



# Evaluation of canine red blood cell quality after processing with an automated cell salvage device

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**Abstract**

**Objective** – To evaluate the properties of RBC concentrate harvested after processing fresh whole blood units from healthy dogs with an automated cell salvage device.

**Design** – Prospective, in vitro, experimental study.

**Setting** – University teaching hospital.

**Animals** – Sixteen healthy, privately owned dogs of various breeds.

**Interventions** – Fresh canine whole blood collected in bags with citrate phosphate dextrose adenine solution was processed with an automated cell salvage device and analyzed in vitro. Laboratory values determined before (baseline, from a catheter sample) and after processing RBCs (procRBCs) included a complete blood count, selected blood chemistry analytes, erythrocyte osmotic resistance, whole blood viscosity, RBC aggregation, and RBC deformability.

**Measurements and Main Results** – Total recovery of RBCs was 80% ± 12%. Hematocrit of the procRBCs yielded by the device was 77% ± 3.7% (mean ± standard deviation). Gross morphology of the RBCs remained unchanged. The mean corpuscular volume, erythrocyte osmotic resistance, RBC deformability, RBC aggregation, and the activity of lactate dehydrogenase showed minor but statistically significant changes from baseline. No differences in the concentrations of free hemoglobin were observed. Whole blood viscosity was less in the procRBCs. Seventy-seven percent (mean) of the platelets were washed out, while a mean of 57% of the leukocytes remained in the procRBCs.

**Conclusions** – Although processing canine blood with this automated cell salvage device leads to slight changes in some properties of RBCs, most of these changes are comparable to changes seen in human blood after processing. Present data indicate that the use of this cell salvage device does not induce changes in canine RBC concentrate that would preclude its use for transfusion.

(*J Vet Emerg Crit Care* 2016; 26(3): 373–383) doi: 10.1111/vec.12472

**Keywords:** autologous transfusion, autotransfusion, dog, hemoabdomen, packed RBC, erythrocytes

**Abbreviations**

CHCM	corpuscular hemoglobin concentration mean
CPDA	citrate phosphate dextrose adenine
fHb	concentration of free hemoglobin

LDH	lactate dehydrogenase
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MPXI	myeloperoxidase index
OR	osmotic resistance
procRBCs	processed RBCs

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The authors declare no conflict of interests.

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 Submitted April 23, 2014; Accepted October 16, 2014.

**Introduction**

The use of blood and blood component products in small animal surgery, emergency, and critical care has increased in recent years.<sup>1,2</sup> Although the potential benefits of allogeneic transfusions are evident, blood products are not always available and can induce

adverse events.<sup>3–10</sup> An alternative or supplement can be transfusion of autologous blood (blood obtained from the patient). Intraoperative cell salvage with automated devices, so called “cell-savers,” and subsequent autologous transfusion has been practiced routinely in people for decades. The system is based on a wash-centrifuge: salvaged blood is processed into units of washed autologous packed RBCs, removing most of the undesirable components of shed blood.<sup>11</sup>

Cell salvage and autologous transfusion can be a life-saving treatment when other blood products are not available, which makes the technique attractive in veterinary practice. Extensive research has been done in people, but only limited studies exist in dogs. A literature search yields only a few case reports on the clinical use of automated cell salvage devices in canine patients<sup>12,13</sup> and some basic science studies using dogs as an experimental model for people, focusing specifically on undesirable effects of cell salvage.<sup>14–17</sup> For this reason, a prospective investigation of the effects of processing canine blood with an automated cell salvage device was warranted.

The aim of the present study was to evaluate the quality of canine processed RBCs (procRBCs) yielded by an automated cell salvage device. The degree of cell damage and hemolysis as well as salvage properties such as RBC recovery rates, separation of platelets, leukocytes, and albumin were assessed and compared with data from people.

## **Materials and Methods**

### **Animals**

The following study was discussed and approved by the institutional Ethics and Animal Welfare Committee (Ethik- und Tierschutzkommission—ETK, experiment number 68.205/0106-II/3b/2012) in accordance with GSP guidelines and national legislation. Sixteen privately owned dogs of various breeds were enrolled in the study. Informed owner consent was obtained prior to inclusion. The animals were deemed healthy based on history and physical examination. Age ranged from 1 year to 8 years (median 5.0 years). Breeds represented included 4 mixed breed dogs and one each of the following: Bavarian Mountain Hound, Belgian Malinois, Doberman Pinscher, Dutch Shepherd Dog, German Shorthaired Pointer, Golden Retriever, Gordon Setter, Irish Setter, Kangal Dog, Newfoundland, Staffordshire Bull Terrier, and Wirehaired Vizsla. Body weights ranged from 16 kg to 46 kg (median 26.5 kg).

### **Sampling and cell salvage procedures**

A 20-Ga catheter was placed in the cephalic vein of each dog to obtain blood samples and administer IV fluids. Baseline samples were taken from the catheter

immediately after placement and prior to any flushing, allowing the blood to free-flow into sample tubes. Immediately afterward, 10 mL/kg of blood was taken from the jugular vein using standard aseptic venipuncture technique. Blood bags of 450 mL capacity<sup>a</sup> containing citrate phosphate dextrose adenine (CPDA) solution (7 mL/50 mL blood) with incorporated needles (16-Ga) were used for collection. In 3 of the dogs, sedation with butorphanol<sup>b</sup> 0.2 mg/kg IV was required to enable smooth donation. Donors received IV infusion of lactated Ringer’s solution (5–10 mL/kg) after the donation to help compensate for blood loss.

Blood samples obtained from the venous catheter were used for baseline measurements of hematology and blood biochemistry after standard anticoagulation with EDTA or heparin, respectively. For baseline values of free hemoglobin, RBC osmotic resistance (OR), RBC deformability, and whole blood viscosity, blood samples were taken from the CPDA bag prior to processing. Baseline sampling and blood collection took place at the same timepoint. Thereafter, the blood unit and blood samples were transported to a different facility where the cell salvage and part of the analysis (OR, deformability, viscosity) were conducted. After completion of the procedure and returning to the principal premises, evaluation of hematology and blood biochemistry of both baseline and procRBC samples took place, approximately 5–6 hours after collection. Baseline samples experienced the same surrounding conditions (storage time, temperature, vibrations due to transport) as the procRBCs to better distinguish the effect of cell salvage. Samples were also taken from the waste bag of the cell salvage device after processing for measurements of hematology and selected blood biochemistry analytes (CBC, platelet count, total solids, albumin, lactate dehydrogenase [LDH], potassium).

