PENTAPHARM

Prionex®

Unique stabilizer for the protection of the biological activity of proteins

Product Information

Registered as DSM Nutritional Products Ltd Branch Pentapharm
Engelgasse 109, Postfach, CH-4002 Basel/Schweiz
Tel: +41 61 706 48 48, Fax: +41 61 319 96 19

Unlimited. DSM

Description

PRIONEX® is a novel polypeptide fraction of highly purified dermal collagen of porcine origin which has excellent protein stabilizing properties. PRIONEX® is therefore an effective alternative to serum albumin [1].

PRIONEX® is available as a sterile, umpreserved aqueous solution containing 10% solids, but no additives or stabilizing agents. The manufacturing process for PRIONEX® comprises a controlled thermic hydrolysis at 145° C followed by sterile filtration through a 0.2μm membrane.

Introduction

Highly flexible polypeptide chains which are essential for biological activity and function cannot form defined, stable configurations. Most biologically active proteins are therefore very sensitive when not in their natural protective environment. To exert their activity also **in vitro**, proteins must be maintained in their native state with correct secondary, tertiary and quaternary structures.

The labile nature of the native state of proteins often presents problems during processing when proteins are exposed to stressful conditions, such as variation in pH or ionic strength, elevated and even cold temperatures, high shear forces and high pressure or chaotropic agents. In practice, this most often results in irreversible, partial or complete inactivation of the active site or denaturation. Moreover, the normally used high protein dilutions can lead to loss of activity by adsorption to walls of containers, membranes and chromatography media or to dissociation of protein subunits. This is not surprising when the high concentration **in situ** of soluble, intracellular proteins of usually more than 100 mg/ml is considered.

To maintain protein activity during processing and storage, an inert compound with a stabilizing effect is usually added. Human and bovine serum albumin (HSA and BSA) are widely recommended and successfully used for this purpose.

However, serum albumins have considerable disadvantages. A potential risk of virus contamination has to be considered. Moreover, BSA may be a carrier of bovine spongiform encephalopahty (BSE) agent, a pathogen which resists pasteurisation. BSA, acting as an antigen to humans, is not a suitable additive for parenteral drugs.

In addition to a possible contamination with pathogens, BSA and HSA frequently contain impurities, e.g. IgG affecting immunological or enzymological test procedures, respectively. PRIONEX® has been developed to overcome these shortcomings.

Properties

General considerations

Due to the chemical nature and the standardized quality PRIONEX® can be used as an inert protein stabilizer in any kind of application, furthermore as an additive for cell culture media, as a diluent for immunoassay and hybridization techniques or for serological tests.

PRIONEX® is prepared from highly purified porcine skin collagen by partial hydrolysis under mild acid conditions. PRIONEX® is free from cartilage, bone and plasma components and can therefore be considered as an extremely pure form of gelatin type A, meeting all purity requirements for gelatin of the Pharmacopoeias USP XXII, Eur.Ph. , DAB 9, Ph.H. VII and Pharmacopoeia Japonica 10th Ed. [2].

Because PRIONEX® is derived from porcine sources, it cannot contain BSE-agent, a potential risk in any bovine-derived material.

PRIONEX® is heat treated at 145°C for one hour. This treatment is known to destroy or inactivate common viruses, BSE- and scrapie-like infective agents [3], [4], [5].

PRIONEX® does neither exert enzyme- nor known enzyme inhibitor activity, nor does it contain immune reactive proteins.

PRIONEX® is free of lipids, polysaccharides and nucleic acids.

Desiccated PRIONEX® is rapidly dissolved in cold water.

PRIONEX® does not gelify, is heat-stable, does not precipitate during heat sterilization (20 minutes at 121°C) and is filterable through 0.2 μm membranes.

The average molecular weight of PRIONEX®, estimated by gel permeation chromatography, is 20 kilo Dalton.

Analytical data

Appearance	clear, yellowish solution
pH	6.0 – 7.5
Dry residue	9.0 – 11.0 % m/V
Relative density (20°C)	1.030 – 1.045
Total nitrogen (TN)	1.50 – 1.90 % m/V
Polypeptide content, calculated from total nitrogen (5.5. x TN)	8.2 – 10.5 % m/V
Sodium chloride	0.55 – 0.70 % m/V
Heavy metals	< 20 ppm
Sterility	sterile
Endotoxin testing (LAL)	< 10 EU/ml

Properties

Solubility

PRIONEX® is freely soluble in water, diluted electrolyte solutions, glycerol and DMSO as well as in ethanol in concentrations below 75% and ammonium sulfate solution below 20% saturation.

