9 ± 0.1	3.4 ± 0.2	3.0 ± 0.2	3.5 ± 0.2
	Significant differences: ns			
pH 37°C	6.991 ± 0.018	6.846 ± 0.017	6.988 ± 0.032	6.844 ± 0.025
	Significant differences: ***, †††, ###, §§§			
Glucose (mmol/l)	29.8 ± 0.6	28.9 ± 0.6	29.7 ± 0.8	28.9 ± 0.8
	Significant differences: ns			
Lactate (mmol/l)	3.8 ± 0.3	3.5 ± 0.3	4.0 ± 0.5	3.6 ± 0.3
	Significant differences: ns			
Adenosine triphosphate (ATP) (μmol/g Hb)	5.8 ± 0.6	5.5 ± 0.4	5.7 ± 0.5	5.1 ± 0.6
	Significant differences: #			
Mean corpuscular volume (fl)	92.6 ± 2.5	92.7 ± 2.6	92.5 ± 2.7	92.6 ± 2.7
	Significant differences: ns			
2,3-diphosphoglycerate (2,3-DPG) (μmol/g Hb)	10.9 ± 1.8	11.6 ± 1.8	11.2 ± 1.7	10.8 ± 1.7
	Significant differences: ns			

Analysis parameter, day 49	DEHT/SAGM Mean ± SD	DEHT/PAGGSM Mean ± SD	DEHP/SAGM Mean ± SD	DEHP/PAGGSM Mean ± SD
Haemolysis (%)	0.39 ± 0.03	0.27 ± 0.03	0.23 ± 0.04	0.18 ± 0.04
	Significant differences: ***, †††, ††††, ###, □□□, §§§			
Red blood cell microvesicles, count (× 10 ³ /μl)	0.73 ± 0.32	0.31 ± 0.18	0.18 ± 0.09	0.12 ± 0.07
	Significant differences: ***, †††, ††††, ###, □□□, §			
Red blood cell microvesicles, phosphatidylserine positive (%)	62.8 ± 8.3	54.2 ± 12.5	52.4 ± 8.2	53.0 ± 11.1
	Significant differences: †, †			
Extracellular K ⁺ (mmol/l)	45.5 ± 1.4	44.7 ± 1.7	48.4 ± 1.7	48.7 ± 1.5
	Significant differences: †††, ††††, ###, □□□			
pH 37°C	6.275 ± 0.039	6.234 ± 0.040	6.291 ± 0.027	6.257 ± 0.029
	Significant differences: ***, ###, §§			
Glucose (mmol/l)	12.9 ± 1.5	11.5 ± 1.4	13.4 ± 1.1	12.3 ± 1.3
	Significant differences: **, ###, §			
Lactate (mmol/l)	33.4 ± 2.3	32.1 ± 2.2	34.0 ± 1.1	32.2 ± 1.3
	Significant differences: *, †, ##, §§			
Adenosine triphosphate (ATP) (μmol/g Hb)	2.9 ± 0.3	3.7 ± 0.2	3.0 ± 0.7	4.0 ± 0.8
	Significant differences: *, †††, #, §§			
Mean corpuscular volume (fl)	102.4 ± 3.3	96.3 ± 3.0	100.7 ± 2.5	94.8 ± 2.4
	Significant differences: ***, ††††, ###, §§§			
2,3-diphosphoglycerate (2,3-DPG) (μmol/g Hb)	Not applicable			

Data is presented as mean ± standard deviation (SD). Significances are shown as *DEHT/SAGM vs. DEHT/PAGGSM, †DEHT/SAGM vs. DEHP/SAGM, ††DEHT/SAGM vs. DEHP/PAGGSM, †††DEHT/PAGGSM vs. DEHP/SAGM, ††††DEHT/PAGGSM vs. DEHP/PAGGSM, †DEHT/PAGGSM vs. DEHP/SAGM, ††DEHT/PAGGSM vs. DEHP/PAGGSM, †††DEHP/SAGM vs. DEHP/PAGGSM. Significance levels are shown as **P* < 0.05, ***P* < 0.01, ****P* < 0.001, ns (no significance).

