Received: 4 July 2022 | Revised: 1 September 2022 | Accepted: 11 September 2022

DOI: 10.1111/trf.17139

BLOOD COMPONENTS

TRANSFUSION

Frozen and freeze-dried solvent/detergent treated plasma: Two different pharmaceutical formulations with comparable quality

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Abstract

Background: OctaplasLG is a frozen solvent/detergent-treated plasma product used for treating complex coagulation factor deficiencies or as substitution therapy in emergency situations where specific factor concentrates are not available. A new freeze-dried (also known as lyophilized) form of OctaplasLG, referred as OctaplasLG Lyo (Octapharma AG, Switzerland) offers rapid reconstitution and more flexible storage conditions, improving logistics and utilization. This study compared the biochemical quality of OctaplasLG Lyo with OctaplasLG and single-donor fresh frozen plasma units.

Study Design and Methods: Three batches of OctaplasLG Lyo, manufactured for production process qualification, and 12 batches of OctaplasLG were provided by Octapharma AB (Sweden). Twelve units of fresh frozen plasma were collected by the local FDA-licensed blood provider. All plasma samples were assessed for global coagulation parameters, coagulation factors and protease inhibitors, activation markers of coagulation and fibrinolysis, and important plasma proteins. Quality control assays were conducted in accordance with European Pharmacopeia requirements.

Results: Frozen and freeze-dried OctaplasLG demonstrated comparable quality profiles upon thawing or reconstitution. All coagulation factor and protease inhibitor activity parameters were in line with levels mandated by the European Pharmacopeia. Fresh frozen plasma units showed comparable coagulation factor activities, with higher protein S and plasmin inhibitor levels than the OctaplasLG products. Fresh frozen plasma parameters showed high lot-to-lot variations.

Discussion: The two pharmaceutical forms of OctaplasLG (frozen and freeze-dried) have comparable biochemical quality. Key features of OctaplasLG Lyo are rapid reconstitution time and storage flexibility, which may improve logistics and utilization, and have particular advantages in emergency situations and pre-hospital settings.

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KEYWORDS
coagulation factor therapy, hemostasis, plasma derivatives

1 | INTRODUCTION

OctaplasLG is a solvent/detergent (S/D) treated coagulation active plasma product, manufactured at Octapharma AB (Stockholm, Sweden), that contains all of the clotting factors and anticoagulants found in circulating human plasma.[1] OctaplasLG is therapeutically indicated for the treatment of complex coagulation factor deficiencies, such as coagulopathy due to severe hepatic failure,[2] or massive transfusion.[3] It can also be used for substitution therapy in emergency situations where precise laboratory diagnosis is not possible, or in situations where specific factor concentrates are not available for the treatment of coagulation factor deficiencies.[1] Studies have confirmed clinical efficacy for OctaplasLG in both adult[2,4–6] and pediatric patients,[7,8] as well as the real-world safety profile in a range of clinical indications.

OctaplasLG is supplied frozen and is suitable for long-term frozen storage at ≤−18°C for up to 4 years.[1] Before use, frozen OctaplasLG bags are thawed using water bath systems with good circulation or dedicated thawing devices such as microwave oven or dry heater.[9] Once thawed, OctaplasLG is stable for up to 5 days when stored refrigerated (1–6°C) or 8 h at room temperature (20–25°C).[1,10–12]

The thawing procedure, using a dry heater or water bath, takes approximately 20–30 min to thaw two units of frozen product, and is often regarded as slow in time critical emergency situations.[9,13] These limitations are important considerations in pre-hospital settings, where the lack of specialized thawing and storage equipment may also limit plasma availability.[14]

Therefore, a new pharmaceutical form was developed, a freeze-dried (also known lyophilized) form of OctaplasLG, referred as OctaplasLG Lyo. OctaplasLG Lyo has the same manufacturing process as developed for the pooled, coagulation active plasma OctaplasLG, including pathogen safety against both viruses (the S/D treatment) and prion proteins (affinity ligand chromatography). OctaplasLG Lyo is filled and lyophilized in glass vials and can be reconstituted within 10–15 min right before use with water for injection (WFI) provided in a flexible bag (the specification for reconstitution time is subject to approval and not yet determined). The freeze-dried product can be stored at room temperature for up to 2 years, reducing product loss by breakage or leakage, improving transport and storage logistics compared to fresh frozen plasma (FFP). With the provision of AB blood type, the product allows a universal coagulation support in pre-hospital and early hospital emergency settings, and also making plasma transfusion possible in remote settings with an insufficient level of medical infrastructure.

This study aimed to provide a comprehensive biochemical analysis of the two pharmaceutical forms of OctaplasLG; OctaplasLG frozen and OctaplasLG Lyo, with single-donor FFP used for comparison.

2 | MATERIALS AND METHODS

2.1 | Manufacturing process

Figure 1 shows the manufacturing process of OctaplasLG and OctaplasLG Lyo. OctaplasLG is produced by pooling of 630–1520 units of single-donor FFP of the same ABO blood group.[15] During the manufacturing process, cells, cell fragments and aggregates are removed by 1.0 μm size-exclusion filtration. S/D treatment is performed in the presence of 1% tri(n-butyl)phosphate (TNBP) as solvent and 1% octoxynol as detergent for 1–1.5 h at 30°C to inactivate any lipid enveloped viruses.[16] S/D reagents are later removed by liquid and solid phase chromatography, respectively. Subsequently, an affinity ligand column is used to remove potentially present pathological prion proteins.[17–19] After sterile filtration, OctaplasLG is aseptically filled into 200 ml bags and rapidly deep-frozen.