Safety

PRIONEX® is non-antigenic and has no allergenic potential as revealed in guinea pigs. No signs of intoxication could be detected after oral administration up to a dose of 1250 g per kilogram body weight in mice. After intravenous injection of 25 ml PRIONEX® per kilogram body weight in mice, no toxic signs were observed. Thus PRIONEX® is not harmful after oral and intravenous administration, respectively.

Amino acid composition

Due to its origin, PRIONEX® has an amino acid composition comparable to that of collagen. For a selected lot of PRIONEX® the following composition was determined according to Spackman [6]:

Ala	11.2	mg/ml
Arg	9.4	mg/ml
Asp & Asn	7.2	mg/ml
Glu & Gln	11.8	mg/ml
Gly	29.7	mg/ml
His	0.8	mg/ml
HyPro	14.7	mg/ml
Ile	1.5	mg/ml
Leu	3.5	mg/ml
Lys	4.3	mg/ml
Met	1.0	mg/ml
Phe	2.5	mg/ml
Pro	16.5	mg/ml
Ser	3.6	mg/ml
Thr	2.1	mg/ml
Tyr	0.4	mg/ml
Val	3.2	mg/ml

UV-spectrum

Due to the low content of aromatic amino acid residues, PRIONEX®, unlike albumin, does not show an absorption maximum at 280 nm, but only a small shoulder (see figure 1).

