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Research paper

Reverse micellar partitioning of Bovine Serum Albumin with novel system

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ABSTRACT

To overcome the difficulties associated with the conventional extraction process like poor selective extraction of biomolecule and scale up of the process, the reverse micellar system consist of AOT/n-heptanol was considered to extract Bovine Serum Albumin (BSA) as a model biomolecule. The maximum forward extraction of BSA from aqueous phase to micelle phase was observed at AOT concentration 160 mM, aqueous phase pH value of 4, NaCl concentration 0.8 M and 95% back extraction of BSA from micelle phase to stripping phase was obtained at 1 M NaCl concentration with the pH of 7.5. HPLC analysis confirmed the stability of BSA during extraction. The size and water content of the reverse micelle was also reported. The obtained results emphasize the application of the AOT/n-heptanol reverse micellar system for the extraction of BSA and may be utilized for the selective extraction of similar hydrophilic proteins from the complex sources.

1. Introduction

Serum albumins are the most generous proteins in the circulatory system of a wide variety of organisms, being the major complex molecules contributing to the osmotic blood pressure [1]. Albumin plays a crucial role in the design of media for the culture of mammalian cells, in both the research and commercial areas due to its antioxidant potential [2]. The high demand for proteins from animal sources around the world has raised the search for new sources of proteins [3]. Therefore need for simplified purification techniques for proteins come into focus. Water in oil emulsion has the ability to solubilize hydrophilic proteins and nucleic acids in its hydrophilic core. This property of inverse emulsion, reverse micelles, makes it promising continuous extraction method for bioseparation [4]. The reverse micelles are formed by mixing the surfactant at specific concentrations with the aqueous solution. The hydrophilic head of surfactant protects the proteins from denaturation by the organic phase and due to which little or no damage to their catalytic activity is reported [4–6]. This selective extraction of a target biomolecules from mixture in to reverse micelles can be achieved by varying parameters both in the organic and the feed phases [7].

A new reversed micelle system is prepared for extraction of BSA from the solution contains 0.5 mg/ml. The effect of different factors like pH value, ionic strength in the aqueous phase, surfactant concentration and phase volume ratio which affects the mechanism of protein transfer in forward and backward extraction of protein was studied and optimum conditions were reported. Whereas, the effect of co-solvent addition was examined for better back extraction of the protein. Reveres micelles water content and radius were also calculated to characterize the reverse micelles during forward extraction.

2. Materials and methodology

2.1. Materials

Bovine serum albumin was obtained from High media, India. Sodium bis-2-ethyl hexyl sulphosuccinate (AOT) of 99% purity and other organic solvents n-heptanol, n-butanol, n-octanol, n-decanol were purchased from Loba Chemie, India. AOT used in all experiments without further purifications. Acetonitrile and Trifluoroacetic acid (TFA) of HPLC grade were procured from Merck.

2.2. Forward extraction

Forward extraction was carried out by mixing equal volumes of aqueous and organic phases (n-heptanol with AOT) using mag-

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Nomenclature

BSA	Bovine Serum Albumin
CMC	Critical Micellar Concentration
HPLC	High Performance Liquid Chromatography
L	Liter
M	Molar
mg	milligrams
min	Minutes
ml	milliliter
mMol	millimolar
nM	Nanometer
pI	Isoelectric point
R_m	Reverse Micellar Core Radius
rpm	Revolutions Per Minute
TFA	Trifluoroacetic acid
W_0	Water Content

netic stirrer for 15 min at 500 rpm at room temperature. For all experiments phase volume and phase volume ratio were maintained as 10 ml and 1:1 respectively (except phase volume ratio study). The organic phase had a known amount of surfactant dissolved in it. The aqueous phase was prepared by maintaining BSA concentration at 0.5 mg/ml. Phase separation was carried out using Remi cooling centrifuge at 3000 rpm for 10 min. The organic phase separated from the mixture and further used for back extraction.

Water content (W_0) of reverse micelles was measured using Metrohm 899 coulometer. Further, these W_0 values were used to calculate reverse micellar core radius (R_m) using Eq. (1) [8];

$$R_m = 0.175 W_0 \quad (1)$$

2.3. Back extraction

Back extraction was carried out by mixing the organic phase obtained from forward extraction with an equal volume of stripping phase in a magnetic stirrer for 30 min at 500 rpm followed by centrifugation at 3000 rpm for 10 min and the resulted two phases were separated for further analysis.

