

THE EFFECT OF FREEZE-DRYING ON THE KEEPING QUALITY OF ANTI-TETANIC SERUM

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Abstract

The purpose of this work is to obtain lyophilized preparation of tetanus antitoxin, and to study the effect of freeze-drying on the keeping quality of the lyophilized products. Six of antitetanic serum (ATS) products were prepared. These products were: liquid ATS with phenol 0.5%, lyophilized ATS with phenol 0.5%, lyophilized antitoxin immunoglobulin (ATI) with phenol 0.5%, liquid ATS with sodium azide 0.2%, lyophilized ATS with sodium azide 0.2%, lyophilized ATI with 0.2% sodium azide. Saccharose 1% and glycine 2% were used as stabilizer during lyophilized process.

Sterility, safety and potency tests were applied for evaluation of different prepared lyophilized products.

The results showed that the lyophilized products were easy reconstituted with distilled water. The products were prepared from ATS with sodium azide 0.2%, ATS with phenol 0.5% and their concentrated antitoxin immunoglobulin (ATI) were bacteriology, mycoplasma and fungal sterile, potent and safe for injection.

This study proved that the lyophilized process of the ATS either lyophilized as it is or lyophilized after concentration of antitoxin immunoglobulin has no effect on the sterility, safety and also has no significant effect on the potency of the freeze-dry products.

INTRODUCTION

Tetanus or lock jaw is highly fatal disease of several species of animals (Blood *et al.*, 1983). The only reliable means of protection of an animal against tetanus is by providing it with at least antitoxin level of 0.1 IU/ml serum (Ahmed, 1991). Anti-tetanic serum is recommended for horses subjected to injury and have unvaccination history, and for mares and foals following parturition (Daniel *et al.*, 1984). This can be done by injecting a horse with about 1500 IU of tetanus antitoxin.

Tetanus antitoxin is prepared from blood of healthy horses, firstly immunized by administration of tetanus toxoid and followed by courses of injections of tetanus toxin and prepared in liquid form (Carpenter, 1975). The storage period of liquid antitetanic serum is one year, while, that of the lyophilized products could be as long as five years (Huang *et al.*, 1981).

The aim of this work is to obtain lyophilized antitetanic serum sterile, safe and potent for injection use.

MATERIALS AND METHODS

1. Experimental animals

- a. Seven groups of Swiss mice each of five (15-20 g weight) and seven groups of guinea pigs each of three (300-400 g weight) were used.
- b. Horse
 - i. Ten adult healthy horses aged 3-5 years were used for production of antitetanic serum.
 - ii. Seven groups of horses each of two were used for safety test.

2. Tetanus toxoid

It was supplied by Burroughs Wellcome, Co., London and standardized to contain 1000 Lf/ml.

3. Tetanus toxin

Tetanus toxin containing 40,000 guinea pigs MLD/ml was prepared in Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.

4. Preparation of liquid and lyophilized antitetanic serum (ATS)

a. Preparation of liquid ATS

Liquid (AST) was prepared according to Marchant and Packer (1983) and contained 300 IU/ml. It was divided into 2 parts, the first one was preserved with 0.5% phenol according to British Pharmacopoeia (1985), and the second part was preserved with 0.2% sodium azide according to Olivier *et al.* (1998).

b. Preparation of lyophilized ATS

Two parts of liquid ATS (200 IU/ml) were distributed in vials 10ml each containing 2000 IU, groups of vials contained ATS preserved with phenol 0.5%, another group (number of vials) was preserved with 0.2% sodium azide. These vials were lyophilized without stabilizer. These vials were reconstituted in 10 ml distilled water during use.

c. Preparation of lyophilized antitoxin immunoglobulin (ATI)

- i. Precipitation and concentration of antitoxin immunoglobulins (ATI) by ammonium sulphate was done according to Talwar (1983).
- ii. Two parts of liquid concentrated antitoxin immunoglobulin (ATI) were distributed in vials 5ml, one part of ATI was preserved with 0.5% phenol, each one contained 320IU/ml and ATI preserved with 0.2% sodium azide each contained 400 IU/ml. Saccharose 1% and glycine 2% were added as stabilizer to each part. These vials were lyophilized according to Gonzalez *et al.* (1984).

5. Evaluation of liquid and lyophilized batches of antitetanic serum (ATS)

Safety and sterility tests, total protein, pH value and potency tests were done according to US Code of Federal Regulation (1987).

6. Standardization of liquid and lyophilized antitetanic serum (ATS)

Potency of different prepared batches were titrated for 12 months by flocculation test described by Norris and Ribbons (1971).

RESULTS AND DISCUSSION

Tetanus antitoxin is produced in horses by courses of injections of tetanus toxoid and toxin (Marchant and Packer, 1983) and prepared in liquid form. This antitoxic serum had an expired date one year, while, lyophilized serum had 5 years expired date (Huang *et al.*, 1981).

In this investigation, we prepared 6 types of ATS which were: liquid ATS, lyophilized ATS, lyophilized ATI with 0.5% phenol, liquid ATS, lyophilized ATS and lyophilized ATI with 0.2% sodium azide. We followed the effect of lyophilizing process on the

keeping quality of each product.

Sterility tests, total protein and pH value, safety and potency tests were studied. Sterility tests according to US Code of Federal Regulations (1987) proved that liquid ATS and lyophilized ATS were sterile and safe for one year.

Topy and Wilson (1998) stated that phenol is bactericidal to gram-positive and gram-negative bacteria and possesses antifungal activity. Olivier *et al.* (1998) reported that sodium azide in concentration of 0.2% was used as a good preservative for purified serum immunoglobulin.