BSA, 0.1% in water

PRIONEX®, 1.0% in water; corresponding to 0.1% solids

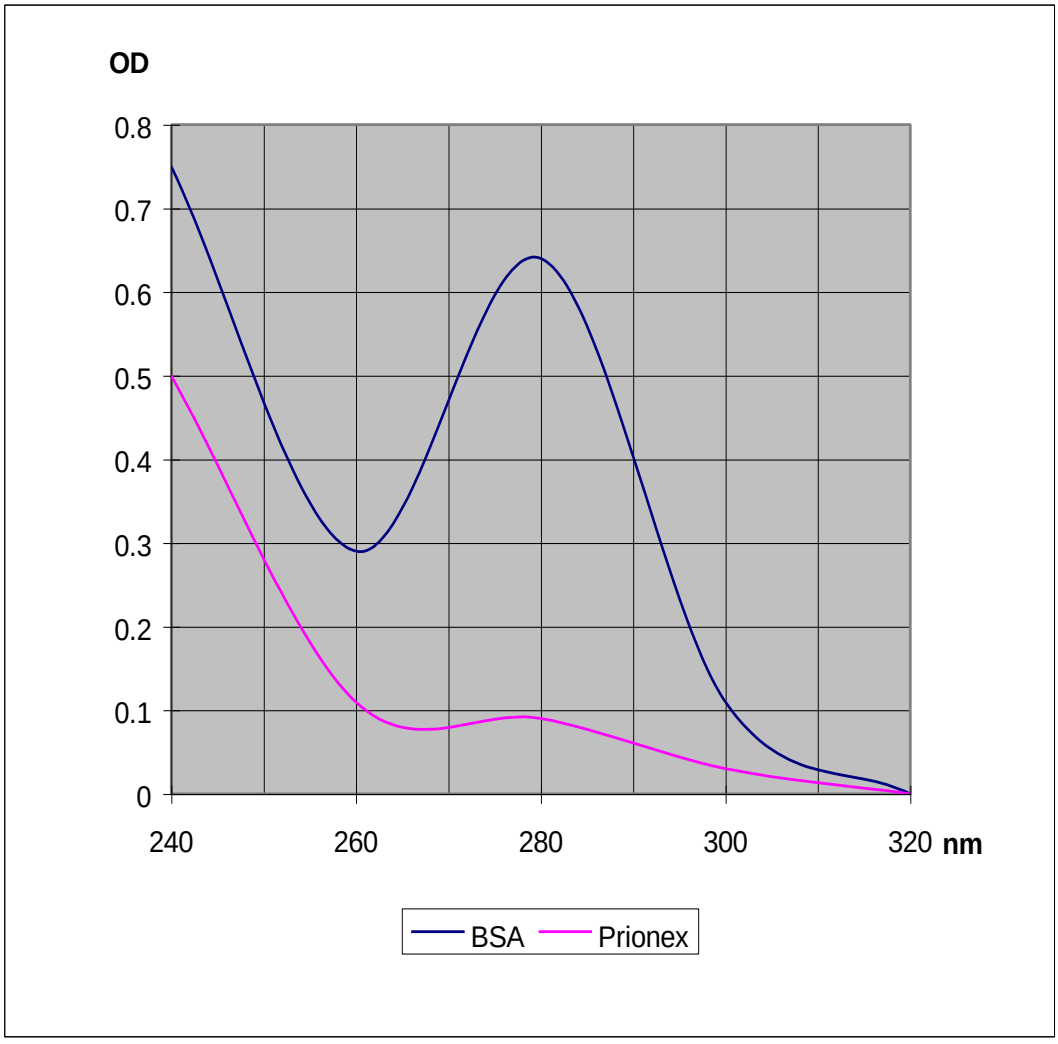

Figure 1

Application Examples

The utility of PRIONEX® in stabilizing the native structure of proteins was examined in several experiments simulating denaturation or adsorption effects on three selected enzymes. In all the test systems, PRIONEX® was compared with a 10% solution of a commercially available BSA recommended for use as an enzyme stabilizer.

The tests demonstrated that PRIONEX® shows protein stabilizing properties at least equal to those of BSA.

Effect on storage

To be of practical interest as a biochemical agent, a diagnostic kit component or a pharmaceutica drug, the isolated protein must have an acceptable shelf-life.

Test procedure

To solutions containing 2 NIH-U/ml of thrombin 1.0% of stabilizer solution was added. The solutions were kept at 37°C. The clotting activity of thrombin was determined on a COBAS FIBRO coagulometer using citrated normal human plasma (CNP). Inactivation of thrombin is expressed as a clotting time prolongation.

Result

PRIONEX® shows a better effect on storage stability of thrombin than does BSA (see figure 2). PRIONEX® may therefore be used to prolong the shelf-life of a protein preparation.

Effect of PRIONEX® and BSA on storage of thrombin

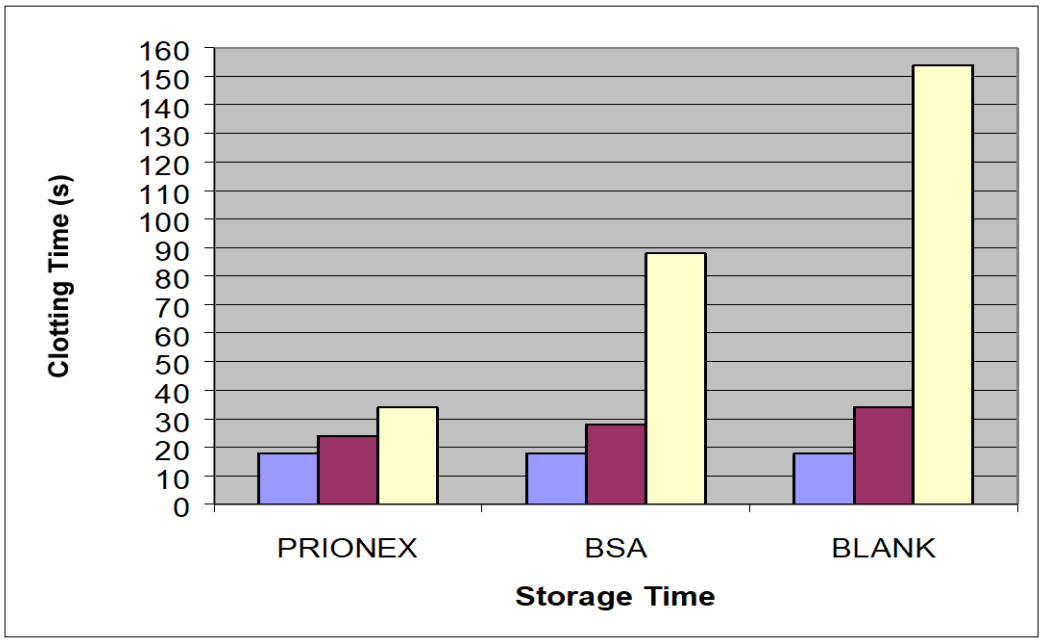

Figure 2

Thermal denaturation

Undesired elevated temperatures often occur during processing. It is also often necessary to protect a protein from denaturation during pasteurization or sanitization by heat. Moreover, protein stability at elevated temperatures is a requirement for long-term storage stability.

Test procedure

Solutions containing 3 batroxobin units/ml of the thrombin-like protease batroxobin (from Bothrops atrox snake venom) were provided with 1.0% of stabilizer solution and heated in a boiling water bath. The clotting activity of batroxobin was determined on a COBAS FIBRO coagulometer using citrated normal human plasma (CNP). Inactivation of batroxobin is expressed as a clotting time prolongation.

