Two solvent/detergent-treated plasma products with a different biochemical profile

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Background and Objectives
The distribution of US-produced solvent/detergent (S/D)-treated plasma (Plas+SD) was discontinued in 2003 due to bleeding and thrombotic events. In this work, we were searching for explanations that might explain these severe side-effects.

Materials and Methods
The activities of coagulation factors and inhibitors and von Willebrand factor (VWF) multimers were tested in three Plas+SD batches and compared with an S/D-treated plasma named OctaplasLG. In addition, the Plas+SD process was investigated in downscale studies.

Results
When compared to OctaplasLG, coagulation factors (FV) and FXI were significantly increased in the Plas+SD product, whereas FIX, protein C (PC), protein S (PS) and plasmin inhibitor (PI) were significantly decreased, leading to a haemostatic imbalance in this product. In addition, loss of high molecular weight VWF multimers was observed in the Plas+SD product compared to both OctaplasLG and normal plasma. Downscale studies of the Plas+SD manufacturing steps revealed significant losses of all parameters tested during the S/D-treatment and removal steps. Except for PS and PI, which remained decreased by 47% and 65%, respectively, these losses were nearly compensated for the other parameters by the subsequent ultrafiltration step performed.

Conclusion
Although both S/D plasma manufacturing processes have the same basic set-up, differences in single process steps are responsible for the different biochemical profiles. The study performed may contribute to a better understanding of the side-effects that occurred with the Plas+SD product.

Key words: biochemical profile, manufacturing process, plasma transfusion, S/D plasma

Introduction
Transfusion of human plasma is essential to treat coagulation disorders and to control bleeding. However, enveloped and non-enveloped viruses such as human immunodeficiency virus (HIV), hepatitis viruses (A, B, C and E), West Nile virus, parvovirus B19 (B19), cytomegalovirus and human T-cell lymphotropic viruses are capable of being transmitted through plasma transfusion [1]. Although the implementation of HIV, HBV and HCV nucleic acid testing (NAT) has improved its safety [2], there are still cases reported in which viruses were transmitted through plasma transfusions [3–5]. Therefore, preventing pathogen transmission through plasma transfusion is of great importance. The solvent/detergent (S/D) inactivation method has been successfully used since 1985 in the manufacturing of coagulation factor concentrates [6]. Also, this inactivation technology has been applied to fresh frozen plasma (FFP) to reduce the risk of enveloped virus transmission through plasma transfusions [7, 8]. Moreover, the virus validation studies showed very effective inactivation of different enveloped viruses, which led to a high safety margin [7, 9].
As S/D-treated plasma is produced from a large donor pool, the transmission risk of a virus that is resistant to this inactivation procedure is increased. However, as discussed by Rollag and colleagues, neutralizing antibodies that occur in the plasma pools function as important barriers against many enveloped and non-enveloped viruses, which might contaminate the plasma pool [10].

Transmission of parasites [11], such as Plasmodium falciparum (causing malaria), Babesia microti (causing babesiosis), Trypanosoma cruzi (causing Chagas disease) as well as various bacteria [12] including Staphylococcus aureus and Escherichia coli, more often occur in cellular blood components. The likelihood of these pathogens to survive the plasma-freezing process that is performed after donation (at temperatures under -18°C) is very low. Furthermore, as S/D-treated plasma products are sterile filtered, bacteria and parasitic infections do not pose a risk [13].

As specific prion proteins, which are associated with Creutzfeldt–Jakob disease, can be transmitted through blood and blood products [14, 15], a prion removal step was added to the manufacturing process of the S/D-treated plasma which is, in most countries, named OctaplasLG (second-generation product). Since several years, this product is manufactured by the company Octapharma and is available in Europe, as well as in Canada and the USA.