Total protein and pH value of each product were shown in Table 1. The total protein of liquid ATS with phenol was 4.7 g/dl, while, liquid ATS with sodium azide was 4.3 g/dl, lyophilized ATS with phenol was 4.87 g/dl, while, lyophilized ATI was 3.59 g/dl lyophilized ATS with sodium azide was 5.28 g/dl, while, the lyophilized ATI was 4.77 g/dl. pH was between 7-8. Total protein must not be more than 17 percent w/v and pH of crude serum from 7-8 (British Pharmacopoeia, 1985).

All the products proved to be safe for mice, guinea pigs and horses.

Potency of the antitetanic serum (ATS) in each product was determined in Table 2. It indicates that liquid ATS with 0.5% phenol was 200IU/ml, while, lyophilized ATS with 0.5% phenol was 180 IU/ml, liquid ATS with 0.2 sodium azide was 200 IU/ml, while, lyophilized ATS with 0.2% sodium azide was 180 IU/ml, ATI with 0.5% phenol before lyophilization was 320 IU/ml, while, the lyophilized ATI of the same product was 280IU/ml. Also, the ATI with 0.2% sodium azide before lyophilization was 400 IU/ml, while, when this product was lyophilized gave 375 IU/ml. Although the freeze-drying process slightly lowered the level of antitoxin in liquid ATS and ATI, yet, it had no significant difference which agreed with Huang *et al.* (1981) and Gonzalez *et al.* (1984).

In conclusion, ATS can be lyophilized without stabilizer and with 0.5% phenol or 0.2% sodium azide. It gave a product of good quality, while, lyophilized concentrated antitoxin immunoglobulin (CATI) was prepared by using 1% saccharose and 2% glycine as stabilizing agent and using either 0.5% phenol or sodium azide 0.2% as preservative.

Table 1. Total protein and pH values in liquid and lyophilized products of ATS.

Types of products	Total protein (g/dl)	pH value
1. Liquid ATS with 0.5% phenol	4.7	7.8
2. Lyophilized ATS with 0.5% phenol	4.87	8.00
3. Lyophilized antitoxin immunoglobulin with 0.5% phenol	Immunoglobulin 3.59	7.5
4. Liquid ATS with sodium 0.2% azide	4.3	7.7
5. Lyophilized ATS with 0.2% sodium azide	5.28	8.0
6. Lyophilized antitoxin immunoglobulin with 0.2% sodium azide	Immunoglobulin 4.77	7.0

pH of crude serum ranges from 7-8 gm/dl according to British Pharmacopoeia (1985).

Table 2. Potency of liquid and different lyophilized products of antitetanic serum determined by (FT) and expressed in Lf/ml after one year of storage.

Types of ATS products	Months during one year storage											
	1	2	3	4	5	6	7	8	9	10	11	12
1. Liquid ATS with 0.5% phenol	200	200	200	200	200	200	200	200	200	200	200	200
2. Lyophilized ATS with 0.5% phenol	180	180	180	180	180	180	180	180	180	180	180	180
3. Lyophilized antitoxin immunoglobulin with 0.5% phenol	280	280	280	280	280	280	280	280	280	280	280	280
4. Liquid ATS with 0.2% sodium azide	200	200	200	200	200	200	200	200	200	200	200	200
5. Lyophilized ATS with 0.2% sodium azide	180	180	180	180	180	180	180	180	180	180	180	180
6. Lyophilized antitoxin immunoglobulin with 0.2% sodium azide	375	375	375	375	375	375	375	375	375	375	375	375

Lf: Limit of flocculation. FT: Flocculation Test

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دراسات على تأثير التجفيد بالتبريد على الجودة النوعية للمصل المضاد للتيتانوس

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الهدف من هذه الدراسة هو الحصول على مستحضر مجفد بالتبريد للمصل المضاد للتيتانوس ودراسة تأثير عملية التجفيد بالتبريد على الجودة النوعية للمستحضرات المجفدة.

تم تحضير ستة أنواع من المصل المضاد للتيتانوس وهى: مصل سائل مضاد للتيتانوس الفينول ٠.٥٪، مصل مضاد للتيتانوس مجفد بالتبريد مضاف إليه ٠.٥٪ فينول، أجسام مناعية مجفدة مضادة للتيتانوس مضاف إليها ٠.٥٪ فينول، مصل سائل مضاد للتيتانوس مضاف إليه صوديوم أزيد ٠.٢٪، مصل مضاد للتيتانوس مجفد مضاف إليه ٠.٢٪ صوديوم أزيد، وأجسام مناعية مجفدة مضادة للتيتانوس مضاف إليها ٠.٢٪ صوديوم أزيد. وتم استخدام السكروز بنسبة ١٪ والجليسين بنسبة ٢٪ كمواد مثبتة فى عملية التجفيد بالتبريد. تم إجراء اختبارات النقاوة، والأمان والقوة العيارية لتقييم هذه المستحضرات السائلة والمجفدة. أثبتت النتائج ان المستحضرات المجفدة بالتبريد سهلة الذوبان فى الماء المقطر وأن المستحضرات السائلة والمجفدة المضاف إليها المواد الحافظة الفينول أو الصوديوم أزيد كانت خالية من الملوثات البكتيرية والفطرية والميكوبلازما وعند الحقن فى حيوانات التجارب لم تعط أية أعراض أو نفوق وكانت صالحة للحقن وكانت المستحضرات عالية القوة العيارية.

أثبتت الدراسة أن عملية التجفيد بالتبريد للمصل المضاد للتيتانوس سواء كان مجفداً كما هو أو مجفداً بعد تركيز الأجسام المناعية لا تؤثر على نقاوة وسلامة المستحضرات المجفدة وكذلك لا تؤثر تأثيراً معنوياً على القوة العيارية للمستحضرات المجفدة.