Result

PRIONEX® exerted a highly significant stabilizing effect on the enzymatic activity of batroxobin (see figure 3). PRIONEX® was shown to be a useful additive for protection of proteins against heat denaturation.

Effect of PRIONEX® and BSA on heat denaturation of batroxobin

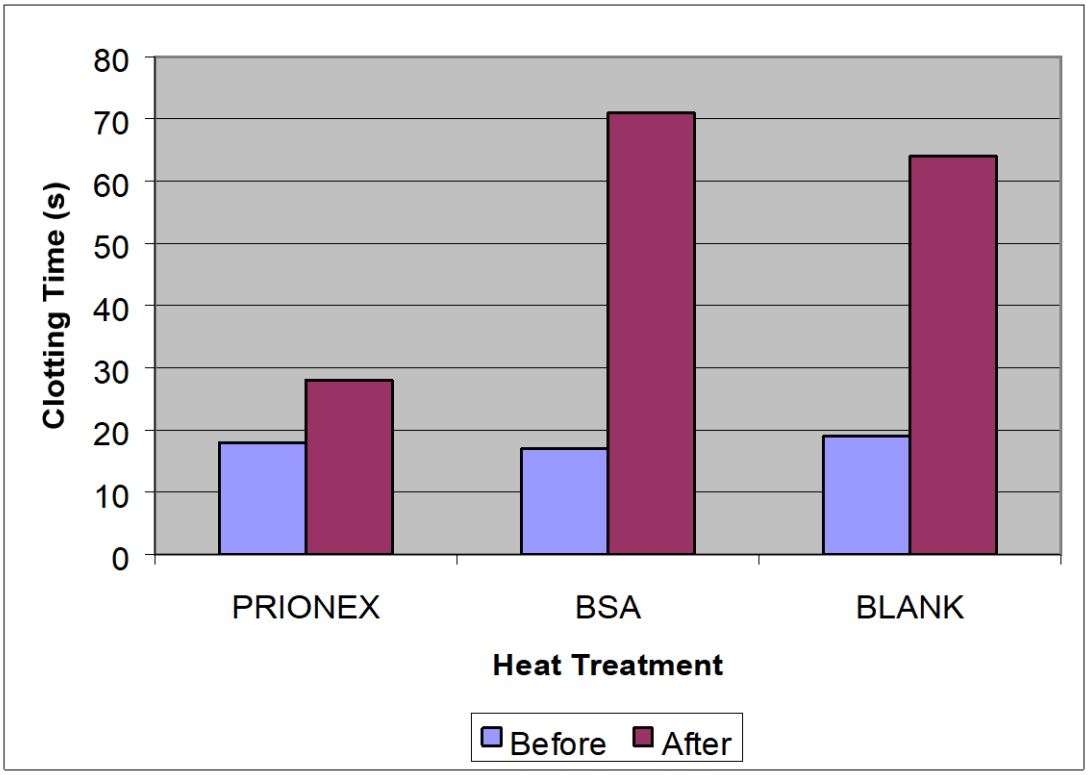

Figure 3

Chemical denaturation by chaotrops

Chaotrops affect the intramolecular hydrogen bound structure between water and a protein. This leads to altered conformation and solution properties.

Ethanol denaturation

Ethanol precipitation is a very common method used for protein fractionation. A well-known example is the Cohn method for blood serum fractionation [7].

Test procedure

To solutions of 40 NIH-U/ml of thrombin and 10% of stabilizer solution, ethanol was added to a final concentration of 25% v/v. In a second step the solutions were incubated at 37°C for 8 hours. Then, the clotting activity of thrombin was determined on a COBAS FIBRO coagulometer using citrated normal human plasma (CNP). Inactivation of thrombin is expressed as a clotting time prolongation.

Result

The thrombin inactivation was more pronounced in the BSA than in the PRIONEX® containing sample (see figure 4). Accordingly, PRIONEX® can be used to protect proteins against denaturation due to salting out processes.