A S/D-treated plasma product, named Plas+SD, manufactured by V.I. Technologies, Inc. (VITEX) received approval in the USA already in 1998 [16] and in Canada in 1999 [17]. Importantly, the distribution of this product was, however, discontinued in both countries some years later due to bleeding and thromboembolic complications [18, 19]. Furthermore, after administration of the first-generation OctaplasLG product (4 h S/D treatment), seven cases with venous thromboembolism (VTE) have been reported [20]. However, as the performed study was retrospective and other known risk factors for VTE were identified, the authors concluded that the number of events is insufficient to assess whether OctaplasLG was the risk factor for the observed VTE cases.

The purpose of this study was to compare the biochemical profiles and manufacturing processes of the two S/D-treated plasma products Plas+SD and OctaplasLG, and to explain the thromboembolic events that occurred following administration of the Plas+SD product.

**Materials and methods**

**S/D-treated plasma products used for biochemical analysis**

Because the Plas+SD product has been discontinued, only three units (blood group 0) from different batches were obtained from VITEX. The products were manufactured in 2000/2001 and stored at ≤-18°C according to the manufacturer instructions. The units were tested for 19 different plasma parameters within the approved 1-year shelf life. The results were compared with the results of three batches blood group 0 plasma, which were produced in 2013/2014 from plasma collected in the USA according to the OctaplasLG process.

**Downscaling of the Plas+SD manufacturing process**

Three different units of blood group 0 plasma pools, produced in 2015, were used for the downscaling experiments of the Plas+SD manufacturing process. As a start volume of 500 ml plasma was used, this equals approximately 1:2000. Residual cells and cell fragments were removed by filtration through 1-μm filters. Calcium chloride at a final concentration of 2 mM was added to stabilize coagulation. S/D treatment was performed in the presence of 1% tri(n-butyl)phosphate (TNBP) and 1% Octoxynol for 4 h at 31°C. Residual S/D reagents were removed by oil extraction (5% soya bean oil for 15 min) and chromatography (100 ml column packed with preparative C18 silica, Waters Corporation). After filtration through 1-μm, 0.45-μm and 0.2-μm filters, respectively, ultrafiltration was performed using a 30K membrane (Pellicon XL, Merck Millipore).

**Measurement of plasma proteins**

Although the sensitivity of the available test systems has improved over time, the principle of each test has not changed. All parameters were tested using validated test methods according to the valid European (EP) and/or US Pharmacopeia (USP) protocols in use at the time of testing.

The total protein content was measured by the BIURET method according to EP (Biuret reagent, Roche Diagnostics GmbH). Fibrinogen levels were measured by one-stage clotting assay according to CLAUS (Fibrinogen C; Instrumentation Laboratory).

Coagulation factors V (FV), IX (FIX) and XI (FXI) as well as protein S (PS) and activated partial thromboplastin time (aPTT) were all determined by one-stage clotting assays according to EP (i.e. by using FV- or FIX-deficient plasma from Precision Biologic, aPTT-SP Kit from Instrumentation Laboratory or STA-Protein S clotting kit from Diagnostica Stago). Factor VIII (FVIII), protein C (PC) and plasmin inhibitor (PI; also known as 2-antiplasmin) were quantified by chromogenic substrate assays according to EP (i.e. COAMATIC-FVIII or COAMATIC-Protein C from Chromogenix; HemosIL Plasmin Inhibitor from Instrumentation Laboratory).

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The pH value (pH-meter 3110, WTW GmbH) and osmolality (freeze point; Osmomat auto, Ekomed GmbH) of the plasma were measured according to EP and USP methods.

Sodium, potassium, calcium and phosphate levels were determined by spectroscopy, while citrate levels were measured by HPLC; all methods according to EP.

Residual S/D reagents TNBP and Octoxynol were determined according to EP methods, by gas chromatography and HPLC, respectively.

Von Willebrand factor (VWF) multimer analyses were performed using 1-2% agarose gel electrophoresis (Sigma-Aldrich Handels GmbH) with subsequent transfer to nitrocellulose membranes (GE Healthcare). For visualization of the VWF bands, membranes were incubated with polyclonal rabbit anti-human VWF antibody A0226, which was conjugated with horseradish peroxidase (DAKO). The film was developed with Super Signal West Pico Chemiluminescent Substrate (Pierce). Densitometric evaluation was performed using an automated imager reader. Normal Plasma Reference Standard (NP, American Diagnostica GmbH) was used as control for VWF multimer pattern.