The automated cell salvage device<sup>c</sup> used in the present study is specifically adapted for processing smaller units of blood (up to 2 L per hour). It features a reservoir from which blood is automatically transferred into a dynamic rotating disc. Alternately adding 0.9% NaCl and separating supernatant fluid through centrifugation yields washed pRBCs. The minimum volume in the reservoir for starting the salvage process is 100 mL. A manual override function, which was used frequently in this study, enables cell salvage from collected volumes smaller than 100 mL and can also be used at the end of salvage to maximize harvest. The system was primed with 100 mL of 0.9% NaCl<sup>d</sup> as recommended by the manufacturer. The CPDA blood was then suctioned into the reservoir of the device by cutting open the bag and introducing a sterile tube connected directly to the reservoir, flushing the bag with sterile 0.9% NaCl at the end to achieve maximum yield. Suction originates from an

external machine (maximum suction 40 mm Hg) but is connected to and applied by the automated cell salvage device. No additional anticoagulant was used. After processing, samples were taken from the bag of procRBCs to reassess hematology, blood biochemistry, RBC OR, RBC deformability, and whole blood viscosity. Samples were also taken from the waste bag of the device after revolving the bag a couple of times to homogenize the content.

## Measurements

### Hematology and clinical biochemistry

Complete blood counts were determined by laser flow cytometry technique<sup>e</sup> and included RBC, leukocyte, and platelet counts; hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), corpuscular hemoglobin concentration mean (CHCM), and myeloperoxidase index (MPXI). MCHC is a calculated value ( $([\text{Hemoglobin}]/\text{RBC})/\text{MCV}$ ) and CHCM is measured directly on a cell-by-cell basis via flow cytometry.<sup>18</sup> The following biochemistry analytes were determined by a fully selective autoanalyzer<sup>f</sup>: total protein, albumin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, glutamate dehydrogenase, bilirubin, LDH, and potassium. In addition, blood films of baseline samples (EDTA blood from the catheter sample) and procRBCs were stained with a modified Wright stain by an automated stainer,<sup>g</sup> and RBC morphology evaluated by light microscopy under oil immersion by a board certified clinical pathologist. Baseline samples experienced the same storage time and temperatures until evaluation of hematology, except for the processing with the device, as all samples underwent laboratory measurements at the same timepoint (after finishing of the experiment and processing). Measurements from waste bag samples included CBC, platelet count, total solids, albumin, LDH, and potassium.

### Free hemoglobin

Free hemoglobin concentration was determined as a marker of hemolysis with an adapted photometric method<sup>h</sup> using a custom-made reagent. Samples for measurements were taken from the CPDA blood bag (baseline) and from the procRBCs bag.

### Standardizing the PCV for determination of OR and whole blood viscosity

Baseline samples were prepared from the CPDA blood: 5 mL of CPDA blood from the collection bag were centrifuged for 5 minutes at  $1,198 \times g$ . Then 75  $\mu\text{L}$  of each supernatant plasma and pRBCs were gently mixed

to result in a standardized PCV of 50%, controlled by microhematocrit tubes and corrected further if necessary. The procRBCs yielded by the automated cell salvage device were centrifuged likewise and 75  $\mu\text{L}$  of pRBCs was mixed with 75  $\mu\text{L}$  of autologous plasma prepared from the baseline sample as described above, resulting in a sample with a PCV of 50%.

### Osmotic resistance

OR was determined as reported previously by Schalm<sup>19</sup> with slight modifications. Samples from the CPDA blood were standardized to a PCV of 50% as described above. Twenty microliter aliquots of the prepared blood samples was added to 2.5 mL of buffered saline (stock solution 10% containing NaCl 90 g/L,  $\text{Na}_2\text{HPO}_4$  34.4 g/L,  $\text{NaH}_2\text{PO}_4$  2.5 g/L) in decreasing concentrations (0.85%; 0.80%; 0.75%; 0.70%; 0.65%; 0.60%; 0.55%; 0.50%; 0.45%; 0.40%; 0.35%; 0.30%; 0.25%; 0.20%; 0.10%; and 0.0%), gently mixed by inversion and allowed to incubate for 30 minutes at room temperature ( $\sim 21^\circ\text{C}$ ). Samples were then centrifuged for 10 minutes at  $300 \times g$  and optical density of the supernatant measured at 540 nm with a spectrophotometer<sup>i</sup> with distilled water as a blank. After conversion to percent hemolysis (tube with pure distilled water/0% saline assumed to be 100% hemolysis) the mean corpuscular fragility was determined, which is the concentration of saline in which 50% hemolysis occurs. Baseline samples acted as a negative control, experiencing the same storage time and temperatures until evaluation, except for the processing with the device. To further assess the effect of storage time, a preliminary trial was carried out in which OR of blood samples stored at room temperature for the study time frame (4–6 hours) was compared.

### Whole blood viscosity

Whole blood viscosity was analyzed by a rheometer<sup>j</sup> using a Searle system with cone and plate symmetry. To abolish the impact of hematocrit and plasma viscosity on whole blood viscosity, PCV was standardized to 50% with autologous plasma in all samples (see above). For the measurements, 0.6 mL of blood was rotated in the measuring system with a fixed plate and a rotating cone. Shear rates between 1 and  $2,000 \text{ s}^{-1}$  were adjusted to obtain flow curves. Isothermal runs at  $37^\circ\text{C}$  were conducted with a logarithmic shear rate ramp. A preshear interval at  $100 \text{ s}^{-1}$  was carried out for 30 seconds followed by a 1 second rest interval before the measurements started at the lowest shear rate. Whole blood viscosity was compared at shear rates of  $1 \text{ s}^{-1}$  and  $214 \text{ s}^{-1}$ . Furthermore, as a common method for evaluating viscosity further, the viscosity ratio was calculated [viscosity ratio

=  $\eta_1(n_1)/\eta_2(n_2)$ ] using a low shear rate and a high shear rate.<sup>20</sup> In the present study shear rate at  $1\text{ s}^{-1}$  and shear rate at  $214\text{ s}^{-1}$  were used, calculating with the mean values of 3 consecutive measurements. For non-Newtonian fluids displaying shear-thinning behavior (eg, blood) a viscosity ratio  $>1$  is typical. Blood with destroyed RBCs containing cell stroma would approximate properties of Newtonian fluids and a viscosity ratio  $<1$ .<sup>20,21</sup> To provide further data enabling statistical comparison, curve fittings using the Casson model ( $y^{1/P} = a + b \cdot x^{1/P}$ ) were conducted with commercial software.<sup>k</sup> This previously described model allows calculation of flow point ( $\tau_0$ ) and infinite viscosity ( $\eta_\infty$ ) with input parameters of shear stress  $\tau$  and shear rate  $d(\gamma)/dt$ .<sup>22</sup>

There is consensus that values of whole blood viscosity at shear rates below  $10\text{ s}^{-1}$  are associated with the amount of RBC aggregates.<sup>23</sup> Therefore, low shear rate viscosity at shear rates of  $1\text{ s}^{-1}$  was used to estimate RBC aggregation, although it was not measured directly.

