



Contents lists available at ScienceDirect

Biologicals

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Biochemical characterization, stability, and pathogen safety of a new fibrinogen concentrate (fibryga[®])

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ARTICLE INFO

Keywords:

Fibryga[®]
Fibrinogen concentrate
Integrity
Safety
Stability

ABSTRACT

Fibryga[®] is a new lyophilized fibrinogen concentrate for intravenous use for the treatment of congenital fibrinogen deficiency. fibryga[®] is produced from pooled human plasma and the final product is characterized by high purity, integrity, and pathogen safety.

Functional activity of fibrinogen was demonstrated by cross-linking studies and thromboelastometry; integrity of the fibrinogen molecule was demonstrated by size exclusion chromatography and the detection of only trace amounts of activation markers in the final product.

Pathogen safety of fibryga[®] was proved by downscaling studies for the two dedicated pathogen inactivation/removal steps, i.e. solvent detergent treatment and nanofiltration.

Fibryga[®] is stable for at least three years when stored at room temperature.

In conclusion, the performed studies demonstrated that fibryga[®] meets the requirements for a state-of-the-art fibrinogen concentrate, such as a satisfactory activity profile combined with a favorable pathogen safety profile and stability.

1. Introduction

Fibrinogen, also known as clotting factor I, plays a critical role in hemostasis and clot formation [1]. Interaction of the fibrinogen with platelet surface receptor molecules supports platelet aggregation. After cleavage of activation peptides from the precursor molecule by thrombin, fibrin monomers are generated that undergo polymerization and subsequent cross-linking by activated factor XIII (FXIII) to form the fibrin network of the clot [2].

The fibrinogen molecule consists of two sets of three subunits each, namely α -, β -, and γ -chains, linked via disulfide bridges forming a rod-shaped molecule with a molecular weight of 340 kDa [2]. The normal plasma concentration of fibrinogen is 2–4.5 g/L [1]. Plasma levels below 1.0 g/L fibrinogen are considered to be critical and are associated with an increased risk of bleeding [3]. Such low fibrinogen plasma levels can be either congenital or acquired. The most severe inherited condition, afibrinogenemia, is a very rare disorder with an estimated incidence of 1–2 cases per million [4]. Patients affected by afibrinogenemia experience a highly variable bleeding tendency. These

bleeding episodes can arise frequently, can be spontaneous or trauma-induced, and can be severe and life threatening [5].

Administration of fibrinogen to achieve a target level of at least 1–1.5 g/L is established as a standard therapy for congenital fibrinogen deficiency [6]. Fibrinogen can be administered via plasma transfusion, but this kind of treatment bears the risk of volume overload. Another treatment option is the administration of cryoprecipitate as used in several countries, but these preparations contain substantial and variable amounts of additional coagulation factors such as Factor VIII and von Willebrand Factor (VWF), and do not undergo pathogen inactivation [7,8].

A fibrinogen concentrate manufactured from cryoprecipitate, for example, provides a highly purified and thus enriched preparation, which undergoes dedicated pathogen safety steps for *in-vivo* use. Furthermore, compared to the administration of fresh frozen plasma or cryoprecipitate, a fibrinogen concentrate exhibits additional considerable advantages, such as rapid availability, lower infusion volumes and protein load, standardization, and importantly, a well-characterized safety profile in terms of composition and pathogen safety and no need

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<https://doi.org/10.1016/j.biologicals.2017.12.003>

Received 31 July 2017; Received in revised form 29 November 2017; Accepted 21 December 2017

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for blood type related cross matching.

For patients with congenital or acquired fibrinogen deficiency, replacement therapy by administration of a human fibrinogen concentrate is the method of choice for treatment. The new fibrinogen concentrate fibryga® (Octapharma AG, Lachen, Switzerland), a state-of-the-art lyophilized fibrinogen product produced from pooled human plasma, has been developed to offer a product, which combines convenient usage and functionality of the target protein with high purity and high pathogen safety for effective treatment of fibrinogen deficiency.

In a pharmacokinetic study performed in congenital afibrinogenemia patients, this new fibrinogen concentrate showed a larger area under the curve (AUC) and a slower clearance versus an active control while increases in clot strength and safety profiles were comparable with the comparator [9]. Additionally, the planned interim analysis of a prospective, multinational phase III study has confirmed the excellent hemostatic efficacy and safety profile of the new concentrate for on-demand treatment of acute bleeding and surgical prophylaxis in congenital afibrinogenemia patients [10].

The new fibrinogen concentrate has recently been approved in European countries, Canada, and the United States.