The manufacturing process of freeze-dried OctaplasLG Lyo is almost an exact copy of the OctaplasLG (frozen product) manufacturing process (Figure 1). The only difference in the production of OctaplasLG Lyo is a pH adjustment step which was implemented, to compensate for a potential pH increase during the downstream freeze-dried process. The pH value of the OctaplasLG Lyo bulk is adjusted in a stepwise manner by using citric acid and phosphoric acid under low pressure conditions. After sterile filtration OctaplasLG Lyo is filled under aseptic conditions into sterile and depyrogenated type I glass vials (200–210 ml plasma per vial) and subjected to a freeze-drying process. Vials are closed under vacuum, sealed, and stored protected from light until final container analysis and release by the quality department.

All OctaplasLG (frozen and freeze-dried) batches are routinely tested on quality test parameters selected in accordance with the European Pharmacopeia (Ph. Eur.) monograph for human plasma (pooled and treated for virus inactivation).[20]

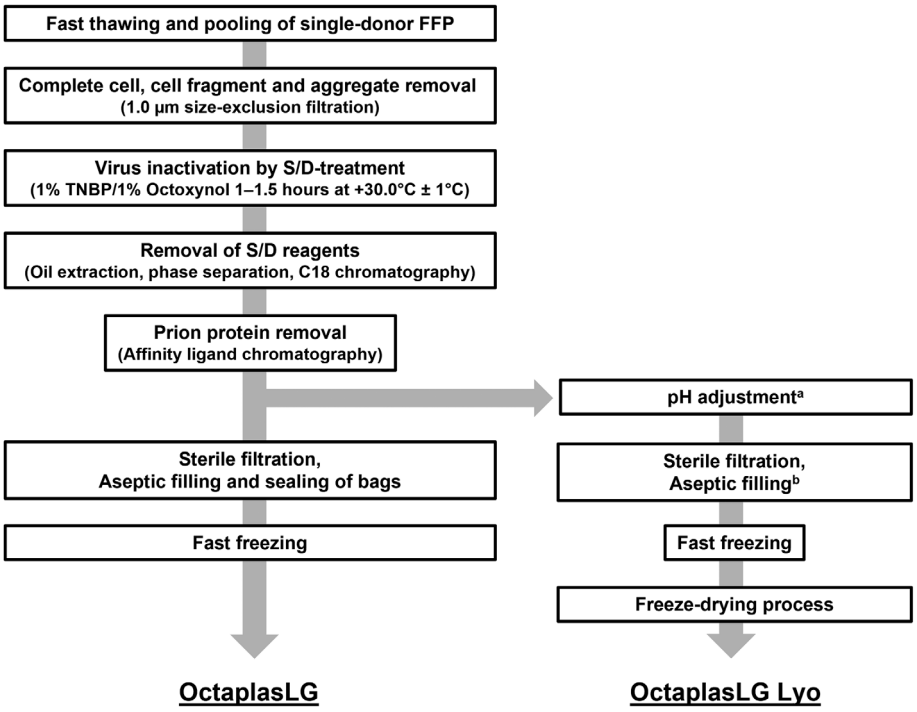

FIGURE 1 Flow-chart of the OctaplasLG and OctaplasLG Lyo manufacturing process. [a]The pH value is adjusted in stepwise manner by using citric acid, phosphoric acid and low pressure. [b]The sterile filtered plasma is filled under aseptic conditions into sterile and depyrogenated type I glass vials (200–210 ml plasma per vial). FFP, fresh frozen plasma; S/D, solvent/detergent; TNBP, tri(n-butyl)phosphate.

2.2 | Materials

Three batches of the freeze-dried OctaplasLG Lyo were produced by Octapharma (Octapharma AB, Sweden) (US plasma of blood groups O and AB) in the frame of production process qualification. Prior to biochemical testing, the vials were optically inspected and then reconstituted with 190 ml WFI supplied in a bag, and the transfer set, according to the instructions stated in the product information.

Twelve batches of frozen OctaplasLG, produced by Octapharma (Octapharma AB, Sweden), were manufactured from US plasma of different blood groups (blood groups A, B, O, and AB, three batches each). Three units of single-donor FFP of blood groups A, B, O, and AB (12 total) were collected by local FDA-licensed blood provider and used for comparison. Prior to testing, OctaplasLG and FFP bags were thawed in a water bath at 37°C according to standard operating procedures.

2.3 | Analytical assays

Quality was assessed for OctaplasLG and OctaplasLG Lyo using established quality control assays interpreted in accordance with the OctaplasLG final product specification and the Ph. Eur. specifications for S/D plasma.[20] Blood coagulation and inhibition, as well as activation markers of fibrinolysis and coagulation, were assessed for both OctaplasLG products and FFP using coagulation parameters interpreted in accordance with the OctaplasLG final product specification and reference range for plasma.