#### Red blood cell deformability

Erythrocyte deformability was assessed by laser diffractometry. This technique consists of shearing a diluted RBC suspension between 2 thermostatic concentric cylinders achieved by rotation of the outer cylinder, applying different fluid shear stresses as deforming force. For analyzing RBC size and shape, diffraction of the laser beam by single RBCs in suspension is monitored by a video camera. For this purpose,  $20\text{ }\mu\text{L}$  of EDTA blood and  $20\text{ }\mu\text{L}$  of procRBCs, in turn, were gently mixed with  $4.7\text{ mL}$  of 5% polyvinylpyrrolidone solution ( $50\text{ g}$  polyvinylpyrrolidone,<sup>1</sup> molecular weight  $360,000\text{ g}\cdot\text{mol}^{-1}$ , in  $1\text{ L}$  of phosphate buffered saline,<sup>m</sup> viscosity  $14\text{ mPa}\cdot\text{s}$ ). The resulting suspension was analyzed with an ektacytometer<sup>n</sup> at predetermined shear stresses between  $0.3\text{ Pa}$  and  $53.06\text{ Pa}$  at temperatures between  $36\text{--}37^\circ\text{C}$ . To facilitate interpretation of the complex shear stress-RBC strain curve, 2 comprehensive parameters were calculated: data were converted into a Lineweaver-Burke plot by a method described elsewhere<sup>24</sup> to calculate the maximum deformation of the RBC at the highest shear stress ( $EI_{\text{max}}$ ) and the shear stress that is needed for half-maximal deformation of the RBC ( $SS_{1/2}$ ).

#### Statistical Analysis

The assumption of normal distribution was confirmed using the Kolmogorov-Smirnov test. All results were reported as mean  $\pm$  standard deviation. The difference between the 2 sample means obtained before and after processing with the automated cell salvage device was analyzed using the paired sample *t*-test. All analyses were performed using commercial statistical software.<sup>o</sup>

For all analyses a *P* value of  $<0.05$  was considered significant.

#### Results

Blood collection for baseline samples was uneventful in all subjects with blood flowing easily from the venous catheter. The volume of blood collected from each dog ranged from  $57\text{ mL}$  to  $397\text{ mL}$  (Table 1). The duration from starting the cell salvage procedure (suctioning of the blood into the reservoir of the automated cell salvage device) until completion of RBC salvage was  $50 \pm 4$  minutes (range  $42\text{--}55$  minutes). Total recovery of RBCs was  $80\% \pm 12\%$  (range  $59\text{--}100\%$ ).

The hematocrit of the procRBCs yielded by the device ranged from  $69\%$  to  $83\%$ . MCV changed significantly ( $P < 0.001$ ) between baseline blood and procRBCs, showing a mean increase of  $2.5\%$ . There were significant decreases in both MCHC ( $P = 0.002$ ) and CHCM ( $P < 0.001$ ; Table 1). The morphology of RBCs evaluated on stained blood films by light microscopy under oil immersion was normal for all but 1 dog. In this dog, anisocytosis and polychromasia were present on smears of baseline samples and could also be seen in the procRBCs. In 1 individual, a low number of acanthocytes were found in baseline blood films but none were present after the cell salvage procedure. Of the total of leukocytes (cells/ $\mu\text{L}$   $\times$  volume of blood or procRBCs), a mean of  $56\%$  (range  $26\text{--}87\%$ ) was found to still be present in the procRBCs. Only an average of  $8\%$  (range  $3\text{--}15\%$ ) of the total of platelets (cells/ $\mu\text{L}$   $\times$  volume of blood or procRBCs) remained in the procRBCs while a mean of  $77\%$  (range  $58\text{--}100\%$ ) was located in the waste bag of the device.

A statistical difference in the concentration of free hemoglobin (fHb) between baseline and procRBCs could not be detected. In 3 of the dogs, fHb measurements could not be completed successfully because of errors in the sampling process. All fHb values from these individuals were excluded from analysis. In  $6/13$  of the dogs, baseline fHb values were higher than in the corresponding samples after processing (Table 2). Only  $5\%$  of the mean albumin concentration in the donated blood was found in the procRBCs. Activity of LDH was significantly greater ( $P = 0.013$ ) in the procRBCs, with a mean increase of  $61\%$ . Bilirubin was significantly lower in procRBCs ( $P < 0.01$ ) than in the baseline blood, and in  $11$  of  $16$  procRBC samples it was below the limit of detection.

OR was significantly decreased ( $P < 0.001$ ) in the procRBCs compared to the baseline blood (mean change  $18.9\%$ ), meaning that erythrocytes hemolyzed more readily after cell salvage (Table 3, Figure 1). No difference in OR was observed in a preliminary trial when determined immediately and after storage at room temperature for the study time frame ( $4\text{--}6$  hours; Figure 2).