Statistical analysis

All results are expressed as mean values ± 1 standard deviation. The Student’s paired t-test was used to assess statistically significant differences in the activities/concentrations of the selected plasma proteins between the Plas+SD and OctaplasLG plasma. A P-value of <0.05 was considered as statistically significant.

Results

Biochemical analysis

The mean activity values, ± 1 standard deviation, of the biochemical analysis of the historical Plas+SD product (VITEX) as well as the recently produced OctaplasLG are shown in Table 1. The percentile mean difference of each parameter in the Plas+SD product compared to the OctaplasLG product, as well as the p-values, is also included in this table.

From all parameters tested, no significant differences were found between the two S/D plasma products in fibrinogen, FVIII levels, aPTT or pH values.

A significant increase of more than 20% was found in the Plas+SD product in total protein, FV and FXI levels, whereas a significant decrease of more than 40% was seen for FIX, PC, PS and PI. In addition, the osmolality was significantly lower in the Plas+SD product when compared to OctaplasLG.

Additionally, the mean of calcium and phosphate values were significantly increased, while sodium, potassium

### Table 1: Biochemical analysis of S/D-treated plasma products

| Parameter                  | OctaplasLG (n = 3) | Historical Plas+SD (n = 3) | Mean difference (%) | P-value |
|----------------------------|--------------------|----------------------------|---------------------|---------|
| Total protein [mg/ml]      | 54.7 ± 0.6         | 68.0 ± 1.0                 | +24.3               | 0.0000  |
| Fibrinogen [mg/ml]        | 3.1 ± 0.1          | 3.1 ± 0.1                  | 0.0                 | n.a.    |
| FV [IU/ml]                | 0.93 ± 0.06        | 1.13 ± 0.06                | +21.5               | 0.0133  |
| FVIII [IU/ml]             | 0.73 ± 0.06        | 0.68 ± 0.05                | -6.8                | 0.3065  |
| FIX [IU/ml]               | 1.20 ± 0.00        | 0.70 ± 0.00                | -41.7               | n.a.    |
| FXI [IU/ml]               | 0.93 ± 0.06        | 1.20 ± 0.00                | +29.0               | 0.0013  |
| PC [IU/ml]                | 1.00 ± 0.00        | 0.58 ± 0.07                | -42.0               | 0.0005  |
| PS [IU/ml]                | 0.70 ± 0.10        | 0.32 ± 0.04                | -54.3               | 0.0035  |
| Plasmin inhibitor [IU/ml] | 0.47 ± 0.06        | 0.17 ± 0.01                | -63.8               | 0.0009  |
| aPTT [sec]                | 29.3 ± 1.2         | 30.3 ± 0.6                 | +1.4                | 0.2508  |
| pH                        | 7.4 ± 0.2          | 7.5 ± 0.0                  | 0.0                 | 0.3475  |
| Sodium [mmol/l]           | 162 ± 2            | 155 ± 0                    | -4.3                | 0.0053  |
| Potassium [mmol/l]        | 3.5 ± 0.0          | 2.0 ± 0.0                  | -42.9               | n.a.    |
| Calcium [mmol/l]          | 0.9 ± 0.1          | 2.2 ± 0.1                  | +144.4              | 0.0000  |
| Citrate [mmol/l]          | 17 ± 1             | 10 ± 0                     | -41.2               | 0.0003  |
| Phosphate [mmol/l]        | 3.4 ± 0.2          | 4.9 ± 0.3                  | +44.1               | 0.0022  |
| Osmolality [mosmol/kg]    | 354 ± 2            | 289 ± 1                    | -18.4               | 0.0000  |
| TNBP [µg/ml]              | < 0.5              | 1.4 ± 0.3                  | +180.0              | 0.0342  |
| Octoxynol [µg/ml]         | < 1.0              | < 1.0                      | 0.0                 | n.a.    |

SD, standard deviation; n.a., not applicable.