Haemagglutinins, pH value, solubility, osmolality, total protein (Biuret), activated coagulation factors (NaPTT), antibodies to Hepatitis A virus (anti-HAV IgG), irregular erythrocyte antibodies, citrate, calcium, potassium, sodium, water, sterility, pyrogens, coagulation factors (F) V, VIII, XI, and protease inhibitors protein C, protein S and plasmin inhibitor (also known as α_2-antiplasmin), as well as phosphate, glycine, TNBP, octoxynol, were all assessed according to Ph. Eur. methods. Water content was implemented as additional quality test parameter for the freeze-dried product.

Protein composition was determined by electrophoresis and fibrinogen by the method according to Clauss. Partial thromboplastin time (aPTT), prothrombin time (PT), thrombin time (TT), reptilase time (RT), factors FII, FVII, FIX, FX, FXII, and FVIIa were assessed by coagulation assays. Chromogenic substrate assays were used to quantify FXIII, plasminogen, antithrombin, heparin cofactor II (HCII), α_1-antitrypsin (A1AT), and C1-inhibitor (C1-INH) activities. Enzyme-linked immunosorbent assay (ELISA) test kits were used for the quantitative determination of a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13), protein S, immunoglobulins (i.e., IgG, IgA, and IgM), prothrombin split product F1 + 2, thrombin-antithrombin complex (TAT), and D-dimer. Albumin levels and A1AT concentrations were detected by nephelometric methods, complement

proteins 1q, 3, and 4 (C1q, C3, C4) as well as factors H and I (FH, FI) were quantified by radial immunodiffusion (RID) methods, whereas lipoprotein (a) was quantitatively determine using enzymatic colorimetric methods. Fibrinogen was determined by both the Clauss and ELISA methods. For more details of the assays see Heger et al.[21]

Thrombin generation capacities in plasma were determined using the Calibrated Automated Thrombogram (CAT) assay and the PPP Low reagent (i.e., 4 μM phospholipids, 1 pM tissue factor).[22] The peak thrombin, the lag time, and the endogenous thrombin potential (also known as the area under the curve, AUC) were measured.[22] Rotational thromboelastometry (ROTEM) measurements were performed using the EXTEM assay as described previously[22] to assess the clotting time (CT) and the maximum clot firmness (MCF). The ristocetin cofactor activity of von Willebrand factor (VWF:RCo) was measured by agglutination method. Von Willebrand factor (VWF) multimeric analyses were performed using 1.2% agarose gel electrophoresis.

2.4 | Statistical evaluation

Results were presented as mean (minimum–maximum) levels (in all tables) or median levels and 25% and 75% percentiles (in all box plot diagrams). Student's paired *t*-test was used to establish statistically significant differences between OctaplasLG frozen and lyophilized products (for quality control assays) or OctaplasLG products versus FFP (for other parameters). *p*-values <.05 were reported as statistically significant.

3 | RESULTS

3.1 | Quality control assays

For both OctaplasLG and OctaplasLG Lyo the quality test parameters for product release were in line with the Ph. Eur. requirements and the approved OctaplasLG final product specifications (Tables S1 and S2). Water content in the OctaplasLG Lyo batches was lower than 1%.

Visual inspection of the OctaplasLG Lyo showed a slightly yellow OctaplasLG Lyo lyophilized powder. The three batches were reconstituted with WFI and ready for use in 12, 14, and 15 min. After reconstitution, all three OctaplasLG Lyo batches were clear and free of any visible particles. Protein composition corresponded to the pattern of normal human plasma, and hemagglutinins corresponded to the blood groups stated on the label for both OctaplasLG and OctaplasLG Lyo (Table S1).

Mean pH level for OctaplasLG Lyo after reconstitution was pH 7.5, with a narrow range between 7.4 and 7.6. This was comparable to that of the frozen product (i.e., pH 7.4 [7.2–7.6]). Osmolality was slightly lower in the freeze-dried versus frozen product, with mean 333 versus 350 mOsmol/kg respectively. Total protein concentrations were 55–56 mg/ml for both standardized products, which were within Ph. Eur. requirements (Table S2). OctaplasLG Lyo showed higher levels of NaPTT compared with OctaplasLG, but within specifications. In addition, anti-HAV IgG levels for OctaplasLG Lyo were within requirements to allow successful immune neutralization. In addition, no irregular erythrocyte antibodies were found in either OctaplasLG product, with all batches sterile and free of pyrogens.

In the batches of freeze-dried OctaplasLG Lyo, mean citrate and phosphate concentrations were higher (citrate, 20 mmol/L; phosphate, 5.3 mmol/L) compared to the frozen product (citrate, 16 mmol/L; phosphate, 3.3 mmol/L) as citrate and phosphate are added to OctaplasLG Lyo for pH adjustment before lyophilization, however, levels remained well within the specification levels and normal plasma levels. There was no significant variation in the concentrations of calcium, potassium, sodium, and glycine in OctaplasLG versus OctaplasLG Lyo. TNBP and octoxynol were below the limits of detection (i.e., <1 μg/ml) for all batches, confirming successful removal of the S/D reagents.