**Table 1:** Results of blood volumes, hematology, and clinical biochemistry before (baseline) and after cell salvage (procRBCs) shown as mean  $\pm$  standard deviation

Analyte (unit)	Baseline	procRBCs	Analyte (unit)	Baseline	procRBCs
Volume of blood (mL)	212.4 $\pm$ 90.1	118.2 $\pm$ 53.4	Total solids (g/L)	63 $\pm$ 4	5 $\pm$ 3
Hematocrit (L/L)	0.53 $\pm$ 0.07	0.77 $\pm$ 0.04	Albumin (g/L)	36.5 $\pm$ 3	1.8 $\pm$ 2
Erythrocytes ( $10^{12}$ /L)	7.44 $\pm$ 1.1	10.6 $\pm$ 0.6	AP (IU/L)	36 $\pm$ 21	2 $\pm$ 2.3
Erythrocytes total ( $10^{12}$ )	1,601.2 $\pm$ 772	1,265.6 $\pm$ 600	AST (IU/L)	29 $\pm$ 8	9 $\pm$ 7.2
MCV (fL)	71.1 $\pm$ 2.6	72.9 $\pm$ 3.1*	ALT (IU/L)	43 $\pm$ 13	3 $\pm$ 2.6
MCHC (g/L)	331 $\pm$ 4	324 $\pm$ 9*	GLDH (IU/L)	2.5 $\pm$ 1.6	0.7 $\pm$ 0.6
CHCM (g/L)	346 $\pm$ 7	334 $\pm$ 13*	Bilirubin ( $\mu$ mol/L)	1.88 $\pm$ 0.68	0.51 $\pm$ 1.71*
Hemoglobin (g/L)	1.7 $\pm$ 0.27	2.49 $\pm$ 0.11	LDH (IU/L)	98 $\pm$ 64	144 $\pm$ 101*
Hemoglobin total (g)	3,666 $\pm$ 1,775	2,959 $\pm$ 1,348	K <sup>+</sup> (mmol/L)	4.3 $\pm$ 0.4	0.75 $\pm$ 0.3
Free hemoglobin (g/L)	0.19 $\pm$ 0.15	0.31 $\pm$ 0.33			
Leukocytes ( $10^9$ /L)	7.56 $\pm$ 1.59	7.03 $\pm$ 2.78			
MPXI	19.7 $\pm$ 3.9	18.7 $\pm$ 5.3			
Thrombocytes ( $10^9$ /L)	277.4 $\pm$ 90.8	41.8 $\pm$ 28.4			
Thrombocytes total ( $10^9$ )	58,938.3 $\pm$ 32,886	5,111.2 $\pm$ 5,524			

Asterisks (\*) indicate a statistically significant difference ( $P < 0.05$ ) between paired sample means.

Total, concentration/mL  $\times$  amount of blood (mL); MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; CHCM, corpuscular hemoglobin concentration mean; MPXI, myeloperoxidase index.

**Table 2:** Results of free hemoglobin (fHb) measurements (g/L) before (baseline) and after cell salvage (procRBCs)

Number	Baseline	procRBCs	Difference
1	0.05	1.38	1.33
2	0.19	0.2	0.01
3	0.19	0.16	-0.03
4	0.30	0.24	-0.06
5	0.08	0.37	0.29
6	0.12	0.03	-0.09
7	0.11	0.19	0.08
8	0.23	0.22	-0.01
9	0.05	0.17	0.12
10	0.06	0.19	0.13
11	0.51	0.21	-0.30
12	0.39	0.35	-0.04
13	0.03	0.33	0.30
Mean	0.19	0.31	0.13
SD	0.15	0.33	0.39

Whole blood viscosity was significantly reduced in the procRBCs (adjusted to a similar hematocrit) when compared to the baseline blood at all statistically investigated shear rates (Table 3); however, the viscosity ratio did not show a statistically significant difference (Table 3, Figure 3). The flow curves before and after processing with the automated cell salvage device did not show a major change (Figure 3). The application of a curve fitting function using the Casson model yielded satisfactory fittings (Figure 4). Whereas flow point ( $\tau_0$ ) was maintained, infinite viscosity ( $\eta_\infty$ ) decreased significantly ( $P < 0.001$ ) following cell salvage, implicating decreased viscosity (Table 3). Low shear viscosity decreased by a mean of 2.2 mPa·s, which equates to a mean reduction of 13%. Maximum deformation of RBCs decreased significantly

**Table 3:** Results of RBC mechanic properties before (baseline) and after cell salvage (procRBCs), shown as mean  $\pm$  standard deviation

Analyte	Baseline	procRBCs	$P$
Osmotic resistance			
MCF (% NaCl)	0.285 $\pm$ 0.08	0.326 $\pm$ 0.08*	<0.001
Deformability			
Elmax	0.6 $\pm$ 0.08	0.54 $\pm$ 0.1*	<0.001
SS <sub>1/2</sub>	3.03 $\pm$ 0.81	2.67 $\pm$ 1.14	
Viscosity			
Low shear viscosity 1s <sup>-1</sup> (mPa·s)	15.89 $\pm$ 4.16	13.62 $\pm$ 3.85*	0.006
$\eta$ at 214 s <sup>-1</sup> (mPa·s)	4.57 $\pm$ 0.57	3.84 $\pm$ 0.6*	<0.001
$\eta$ at 523 s <sup>-1</sup> (mPa·s)	3.8 $\pm$ 0.66	3.18 $\pm$ 0.6*	<0.001
$\eta$ at 1,280 s <sup>-1</sup> (mPa·s)	3.72 $\pm$ 0.4	3.13 $\pm$ 0.5*	<0.001
VR 1/214 s <sup>-1</sup>	3.47 $\pm$ 0.67	3.49 $\pm$ 0.6	0.86
VR 1/523 s <sup>-1</sup>	4.21 $\pm$ 1.08	4.32 $\pm$ 1.1	0.498
VR 1/1,280 s <sup>-1</sup>	4.23 $\pm$ 0.80	4.32 $\pm$ 0.7	0.617
Flow point $\tau_0$ (mPa)	13.67 $\pm$ 4.94	15.2 $\pm$ 4.8	0.208
Infinite viscosity $\eta_\infty$ (mPa·s)	3.47 $\pm$ 0.39	2.72 $\pm$ 0.37*	<0.001

