Available online at www.sciencedirect.com
SCIENCE DIRECT
International Journal of Pharmaceutics 313 (2006) 1–4
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Stability of Beriplast® P fibrin sealant: Storage and reconstitution

Ulrich Eberhard *, Martin Broder, Günther Witzke

ZLB Behring GmbH, P.O. Box 1230, 35002 Marburg, Germany

Received 16 November 2005; received in revised form 16 November 2005; accepted 4 December 2005
Available online 10 March 2006

Abstract

This study was performed to investigate the stability of Beriplast® P fibrin sealant (FS) across a range of storage conditions, both pre- and post-reconstitution. Storage stability of the FS was evaluated during long-term refrigeration (24 months) with or without interim storage at elevated temperatures (40 °C for 1 week and 25 °C for 1 and 3 months). Stability of individual FS components was assessed by measuring: fibrinogen content, Factor XIII activity (FXIII), thrombin activity and aprotinin potency. The package integrity of each component was also checked (sterility testing, moisture content and pH). Storage stability was also evaluated by testing the reconstituted product for adhesion (tearing force testing after mixing the solutions) and sterility. Reconstitution stability was evaluated following 3-months' storage, for up to 50 h post-reconstitution using the same tests as for the storage stability investigations. Pre-defined specifications were met for fibrinogen content, Factor XIII activity, and thrombin activity, demonstrating storage stability. Package integrity and the functionality and sterility of the reconstituted product were confirmed throughout. Reconstitution stability was demonstrated for up to 50 h following reconstitution, in terms of both tearing force and sterility tests. In conclusion, the storage stability of Beriplast® P was demonstrated over a range of 24-month storage schedules including interim exposure to elevated temperature, and the reconstituted product was stable for up to 50 h.

Keywords: Fibrin tissue adhesive; Fibrin sealant; Drug storage; Drug stability

1. Introduction

Fibrin sealants (FS), also known as fibrin adhesives or glues, mimic the final steps of the blood coagulation process, forming a stable, physiological fibrin clot (Probst, 1989). FS are most commonly used to assist haemostasis, to support sutures in surgical procedures, and for tissue adhesion. These applications have enabled the use of FS in a variety of surgical settings, including cardiovascular, thoracic, vascular, neurological and abdominal surgery (Dunn and Goa, 1999; Witzke and Demertzis, 2003).

FS preparations typically contain fibrinogen, varying levels of Factor XIII (FXIII), thrombin, an anti-fibrinolytic agent (i.e. aprotinin), and calcium chloride. All these components actively contribute to the function of FS (Dickneite et al., 2003) so long-term storage stability of FS is dependent on retaining activity of the individual components, as well as the adhesive properties of the reconstituted product. Package integrity is required not only to retain the components' activity, but also to maintain sterility.

Beriplast® (ZLB Behring) preparations have been used for many years in Europe, Asia and the Americas. In order to improve packaging integrity and ease of use, the presentation of this FS has evolved over time. The current presentation, Beriplast® P Combi-Set, is supplied as two pairs of vials, each pair being contained within a blister pack designed for straightforward reconstitution within a sterile environment. The blisters only need to be opened when the application syringes are to be filled.

Storage recommendations for Beriplast® P are based on stability studies that have been performed over a number of years. Data from these studies indicate a degree of flexibility in terms of product storage and reconstitution. For Beriplast® P, storage at 2–8 °C for up to 24 months is recommended, with the exception of Japan where the storage temperature is ≤10 °C without freezing (ZLB Behring, 2005). This variation is because of the different regulatory recommendations for biological products requiring refrigeration (Association of Biologicals Manufacturing of Japan, 1993; Committee for Proprietary Medicinal Products, 2001). However, the implications of temporary storage at higher temperatures are not clarified. As with storage stability, recommendations for using the reconstituted product vary between

* Corresponding author. Tel.: +49 6421 392686; fax: +49 6421 392243.
E-mail address: ulrich.eberhard@zlbbehring.com (U. Eberhard).

0378-5173/$ – see front matter © 2006 Published by Elsevier B.V.
doi:10.1016/j.ijpharm.2005.12.001

different regions. In Europe and Asia, though not Japan, a conservative figure of 24 h post-reconstitution stability at 20–25 °C is stated (8 h if outside the sterile blister), whereas in Brazil the product may be used up to 36 h after reconstitution (8 h if outside the sterile blister) (ZLB Behring, 2005). There are no differences in the composition of the components of Beriplast® P in different countries.

In order to satisfy European and Japanese and other regulatory authority requirements, and to answer a number of practical questions relating to the use of the product, the stability of Beriplast® P across a range of different storage conditions, both pre- and post-reconstitution, has been investigated for many years.