Effect of PRIONEX® and BSA on ethanol denaturation of thrombin

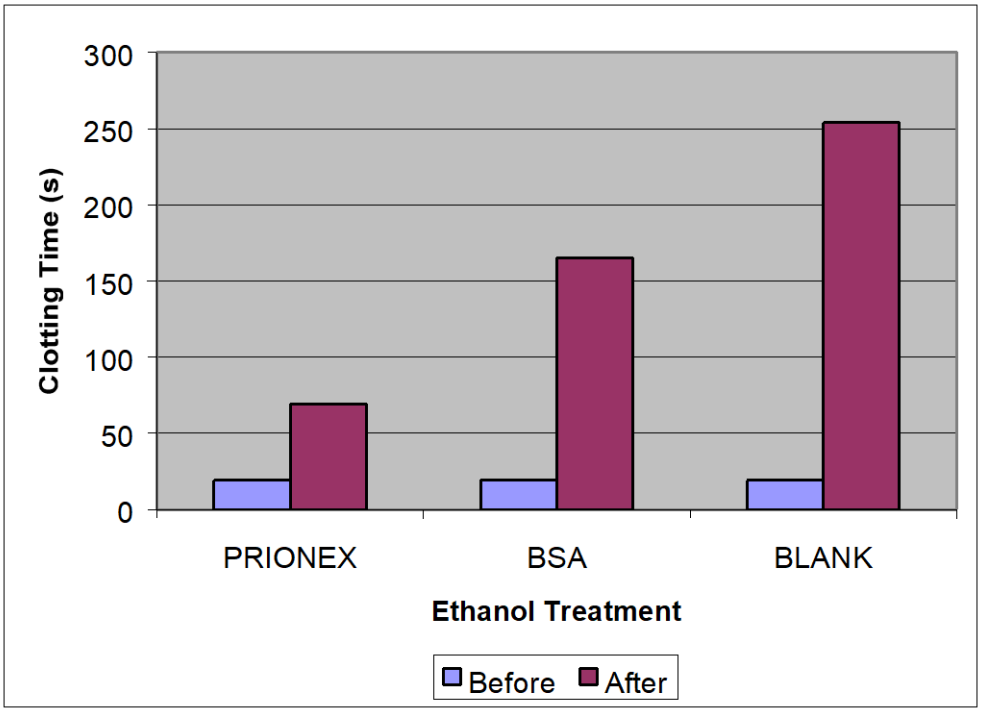

Figure 4

Urea denaturation

In protein chemistry, urea is well known for its universal destabilizing action. Urea is used to enhance the solubility of most proteins in water by salting in effects.

Test procedure

Solutions consisting of 5000 U/ml trypsin, 50% of stabilizer solution and 6 M urea were incubated at room temperature for 90 seconds. The activity of trypsin was colorimetrically determined at 405 nm using Pefachrome® Try (Bz-Val-Gly-Arg-pNA · AcOH) as a chromogenic substrate.

Result

The sample containing PRIONEX® was still more active after this treatment than that containing BSA (see figure 5). PRIONEX® increases protein stability in presence of urea.

