International Journal of
Analytical, Pharmaceutical and Biomedical Sciences

Research Article

Volume: 1: Issue-2: April-June2012
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Received: 25th Jan-2012 *Revised: 29th Jan-2012* *Accepted: 12th June-2012*

Coden : IJAPBS *www.ijapbs.com*

APPLICATION OF SODIUM C APRYLATE AS A STABILIZER DURING PASTEURIZATION OF INFUSIBLE PLATELET MEMBRANE AND EVALUATION OF ITS EFFECTIVENESS BY TURBIDITY ASSAY

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ABSTRACT : For many years, sodium caprylate has been used as a suitable stabilizer to retain biological activity of pharmaceutical proteins such as human albumin solutions during pasteurization. A simple method of turbidity assay was applied for evaluation of sodium caprylate as a stabilizer to protect biological activity of infusible platelet membrane product during pasteurization at 60°C for 20 h. Concentration of sodium caprylate of samples were 0.4, 0.2, 0.1 and 0.05 M. After pasteurization, turbidity of samples was measured with spectrophotometer at optical density of 450 nm. The results were found 0.662, 1.890, 2.300, 2.365 respectively and it was concluded that sodium caprylate concentration of 0.4 M is suitable for pasteurization of infusible platelet membrane product and also turbidity assay is simple and efficient method for evaluation of this process.
Key words: Sodium caprylate stabilizer, turbidity assay, Infusible platelet membrane.

INTRODUCTION

Infusible platelet membrane (IPM) is prepared from outdated human platelets by fragmentation of the platelet membrane and removal of intracellular components with resulting potential clinical use in the treatment of bleeding due to thrombocytopenia [1-3].

Stabilizers, like preservatives are used in pharmaceutical products as excipients. Such substances are added to pharmaceutical formulations to prevent the active substance from loosing its biological activity [4-5]. Sodium caprylate is the most frequently used stabilizer that protect the albumin from aggregation or heat induced denaturation [6-7].

Turbidity test was used as a measurement of thermal denaturation of protein. Maclean and et al showed that a simple turbidity assay can be employed for detection of protein aggregation during stabilization of proteins by low molecular weight multi-ions [4].

The objective of this study was to show that sodium caprylate is a suitable stabilizer for pasteurization of IPM which can be monitored effectively by a simple turbidity assay.

MATERIALS AND METHODS

Preparation of IPM

IPM is prepared from 8 outdated platelet units of Tehran Blood Transfusion Center. The units were pooled and centrifuged for 15 min at 1000 RPM to remove contaminating red cells and white cells. The supernatant was centrifuged for 30 min at 2500 RPM to remove plasma. The precipitate was resuspended in 25 ml physiological saline solution (0.9 g%). For lysis and disruption of platelets, freeze-thaw procedure was repeated three times at -80°C and room temperature for 6 and 2 h respectively. The solution was washed twice with physiological saline solution for removing of intracellular components by centrifugation (30 min at 2500 RPM). The precipitate resuspended in 45 ml of the same solution.

Pasteurization of samples

The samples of IPM with various sodium caprylate concentrations of 0.4, 0.2, 0.1 and 0.05 M were prepared and heated at 60°C for 20 h to inactivate possible viral or bacterial contaminants. Non-heated and non-added stabilizer controls were applied.

Turbidity assay

The absorbance of the samples and controls were measured at 450 nm against physiological saline solution by UNIC UV-2100 spectrophotometer.

RESULTS AND DISCUSSION

The results of turbidity assay was found and shown in Table-1. With regard to optical characteristics of the proposed method of turbidity assay it was found that at the concentration range of 0.05-0.4 M sodium caprylate , turbidity of samples were greatly affected during heat treatment. The results showed that the OD of sodium caprylate at 0.4 M is nearly similar to non-heated control which implies of good protection of IPM during pasteurization process (Fig.1).The mechanism of its stabilization action on IPM has not been fully elucidated, however previous studies have shown that the stability of proteins against heat stress can be increased by the additives which increase the melting temperature of the proteins [8-9]. Alternatively

stabilizers can increase the stability against heat stress of the proteins by increasing reversibility of minimizing the irreversible reaction following thermal unfolding [10-12].

Table 1: Results of turbidity assay

Test samples	Sodium caprylate concentration (molar)	Optical density (OD)
1	0.05	2.365
2	0.1	2.300
3	0.2	1.890
4	0.4	0.662
Non-heated control	0.4	0.645
Non-added stabilizer control	0.0	2.737

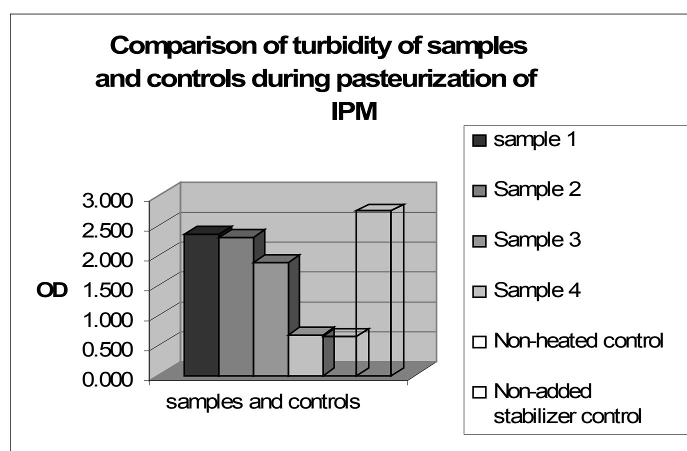

Fig.1: Comparison of turbidity of samples containing sodium caprylate at concentrations of 0.05, 0.1, 0.2 and 0.4 M (sample 1to 4) with non-heated and non-added stabilizer controls.

In this research, turbidity of samples were increased at the lower stabilizer concentrations of 0.2 to 0.05 M respectively. This indicates more loss of biological activity of pharmaceutical protein, because previous studies have shown turbidity as a parameter can be used to evaluate protein aggregation, crystallization and denaturation [13-15].

CONCLUSION

It was concluded that: 1) turbidity assay is a simple and efficient method for determination of biological status of IPM during its pasteurization at 60°C for 20 h. 2) Sodium caprylate at 0.4 M is the favorite concentration to preserve IPM during heat treatment and can be applied for this process.

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