The manufacturing process of this new fibrinogen concentrate was designed with a focus on maintaining functionality of the target protein while increasing the purity and safety of the product. The manufacturing process yields a highly purified fibrinogen concentrate as a freeze-dried product, which does not require the addition of protein stabilizers such as albumin. Two dedicated, synergistic, pathogen inactivation/removal steps were implemented to ensure pathogen safety, namely solvent/detergent (S/D) treatment and nanofiltration with filters of a nominal pore size of 20 nm.

In this study, the biochemical and functional characteristics of the new fibrinogen concentrate were assessed in final product batches. Using downscaling models for S/D-treatment and nanofiltration, the pathogen safety of the final product was validated.

2. Materials and methods

2.1. Manufacturing process of the new fibrinogen concentrate

The new fibrinogen concentrate (fibryga®, Octapharma AG, Lachen, Switzerland) is produced starting from cryoprecipitate of pooled human plasma. The manufacturing process of fibryga® comprises adsorption, precipitation, and ion-exchange chromatography steps. A schematic presentation of the manufacturing process is shown in Fig. 1. S/D-treatment and nanofiltration with a nominal pore size of 20 nm are used to ensure pathogen safety. The product at a filling-size of 1 g per vial is supplied as a freeze-dried powder for reconstitution with 50 mL of water for injection, resulting in a solution with 20 mg/mL fibrinogen, which is formulated with 6 mg/mL sodium chloride, 1.5 mg/mL trisodium citrate dihydrate, 10 mg/mL glycine, and 10 mg/mL L-arginine hydrochloride.

2.2. Biochemical investigations

2.2.1. Fibrinogen final product

Final product samples of fibryga® were taken from batches manufactured at production scale.

2.2.2. Reconstitution of lyophilizates and sample handling

Reconstitution of the lyophilized product was performed with one of two methods: either using the new developed transfer device Octajet according to the manufacturer's instructions or using a syringe and adding 50 mL of water for injection.

Samples were tested immediately after reconstitution for clottable protein and activity and aliquots thereof were stored at -70°C for further analysis. Repeated freezing/thawing cycles for the samples was

Manufacturing process for fibryga®

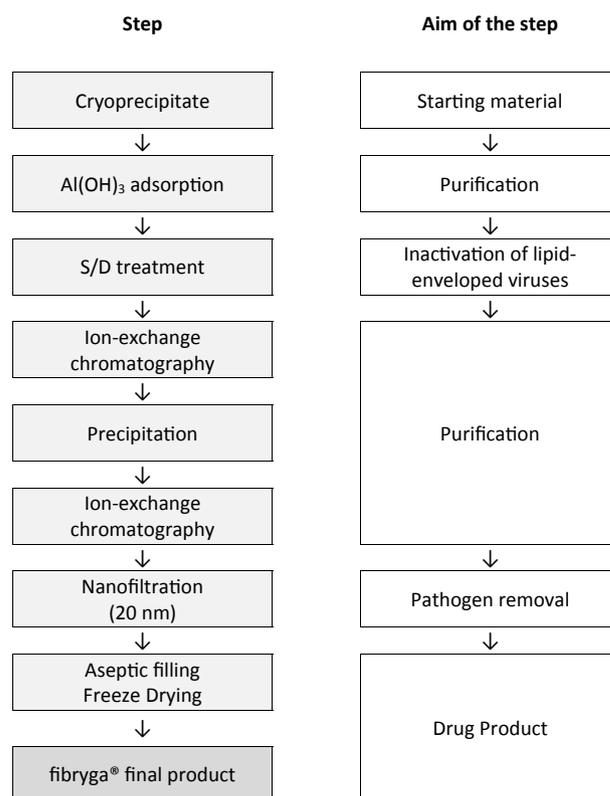


Fig. 1. Manufacturing process for fibryga®.

avoided.

2.2.3. Biochemical parameters

Fibrinogen activity in terms of clottable protein was determined according to European Pharmacopeia monograph 0024 [11]. Fibrinogen activity according to Clauss [12] was analyzed by using the Fibrinogen STA Liquid Fib Reagent on an STA-R Instrument (Diagnostica Stago SAS, Asnières sur Seine, France). Calibration was done with a secondary calibrator referenced to the 2nd International Standard for Fibrinogen Concentrate, NIBSC Code 09/242.

Fibrinogen antigen was analyzed by a nephelometric method (Siemens System, Erlangen, Germany).

Total protein concentrations were determined by the method according to Biuret [13] calibrated against an in-house standard for fibrinogen.