Mean levels and standard deviation are shown for three batches Plas+SD and OctaplasLG. Mean difference between Plas+SD and OctaplasLG is indicated in percentage and P-value.
and citrate were significantly decreased at varying degrees in the Plas+SD in comparison with the OctaplasLG.

Regarding the S/D reagents, Octoxynol was below detection limit in both products, whereas a small amount of TNBP was detectable in the Plas+SD, but was below the detection limit in OctaplasLG.

The mean values of all parameters which showed a difference between the two S/D plasma products are shown in Fig. 1.

In addition to the quantitative analysis of the coagulation factors and inhibitors, the VWF multimeric pattern was also analysed in Plas+SD and OctaplasLG and compared with the normal plasma reference standard (NP, Fig. 2). The loss of the high molecular weight VWF multimers was detected in the Plas+SD, whereas the VWF multimeric pattern found in OctaplasLG was comparable with the NP pattern.

Comparison of the two manufacturing processes

As the biochemical profiles of the two S/D plasma products were different, the single steps used for the two manufacturing processes were more closely examined (Table 2). The main differences in the manufacturing processes (highlighted in bold) are the prolonged S/D treatment time and the shorter extraction time used for the removal of the S/D reagents, as well as the concentration step (ultrafiltration) utilized in Plas+SD when compared to the OctaplasLG production method.

Downscaling of the Plas+SD manufacturing process

The data of the downscale experiments of the Plas+SD manufacturing process are shown in Fig. 3. Compared to the amount detected in the initial plasma pool, a decline of the mean activity levels to various extents was found for all parameters tested following the S/D treatment and removal steps. Accordingly, the aPTT was prolonged.

Fibrinogen (data not shown) was the only parameter that was increased during the concentration step (ultrafiltration) to the same extent as measured in the plasma pool. Compared to the plasma pool levels, an increase was shown in the amount of total protein, FIX and PC, whereas a reduced amount was still detected for FV.
FVIII, FXI, PS and PI (Fig. 3). aPTT was still prolonged when compared to the plasma pool (data not shown).

A possible influence of the S/D treatment and ultrafiltration steps performed during Plas+SD manufacturing on the VWF multimer pattern was investigated by densitometric analysis. The results of this analysis are shown in Fig. 4 and demonstrate that the S/D treatment had no impact on the VWF multimer pattern, whereas the ultrafiltration step contributed to the significant loss of the high molecular weight VWF multimers.

**Discussion**

The main limitation of this study is the sample size. However, the biochemical data are supported by a previous study performed by Solheim and Hellstern [21], who investigated 12 units of Octaplas (the first-generation product from Octapharma) and 8 units of Plas+SD, which showed lower concentrations of citrate, PS and PI in the historical Plas+SD product. Similar results were found by Salge-Bartels and colleagues [22], who compared coagulation factors and inhibitors in 8 units Octaplas and 2 units Plas+SD. Furthermore, Dr. Bernard Horowitz kindly provided unpublished data showing the mean values of 124 units Plas+SD and found levels of total protein (61.7 mg/ml), fibrinogen (2.6 mg/ml), FV (0.82 IU/ml), FX (1.08 IU/ml), FXI (0.93 IU/ml), FXIII (1.1 IU/ml), as well as single measurements of PC (0.36 IU/ml), PS (0.15 IU/ml) and PI (0.25 IU/ml), which are comparable with the results of our study.