2. Materials and methods

The stability testing program was established in accordance with the International Conference on Harmonisation (ICH) Guidelines and/or other applicable guidelines for stability testing.

2.1. Storage stability

A number of storage schedules of the marketed product before reconstitution were investigated, incorporating long-term refrigeration with and without interim storage at elevated temperatures. The storage schedules are detailed in Fig. 1; briefly, the conditions were:

Schedule 1: 5 °C up to 24 months (constant temperature throughout).
Schedule 2: 10 °C up to 24 months with interim storage at 40 °C for 1 week after 12 months.
Schedule 3: 2–8 °C up to 24 months with interim storage at 25 °C for 1 month after 11 months.
Schedule 4: 2–8 °C up to 24 months with final storage at 25 °C for 3 months after 21 months.

Stability assessments were performed at 0, 3, 6, 9, 12, 18 and 24 months during schedule 1; and at 0, 12 and 24 months for schedules 2–4 inclusive. Stability was usually measured by assessment of each individual component as well as the reconstituted product. The test methods employed for the individual components were: fibrinogen content (clottable protein following addition of thrombin, according to European Pharmacopoeia); FXIII activity (chromogenic substrate assay); thrombin activity (clotting assay); aprotinin potency; and calcium chloride content (atomic absorption spectrometry). Predefined manufacturer's specifications were applied. The package integrity of all the components was also tested via sterility (membrane filtration method), moisture content (Karl Fischer titration) and pH (potentiometric determination). According to European Pharmacopoea, activity of the product is proved by the potency of the components of the sealant. As the integrity of the packaging materials is required even after reconstitution of the components, sterility was investigated after the reconstitution.

To investigate potential influences on product stability, all filling sizes (0.5, 1, 3 ml) and different lots were tested.

Analytical testing was performed under conditions of temperature and humidity monitored according to the manufacturer's standard operating procedures.

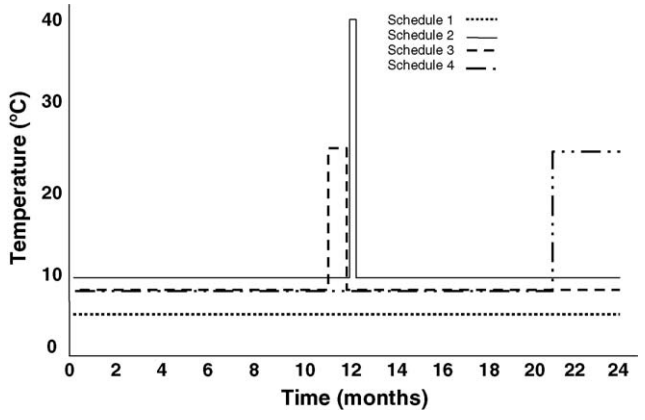

Fig. 1. Storage conditions investigated (temperature vs. timescale) in schedules 1–4.

2.2. Reconstitution stability

The stability of the reconstituted components of Beriplast® P was assessed after initial storage of the unreconstituted product at 2–8 °C for 3 months.

Following reconstitution at 20–25 °C the product was kept at that temperature, both within and outside the blister pack, with stability assessments performed at 0, 18, 26 and 50 h. For all timepoints except 0 h, the reconstituted product was stored within the application syringes for the final 2 h prior to assessment. Stability investigations comprising tearing force testing (in some cases according to Minimum Requirements for Biological Products Japan [MRBPJ]) and sterility testing using the same procedures as for the storage stability evaluations were performed.

3. Results

3.1. Storage stability

Fibrinogen content and FXIII activity were maintained throughout the long-term refrigerated storage, schedule 1 (Fig. 2a and b). At all timepoints, the pre-defined specifications for fibrinogen content and FXIII activity were met. Consistent with these findings, thrombin activity, aprotinin potency and calcium chloride content were also stable throughout the study. Package integrity, as determined by sterility, moisture content and pH, was maintained for the duration of the study. Based on data from the 3 ml filling size of Beriplast® Combi-Set, similar outcomes were recorded with all of the storage schedules investigated. The lack of any apparent decreases in activity measurements indicates that interim storage for 1 week at 40 °C, or up to 3 months at 25 °C, does not affect the function of Beriplast® P. A comparison of analytical data obtained after