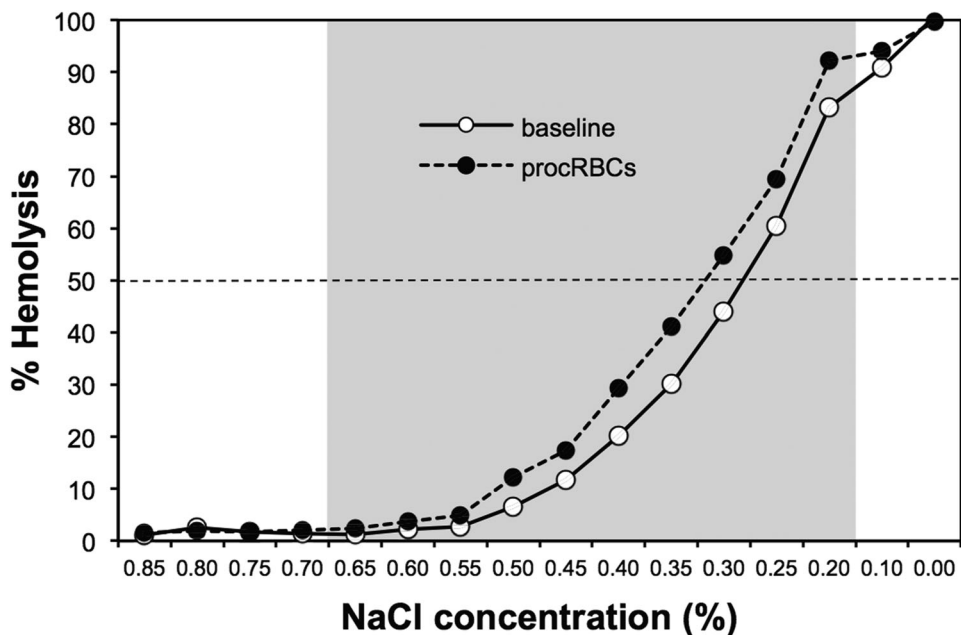
Asterisks (\*) indicate a statistically significant difference ( $P < 0.05$ ) between paired sample means.

MCF, mean corpuscular fragility; Elmax, maximum deformation; SS<sub>1/2</sub>, shear stress at half-maximum deformation;  $\eta$ , apparent viscosity; VR, viscosity ratio.

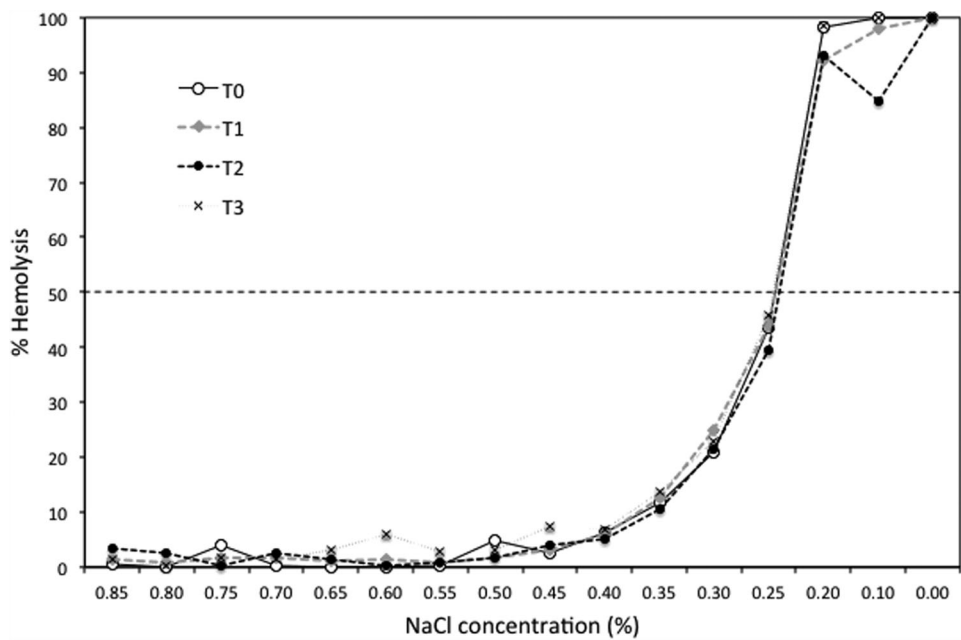
after automated cell salvage ( $P < 0.001$ ), with a mean reduction of 9.8%, while shear stress at half-maximal deformation was maintained (Table 3, Figure 5).

## Discussion

The present study aimed to assess the effect of an automated cell salvage device on the mechanical properties of canine blood and to evaluate the quality of the



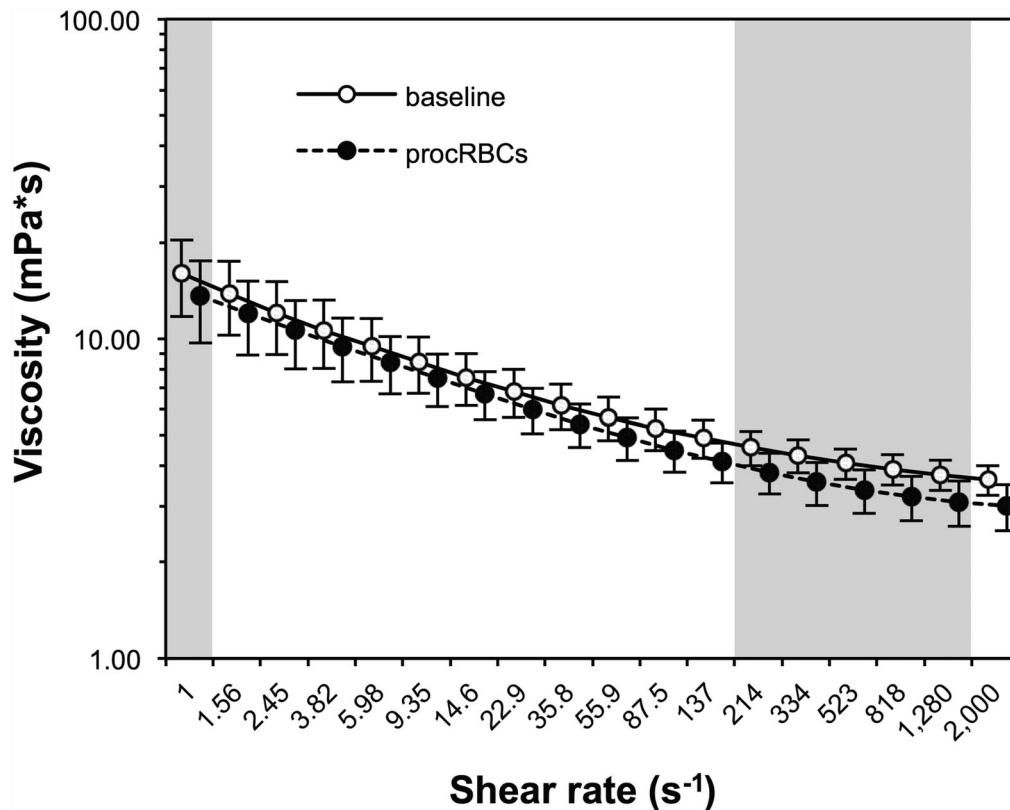
**Figure 1:** Results of osmotic resistance testing before (baseline) and after cell salvage (procRBCs), shown as mean for each saline concentration. Horizontal dashed line indicates mean corpuscular fragility (MCF). Gray area indicates a statistically significant difference ( $P < 0.05$ ) between paired sample means.