3.2 | Screening tests of blood coagulation and inhibition

The new freeze-dried OctaplasLG Lyo demonstrates unaltered blood coagulation and inhibition parameters compared with the frozen OctaplasLG (Table 1). In the batches of OctaplasLG, OctaplasLG Lyo, and FFP, aPTT, and PT were comparable between all three groups, with no prolongation observed in OctaplasLG Lyo. RT and TT for both OctaplasLG products and FFP were within the reference range for plasma, with the exception of the TT for OctaplasLG Lyo which was slightly below the reference range of 14–20 s.

Thrombin parameters (Lag time and AUC) and ROTEM parameters were comparable between OctaplasLG Lyo and OctaplasLG frozen batches. In some batches of FFP thrombin concentration, and AUC were significantly lower, and lag time was significantly higher compared to OctaplasLG ($p < .05$) and OctaplasLG Lyo ($p < .05$), however, overall overlapping hemostatic potentials were found between the different plasma groups.

Coagulation factor activities (Table 1) for OctaplasLG and OctaplasLG Lyo were within the reference range for

TABLE 1 Screening tests of blood coagulation and inhibition

Parameters	OctaplasLG final product specification	Reference range plasma	Frozen OctaplasLG ($N = 12$)	Freeze-dried OctaplasLG Lyo ($N = 3$)	FFP ($N = 12$)
aPTT (s)	23–40	28–40	28 (27–31)	29 (28–31)	30 (26–35)
PT (s)	n.s.	12.5–16.5	11.8 (11.2–12.6)	11.4 (11.4–11.5)	11.4 (10.0–14.1)
RT (s)	n.s.	<20	14.6 (14.2–14.9)	13.9 (13.1–14.3)	17.4 (14.7–19.7)[a,b]
TT (s)	n.s.	14–20	14.7 (13.9–16.6)	12.4 (12.1–12.8)	14.1 (11.9–15.7)[b]
Thrombin (nM)	n.s.	n.s.	147 (111–175)	163 (161–166)	66 (33–118)[a,b]
Lag time (min)	n.s.	n.s.	6.3 (5.5–7.5)	6.0 (6.0–6.0)	8.0 (5.5–11.0)[a,b]
AUC (nM × min)	n.s.	n.s.	1376 (1219-1528)	1476 (1438-1525)	1116 (803-1358)[a,b]
Clotting time (min)	n.s.	n.s.	417 (206–465)	342 (311–374)	379 (330–451)
MCF (mm)	n.s.	n.s.	20 (18–23)	22 (21–22)	22 (16–28)
Factor II (IU/ml)	n.s.	0.65–1.54	1.08 (0.98–1.14)	1.15 (1.12–1.21)	1.29 (1.05–1.51)[a]
Factor V (IU/ml)	≥0.5	0.54–1.45	0.93 (0.90–1.00)	0.90 (0.90–0.90)	0.99 (0.80–1.25)
Factor VII (IU/ml)	n.s.	0.62–1.65	1.07 (0.97–1.21)	1.13 (1.10–1.20)	1.35 (0.85–1.74)
Factor VIII (IU/ml]	≥0.5	0.45–1.68	1.08 (0.80–1.30)	0.93 (0.80–1.00)	1.32 (0.61–1.80)[a]
Factor IX (IU/ml)	n.s.	0.45–1.48	1.15 (0.99–1.27)	1.20 (1.10–1.37)	1.32 (1.06–1.54)
Factor X (IU/ml)	n.s.	0.68–1.48	1.09 (0.98–1.12)	1.25 (1.22–1.29)	1.19 (1.08–1.52)
Factor XI (IU/ml)	≥0.5	0.42–1.44	0.93 (0.90–1.00)	0.80 (0.80–0.80)	0.99 (0.69–1.20)[a]
Factor XII (IU/ml)	n.s.	0.40–1.52	1.19 (1.03–1.40)	1.00 (0.95–1.03)	0.98 (0.54–1.37)[a,b]
Factor XIII (IU/ml)	n.s.	0.65–1.65	0.92 (0.85–0.98)	0.90 (0.88–0.92)	0.88 (0.64–1.20)
VWF:RCo (IU/ml)	n.s.	0.45–1.75	0.87 (0.78–0.94)	0.95 (0.89–1.07)	1.08 (0.57–1.42)[a]
ADAMTS13 (IU/ml)	n.s.	n.s.	0.99 (0.83–1.24)	0.92 (0.88–0.94)	0.92 (0.58–1.38)
Antithrombin (IU/ml)	n.s.	0.80–1.25	0.96 (0.91–1.02)	1.06 (1.02–1.11)	1.14 (0.91–1.36)[a]
HCII (IU/ml)	n.s.	0.65–1.35	1.23 (1.14–1.36)	1.18 (1.12–1.24)	1.27 (0.67–1.71)
Protein C (IU/ml)	≥0.7	0.58–1.64	1.00 (1.00–1.00)	1.03 (1.00–1.10)	0.97 (0.76–1.16)
Protein S activity (IU/ml)	≥0.3	0.65–1.45	0.66 (0.60–0.80)	0.67 (0.60–0.70)	1.02 (0.69–1.40)[a,b]
Protein S antigen (IU/ml)	n.s.	0.65–1.45	0.87 (0.81–0.93)	0.81 (0.80–0.82)	1.05 (0.73–1.39)[a]
Plasmin inhibitor (U/ml)	≥0.2	0.72–1.32	0.43 (0.40–0.50)	0.47 (0.40–0.50)	1.33 (1.11–1.49)[a,b]
Plasminogen (IU/ml)	n.s.	0.68–1.44	0.87 (0.82–0.90)	0.86 (0.83–0.88)	0.96 (0.70–1.18)
A1AT (mg/ml)	n.s.	n.s.	1.32 (1.02–1.47)	1.26 (1.15–1.42)	1.48 (0.93–1.71)
A1AT Ag (mg/ml)	n.s.	1.10–2.60	1.17 (1.04–1.24)	1.16 (1.16–1.17)	1.20 (0.80–1.46)
C1-INH (IU/ml)	n.s.	0.60–1.24	1.18 (1.02–1.45)	1.17 (1.08–1.22)	1.56 (1.17–1.97)[a,b]