Effect of PRIONEX® and BSA on urea denaturation of trypsin

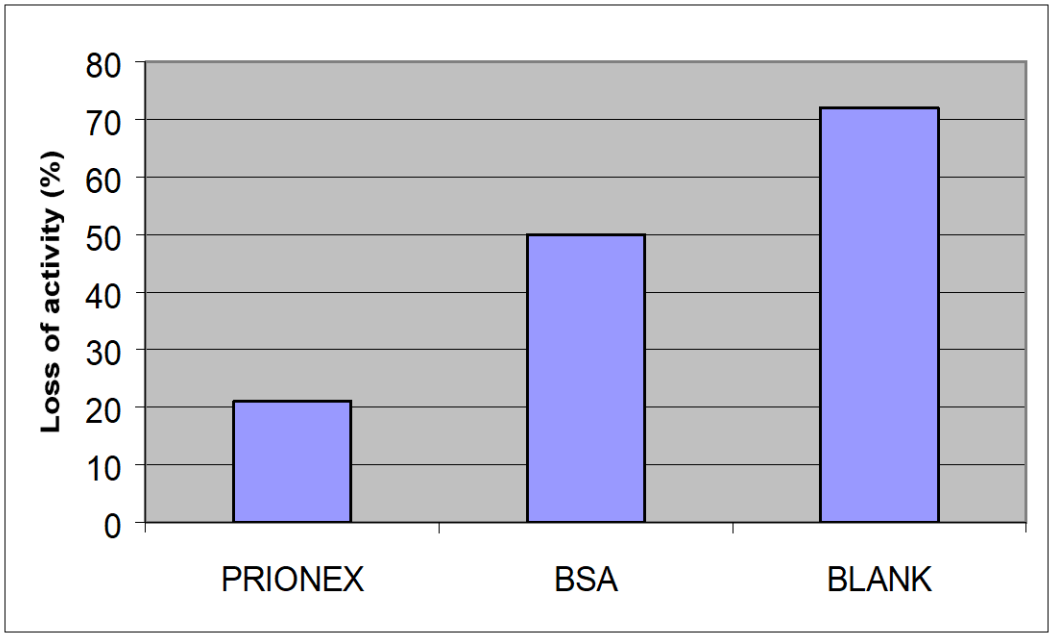

Figure 5

Adsorption effects

The processing of highly diluted protein solutions is often connected with a remarkable loss of activity due to adsorption effects. e.g. on walls of containers, membranes, filter aids or chromatography media.

Test procedure

To solutions containing 20 U/ml of trypsin and 1.0% of stabilizer solution, 10% of glass powder were added. After shaking (160 rpm) at room temperature, the trypsin activity was colorimetrically determined at 405 nm using Pefachrome® Try (Bz-Val-Gly-Arg-pNA·AcOH) as a chromogenic substrate.

Result

The loss of activity was reduced by PRIONEX® and BSA to a comparable, significant extent while over 95% of potency was lost in the stabilizer-free sample (see figure 6). PRIONEX® prevents protein adsorption.

Effect of PRIONEX® and BSA on glass adsorption of trypsin

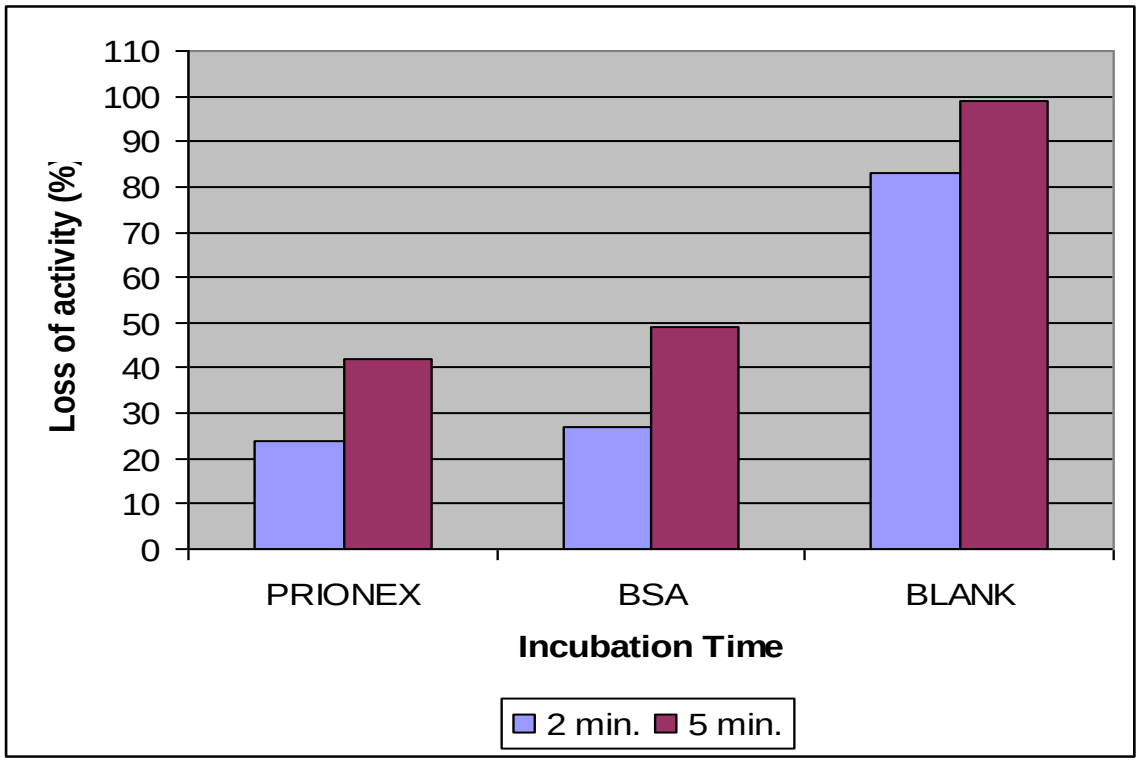

Figure 6

Freeze denaturation

The main protein damaging effect of freezing is not due to the low temperature but rather the concomitant concentration of all soluble substances as ice separates from the mixture as a pure phase. As a result, freezing increases the concentrations of dissolved buffers and other additives leading to precipitation of acids and / or salt. Associated changes (e.g. pH changes) can cause protein denaturation.