**Figure 2:** Results of osmotic resistance testing of whole blood over a timespan of 6 hours (T0–3) in a preliminary trial. Horizontal dashed line indicates mean corpuscular fragility (MCF).

procRBCs yielded by the device. An important feature of automated cell salvage devices is the effectiveness of cell salvage. The results of this study show an RBC recovery rate similar to that reported for human banked blood processed by the same device ( $80\% \pm 12\%$  vs.

$86.8\% \pm 5.9\%$  for human blood with predilution),<sup>25</sup> and therefore indicates that no profuse hemolysis of healthy canine RBCs occurs due to processing. This supports the hypothesis that this device may be successfully used in dogs, although clinical trials are warranted.



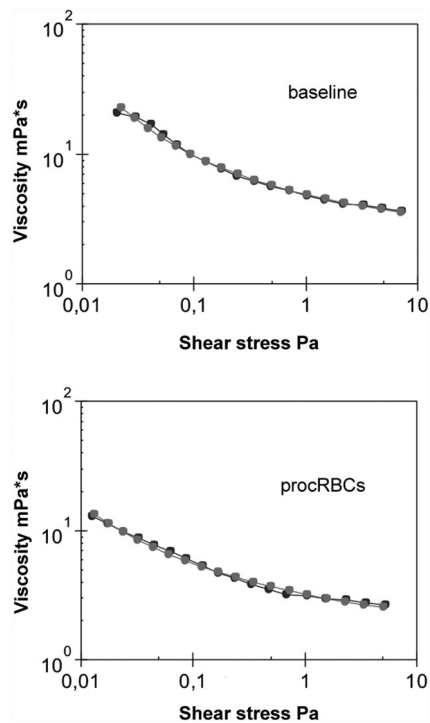
**Figure 3:** Whole blood viscosity before (baseline) and after cell salvage (procRBCs) shown as mean for each shear rate; all measured at standardized Hct 50 L/L (50%). Error bars indicate standard deviation. Gray areas indicate statistically significant differences ( $P < 0.05$ ) between paired sample means. Only shear rates of 1, 214, 532, and 1,280  $s^{-1}$  were submitted to statistical analysis.

Of the hematological properties, the MCV showed a significant increase after the cell salvage procedure. Although this increase was small, it indicates some degree of cell swelling. This is supported by the significant decreases in MCHC and CHCM also observed. Cell swelling was not observed in the baseline blood samples, which experienced the same storage time and temperature until evaluation; therefore, the minor increase in RBC volume was interpreted as a result of the processing with the automated cell salvage device.

OR was measured as a marker of red blood cell membrane integrity, and was found to be significantly reduced in the procRBCs. OR is influenced by pH and temperature, as well as species, breed, and age of the animal.<sup>19</sup> Prolonged storage also affects OR, with erythrocyte suspensions showing decreased OR after storage for 42 days.<sup>19,26</sup> It is unclear whether storage for a short period, as is the case in the present study, has a noticeable effect on RBC OR. In a preliminary trial incorporated in this study, no difference in OR was observed when determined immediately and after storage at room temperature for the study time frame. Furthermore, baseline samples acted as negative control,

experiencing nearly the same storage time as the processed blood until determination of OR. It was therefore concluded that the changes in OR seen in this study, from baseline to after the automated cell salvage process, were a consequence of the cell salvage process itself.

OR is further influenced by RBC MCV, as cells with greater MCV are lysed at higher concentrations of saline due to a reduced surface area to volume ratio. Other mechanisms leading to reduced OR are also described. One of them is cell swelling due to accumulation of osmotically active substances in the RBCs (eg, lactate and chloride as substitutes for 2,3-BPG). This process has been described in RBCs stored for prolonged periods.<sup>19,26</sup> In the present study an increase in MCV was observed, which indicates cell swelling and could explain the observed decrease in OR. However, other mechanisms cannot be ruled out, as intracellular 2,3-BPG and osmotically active substances were not measured. However, in another study investigating cell salvage in dogs after 100% exchange transfusion, ATP and 2,3-BPG remained stable.<sup>17</sup> In contrast to the results of the present study, a study conducted in people scheduled for prostatectomy found no decrease in OR of



**Figure 4:** Example of whole blood viscosity Casson model fitting of flow curves from baseline and pRBCs samples using specialized software (Rheoplus Version 3.40, 2009): dark gray line, sample; light gray line, fitting.

RBCs after cell salvage.<sup>27</sup> Therefore, the minor changes in RBC OR observed here could be due to species-related differences, as RBCs of different species display variable susceptibility to osmotic stress.<sup>19</sup> If cell swelling occurs as a consequence of the cell salvage process, as indicated by the increased MCV and reduced OR, this might lead to a decreased lifespan of procRBCs, though clinical trials would be required to confirm this hypothesis.

A statistical difference between the amount of fHb at baseline and after processing with the cell salvage device was not detected. However, in 6 of 13 dogs, baseline values were higher than in the corresponding samples after processing. The most likely explanation for this phenomenon is RBC damage occurring during jugular venipuncture and blood collection. Furthermore it underscores that fHb was washed out during processing, as stated by the manufacturer of the device and demonstrated in previous studies.<sup>25</sup> The RBC recovery rates with a mean of 80%, comparable to values in people, indicate that some cells are lost in the process. This could be due to some degree of hemolysis or because RBCs remain in the tubing, pump, and other parts of the device. The latter mechanisms were not investigated in the present study; therefore, the extent of both is unclear. If however cells were lysed, the free hemoglobin, which

could be harmful for the patient, was apparently washed out. In human literature, values for fHb of up to 0.4 g/L are referenced as normal for salvaged, processed blood.<sup>28</sup> This value was not exceeded in all proRBC samples but one, demonstrating acceptable quality of the product.