Note: Mean (minimum–maximum) levels are presented.

[a]Statistically significant differences between OctaplasLG and FFP are indicated, that is, p-value < .05.

[b]Statistically significant differences between OctaplasLG Lyo and FFP are indicated, that is, p-value < .05.

Abbreviations: A1AT, α_1-antitrypsin; ADAMTS-13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; Ag, antigen; aPTT, activated partial thromboplastin time; AUC, area under the curve; C1-INH, C1-inhibitor; FFP, fresh frozen plasma; HCII, heparin cofactor II; IU, international units; MCF, maximum clot firmness; N, number of batches; n.s., not specified; PT, prothrombin time; RT, reptilase time; TT, thrombin time; VWF:RCo, ristocetin cofactor activity of von Willebrand factor.

plasma, and in agreement with the OctaplasLG final product specifications. FFP had significantly higher levels of FII, FVIII, FXI, and significantly lower levels of FXII in comparison to OctaplasLG and OctaplasLG Lyo ($p < .05$), but also a higher level of variability was observed (e.g. 0.5–1.4 IU/ml for FXII in FFP; see Figure 2). As expected, the lowest activities were found for FVIII in blood group O plasma (i.e., 0.8 IU/ml) and for FXI in all batches (i.e., 0.8 IU/ml). The highest activities were measured for FIX (i.e., 1.1–1.4 IU/ml). Levels of VWF:RCo and ADAMTS13 were similar in both OctaplasLG products (Table 1). In addition, high-resolution 1.2% agarose gel