No differences in FFP coagulation factors/inhibitor preservation

The results of the plasma factors were consistent: similar levels were observed for both DEHT and DEHP units at all measured time points (Table 2).

The expected decrease of factor VIII was observed in both types of bags to a similar extent: DEHT: 72 ± 19 and DEHP: 68 ± 14 IU/100 ml directly after freezing/thawing, which was tolerable compared to the European limit (≥70 IU/100 ml [23]). At d14 post-thawing, both had decreased to 57 IU/100 ml (Fig. 5a). None of the

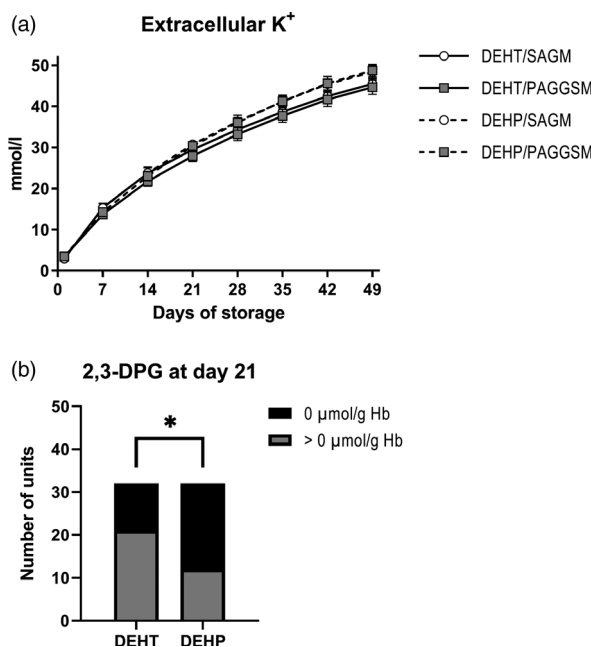


Figure 3 DEHT was slightly superior to DEHP for RBC storage independent of additive solution for the parameters (a) extracellular potassium (K^+) and (b) 2,3-DPG. Values are displayed as (a) mean \pm standard deviation and (b) number of non-depleted (DEHT: 21, DEHP: 12) and depleted (DEHT: 11, DEHP: 20) units. Significance in (b) was tested with Fisher's exact test ($*P < 0.05$).

other parameters was noticeably affected over time with either plasticizer. Complementary details are available in Supporting Information 4.

Similar platelet reactivity, activation and metabolism

The platelets were exposed to DEHT or DEHP until preparation of the PCs and then again during sampling. There were no significant differences between the properties of platelets produced in DEHT or DEHP systems (Table 3). The collective assessment of the platelet parameters demonstrated an overall high quality, independent of plasticizer exposure.

No alterations in MPV were observed. Extracellular LDH levels implied normal platelet lysis rate in both plasticizers. Glucose consumption and lactate production correlated well, and a viable metabolism was confirmed by sufficient bicarbonate buffering capacity and ATP content [24, 25]. Mitochondrial membrane potential (JC-1) was well preserved in both study arms. Relative expression of surface receptor CD42b, containing vWF binding site, and PECAM-1, regulator of collagen stimulation, remained equally stable during storage for both study arms, with an expected moderate decrease of CD42b and increase in

PECAM-1. Assessment of CD62P indicated no abnormal activation in either study arm (Fig. 5b). Response capacity to agonist stimulation was similarly reduced during storage (Fig. 5c). Complementary details are available in Supporting Information 5.

Discussion

This study presents a collection of *in vitro* evidence indicating that DEHT is a realistic candidate to replace DEHP in blood bags. The RBC quality markers were well within regulatory limits, and no adverse effects were seen for plasma or platelets.