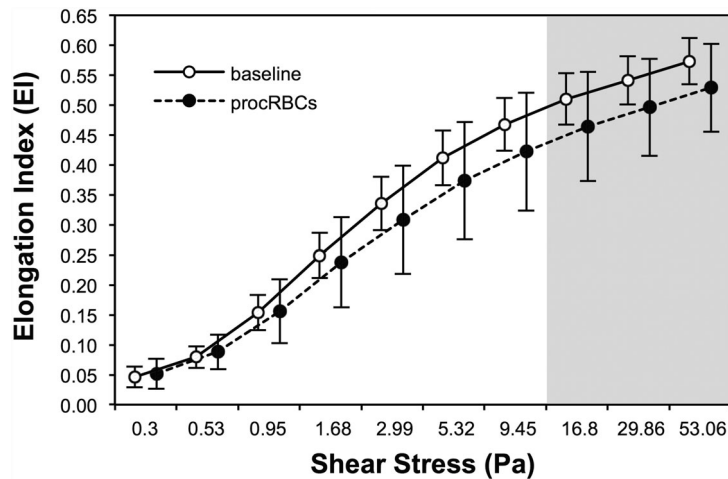
Activity of LDH is considered a marker of hemolysis, especially when measured *in vitro* in blood samples separated from other enzyme sources than RBCs.<sup>29</sup> The increased LDH activity found in the procRBCs indicates some degree of cell injury, which is consistent with the reduced OR found.

As described in the literature, platelets were washed out by the automated cell salvage device.<sup>30,31</sup> Therefore, autotransfusion of large volumes of blood salvaged with an automated cell salvage device may lead to thrombocytopenia and a primary coagulopathy.

As expected, the units of procRBCs generated by the automated cell salvage device still contained a large number of WBCs. Activated WBCs in blood products can be the cause of adverse effects from transfusions.<sup>32,33</sup> Therefore, the use of a leukocyte reduction filter in the system is generally advised for clinical use. The MPXI, which expresses the myeloperoxidase concentration of a neutrophil population compared to a “standard neutrophil,” was determined in the present study. Myeloperoxidase is a microbicidal enzyme released from activated neutrophil granulocytes and monocytes.<sup>34</sup> Lower values of MPXI indicate enzyme depletion that occurs after activation of neutrophils.<sup>35</sup> A study regarding myeloperoxidase deficiency in dogs, in which values were measured with the same device used in the current study, reported markedly lower MPXI values than those found in the present study.<sup>36</sup> However, the authors state that the use of MPXI as a marker of depletion is impaired due to high standard deviation in dogs and consequently might not be reliable *in vivo* for diagnostic purposes.<sup>36</sup> Present data show no significant difference in MPXI values before and after processing, and although the conclusion may be impaired due to the high standard deviation, this suggests that no profound release of myeloperoxidase occurred in the present study. This finding suggests that neutrophils are not activated due to processing. However, further studies are warranted to investigate this possibility and to determine potential adverse effects of transfused leukocytes.

Plasma, and hence albumin, are reportedly separated from the RBCs during the cell salvage process in humans.<sup>37</sup> The present study confirmed that this is also true for canine blood, as only a small amount of albumin was detected in the procRBCs. Remarkably, only 40% of the calculated total amount of albumin present in the whole blood collection bag was found in the waste bag of the device. This could be due to adhesion of proteins to plastic material in the system, though this theory was not





**Figure 5:** Results of RBC deformability before (baseline) and after cell salvage (procRBCs), shown as mean for each shear stress. Error bars indicate standard deviation. Gray area indicates a statistically significant difference ( $P < 0.05$ ) between paired sample means.

investigated. Because the procRBCs have low albumin concentration, similar to allogeneic pRBCs, transfusion of larger volumes can lead to hypoalbuminemia.

Bilirubin, although it may not be meaningful as a marker of hemolysis in an in vitro study, was measured to investigate whether it would be washed out by the cell salvage device. Present data support the theory that it is indeed removed. This may be of interest in clinical situations where hemolysis has taken place to some degree before collection and bilirubin is already present in the accumulated blood, but is not desirable as a component in blood salvaged for retransfusion.

In the present study, viscosity measurements at increasing shear rates resulted in continuous flow curves, both before and after cell salvage. This indicates that no relevant RBC aggregates or clusters were formed, as flow curves of whole blood are sensitive to inhomogeneities. Flow curves are generally used to describe a set of mechanical properties of viscoelastic materials and always focus on the characteristic of a bulk sample, although a relation to the behavior of single components exists as well.<sup>21</sup> If shear thinning in a material is present, it results from the organization and deorganization of structures within the sample in association with the shear forces. Blood is a shear thinning viscoelastic fluid that possesses a species-specific degree of thixotropy, which is the time-dependent change of viscosity at constant shear stress. Both the degree of shear thinning and the degree of thixotropy are diverse in the animal kingdom and depend on the quantitative hematocrit, the qualitative (eg, aggregability, deformability) mechanical properties of the RBC, and the quantitative and qualitative composition of the plasma.<sup>38,39</sup> A change of one of those parameters will change the shape of the flow curve. First of all, viscosity ratio (ie, shear thinning

index) was calculated to determine the degree of shear thinning within relevant physiologic shear rates. Viscosity ratio, calculated from the apparent viscosity at  $1 \text{ s}^{-1}$ ,  $214 \text{ s}^{-1}$ ,  $523 \text{ s}^{-1}$ , and  $1,280 \text{ s}^{-1}$ , showed no change following cell salvage, indicating that shear thinning was preserved and the bulk blood samples did not lose their characteristic mechanical properties. Whole blood viscosity was significantly decreased at all statistically analyzed shear rates:  $214 \text{ s}^{-1}$ , which represents larger vessels since viscosity in the femoral artery is reported to be  $150 \text{ s}^{-1}$ ;  $523 \text{ s}^{-1}$ , relating to arterioles where wall shear rates of  $400 \text{ s}^{-1}$  are described; and  $1,280 \text{ s}^{-1}$ , which covers conditions found in capillary beds.<sup>40</sup> Wall shear rates fluctuate in vivo because they are dependent on vessel diameter and blood flow, which change dynamically because they are influenced by cardiac output, pulsation, and vessel tension. In this study, flow curves showed an overall decrease, displaying a reduced viscosity at shear rates relevant for microcirculation. Furthermore, applying a curve fitting function using the Casson model enabled determination of flow point ( $\tau_0$ ) and infinite viscosity ( $\eta_\infty$ ) of the bulk samples, which are classical approaches to describe viscoelastic fluids. While  $\tau_0$  was maintained, the decrease in  $\eta_\infty$  following cell salvage implicates reduced viscosity. This decrease in viscosity in salvaged pRBCs could mean less resistance to blood flow, which could improve microcirculatory flow in the transfused patient.