Accompanying proteins were quantified with commercially available ELISA test kits for the content of fibronectin (Technozym® Fibronectin ELISA, Technoclon, Vienna, Austria), von Willebrand Factor (VWF-Enzym-Immunoassay-Testkit VWF:Ag Asserachrom, Diagnostica Stago SAS, Asnières sur Seine, France), and vitronectin (Imubind Vitronectin ELISA Kit, Sekisui Diagnostics GmbH, Pfungstadt, Germany). An in-house ELISA method was performed for albumin. FXIII activity was measured using the Berichrom FXIII chromogenic ammonia release assay (Siemens Healthcare Diagnostics, Marburg, Germany).

Activation and fibrinolysis markers tested were fibrinopeptide A (Zymutest human FPA, Hyphen Biomed, Neuville-sur-Oise, France), D-Dimer (Asserachrom D-Dimer Kit, Diagnostica Stago SAS, Asnières sur Seine, France), and plasminogen (HemosIL Plasminogen, Instrumentation Laboratory Company, Bedford, MA, USA).

Molecular size distribution of proteins in fibrinogen concentrates

was analyzed by size exclusion chromatography. A Superdex 200 column (0.5 cm × 30 cm; GE Healthcare, Uppsala, Sweden) was used. Phosphate buffered saline was used as running buffer; 20 µL of sample were injected and the elution pattern was followed by UV detection at 280 nm.

Fibrinogen cross-linking studies were performed according to the method previously described by Ariëns [14].

For each fibrinogen sample and each reaction time (0, 1, 2, 5, 10, 20, 30, 60, and 120 min) a fibrin clot was produced as follows: 3 µL fibrinogen concentrate (20 mg/mL) was incubated with 3 µL human thrombin (10 U/mL; T6884, Sigma, St. Louis, USA) and 6 µL CaCl₂ (100 mM) in 48 µL 50 mM Tris-HCl, 150 mM NaCl, pH 7.5 at room temperature and with mixing. The reaction was stopped after the respective reaction time by addition of an equal (1:1) volume of reducing loading buffer (300 mM Tris-HCl, 430 mM SDS, 50 mM DTT, 50% glycerol, and Brilliant Blue G250), with immediate boiling for 5 min 20 µL of each sample were subjected to SDS-PAGE and stained with Coomassie blue. The fibrin γ -chain and the γ - γ -chain were quantified by densitometric analysis using the program Multi Gauge V3.2 (Fujifilm, Düsseldorf, Germany).

Thromboelastometry (ROTEM[®]) for determination of maximum clot firmness (MCF) was performed using the ROTEM[®] delta instrument (instrument and reagents from Tem International GmbH, Munich, Germany). Whole blood donated with written consent by healthy volunteers (Red Cross, Vienna, Austria) was used for spiking experiments. 50 µL of fibryga[®] samples prediluted with 0.9% NaCl was added to 950 µL of whole blood (premixed with equal parts of Ringer's-acetate-solution) and 300 µL thereof added to 20 µL of reagent and 20 µL star-tem or 20 µL of fib-tem reagent into the cuvette and clotting initiated for EXTEM and FIBTEM tests. Determination of MCF running the NATEM test was done by recalcification using 20 µL star-tem reagent. Progress of clot formation was followed for one hour and MCF was determined by the instrument software.

2.3. Assessment of stability

A stability study for fibryga[®] was performed according to the current guidelines of the International Conference on Harmonisation for stability testing [15,16]. Three batches of fibryga[®] were put on stability under long-term conditions at +5 °C ± 3 °C and +25 °C ± 2 °C (60% ± 5% relative humidity) for up to 36 months. In addition six batches were stored under the same conditions for 24 months. Clottable protein indicative for functionality was tested at batch release and after 3, 6, 9, 12, 18, 24, and 36 months. Values at starting time point were set to 100%. For the following time points, concentrations in clottable protein were calculated relative to the respective starting value. Mean values and standard deviations (SD) were calculated.

2.4. Assessment of pathogen safety

The scaled-down model employed in the pathogen safety studies was validated previously to comply with the commercial scale process in all relevant parameters, and was implemented successfully at the test facility. The following pathogens were selected for testing of both safeguarding steps S/D-treatment and 20 nm nanofiltration: human immunodeficiency virus type 1 (HIV-1), pseudorabies virus (PRV), and bovine viral diarrhoea virus (BVDV). In addition, hepatitis A virus (HAV), porcine parvovirus (PPV), and prion protein (strain 263K hamster-adapted scrapie; PrP^{Sc}) were investigated to specifically test the nanofiltration step. Furthermore, the removal of prion protein was also investigated by two subsequent precipitation steps of fibryga[®] manufacturing, namely cryoprecipitation and aluminum hydroxide adsorption. All pathogen safety studies were performed in duplicate. Cytotoxicity and interference tests were performed to determine necessary dilutions to avoid matrix effects. A validated assay system for endpoint titration and large-volume plating was used. PrP^{Sc} was

Table 1

Biochemical characterization of fibryga[®]. For standardization, ratios of respective parameters versus clottable protein are shown.