2.4. Protein content measurement

BSA concentration was measured at 280 nm using Lab India Analytical UV spectrophotometer before and after forward and back extraction. Extraction efficiency was calculated by using the Eqs. (2) and (3):

$$\textit{Forward Extraction Efficiency}\ (\%) = [\textit{Protein concentration in organic phase}\ (\mathrm{mg/ml}) / \textit{protein concentration in aqueous feed phase}\ (\mathrm{mg/ml})] * 100 \quad (2)$$

$$\textit{Back Extraction Efficiency}\ (\%) = [\textit{Protein concentration in stripping phase}\ (\mathrm{mg/ml}) / \textit{protein concentration in organic phase after forward extraction}\ (\mathrm{mg/ml})] * 100 \quad (3)$$

2.5. HPLC analysis

Reverse phase HPLC was performed to confirm the stability of BSA after the back extraction process. Reverse phase C18 column was used with mobile phase Water/Trifluoroacetic acid (0.1%) and Acetonitrile/ Trifluoroacetic acid (0.1%). The flow rate was maintained at 0.4 ml/min at column temperature 25 ± 0.2 °C.

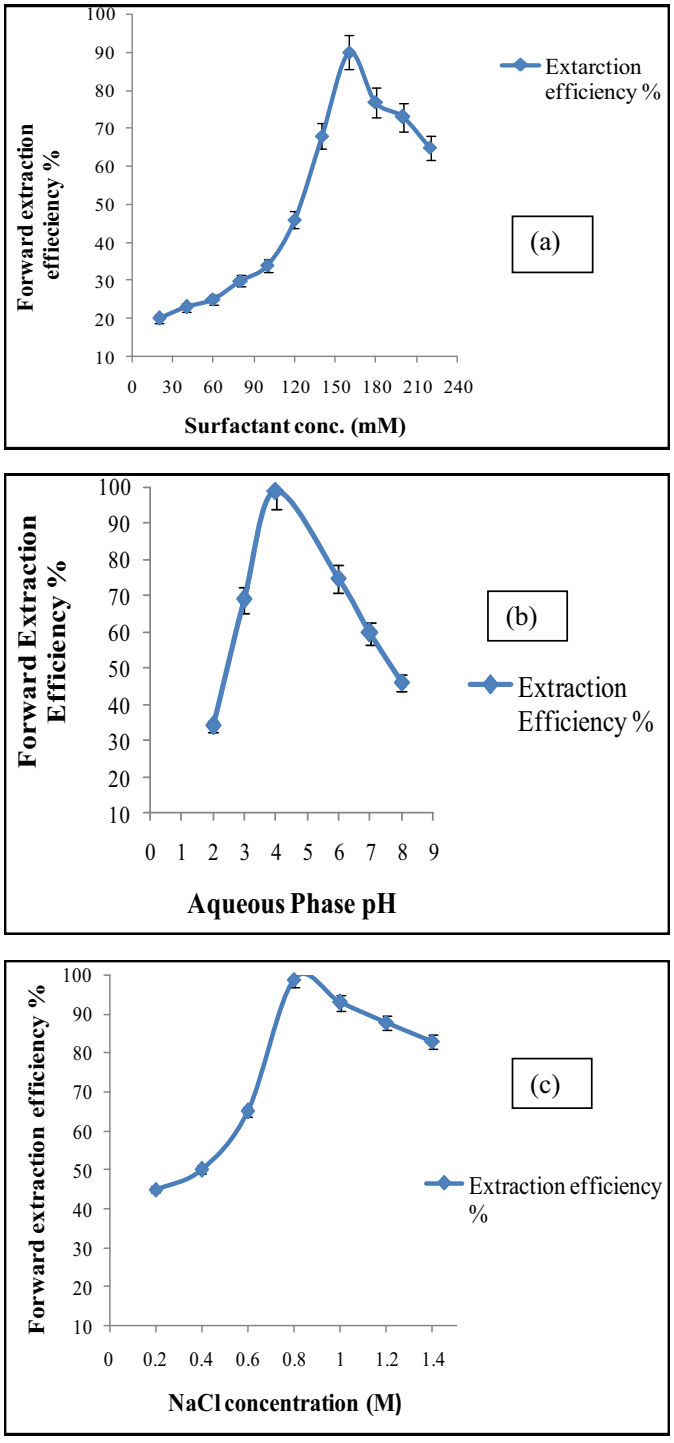

Fig. 1. Effect of (a) surfactant concentration (b) aqueous phase pH and (c) salt concentration on forward extraction of BSA.

3. Results and discussion

3.1. Forward extraction

3.1.1. Effect of AOT concentration

The Critical Micellar Concentration (CMC) of the n-heptanol/AOT system was found to be 6.6 mMol L^{-1} of AOT. The AOT concentration was varied from 20 mMol L^{-1} to 220 mMol L^{-1} (Fig. 1a), which was 3 to 33 times than the CMC concentration of surfactant. The extraction efficiency of the system was ob-