All measurements of viscosity have to be performed at standardized conditions to exclude the influence of hematocrit and plasma composition. Therefore, the present finding indicates a change in the mechanical properties of blood during cell salvage. Whether or not the change in RBC deformability alone accounts for this modification cannot be determined.

Like viscosity, aggregability of RBCs is an important factor for microcirculation, affecting vascular resistance, and endothelial function.<sup>41,42</sup> Red blood cell aggregation was inferred from whole blood viscosity at low shear rate ( $1 \text{ s}^{-1}$ ), as low shear viscosity has been suggested as a reliable index of RBC aggregation.<sup>23</sup> The decrease in low shear rate viscosity by 13% after cell salvage indicates reduced RBC aggregation. This finding supports the argument that no aggregate formation occurred.

Deformability describes the ability of the RBC to adapt its shape in response to a deforming force, physiologically enabling passage of RBC through narrow tubes. Following cell salvage, maximum deformation was significantly reduced, whereas shear stress for achieving half-maximum deformation remained constant. The reduction in maximum deformation indicates increased rigidity of cells at high shear stresses. Reduced deformability is also reported in human literature;<sup>31</sup> however, the mechanism remains unclear. It could be the result of ATP and 2,3-BPG loss or oxidative stress.<sup>43,44</sup> Since ATP, 2,3-BPG, and factors marking oxidative stress were not measured, the cause in this case remains unknown. However, it is considered unlikely that depletion of ATP and 2,3-BPG occurred over the short study time frame; also, human data report maintenance of 2,3-BPG after cell salvage.<sup>45</sup> Higher rigidity of RBCs could lead to sequestration in the spleen or other organs, or decreased cell survival depending on the degree of alteration.<sup>46</sup> Thus, further investigation into salvaged RBC survival time after transfusion would be valuable.

The results of the present study may be limited due to the experimental setting. Baseline samples were taken from a newly placed venous catheter rather than out of the collection bag, which was further processed (except for OR and fHb, which were compared directly between CPDA blood and procRBCs). This approach was chosen to enable evaluation of clinical biochemistry values from heparin plasma and hematology from EDTA samples, which generally deliver the best results. The collection of the blood for processing took place at the same time but from a different route, which might theoretically have altered results slightly. However, no or only slight changes are expected since collection was carried out similarly to donations for blood banking, where only minimal alterations are acceptable because longer storage is intended. Furthermore, the blood donors in this study were healthy dogs, and therefore RBCs undergoing salvage were presumably healthy at the time of collection. The whole blood collected for salvage was kept in preservative (CPDA) to maintain cell viability and processed rapidly. Under clinical circumstances, RBCs salvaged from a hemoabdomen or other source of bleeding may already be compromised due to longer contact with serosal surfaces, other surfaces, or rinsing

solutions as well as microbial agents or other deleterious effects of underlying disease. These circumstances might render RBCs more susceptible to damage through the salvage process, resulting in a higher degree of cell lysis and therefore lower recovery rate in clinical patients. Finally, further trials on cell viability, such as RBC survival time post transfusion are necessary to fully investigate the effects of salvage on canine RBCs.

In summary, the recovery rate and the hematocrit of the procRBCs as well as the content of fHb were comparable to values obtained in people, suggesting the absence of clinically important hemolysis. Present data show RBC swelling (increased MCV), a mild degree of hemolysis (increased LDH activity), and a reduction in OR and deformability after cell salvage. The gross morphology of RBCs remained physiologic and whole blood viscosity of the procRBCs was slightly reduced compared to baseline blood.

## Conclusion

Although cell salvage with an automated cell salvage device leads to minor changes in canine hematology, RBC OR, and deformability, properties of the procRBCs yielded by the device are similar to what is seen in people. Therefore, the alterations can be considered to be in an acceptable range. A detrimental effect on the patient upon transfusion seems to be unlikely, although further controlled studies and clinical trials are warranted to determine the safety and effectiveness of salvaged blood.

## Acknowledgments

The University of Veterinary Medicine, Vienna, Austria, provided funding for this study. The authors would like to thank PD Dr. Ulrike Auer for her support with this project and Prof. Dr. Erich Möstl and Dr. Michael Püringer-Lepschy from the Division of Biochemistry for their technical assistance in photometric measurements. The authors would also like to thank Haemonetics Corporation, Austria for providing the OrthoPAT device.

## Footnotes

- <sup>a</sup> Terumo Blood Bag, CPDA-1 63 mL, Terumo Europe N.V., Leuven, Belgium.
- <sup>b</sup> Butomidol, Richter Pharma AG, Wels, Austria.
- <sup>c</sup> OrthoPAT, Mod. 1050–240, Haemonetics Corporation, Braintree, MA.
- <sup>d</sup> Kochsalz „Braun“ 0.9% - Infusionslösung, B. Braun Melsungen AG, Melsungen, Germany.
- <sup>e</sup> ADVIA 2120i™, Siemens AG, Erlangen, Germany.
- <sup>f</sup> Cobas c501™, Roche Diagnostics GmbH, Mannheim, Germany.
- <sup>g</sup> Hematek™, Siemens AG.
- <sup>h</sup> Cobas C311, Roche Diagnostics GmbH.
- <sup>i</sup> Novaspec II, Amersham Biosciences, Tokyo, Japan.
- <sup>j</sup> Physica MCR301, A. Paar, Graz, Austria.
- <sup>k</sup> Rheoplus Version 3.40, A. Paar.
- <sup>l</sup> P-5288, Sigma-Aldrich Chemie GmbH, Steinheim, Germany.

- <sup>m</sup> P-3813, Sigma-Aldrich Co, St. Louis, USA.  
<sup>n</sup> LORRCA, R&R mechatronics, Hoorn, The Netherlands.  
<sup>o</sup> IBM SPSS Statistics for Windows, Version 20.0, IBM Corporation, Armonk, NY.

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