Parameter		fibryga [®] (n = 6) Mean ± SD
Fibrinogen activity		
Specific activity	[%]	98 ± 7
clottable protein/total protein		
Fibrinogen Clauss/clottable protein	[mg/mg]	1.08 ± 0.09
Fibrinogen antigen/clottable protein	[mg/mg]	1.05 ± 0.09
Activation and fibrinolysis marker proteins		
Fibrinopeptide A	[ng/mg]	0.3 ± 0.1
D-Dimer	[ng/mg]	3.7 ± 0.9
Plasminogen	[mU/mg]	1.8 ± 0.3
Accompanying proteins		
Factor XIII activity	[IU/mg]	0.20 ± 0.01
Fibronectin	[µg/mg]	0.8 ± 0.3
Albumin	[mg/mg]	0.02 ± 0.01
Von Willebrand Factor	[IU/mg]	0.01 ± 0.002
Vitronectin	[ng/mg]	< 0.001
Size distribution (Size exclusion chromatography)		
High molecular weight proteins ^a	[%]	2.8 ± 0.5

^a Expressed as percentage of the total chromatogram area.

investigated using Western blot analysis.

The obtained logarithmic reduction factors were determined using the method of Spearman and Kaerber and, where applicable, the Poisson distribution at 95% upper confidence limits ($p = .05$).

3. Results

fibryga[®] is presented as a freeze-dried concentrate of human fibrinogen. It is reconstituted with water for injection to a final concentration of nominal 20 mg/mL clottable protein. Using the Octajet transfer device for reconstitution, an average reconstitution time of 04:27 ± 03:37 min was achieved (mean value ± SD of 87 determinations performed by 25 participants).

3.1. Biochemical and functional characterization

Results of the biochemical characterization of six batches of fibryga[®] are summarized in Table 1. Results are shown as mean values ± SD, values are standardized for clottable protein as the leading parameter.

3.1.1. Purity and accompanying plasma proteins

fibryga[®] is a highly purified fibrinogen preparation as shown by a high specific activity of about 98 ± 7% clottable protein based on total protein.

The high purity and integrity is further underlined by the ratio of fibrinogen antigen to clottable protein. The mean calculated value with 1.05 ± 0.09 mg/mg in fibryga[®] is within the variance of the analytical methods applied, and almost identical to the theoretical value of 1.0 for a fully native fibrinogen preparation. The same applies to the ratio of Fibrinogen Clauss in relation to clottable protein calculated with 1.08 ± 0.09 mg/mg.

Fibrinopeptide A, which is cleaved from the fibrinogen molecule during activation by thrombin, is present in trace amounts of about 0.3 ± 0.1 ng/mg clottable protein in fibryga[®]. A low amount of fibrinopeptide A can be taken as indicative that undesirable activation does not occur during the manufacturing of fibryga[®] and there is also no further activation of fibrinogen after reconstitution, thus demonstrating a stable fibrinogen product. This finding is also supported by a very low D-Dimer content (3.7 ± 0.9 ng/mg clottable protein), an indicator for fibrinolysis. Plasminogen was quantified with 1.8 ± 0.3 mU/mg.

Factor XIII is essential during formation of a stable clot. As a final step in the fibrin polymerization, the fibrin strands are covalently crosslinked by activated FXIII. This protein is co-purified in the fibryga[®]