A better understanding of which manufacturing steps might have contributed to the different biochemical profiles of the two S/D-treated plasma products is provided by a stepwise comparison of the two manufacturing processes, as well as the downsampling experiments performed using Plas+SD. Within the Plas+SD manufacturing process, the S/D treatment time was much longer (4 h) and the S/D reagent (TNBP) removal time was much shorter (15 min) when compared to the OctaplasLG process (1–1.5 h S/D treatment time and 60–70 min TNBP removal time). Compared to the start material (plasma pool), the prolonged S/D treatment time used for Plas+SD led to a reduction of all protein levels tested except VWF. The lack of impact of the
S/D treatment on high molecular weight VWF multimers has already been shown earlier [23]. Furthermore, previous studies already demonstrated that a shorter S/D treatment time used for OctaplasLG almost doubles PI levels and also increases PS levels [23, 24], as it has been shown that S/D treatment using TNBP and Triton X–100 (Octoxynol) is responsible for the loss of activity of these plasma inhibitors [25, 26].
The concentration step (ultrafiltration) implemented into the Plas+SD manufacturing process, which is not included in the OctaplasLG production process, compensated for the reduced activity of fibrinogen, FV, FVIII and FXI as well as for the aPTT prolongation, which occurred following S/D treatment leading to increased levels of total protein, FIX and PC activities. In addition, after the ultrafiltration step the mean values of PS activities were further reduced and significant losses of the high molecular weight VWF multimers were detected.

The very low PI activity in the Plas+SD product might have contributed to the reported bleeding events, as it has been earlier demonstrated that an increased risk of bleeding is associated with very low amounts of PI resulting in uncontrolled plasmin-mediated breakdown of the fibrin clot [27], and can lead, under certain clinical conditions, to unregulated excessive fibrinolysis [21].

Furthermore, the reduced high VWF multimers in the Plas+SD product might have contributed to a defective haemostasis especially as mainly the high VWF multimers are crucial in supporting platelet adhesion and aggregation [28].

The thrombotic events which occurred following the administration of the historical Plas+SD product might be related to the significantly reduced amount of PS activity in the final product, as it has already been shown that the loss of functional PS is associated with an increased risk for arterial/venous thrombosis [21, 22, 29]. In addition, the low citrate concentration in the Plas+SD product may have increased the risk for clot formation, especially in combination with high levels of ionized calcium. A previous study has demonstrated that a final citrate concentration lower than 10 mM led to a marked rise in fibrinopeptide A, indicating coagulation activation and fibrin formation [30].

In conclusion, although both manufacturing processes comprise an S/D treatment step for inactivation of enveloped viruses, the biochemical profile of the historical Plas+SD product is significantly different in regard to the levels of coagulation factors and inhibitors when compared to the currently available OctaplasLG. The imbalance of coagulation factors and inhibitors in the historical Plas+SD final product has the potential to be clinically relevant and might have been responsible for the adverse events that occurred with this product.

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Conflict of interest
Andrea Neisser-Svae and Andrea Heger are employed by Octapharma.

