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Quality evaluation of human serum albumin prepared by heat denaturation in Iran: An experience for developing countries

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ABSTRACT

Blood and plasma are unique resources and access to these products save life. In this study, albumin demand and surplus plasma makes it possible to use local experiences in plasma industry for preparation of albumin so plasma was heated after stabilization; afterward denatured proteins were precipitated and separated by continuous centrifuge system. The supernatant contained albumin was filtrated, diafiltrated, ultrafiltrated, formulated and pasteurized.

Albumin preparation in pilot scale with heat denaturation was performed for the first time in Iran. This method using surplus plasma is recommended for all countries that have no access to plasma fractionation industry. Therefore with more attention it has potential for use in the production of safe plasma derived products and thereby it can be used as a safe product in clinic.

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1. Introduction

Access to blood and blood components is an essential part of any healthcare system. These components save life; thereby availability to enough amounts of them is the right of patients who need them [1].

Iranian Blood Transfusion Organization (IBTO) was established in 1974 as a national centralized transfusion system [1] and production of plasma derived products were begun in 1977 [2]. Subsequently a joint company called Blood Research and Fractionation Company was established which was capable to fractionate 80,000 l plasma per year; but it was ended in 1990s, because of some concern about plasma products safety [3].

Obviously if there is excess and high quality plasma in a country, but there are not suitable conditions for native plasma fractionation, Toll manufacturing can be a good choice between that country and a fractionation center, although the resulted products are not cheaper than imported ones [4].

According to statistic study in 2007 about 23–28 million l of human plasma are fractionated each year in the world, from which 35% is from recovered plasma [5] and it is estimated that about 5.8 million l plasma is discarded annually especially in developing countries [6].

In the Iranian Blood Transfusion organization (IBTO) about 2 million blood Units is collected in 2012 from voluntary, non-remunerated donors. In recent years, Iran has been self-sufficient in whole blood and blood components [7]. According to number of donations, Iran can produce more than 300,000 l plasma per year, considering the internal need to plasma; it is possible to store 150,000 l plasma for fractionation [1]. Toll manufacturing was begun since 2005 in IBTO from surplus plasma [3] and this Iranian plasma is sent to fractionation center and manufactured

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products are returned to country. Although more is needed than is currently available, this is overcome by purchasing from pharmaceutical companies.

Albumin is one of the products that its demand is more than its supply. The high price of this product makes it impossible for all patients to use it and also there is a extra amount of plasma which are not sent to fractionation companies, therefore albumin was prepared for the first time in Iran at 2 concentrations of 5 or 20 g% with heat denaturation method in semi-industrial scale with a cost-effective process, simple equipments and local experiences in plasma industry.

This method is applicable in developing countries with different kind of plasma such as fresh frozen plasma (FFP), cryo poor plasma (CPP) or platelet poor plasma (PPP) without any expensive equipment even though in countries with low plasma sources.

Albumin is a heat resistant protein which can be prepared by heat. The heat denaturation method is only used when other plasma proteins are not considered and only albumin with high purity is needed.

Therefore the heat denaturation method was studied and few modifications were applied. We did not use ethanol in the process and production cost and process time was decreased. Also filtration in acidic pH at cold condition helped to more clarification of supernatant. Accordingly the required condition for plasma fractionation in semi-industrial scale was developed.

2. Material and methods

2.1. Installation and operation of equipments in pilot scale

A pilot plasma fractionation plant was established by the use of the main equipments such as a continuous centrifuge (CEPA Z-41-Germany), 2 jacketed vessels with 60 and 100 l capacity (KHS-Germany) that each one equipped to a temperature probe and an agitator. Cooling (forma scientific-2325-USA) and heating (frigeser-COMBI KWq-France) system connected to mentioned equipments and automatic control of temperature profile was set up. At last all the connectors were insulated for prevention of energy loss.

Afterward peristaltic pump (Heidolph-Germany) was installed for liquid flow between centrifuge and tanks and necessary facilities for recording, control and monitoring of tank temperature was created. Dosing pump system enabled that acid/base solution injection flow rate into tanks with slowly and constant manner. Sampling from the tank contents during the process carried out under nitrogen gas pressure in the closed system. The performance of the equipment was qualified and the required equipments related to filtration system (Millipore Pellicon Cassette-USA) were installed (see Fig. 1).

2.2. Albumin preparation

2.2.1. Plasma preparation

Fresh frozen plasma (FFP), from all blood groups (A, B, O, AB), volume range between 210 and 240 ml (mean 215 ml), individually screened according to mandatory regulations of WHO for HBs Ag, HCV Ab and HIV Ab as infectious markers were used for this study [8]. Therefore 70 plasma bags taken out of freezer (−20 °C) and prethawed in refrigerator at +4 °C for 24 h. The bags were opened and the pooled frozen plasma equal to 15 l thawed to +25 °C in a tank equipped with a mixer and temperature controlling.

All steps of the process were monitored through a sight glass. In order to pH adjustment, acid/base solution was injected into tank at a flow rate of 1 l per hour by dosing pump. To increased purity and yield a few modifications carried out including filtration in low temperature 4 °C and acidic pH 4.5. Also ethanol was eliminated in the process for affordability.

Finally the optimal process was selected for obtaining highest purity and yield and robustness of the process were confirmed.

Albumin stabilization against heating: sodium caprylate 0.03 M (SD Fine-Chem Ltd. India) was added to stabilize of albumin and pH was adjusted to 6.8 with HCl 2M.

2.2.2. Heat denaturation

The temperature is raised to +70 °C for 60 min under continuous stirring.

To evaluate albumin purity progress during the process, acetate cellulose electrophoresis was performed and the ratio of total protein to albumin was considered as progress factor in various experiments.

Stabilized albumin was intact but the most of plasma proteins were denatured and precipitated. Immediately the temperature was decreased to +25 °C by passing tap water through the double jacketed tank and pH was adjusted to 4.5 with HCl 2M and kept overnight in order to complete the precipitation of denatured proteins.

Separation of soluble phase from insoluble phase: Soluble phase rich in albumin was separated from insoluble phase containing precipitated proteins by continuous centrifugation at +4 °C. In this step about 4 kg discarding paste was obtained, this paste is contained most of denatured proteins and trace amounts of trapped albumin (Fig. 2).

2.2.3. Clarification

The temperature of supernatant rich in albumin decreased to 4 ± 2 °C and then passed through a series of

Fig. 1. Pilot scale plasma fractionation.

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