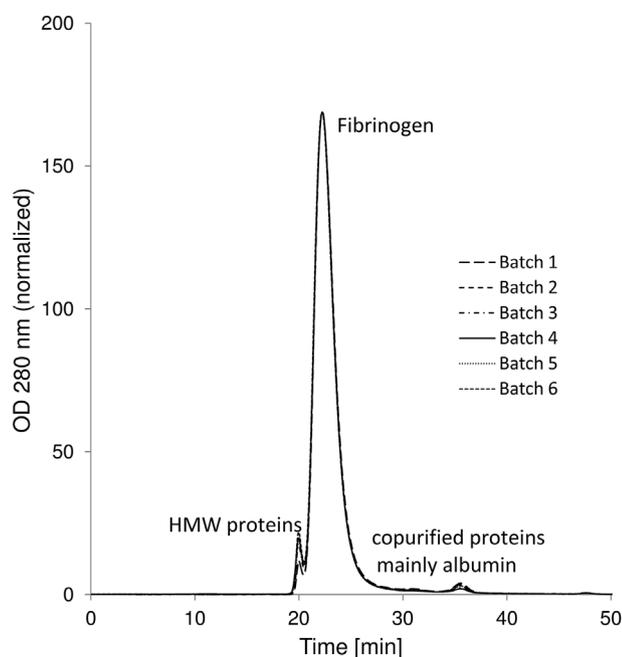


Fig. 2. Size exclusion profiles of six batches fibryga[®] analyzed by analytical SE-HPLC.

manufacturing process to a certain extent, yielding a ratio of 0.20 ± 0.01 IU FXIII per mg of fibrinogen.

3.1.2. Physicochemical characteristics

Possible denaturation, aggregation, or fragmentation of the fibrinogen molecules in fibryga[®] were assessed by analytical size exclusion chromatography under native conditions. A low content of high molecular weight (HMW) proteins is regarded as a quality attribute for a fibrinogen preparation. The molecular weight distribution profiles obtained by analytical SE-HPLC for six batches of fibryga[®] are shown in Fig. 2 as an overlay of the chromatograms. The HMW peak, eluting in front of the fibrinogen monomer peak, represents $2.8 \pm 0.5\%$ of the total peak area in the six batches fibryga[®] included in this study. Traces of co-purified proteins are detected by a peak eluting at a lower molecular weight, representing mainly albumin.

3.1.3. Biological integrity and functionality

To demonstrate the integrity and biological activity of fibryga[®], the kinetics of γ - γ chain cross-linking were determined. Final product samples were allowed to react with thrombin for several defined incubation times. Generation of cross-linked γ - γ chain pairs over time as well as the respective decrease in single γ -chains was followed by densitometric analysis of Coomassie-stained protein bands. In Fig. 3, a representative Coomassie-stained SDS-PAGE for analysis of one of the six batches included in this study is shown. In the first lane the respective final product before reaction with thrombin was analyzed. A typical band pattern [17] for fibrinogen in SDS-PAGE under reducing conditions was obtained: α -chains at a molecular weight of approximately 63.5 kDa, β -chains at 56 kDa, and γ -chains at 47 kDa. Reaction of fibrinogen with thrombin and cross-linking of γ - γ chains was monitored for an incubation time of 120 min and resulting bands quantified by densitometry. Most importantly, the single γ -chain almost completely disappears, i.e. is almost completely cross-linked to form γ - γ chains for all fibryga[®] samples investigated.

The kinetics of γ - γ chain cross-linking for six batches fibryga[®] is shown in Fig. 4 as mean values \pm SD. The kinetic of γ - γ chain cross-linking is virtually identical for all samples from the fibryga[®] batches, indicating full functional integrity and comparability of fibrinogen in the fibryga[®] batches investigated in this study.

Determination of maximum clot firmness by thromboelastometry

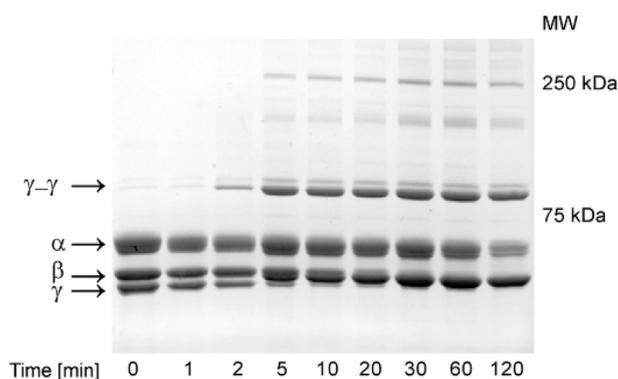


Fig. 3. Cross-linking of fibrin γ -chains after addition of thrombin. A representative Coomassie-stained SDS-PAGE for one fibryga[®] batch is presented. The first lane (0) shows the respective fibrinogen product batch before incubation with thrombin. The following lanes show the incubation of the fibrinogen preparation with human thrombin and CaCl_2 for the different reaction times (minutes) as indicated in the figure. The arrows indicate the α -, β -, γ -chains, and the cross-linked γ - γ chains, respectively. Indicated molecular weights were estimated by parallel analysis of marker proteins with known molecular weights.

