Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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To cite this Article Li, Xiangcun , He, Gaohong , Lin, Chang and Liu, Hongjing(2007) 'Study on the Extraction and Back Extraction of Bovine Serum Albumin using Reversed Micelles', Separation Science and Technology, 42: 16, 3741 — 3757
To link to this Article: DOI: 10.1080/01496390701626750
URL: http://dx.doi.org/10.1080/01496390701626750

Separation Science and Technology, 42: 3741–3757, 2007

ISSN 0149-6395 print/1520-5754 online
DOI: 10.1080/01496390701626750

Study on the Extraction and Back Extraction of Bovine Serum Albumin using Reversed Micelles

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Abstract: The extraction and back extraction of Bovine Serum Albumin (BSA) using a new reversed micelle system of CTAB/isooctane/1-pentanol have been investigated. Under the optimal operating conditions in this study, the extraction ratio of BSA reaches as high as 98%. The back extraction ratio can reach 80% as pH in stripping phase approaches to the isoelectric point (pI) of BSA. UV-spectra indicated that there was no significant change in the structure of BSA enriched in stripping phase. Furthermore, the reversed micelle system was repeatedly used to extract BSA from a mixed solution of protein.

Keywords: BSA, revered micelles, extraction ratio, selectivity

INTRODUCTION

The separation and purification of protein is attracting more and more attention due to its important role in the whole bio-production process. Reversed micelle, which is easy to scale up and to operate continuously, is a promising separation method. A reversed micelle has a polar core which can host proteins and amino acids to maintain their activities. The extraction of proteins by reversed micelles has been extensively investigated in recent

Received 25 January 2007, Accepted 13 June 2007
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years. Somnuk et al. (1) managed to separate a mixture of three proteins from a filtered broth with high yields (70–97%) and high purity. Goto et al. (2) synthesized and studied a series of dialkyl phosphoric acid surfactants (DOLPA, DTDPA, DEPTA), and reported that cytochrome-c or lysozyme was completely extracted into the reversed micelles with DTDPA as a surfactant. Kinugasa et al. (3) found that the mixed reversed micelles formed with AOT (sodium bis-(2-ethylhexyl) sulfosuccinate) and D_2EHPA (di-2-ethylhexyl phosphoric acid) could be used to extract hemoglobin and the transfer ratio could reach up to 80%. Some researchers incorporated an affinity ligand in the polar core of reversed micelles to enhance the extraction selectivity (4, 5).

For the recovery of proteins, two steps are required. The first one is the extraction of protein from feed phase into reversed micelles (forward extraction), and the other is the transfer of protein from the reversed micelles to an aqueous stripping solution (backward extraction). Dong-pyo Hong (6) found both the back extraction ratio and the activity of β-lactoglobulin increased with the presence of alcohol in the micelle phase. Dekker et al. (7) reported that the water content of the organic phase decreased with the increase of operating temperature, and subsequently the back extraction ratio was improved. Leser and Luisi (8) added silica gel to adsorb the water in the reversed micelles and obtained satisfactory back transfer efficiency. It was found that different proteins respond differently to the properties of stripping solution such as the pH value and the ionic strength. In some previous studies, the solubilized proteins were often difficult to release in a large amount from the reversed micelles to the aqueous stripping phase (9, 10).

Up to now, the anion surfactant AOT has been widely investigated in micelle extraction process (9, 11–13), and the micelle system was generally used to extract low molecular weight proteins, such as bacteriolysin, thaumatin, and so on. In the present study, a new reversed micelle system was prepared for extraction of BSA, which is a protein with a large molecular weight, and the parameters affecting the extraction and back extraction ratio of BSA were examined and the optimal condition was obtained. Furthermore, the selectivity of the reversed micelles to BSA and the conformational change of recovered BSA were also investigated.

EXPERIMENTAL

Materials

BSA (MW 67000, pI 4.9), α-amylase (MW 55000, pI 5.3), and lysozyme (MW 13600, pI 10.9) are biochemical reagents, which were purchased from Beijing Aoboxing Biochemical Co. Ltd. Blood Serum solution containing BSA (0.78 mg/ml), globulin (0.025 mg/ml), endotoxin (25 EU/ml), and

other minor components was used as received. CTAB (>99%, analytical grade), a cationic surfactant, was used without further purification. Isooctane (>99%, chemical grade) and 1-pentanol (>98%, chemical grade) were both commercially available reagents. All other inorganic reagents were analytical grade.