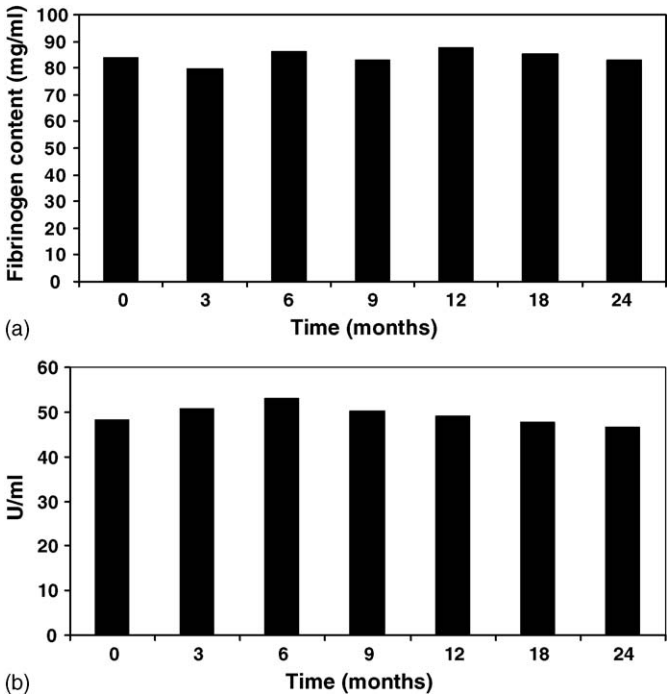

Fig. 2. (a) Fibrinogen content over time during storage at 5 °C over 24 months (clottable protein according to European Pharmacopoeia). Values are mean of three lots; vial filling size of 3.0 ml (schedule 1)—this vial volume is representative for all filling sizes. (b) FXIII activity over time during storage at 5 °C over 24 months (chromogenic substrate assay). Values are mean of three lots; vial filling size of 3.0 ml (schedule 1)—this vial volume is representative for all filling sizes.

a storage period of 24 months at the different storage schedules is given in Tables 1 and 2.

Vial filling size appeared to have no relevant influence on study outcomes, with the pre-defined specifications met by all three volumes at all timepoints. In addition, there was no evidence of any impaired activity among any of the lots tested, and comparable outcomes indicated a high degree of product batch consistency.

3.2. Reconstitution stability

Constant potency of the components demonstrated stability over the entire observation period for the reconstituted and mixed FS at 0 and 50 h following reconstitution (see data given in Table 2).

Importantly, product sterility was maintained at all timepoints, regardless of whether the components were kept inside or outside the blister pack. As with storage stability, vial filling size appeared to have no effect on reconstitution stability.

4. Discussion

Up to the present date there are very few reports of data describing the storage stability of a FS preparation (Hock et al., 1995; Immuno AG, 2004; Quixil/Crosseal, 2004/2005). The results of the present investigation indicate a high degree of stability for Beriplast® P over a range of different storage schedules, including interim short-term storage (1 week) at 40 °C and final long-term storage (up to 3 months) at 25 °C. Furthermore, Beriplast® P remains functional for over 50 h following reconstitution at 20–25 °C.

These findings could have important practical implications for storing and using Beriplast® P. Firstly, the investigations show that the product would not be adversely affected by short-term exposure to elevated temperatures (≤40 °C) which may occur during transportation or storage. Unreconstituted Beriplast® P, for example, may be stored in the operating room for 1 week at room temperature prior to reconstitution. Secondly, there is no requirement for the product to be used immediately following reconstitution. This allows a high degree of flexibility for application of Beriplast® P; such that any delays in treatment

Table 1
Fibrinogen, FXIII, aprotinin, thrombin and calcium chloride data following 24-month storage

Schedule	Fibrinogen content (mg/ml)	FXIII activity (U/ml)	Aprotinin		Thrombin		Calcium chloride	
			Activity (KIU/ml)	pH	Activity (IU/ml)	Residual moisture (%)	Content (mg/ml)	pH
1 (24 months at 5 °C)	83	47	983	7.2	502	3	4.5	6.4
2 (24 months at 10 °C including 1 week at 40 °C)	89	63	1008	6.1	534	3	4.5	6.8
3 (24 months at 2–8 °C including 1 month at 25 °C)	90	52	1030	6.2	512	2	4.5	7.1
4 (24 months at 2–8 °C including 3 months at 25 °C)	91	49	1038	6.3	521	2	4.5	7.1

Table 2
Fibrinogen, FXIII and thrombin data following reconstitution

Storage period (20–25 °C) (h)	Fibrinogen content (mg/ml)	FXIII activity (U/ml)	Thrombin activity (IU/ml)	Tensile strength (g)	Sterility
0	74	53	540	141	Not done
50	80	51	545	146	PASS

(i.e. due to unforeseen circumstances during surgery) would not impair the effectiveness of Beriplast® P.