Test procedure

Test solutions containing 2 NIH-U/ml of thrombin and 1.0% of stabilizer solution were prepared. One freeze-thaw cycle consisted in an overnight freezing and a thawing procedure on the following morning at room temperature for approx. 4 hours. The solutions were frozen again to start the next cycle. The clotting activity of thrombin was determined on a COBAS FIBRO coagulometer using citrated normal human plasma (CNP). Inactivation of thrombin is expressed as a clotting time prolongation.

Result

PRIONEX® as well as BSA showed good stabilizing properties in this experiment (see figure 7). PRIONEX® was judged to be a useful cryoprotectant because it does not crystallize from aqueous solutions. PRIONEX® may be added to thermolabile protein preparations to prevent denaturation by freezing and thawing e.g. during shipment or repeated freeze-thaw cycling during sample subdivision.

Effect of PRIONEX® and BSA on freeze-thaw cycling of thrombin

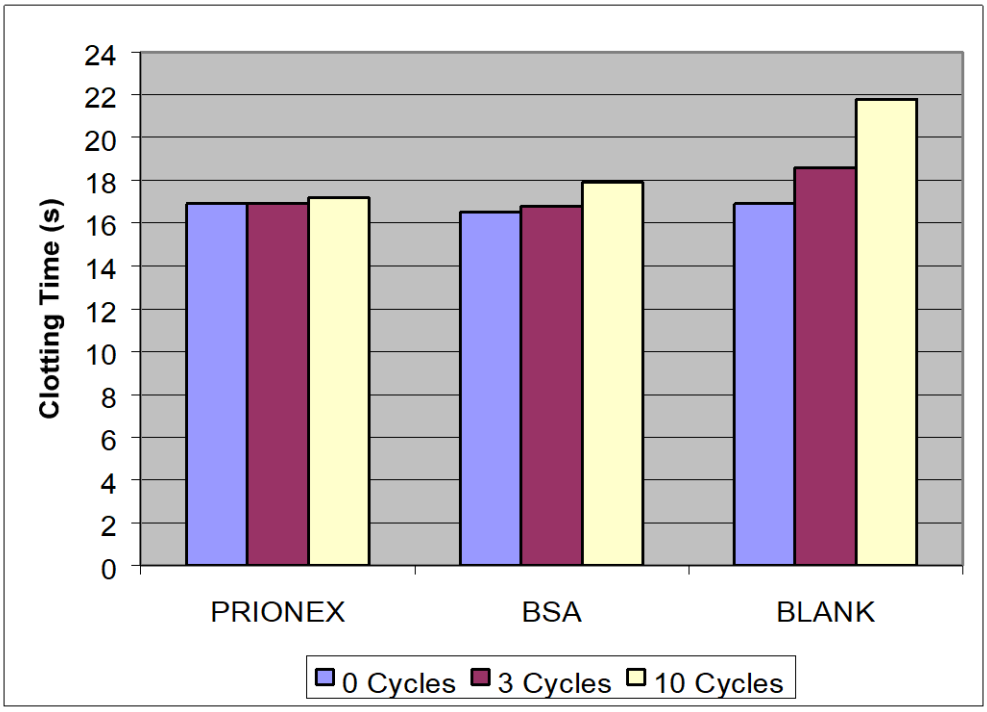

Figure 7

Effect of freeze-drying

Freeze-drying is a standard method for stabilization of labile proteins which are too unstable for handling and storage as a solution. However, the freeze-drying process is very complex. Physical and chemical changes occur which often lead to denaturation if the process and the formulation are not fully optimized.

Test procedure

Solutions containing 10 NIH-U/ml of thrombin and 10% of stabilizer solution were lyophilized. The clotting activity of thrombin was determined after reconstitution on a COBAS FIBRO coagulometer using citrated normal human plasma (CNP). Inactivation of thrombin is expressed as a clotting time prolongation.

Result

No inactivation of enzyme activity was observed in the sample freeze-dried with PRIONEX®, whereas the activity of the BSA containing sample was slightly reduced (see figure 8). On one hand PRIONEX® seems to be a useful lyoprotectant and on the other hand it leads to a firm, easily reconstitutable lyophilisation cake.