References
1 Bihl F, Damiano C, Marincola F, et al.: Transfusion-transmitted infections. J Transl Med 2007; 6:25
2 Stramer SL, Dodd R: Transmission-transmitted emerging infectious diseases: 30 years of challenges and progress. Transfusion 2013; 53:2375–2383
3 Centers for Disease Control and Prevention: HIV Transmission Through Blood Transfusion - Missouri and Colorado, 2008. MMWR October 22, 2010; 59(41):1335–1339
4 Hauser L, Roque-Afonso AM, Beyloune A, et al.: Hepatitis E transmission by transfusion of Intercept blood system-treated plasma. Blood 2014; 123:796–797
5 Hughes JA, Fontaine MJ, Gonzalez CL, et al.: Case report of a transfusion-associated hepatitis A infection. Transfusion 2014; 54:2202–2206
6 Horowitz B, Wiebe ME, Lippin A, et al.: Inactivation of viruses in labile blood derivatives. Transfusion 1985; 25:516–522
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7 Horowitz B, Bonomo R, Prince AM, et al.: Solvent/detergent-treated plasma: a virus-inactivated substitute for fresh frozen plasma. Blood 1992; 79:826–831
8 Sandler G: It is time to bring back solvent-detergent plasma. Curr Opin Hematol 2007; 14:640–641
9 Biesert L, Suhartono H: Solvent/Detergent treatment of human plasma – A very robust method for virus inactivation. Validated virus safety of Octaplas. Vox Sang 1998; 74: 207–212
10 Rollag H, Solheim BG, Svennevig JL: Viral safety of blood derivatives by immune neutralization. Vox Sang 1998; 4:213–217
11 Kitchen AD, Chiodini PL: Malaria and blood transfusion. Vox Sang 2006; 90:77–84
12 Brecher ME, Hay SN: Bacterial contamination of blood components. Clin Microbiol Rev 2005; 18:195–204
13 WHO Technical Report, Series No. 924, 2004, Annex 4: Guidelines on viral inactivation and removal procedures. Clin Microbiol Rev 2005; 18:195–204
14 Andréolletti O, Litaise C, Simmons H, et al.: Highly efficient prion transmission by blood transfusion. PLoS Pathog 2012; 8: e1002782
15 Brown P: Creutzfeldt Jakob disease: reflection on the risk from blood product therapy. Haemophilia 2007; 13:33–40
16 http://www.fda.gov/BiologicsBloodVaccines/DevelopmentApprovalProcess/BiologicalApprovalsbyYear/ucm180084.html [Last accessed 8/18/2015]
17 http://www.prnewswire.com/news-releases/vi-technologies-inc-vitex-receives-canadian-approval-for-plasdd-74845387.html [Last accessed 12/2/2015]
18 Flamholz R, Jeon HR, Baron JM, et al.: Study of three patients with thrombotic thrombocytopenic purpura exchange with solvent/detergent-treated plasma: is its decreased protein S activity clinically related to their development of deep venous thromboses? J Clin Apheresis 2000; 15:169–172
19 Coignard BP, Colquhoun SD, Nguyen GT, et al.: Intra-operative deaths in liver transplantation recipients associated with the use of solvent/detergent plasma. Hepatology 2002; 36:209A
20 Yarranton H, Cohen H, Pavord SR, et al.: Venous thromboembolism associated with the management of acute thrombotic thrombocytopenic purpura. Br J Haematol 2003; 121:778–785
21 Solheim BG, Hellstern P: Composition, efficacy, and safety of S/D-treated plasma. Transfusion 2003; 43:1176–1178
22 Salge-Bartels U, Breitner-Ruddock S, Hunfeld A, et al.: Are quality differences responsible for different adverse reactions reported for SD-plasma from USA and Europe? Transfus Med, 2006; 16:266–275
23 Heger A, Svae TE, Neisser-Svae A, Vox Sang: Biochemical quality of the pharmaceutically licensed plasma OctaplasLG after implementation of a novel prion protein (PrPsc) removal technology and reduction of the solvent/detergent (S/D) process time. Vox Sang 2009; 97:219–225
24 Lawrie AS, Gree L, Canciani MT, et al.: The effect of prion reduction in solvent/detergent-treated plasma on haemostatic variables. Vox Sang 2010; 99:232–238
25 Mast AE, Stadanlick JE, Lockett JM et al.: Solvent/detergent-treated plasma has decreased antitrypsin activity and absent antiplasmin activity. Blood 1999; 94:3922–3927
26 Burnouf T, Goubran HA, Radosevich M, et al.: Impact of Triton X-100 on alpha-2-antiplasmin (SERPINF2) activity in solvent/detergent-treated plasma. Biologics2007; 35:349–353
27 Lijnen HR, Collen D: Congenital and acquired deficiencies of components of the fibrinolytic system and their relation to bleeding or thrombosis. Blood Coagul Fibrinolysis 1989; 3:67–77
28 Stokschlaeder M, Schneppenheim R, Budde U: Update on von Willebrand factor multimers: focus on high molecular-weight multimers and their role in hemostasis. Blood Coagul Fibrinolysis 2014; 25:206–216
29 Hellstern P: Solvent/detergent-treated plasma: composition, efficacy, and safety. Curr Opin Hematol 2004; 11:346–350
30 Rock G, Tittley P, Fuller V: Effect of citrate anticoagulants on factor VIII levels in plasma. Transfusion 1988; 28:248–252

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