Today, approximately 10 fibrin sealant products are commercially available worldwide, and these differ in their composition, presentation and properties (Dickneite et al., 2003). Differences in their storage stability, before and after reconstitution, are therefore likely.

The normal labelled storage recommendations for some other commercially available FS preparations that are stored under refrigeration are broadly similar to those for Beriplast® P (e.g. Tisseel VH Kit (Baxter/Hyland Immuno, USA) and Tissucol® Kit (Baxter/Hyland Immuno, Germany and other countries; also known under the trade mark Tisseel®)): storage for 2 years at 2–8 °C, following reconstitution at 37 °C the two components have to be used within 4 h and the solutions have to be kept at 37 °C; Tisseel® (Japan and Bolheal®): at 10 °C or lower avoiding freezing for up to 2 years (Baxter, 2003; Kaketsuken, 2003). A few FS are provided frozen, and these have clearly different requirements (e.g. Quixil® (Omrix/J&J) and Crosseal™ (J&J Wound Management/Omrix, USA only): long-term storage at −18 °C or lower for up to 2 years, short-term storage of the unopened vials at 2–8 °C for up to 30 days, the thawed product must not be refrozen (Omrix Quixil, 2005, J&J Wound Management Crosseal, 2004); Tissucol® Duo (Baxter/Hyland Immuno, Germany) and other countries: storage at −18 °C or lower for up to 2 years, the cold storage chain must not be interrupted until use, both components should be thawed at 37 °C and the thawed product must not be refrozen or stored in a refrigerator, thawed solutions may be stored at room temperature for up to 36 h) (Baxter, 2003).

Documented information regarding the effect of exposure to elevated temperatures during storage or elongated time elapsing post-reconstitution is scarce with most FS, implying that a deviation from recommended conditions would require the product to be discarded. In contrast, the present results demonstrate a high degree of flexibility with Beriplast® P, and may allow operating room nurses and physicians to exercise their judgment and experience in storing and using a batch of the product even if strict adherence to the labeled storage recommendations cannot be guaranteed. Nevertheless, we would not suggest changing the storage recommendations for Beriplast® P, as a conservative approach is appropriate.

In conclusion, the present study confirms the stability of Beriplast® P over a range of storage conditions (i.e. interim storage at 40 °C for 1 week or at 25 °C for up to 3 months). Furthermore, the reconstituted Beriplast® P demonstrated stability at 20–25 °C for 50 h following reconstitution. As fibrin sealants differ in their composition, these results cannot be applied to other fibrin sealants.

References

Association of Biologicals Manufacturing of Japan, 1993. Minimum Requirements for Biologic Products.

Baxter, 2003. Baxter Hyland Immuno Summary of Product Characteristics. Available from: http://www.tissuesealing.com/uk/products/biologicals/prescinfo.cfm [accessed May 7, 2003].

Committee for Proprietary Medicinal Products, 2001. Note for Guidance on Declaration of Storage Conditions for Medicinal Products in the Products Particulars and Active Substances. European Agency for the Evaluation of Medicinal Products, London.

Crosseal, Fibrin Sealant (Human), J&J Wound Management, September 2004. Available from: http://www.omrix.com/Products PDF/Crosseal PI-230904-FDA approved.pdf (accessed Sepember 17, 2005).

Dickneite, G., Metzner, H., Pfeifer, T., Kroez, M., Witzke, G., 2003. A comparison of fibrin sealants in relation to their in vitro and in vivo properties. Thromb. Res. 112, 73–82.

Dunn, C.J., Goa, K.L., 1999. Fibrin sealant: a review of its use in surgery and endoscopy. Drugs 58, 863–886.

Hock, J., Ronneberger, H., Diehl, K.H., 1995. Increased stability of Beriplast® after reconstitution and a new device for manual spray application. Thromb. Haemost. 73, 1325.

Immuno AG (ÖIH), 2004. Tisseel VH Kit SBA. Available from: http://www.fda.gov/cber/sba/fiboih050198sba.pdf [accessed March 29, 2004].

Kaketsuken, 2003. Package Insert for Bolheal.

Probst, M., 1989. Fibrin adhesives in modern surgery. Biomed. Prog. 1, 13–16.

Quixil, Zusammenfassung der Merkmale des Arzneimittels, Pharmazeutischer Unternehmer: Omrix Biopharmaceuticals S.A., April 2005. Available from: www.omrix.com/products PDF/Quixil SmPC-UK April 2005.pdf (accessed September 17, 2005).

Witzke, G., Demertzis, S., 2003. Fibrin sealing and hemostasis in cardiovascular and thoracic surgery. Biomed. Prog. 16, 1–5.

ZLB Behring, 2005. Package Inserts for Beriplast® P Combi-Set.