In most aspects, DEHT-stored RCCs showed similar patterns to traditional DEHP storage [26, 27], likely attributed to a favourable WB processing strategy. Haemolysis levels of 0.29% (DEHT/SAGM) and 0.22% (DEHT/PAGGSM) at d42, common storage end time, are considered fully normal levels also in DEHP (Supporting Information 3). It is well established that DEHP migrates into and stabilizes the RBC membrane [3]. In a previous study conducted at our centre where RBCs were stored in polyolefin plastics [4], RBCs in PAGGSM exceeded 0.8% beyond 3 weeks storage. The PAGGSM results in our study may indicate that DEHT has a similar stabilizing mechanism to DEHP, although perhaps not as strong, since measurements of leached DEHT in RCCs yield markedly lower free levels than DEHP [9].

In two perspectives, DEHT may even be preferential to DEHP. Slightly lower K^+ levels in older DEHT-stored RCCs (Fig. 3a) and fewer 2,3-DPG depleted units at d21 during DEHT storage (Fig. 3b) were observed. K^+ has been associated with cardiac arrest [28], whereas 2,3-DPG facilitates oxygen unloading [29]. 2,3-DPG is dependent on pH, a metabolic factor that is influenced by AS composition [30–32]. Our results carefully suggest that plasticizer composition may be an additional variable impacting the 2,3-DPG depletion rate, though the possible mechanism, as well as clinical impact, requires further exploration.

The choice of AS clearly influenced the RBC storage lesion. Also with DEHT, PAGGSM offered a better membrane-protective storage environment than SAGM, visualized by reduced haemolysis, RMV count and percentage of RMV apoptosis marker (Fig. 2a–d), a pattern previously described in DEHP studies [30–32]. PAGGSM also implicated better ATP preservation (Fig. 4b) and less cell swelling (Fig. 4c). This indicates that PAGGSM would probably be the first-hand recommendation to be paired with DEHT, although, since overall differences were small, SAGM remains a satisfactory option.

We wanted to confirm that DEHP removal/DEHT addition did not detrimentally affect plasma and platelets, as has been previously investigated for DINCH [10]. For

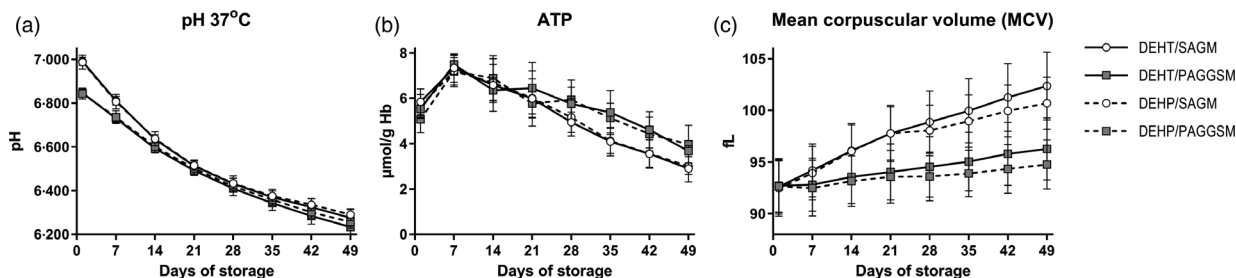


Figure 4 Plasticizer did not impact RBC metabolism parameters (a) pH, (b) ATP or (c) MCV. Graphs show storage over time in DEHT or DEHP combined with SAGM or PAGGSM. Values are displayed as mean \pm standard deviation.

Table 2 Storage analysis results for plasma stored in DEHT or DEHP (N = 16 of each) on day 0 pre-freezing and day 7 post-thawing (day 0 post-thawing: available in Supporting Information 4).