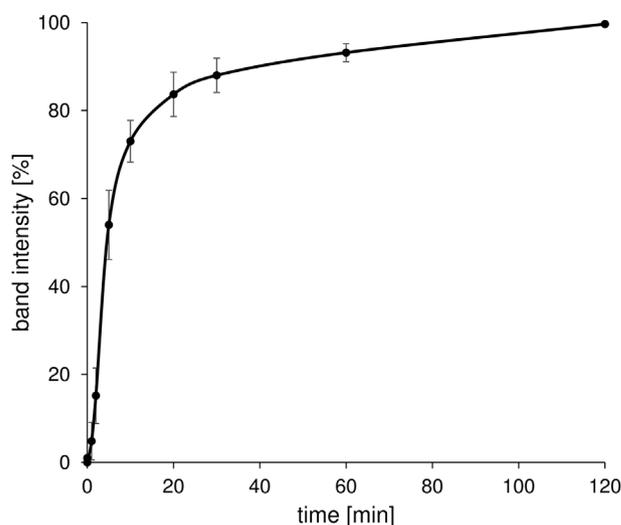


Fig. 4. Densitometric analyses for the kinetics of γ - γ chain cross-linking. Mean values \pm SD of six batches fibryga[®] are shown.

(ROTEM) was performed as a complementary analytical method to demonstrate the functionality of fibryga[®].

In the Clauss assay, determination of clottable protein functionality of fibrinogen was assessed in an artificial, purified system: Thrombin was added in excess and the time to clot formation was measured. For determination of clottable protein, thrombin was also added in excess and the amount of protein present in the clot formed was then determined.

In the present study, the functionality of fibryga[®] was studied additionally in an *ex-vivo* approach using a whole blood system mimicking the patient's hemostatic status after supplementation of fibrinogen for the treatment of fibrinogen deficiency. In contrast to the above mentioned conventional analytical methods for determination of fibrinogen, ROTEM analysis allows generation of the clot and the increase in clot firmness over time to be monitored.

Whole blood, premixed with an equal part of Ringer's-acetate-solution, was spiked with fibrinogen concentrate of three individual final product batches to increase the fibrinogen concentration by 1 mg/mL. MCF was measured in the EXTEM test after initiation of clotting by addition of tissue factor, phospholipids, and CaCl_2 (r ex-tem reagent). In parallel, MCF in the FIBTEM test was analyzed. To exclude the influence of platelets in the MCF readout, the contribution of platelets

Table 2

Increase of Maximum Clot Firmness (MCF) after spiking of fibryga[®] into whole blood of healthy donors in the EXTEM, FIBTEM, and NATEM test. Data from three batches fibryga[®] spiked in whole blood from four (EXTEM, FIBTEM) or two (NATEM) individual donors are shown.

ROTEM test	Increase in MCF (mean \pm SD)
EXTEM	11 \pm 1.4 mm
FIBTEM	8 \pm 0.6 mm
NATEM	15 \pm 1.0 mm

during clot formation was blocked by addition of cytochalasin D (fibrinogen reagent). In addition, MCF was assayed by the NATEM test, i.e. after recalcification without addition of activators. The increase of MCF was assessed by using respective whole blood samples without spiking of fibrinogen concentrate, but after volume compensation using 0.9% NaCl.

An increase in clot firmness was obtained after spiking of fibryga[®] into whole blood in a dose-dependent manner. The relative increase in the EXTEM test when adding 1 mg/mL of fibryga[®] into the whole blood sample was 11 \pm 1.4 mm, in the global NATEM system 15 \pm 1.0 mm, whereas in the FIBTEM assay a lower increase, i.e. 8 \pm 0.6 mm, was detected (Table 2). Upon activation, fibrinogen is converted to fibrin, building the mesh, which is further cross-linked by activated FXIII. In addition, fibrinogen interacts with platelets, building a more stable clot. As in the FIBTEM system interaction of fibrinogen with platelets is inhibited, the contribution of interaction of fibrinogen with platelets added is observed when measuring the MCF in the EXTEM system for comparison in parallel.

3.2. Stability of fibryga[®]

Long-term stability studies of fibryga[®] demonstrated a stable product for at least 36 months at storage temperatures of +5 \pm 3 °C and +25 \pm 2 °C (Fig. 5). Stability of three batches was assessed up to 36 months and data for an additional six batches for time points up to 24 months. The parameter clottable protein, indicative for functionality of a fibrinogen concentrate, remained virtually unchanged throughout the whole storage period within the variance of the analytical method.