Effect of PRIONEX® and BSA on freeze-drying of thrombin

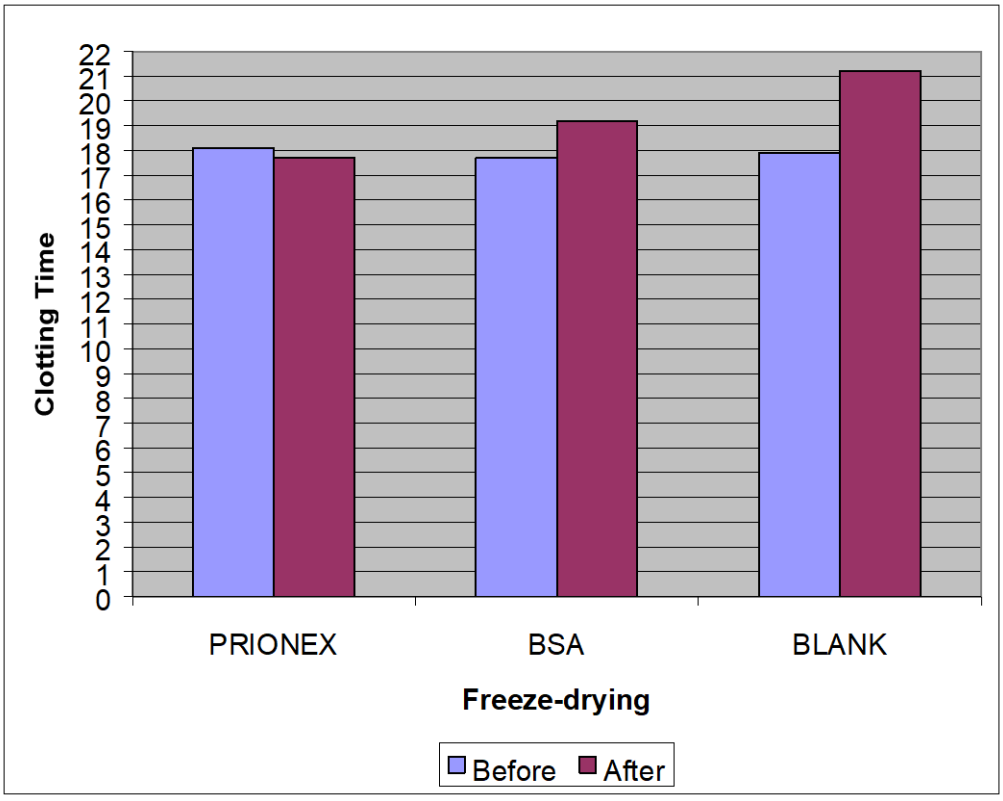

Figure 8

Storage

It is recommended to store PRIONEX® at room temperature, protected from light. As PRIONEX® does not contain any preservative, it should be processed under aseptic conditions. In order to avoid microbial contamination, once opened containers should be handled with special care and, if possible, used up in one operation.

Packaging

PRIONEX® is available in 100-mL and 1-L glass bottles. Larger quantities can be obtained on special request.

Remarks

Although the above data and information have been elaborated with the utmost possible care, technically related deviations or changes may occur.

References

[1] P.J. Gaffney, T.A. Edgell, P.J. Dawson, A.W. Ford, K. Stocker
A pig collagen peptide fraction A unique material for maintaining biological activity during lyophilization and during storage in the liquid state.
J. Pharm. Pharmacol 48: 896-898 (1996)

[2] Martindale
The Extra Pharmacopoeia, 28th Ed. pp. 953-954
Pharmaceutical Press, London (1982)

[3] R.H. Kimberlin et al.
Disinfection studies with two strains of mouse passaged scrapie agent.
J. Neurol. Sci. 59, 355-369 (1983)

[4] D.M. Taylor
Scrapie agent decontamination; implications for bovine spongiform encephalopathy.
Vet. Rec. 124, 291-292 (1989)

[5] Department of health and social security (GB/UK)
BSE: Guidance on GMP and request for information
March 1989

[6] D.H. Spackman, W.H. Stein and S. Moore
Anal. Chem. 30, 1190-1206 (1958)

[7] E.J. Cohn et al.
J. Am. Chem. Soc. 68, 459-475 (1946)

Further Readings

- G.E. Schulz and R. H. Schirmer
Principles of protein structure
Springer, New York (1979)

- R.K. Scopes
Protein purification, principles and practice
Springer, New York, 2nd Ed. (1987)

- M.P. Deutscher
Guide to protein pruification
In: Methods in Enzymology, Vol. 182
Ed. M.P. Deutscher
Academic Press, San Diego (1990)

- F. Franks
Characterization of proteins
Humana Press, Clifton (1988)