Analysis parameter	Day 0 (pre-freezing)		Day 7 (post-thawing)	
	DEHT Mean \pm SD	DEHP Mean \pm SD	DEHT Mean \pm SD	DEHP Mean \pm SD
Protein S (IU/100 ml)	93 \pm 10	92 \pm 14	94 \pm 09	94 \pm 10
Protein C (IU/100 ml)	112 \pm 13	109 \pm 11	110 \pm 12	106 \pm 13
von Willebrand factor GP1bA (IU/100 ml)	104 \pm 34	99 \pm 31	101 \pm 35	100 \pm 34
Factor V (IU/100 ml)	104 \pm 14	99 \pm 12	90 \pm 08	94 \pm 09
Factor VII (IU/100 ml)	112 \pm 14	103 \pm 14	84 \pm 18	81 \pm 14
Factor VIII (IU/100 ml)	105 \pm 31	96 \pm 19	57 \pm 16	55 \pm 11
Factor X (IU/100 ml)	100 \pm 13	95 \pm 11	95 \pm 13	88 \pm 09
Factor XI (IU/100 ml)	97 \pm 17	92 \pm 09	84 \pm 18	86 \pm 09
Factor XIII (IU/100 ml)	125 \pm 13	119 \pm 15	128 \pm 14	119 \pm 14
Fibrinogen (g/l)	2.58 \pm 0.34	2.52 \pm 0.34	2.38 \pm 0.33	2.39 \pm 0.30
Triglycerides (mmol/l)	1.00 \pm 0.29	1.08 \pm 0.45	1.05 \pm 0.30	1.10 \pm 0.45
Alpha-1-antitrypsin (g/l)	0.95 \pm 0.12	0.94 \pm 0.13	0.92 \pm 0.12	0.92 \pm 0.11

Data are presented as mean \pm standard deviation (SD). There were no significant differences (ns).

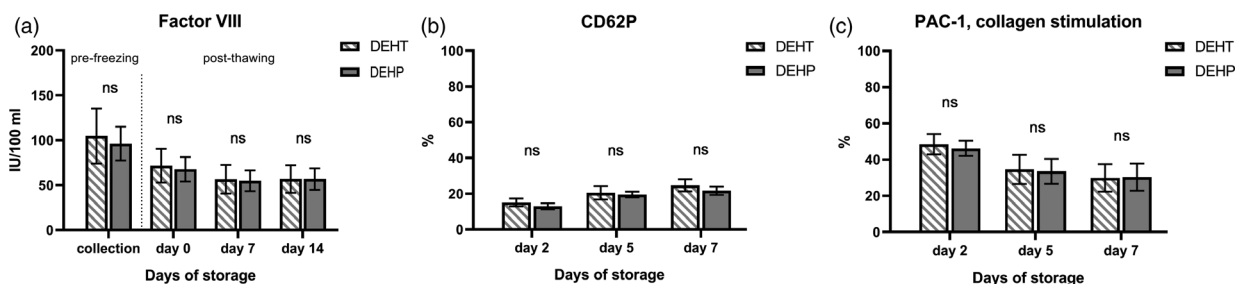


Figure 5 Effect of DEHT or DEHP plasticizer on fresh frozen plasma (FFP) through (a) coagulation factor VIII, and platelet concentrates through (b) activation marker CD62P and (c) response to agonists through PAC-1 collagen stimulation. Values are displayed as mean \pm standard deviation. Significance was tested using unpaired t-test with Holm-Sidak's correction for multiple comparisons (ns: not significant).

plasma, we did not observe any difference in the levels of clotting factors/inhibitors in FFP stored in DEHT compared to DEHP at any of the investigated time points. Likewise, platelets did not show any inferior reactivity, activation or metabolism from DEHT processing and sampling.

The limitations of this study include the inability to completely exclude donor variability impacting

comparisons as, to avoid plasticizer cross-contamination, a pool-and-split model was not possible between the two plasticizers. Another limitation is that all PCs were briefly in contact with DEHP when passing through the IPP system; we do not know how this affected the results. Complementing *in vivo* studies would also be valuable to obtain a complete picture. Furthermore, this is a single-centre study.