3.3. Pathogen safety

In-process material was supplemented with 0.3% tri-(n-butyl)-phosphate (TnBP) and 1% Octoxynol and incubated at +25.5 \pm 2.5 °C for 240 min, according to state-of-the-art S/D virus inactivation practice. The S/D-treatment in the fibryga[®] manufacturing process demonstrated its effectiveness against enveloped viruses. All virus safety studies showed reduction factors to below or close to the limit of detection (Table 3).

The fibryga[®] purification process has implemented a nanofiltration step with a filter pore size of nominal 20 nm. Downscaled pathogen safety studies must be performed in a high biosafety level laboratory in order to validate the actual removal capacity of the selected nanofiltration and other process steps. In the safety studies, it was demonstrated that nanofiltration reduces prion proteins, enveloped viruses, and the non-enveloped virus HAV to below the limit of detection.

In addition, PPV was very effectively removed (Table 3) by nanofiltration. Furthermore, from purifying fibryga[®], it is well known that precipitation steps also contribute to the pathogen safety of the final product. Sequential precipitations (cryoprecipitation and aluminum hydroxide adsorption) implemented into the manufacturing process of fibryga[®] demonstrated that they were effective in reducing prion protein (Table 3).

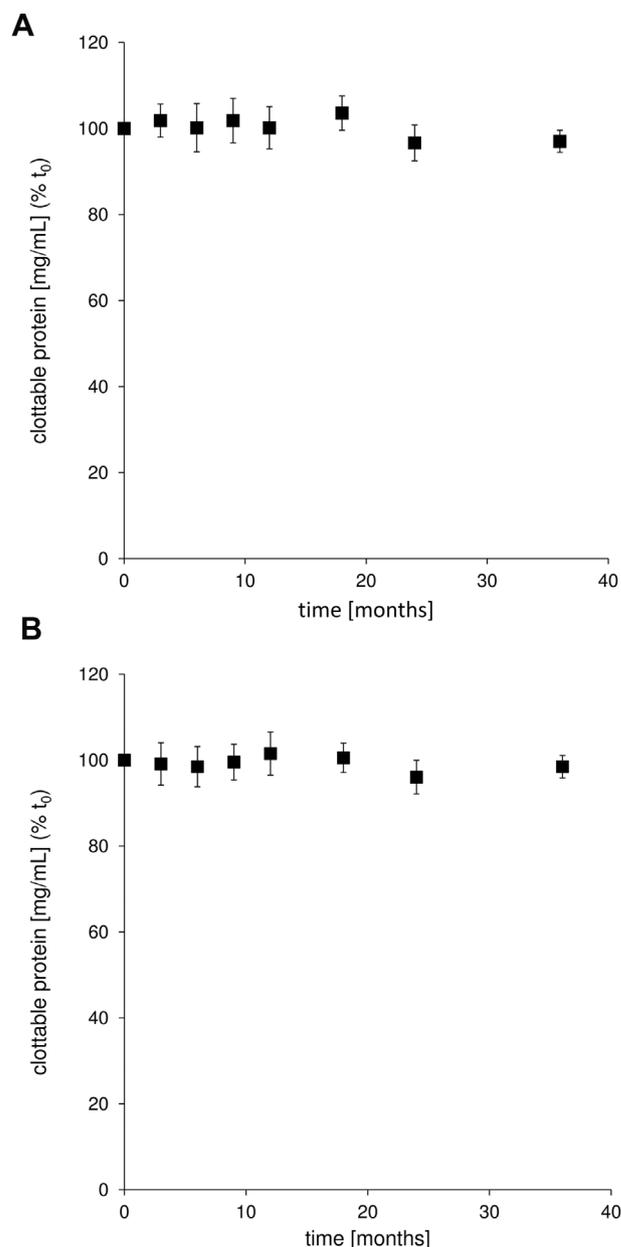


Fig. 5. Stability of clottable protein during storage for up to 36 months at (a) +5 \pm 3 °C and (b) +25 \pm 2 °C. Three batches were assessed up to 36 months, and data for an additional six batches for time points up to 24 months. Concentration for clottable protein was set to 100% at time point 0 for each individual batch and relative changes calculated. Arithmetic mean and SD are shown.

4. Discussion

Fibryga[®] is produced from pooled human plasma and presented as a lyophilized preparation to be reconstituted with water for injection. The final product is stable for at least 36 months without the need to add proteinaceous stabilizers when stored at room temperature. This renders a conditioning step prior to reconstitution unnecessary. By using the exclusively designed and developed transfer device Octajet, reconstitution is performed on average within less than five minutes, facilitating a prompt availability of the solution for injection in emergency situations. If an alternative transfer system such as a syringe is used, the reconstitution time may be longer.