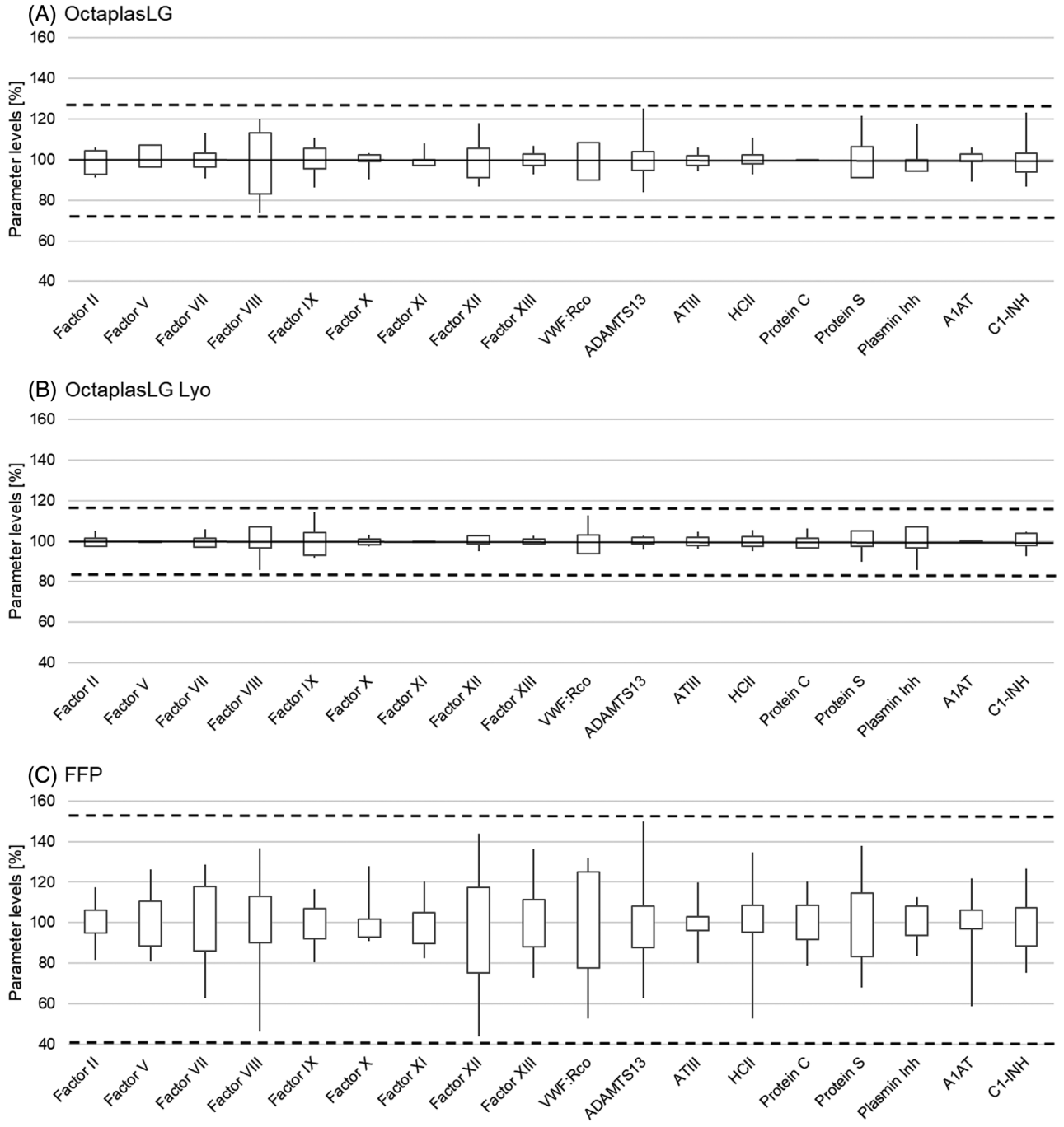

FIGURE 2 Comparison of OctaplasLG (A), OctaplasLG Lyo (B) and FFP (C) on coagulation factors and protease inhibitors. Activities are presented in percent as median levels, 25% and 75% quartiles and minimum-maximum levels. The dashed line shows the maximum standard deviation between the different batches (for OctaplasLG frozen and Lyo) and lots (for FFP). Data presented are normalized to 100%. A1AT, α_1-antitrypsin; ADAMTS-13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; ATIII, antithrombin III; C1-INH, C1-inhibitor; FFP, fresh frozen plasma; HCII, heparin cofactor II. VWF:Rco, ristocetin cofactor activity of von Willebrand factor.

electrophoresis results showed unchanged VWF-multimeric pattern in OctaplasLG frozen, OctaplasLG Lyo, and FFP samples to that of normal plasma (Figure 3).

For the protease inhibitors (Table 1); Antithrombin, HCII, protein C, A1AT, and C1-INH levels in all OctaplasLG Lyo batches were within the normal ranges for plasma and comparable to that of the OctaplasLG batches. Antithrombin levels in FFP were above the reference range for plasma and were significantly higher compared with OctaplasLG ($p < .05$), with HCII levels in

FFP also above the reference range for plasma but also with expressed higher levels of variability. Plasminogen levels were in the physiological range in all three plasma groups. Both OctaplasLG products had protein S levels at the lower limit and plasmin inhibitor levels below the lower limit of the reference range for plasma, but in keeping with the OctaplasLG final product specification. Mean plasmin inhibitor levels in the batches of FFP exceeded the upper limit of the reference range for plasma, and were significantly higher compared with both OctaplasLG products ($p < .05$). Levels of A1AT were also comparable across the groups, while C1-INH levels were higher in FFP.

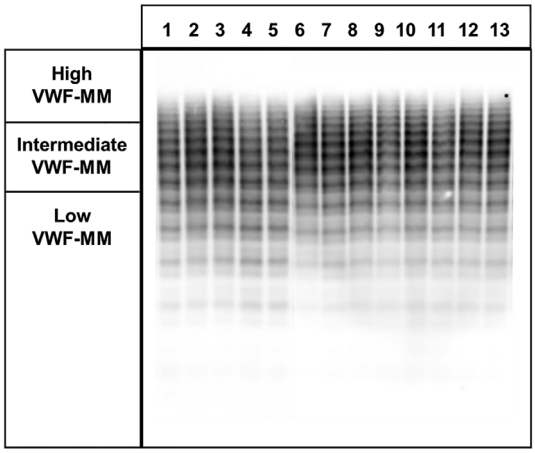

FIGURE 3 Comparison of VWF multimeric pattern in different plasma products. Low (1–5), intermediate (6–10), high (11–15) molecular weight VWF multimers are indicated in OctaplasLG (lanes 2–5), OctaplasLG Lyo (lanes 6–8) and FFP (lanes 9–12). Normal Plasma Reference Standard (lanes 1, 13) was used as control sample. VWF-MM, von Willebrand factor multimers.

Figure 2 shows the levels of coagulation factors and protease inhibitors in OctaplasLG (**A**), OctaplasLG Lyo (**B**), and FFP (**C**), with data normalized to 100%. Generally, in FFP, lot-to-lot variations of all factors and inhibitors were significantly higher than in both OctaplasLG preparations.