Table 3 Storage analysis results for platelet concentrates on day 2 and 7 (day 5: available in Supporting Information 5), prepared from whole blood stored in DEHT or DEHP (N = 8 of each).

Analysis parameter	Day 2		Day 7	
	DEHT Mean ± SD	DEHP Mean ± SD	DEHT Mean ± SD	DEHP Mean ± SD
Mean platelet volume (fL)	9.3 ± 0.2	9.3 ± 0.6	9.5 ± 0.3	9.4 ± 0.5
Extracellular lactate dehydrogenase (%)	4.0 ± 1.6	3.6 ± 1.6	4.4 ± 1.3	4.5 ± 1.6
Glucose (mmol/l)	7.0 ± 0.4	7.1 ± 0.9	3.3 ± 0.6	3.9 ± 1.3
Lactate (mmol/l)	9.9 ± 1.1	9.7 ± 1.5	16.6 ± 1.8	15.6 ± 2.4
pH 22°C	7.293 ± 0.032	7.289 ± 0.060	7.403 ± 0.034	7.431 ± 0.077
Bicarbonate (mmol/l)	6.9 ± 0.4	7.0 ± 0.7	5.2 ± 0.5	5.5 ± 0.9
JC-1 (%)	97.4 ± 1.5	98.2 ± 0.5	96.1 ± 0.9	96.8 ± 0.5
CD62P (%)	15.2 ± 2.2	13.0 ± 1.7	24.7 ± 3.4	21.7 ± 2.3
ATP (µmol/10 ¹¹ platelets)	7.0 ± 0.5	7.3 ± 0.8	6.6 ± 0.8	6.7 ± 0.8
CD42b (MFI)	69.1 ± 9.3	68.4 ± 11.2	59.8 ± 11.9	58.5 ± 11.7
PECAM-1 (MFI)	15.6 ± 1.6	14.7 ± 1.1	15.6 ± 0.6	15.0 ± 1.0
PAC-1 collagen stimulation (%)	48.5 ± 5.6	46.2 ± 4.2	30.0 ± 7.6	30.4 ± 7.5
PAC-1 ADP stimulation (%)	55.2 ± 7.6	55.0 ± 9.4	26.9 ± 7.3	26.6 ± 7.6
PAC-1 thrombin stimulation (%)	48.2 ± 7.0	46.6 ± 7.9	17.5 ± 5.4	16.6 ± 5.5

Data are presented as mean ± standard deviation (SD). There were no significant differences (ns).

It is well known that, for instance, haemolysis is influenced by multiple processing factors, for example WB holding time, RBC filtration, centrifugation force and sampling [30, 33–35], of which our centre fulfils numerous criteria favourable for low haemolysis with small standard deviation. Likely, differences in production processes will lead to different storage lesion results. In addition to the potential of alternative AS compositions [4, 10, 30–32], changes in processing may be another key factor to counteract disadvantageous RBC quality effects of DEHP removal [13]. As a future aspect, the individual contribution of different production parameters should be further explored.

In conclusion, this study demonstrates that PVC blood bags plasticized with DEHT provide adequate red blood cell quality during 49 days of storage. Furthermore, DEHT does not significantly alter the lesion profile of platelets or the degeneration of coagulation factors in plasma. PVC-DEHT bags hold as high physical and functional quality as bags plasticized with DEHP. Therefore, we consider PVC-DEHT blood bags a recommendable non-phthalate candidate for future blood component collection and storage.

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Conflict of interest

This study was conducted in collaboration with Macopharma; NF, SR and SC are Macopharma employees. All other authors declare no conflicts of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Supporting Information 1 Study blood bag prototypes

Supporting Information 2 Component acceptance criteria

Supporting Information 3 Red blood cells: extended results

Supporting Information 4 Plasma: extended results

Supporting Information 5 Platelets: extended results