Biochemical and functional analysis of fibryga[®] revealed that the manufacturing process leads to a consistent product composition with preserved functionality of fibrinogen, which was shown by a high

Table 3
Global reduction factors for pathogens during fibryga[®] manufacturing.

Production step	In vitro reduction factor [log ₁₀]					
	Enveloped viruses			Non-enveloped viruses		Prion
	HIV-1	PRV	BVDV	HAV	PPV	HAS 263 K
Sequential precipitations	n.d.	n.d.	n.d.	n.d.	n.d.	1.69
S/D treatment	≥ 5.15	6.81	≥ 5.80	n.a.	n.a.	n.a.
Nanofiltration	≥ 3.89	≥ 6.34	≥ 4.97	≥ 5.21	4.53	≥ 2.70
Global reduction factor	≥ 9.04	≥ 13.15	≥ 10.77	≥ 5.21	4.53	≥ 4.39

n.d. not done.

n.a. not applicable.

HIV-1 human immunodeficiency virus type 1, PRV porcine pseudorabies virus, BVDV bovine viral diarrhoea virus, HAV hepatitis A virus, PPV porcine parvovirus, HAS 263 K hamster-adapted scrapie strain 263 K.

specific activity and a nearly 1:1 ratio in fibrinogen activity to antigen.

To ensure lot-to-lot consistency and high purity of the product, for batch release the indicating parameters chosen are functionality in terms of clottability of fibrinogen and specific activity as also requested by the respective monograph [11]. Only traces of activation markers were detected in fibryga[®] after reconstitution, demonstrating a gentle purification process and a stable product.

Functional properties like fibrin assembly, stability of the clot, and viscoelastic properties are facilitated by alignment of γ -chain pairs and subsequent covalent γ - γ cross linking by activated FXIII. Fibryga[®] contains co-purified FXIII for γ -chain cross-linking in sufficient amounts, so no external FXIII has to be added for *in-vitro* cross-linking studies. The FXIII amount available within the concentrate could be beneficial to the patient in bleeding situations.

Further related to the functional properties of fibryga[®], the addition of the concentrate to the whole blood samples led to consistent, reproducible increase in the clot strength of both the whole blood clot and the fibrin-based clot, as investigated by means of thromboelastometry tests, EXTEM and FIBTEM, respectively. This observation concurs with the increase documented in the fibrin-based thromboelastometry test performed in plasma of patients with congenital deficit of fibrinogen, and described in both the context of a pharmacokinetics study [9], and within a study investigating the clinical efficacy of fibryga[®] [10]. The latter study, named FORMA-02, showed that the increase in MCF following administration for treatment of bleeding events or for prophylaxis of bleeding in surgery corroborated the excellent clinical efficacy outcome observed in the interim analysis of that study. Consequently, the results of the spiking experiments support the expectation of efficacious clot formation for bleeding control in clinical settings where fibrinogen is deficient.

Pathogen safety of fibryga[®] is ensured by the use of two dedicated pathogen safeguarding steps. The S/D treatment used in the manufacturing process of fibryga[®] is known for its extremely fast inactivation kinetics, robustness, and superiority in terms of inactivating enveloped viruses based on the lipid-membrane destroying properties of TnBP (solvent) and Octoxynol (detergent). Nanofiltration (20 nm) is employed as a synergistic effective step to remove pathogens, enveloped and non-enveloped viruses, as well as infectious prion protein. The choice of filter depends on the molecular size of the product. Pathogen removal depends not only on the pore size of the nanofilter, but also on the process conditions, size of the molecule, and protein concentration. In general, the smaller the pore size of the nanofilter, the greater the potential for pathogen removal [18]. Effective reduction of pathogens was demonstrated in downscaling studies at process conditions.

Beside the pathogen safeguarding steps in the manufacturing process of fibryga[®] the overall pathogen safety strategy for fibryga[®] includes selection of qualified donors and testing of plasma donations and plasma pools for serological and viral markers (e.g. HIV, HBV and HCV) according to current guidelines for plasma-derived medicinal products [11].

In conclusion, fibryga[®] represents a novel fibrinogen concentrate meeting the requirements of a state-of-the-art product regarding fast reconstitution, safety, purity, and functionality for the treatment of patients where fast and efficacious replacement of fibrinogen is needed.

Acknowledgements

The authors would like to thank Andrea Neisser-Svae, Guido Kohla and Oliver Hegener for review of the manuscript, Martina Bayer for technical support and Sandra Janisch and Caroline Leitinger for excellent technical and analytical assistance.

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