3.3 | Activation markers of the fibrinolytic and coagulation system, plasma proteins, and complement proteins

FVIIa, TAT, F1 + 2, and D-dimer were tested as indicators of coagulation activation and fibrinolysis, with the

TABLE 2 Activation markers of the fibrinolytic and coagulation system, plasma proteins and complement proteins

Parameters	OctaplasLG final product specification	Reference range plasma	Frozen OctaplasLG ($N = 12$)	Freeze-dried OctaplasLG Lyo ($N = 3$)	FFP ($N = 12$)
Factor VIIa (mIU/ml)	n.s.	25–170	64 (54–76)	77 (68–83)	82 (42–145)
TAT (μg/L)	n.s.	1.0–4.1	<2.0	<2.0	<2.5 (<2.0–4.3)[a]
F1 + 2 (nmol/L)	n.s.	0.4–1.1	0.17 (0.14–0.21)	0.28 (0.27–0.29)	0.22 (0.13–0.29)[a]
D-Dimer (ng/ml)	n.s.	<400	124 (106–147)	108 (101–115)	157 (56–429)
Total protein (mg/ml)	45–70	48–64	56 (54–62)	55 (55–56)	56 (49–64)
Albumin (mg/ml)	n.s.	28–41	31 (27–32)	31 (30–32)	32 (28–35)
Fibrinogen Clauss (mg/ml)	1.5–4.0	1.45–3.85	3.0 (2.7 –3.2)	3.1 (2.9–3.2)	3.5 (2.3–4.6)[a]
Fibrinogen (mg/ml)	1.5–4.0	1.45–3.85	2.9 (2.5–3.3)	3.2 (3.1–3.2)	3.1 (2.2–4.0)
IgG (mg/ml)	n.s.	6.6–14.5	6.6 (6.0–7.2)	6.5 (6.2–6.7)	5.9 (3.2–8.7)
IgA (mg/ml)	n.s.	0.75–4.20	1.4 (1.3–1.5)	1.4 (1.3–1.5)	1.6 (0.4–5.8)
IgM (mg/ml)	n.s.	0.40–3.10	0.42 (0.37–0.55)	0.43 (0.41–0.45)	0.52 (0.21–2.06)
Lipoprotein(a) (mg/dl)	n.s.	0–25	24 (21–30)	24 (23–25)	23 (13–36)
C1q (μg/ml)	n.s.	n.s.	122 (114–130)	123 (122–125)	112 (82–139)
C3 (mg/dl)	n.s.	40–72	101 (93–108)	97 (94–101)	104 (70–150)
C4 (mg/dl)	n.s.	12–34	23 (18–28)	22 (21–23)	25 (10–40)
Factor H (mg/ml)	n.s.	n.s.	0.46 (0.42–0.50)	0.49 (0.41–0.53)	0.53 (0.35–0.63)[a]
Factor I (mg/ml)	n.s.	n.s.	0.021 (0.020–0.029)	0.023 (0.021–0.026)	0.029 (0.025–0.036)[a]

Note: Mean (minimum–maximum) levels are presented.

Abbreviations: C1q, complement protein 1q; C3, complement protein 3; C4, complement protein 4; F1+2, prothrombin split product 1+2; FFP, fresh frozen plasma; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IU, international units; N, number of batches; n.s., not specified; TAT, thrombin anti-thrombin complex.

[a]Statistically significant differences between OctaplasLG and FFP are indicated, i.e., p-value <.05.

results shown in Table 2. FVIIa mean levels were within the reference range for plasma and comparable for both OctaplasLG products and FFP. TAT, F1 + 2 and D-dimer levels in all OctaplasLG Lyo batches were within the normal ranges, however some batches of FFP exceeded the upper limit for the reference range for plasma, with TAT levels in FFP significantly higher than OctaplasLG ($p < .05$). Some single units of FFP also showed elevated D-dimer levels.

Mean concentrations of the major plasma proteins (including total protein, albumin, immunoglobulins, and lipoprotein[a]) were within the reference range for plasma and comparable between OctaplasLG and OctaplasLG Lyo. In addition, fibrinogen levels obtained with two different methods, (Clauss assay and by ELISA), were unaltered between the two OctaplasLG products.

Finally, complement proteins C1q, C3, and C4 were within the reference range for plasma for both OctaplasLG products (Table 2). FH and FI levels were comparable for both the frozen and freeze-dried forms, with significantly higher levels seen in FFP compared to OctaplasLG ($p < .05$).

All other activation markers, plasma proteins, and complement proteins were comparable between the different product groups, with higher levels of variability seen in FFP.

4 | DISCUSSION

This study compared the biochemical quality of two pharmaceutical forms of OctaplasLG; OctaplasLG, and OctaplasLG Lyo, with single-donor units of FFP used for comparison. This analysis demonstrates that frozen OctaplasLG and freeze-dried OctaplasLG Lyo have comparable quality profiles upon thawing and reconstitution, respectively, in accordance with Ph. Eur. requirements. Blood coagulation and inhibition parameters were comparable for both the frozen and freeze-dried forms and were in line with the OctaplasLG final product specification and reference range for plasma. The same was true for activation markers of the fibrinolytic and coagulation system, plasma proteins and complement factors, showing both OctaplasLG products to have an equally standardized quality profile in composition and function with a very low level of variability in comparison to single donor FFP.

The freeze-drying process for plasma preparation was first developed during World War II as a method of preserving blood plasma for battlefield emergencies without requiring refrigeration or damaging the organic nature of plasma. Currently, freeze-dried plasma is manufactured in for example, Germany (LyoPlas N-w; German Red Cross), France (FLYP, French Military Blood Institute) and South-Africa (Bioplasma FDP, National Bioproducts Institute).[23] The products differ in the starting plasma (i.e., a single-donor FFP for LyoPlas N-w, or [mini-] pooled plasma for FLYP and Bioplasma FDP), pathogen reduction (i.e., not valid for LyoPlas N-w), freeze-drying process, water content, storage conditions, and stability, and consequently in functional profile and biological composition.[24] These products are not all pharmaceutically licensed (FLYP, LyoPlas-N-w) but commercialized under blood product regulations. OctaplasLG Lyo undergoes regulatory assessment for marketing authorization in selected countries of the European Union at the time this publication is written.

It is well known that freeze-drying of plasma leads to pH increase by 0.8–1.0 units, due to the evaporation of carbon dioxide (CO_2).[25] For OctaplasLG Lyo, prior to freeze-drying, a pH adjustment by a combination of citric acid, phosphoric acid and an evacuation step at low pressure was implemented into the manufacturing process, to compensate for the pH increase caused by losses of plasma CO_2 during freeze-drying. The results of this analysis indicate that due to these additional measures taken up front, the evaporation of CO_2 during the freeze-drying of plasma was well compensated by pH-adjustment, meaning the pH value in the freeze-dried product was comparable to that of the corresponding frozen product and remains within the physiological range. Citrate and phosphate levels in OctaplasLG Lyo also remained within the product specification levels.

Stabilizing additives are frequently used to protect plasma proteins from denaturation during both the freezing and the dehydration steps of the freeze-drying process. A wide range of compounds, including sugars (e.g., glucose, sucrose, trehalose), sugar alcohols (e.g., mannitol sorbitol), amino acids (e.g., glycine, arginine), surfactants (e.g., polysorbate 80 or 20), buffers and polymers may be used for this reason.[25,26] In the OctaplasLG manufacturing process, glycine is added at 5 mg/ml final concentration to stabilize plasma proteins during freezing. For OctaplasLG Lyo, no additional stabilization during the freeze-drying step was required.

The comprehensive biochemical investigation study confirmed that the quality and biological function of OctaplasLG Lyo is not impaired by the freeze-drying step. OctaplasLG and its freeze-dried sibling are pathogen reduced, standardized products with comparable profiles in quality and function upon thawing or reconstitution, based on identical controlled starting material, careful pH adjustment, an optimized lyophilization technique, and an adjusted amount of solvent (WFI) for reconstitution of OctaplasLG Lyo. The results showed comparable aPTT and PT results between the products, indicating no

relevant depletion or alterations of coagulation factors due to freeze-drying, and no prolongations of TT and RT in the OctaplasLG Lyo which could indicate decreased or dysfunctional fibrinogen.[27,28] FVIIa levels for OctaplasLG Lyo were also within the reference range for plasma, indicating that there was no activation of FVII during the freeze-drying step of the manufacturing process.[29] Protein S and plasmin inhibitor activities were higher in FFP compared to both OctaplasLG products, however this is a known effect of the S/D treatment, with levels of protein S and plasmin inhibitor being well within the OctaplasLG final product specifications.[22,30] In some FFP lots, thrombin concentrations were significantly lower compared to OctaplasLG and OctaplasLG Lyo, but overlapping hemostatic potentials were found between the different plasma groups, in both thrombin generation and ROTEM assay, similar to that observed in earlier data.[22,31] The significant differences in mean coagulation factor and protease inhibitor levels between the FFP and OctaplasLG products could be attributed to an expressed lot-to-lot variation for FFP.

In conclusion, OctaplasLG and OctaplasLG Lyo are two pharmaceutical formulations of the same pooled and pathogen reduced plasma material, with equally high biochemical quality and function profiles, developed for standardized replacement of physiological coagulation function. Compared to the frozen product, the key attributes of the robust freeze-dried OctaplasLG Lyo are the storage conditions up to room temperature (i.e., at 2–25°C), the reasonable shelf life of up to 24 months, and the fast reconstitution which allows a fast reaction time in emergency situations. These features finally allow a practical implementation of pre-hospital use with long transportation times, enables plasma transfusion in regions with underdeveloped infrastructure, and also may serve military missions where cooling infrastructure, product breakage, and leakage during transportation of deep-frozen blood products is a known burden of logistics. In addition, with the potential to reduce blood product wastage by unused pre-thawed materials in rescue vehicles, helicopter services, and in early in-hospital trauma units the introduction of OctaplasLG Lyo may enable cost savings[32] when applied to the small but relevant number of coagulopathic patients where fast and effective resuscitation may be life-saving.[33,34]

ACKNOWLEDGMENTS

The authors thank the laboratory staff of Octapharma's Research & Development Department for the excellent analytical assay work. We thank Manfred Karlovits at Research & Development, for his outstanding scientific guidance during the development of the lyo-cycle. In addition, we gratefully acknowledge the collaboration with Ann-Charlotte Hinz at Quality Control as well as Gisela Bengtsson and Roya Moezzifard at Operation Support Department from Octapharma AB, during the technology transfer and production of OctaplasLG Lyo pilot batches. Editorial assistance was provided by Portland Medial Communications Ltd, funded by Octapharma AG.

FUNDING INFORMATION

This study was sponsored and funded by Octapharma AG.

CONFLICT OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Heger A, Gruber G. Frozen and freeze-dried solvent/detergent treated plasma: Two different pharmaceutical formulations with comparable quality. Transfusion. 2022;62(12):2621–30. https://doi.org/10.1111/trf.17139