Methods

Reversed micelle solutions were prepared by dissolving a prescribed amount of CTAB in mixture solution of isooctane and 1-pentanol (4:1 v/v). The extraction procedure of BSA was conducted by mixing the organic solution with an aqueous solution containing 1.0 mg/ml of BSA in a taper flask. Volume ratio of the two phases was 1:1. After reaching an equilibrium (about 6 min), the mixture was separated into the micelle phase and the aqueous phase by centrifugation at 3000 rpm for 10 min. For the back extraction process, the protein-loaded micelle solution was mixed with a fresh aqueous stripping phase at an agitating rate of 300 rpm, other steps were the same as those of the extraction process mentioned above. The concentration of BSA in each phase was determined at 280 nm by a Xinmao UV-7504 UV/Visible spectrophotometer. Red shift of the characteristic peak at 280 nm denotes the conformation change of BSA. The pH value of aqueous phase was adjusted by either hydrochloric acid or potassium hydroxide solution, the ion concentration was adjusted using different salts such as KCl, NaCl, KBr, and KI.

The extraction ratio and back extraction ratio of BSA were calculated by the two following (Eqs. (1) and (2), respectively. Experiments were carried out at room temperature ($25 \pm 2^{\circ}$C). Every performed experiment was repeated three times at least. Each data point in the figures is an average of three experiment data and the error bars show the standard deviation (less than 5%).

$$E\% = C_{RM}V_{RM}/C_0V_0 \times 100\% \quad (1)$$

$$E_b\% = C_{BE}V_{BE}/C_0V_0 \times 100\% \quad (2)$$

RESULTS AND DISCUSSION

Extraction of BSA

Effects of Surfactant and pH Value

Figure 1 shows the effects of the surfactant concentration and the pH value on the extraction of BSA. It is found that the extraction ratio increases

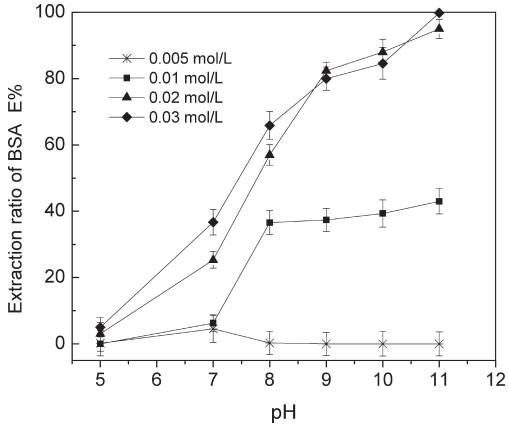


Figure 1. Effects of CTAB concentration and pH on the extraction ratio of BSA, aqueous feed phase KCl 0.1 mol/L, 25 $\pm$ 2°C, 300 rpm.

dramatically with the increase of CTAB concentration at the same pH value. In the figure, at pH values of aqueous feed phase around pI (4.9) of BSA, none or little protein is extracted into the micelle phase, while at pH values higher than the pI, the extraction ratio of the protein enhances drastically. It is because the protein has a net negative charge at pH above its pI and was attracted by the positively charged head groups of CTAB molecules. The results indicate that electrostatic interaction between BSA and CTAB plays an important role in the extraction process. However, a gel-like substance (denatured BSA) was observed at the oil-water interface when pH value in aqueous phase was higher than 11.0. According to mass conservation of BSA, it was proved that the white gel like substance was aggregate of the denatured BSA and CTAB. The phenomenon shows that too strong attraction between BSA molecules and CTAB is disadvantageous to the extraction process. Goto et al. (2) obtained similar results for hemoglobin extraction using the AOT reversed micelle system. They tested the components by elemental analysis and proved that the aggregate was a mixture of denatured hemoglobin and AOT molecules. Figure 2 indicates the absorption of extracted BSA in reversed micelles shifts from 280 nm to 290 nm at pH 12.0, and the changes also give evidence of conformational transition in protein structure.

It can also be seen that the extraction ratio of BSA with 0.005 mol/L of CTAB is lower in Fig. 1, it is considered that there were almost no reversed micelles formed in organic phase at this concentration, and the BSA molecules were not transferred into the micelle phase. It shows that the extraction ratio is about 5% higher at pH 11.0 with 0.03 mol/L of CTAB than that at pH 11.0 with 0.02 mol/L of CTAB. However, due to its limited solubility in isooctane/1-pentanol (4:1 v/v) mixture solution, some CTAB powders were observed in the organic phase during the extraction

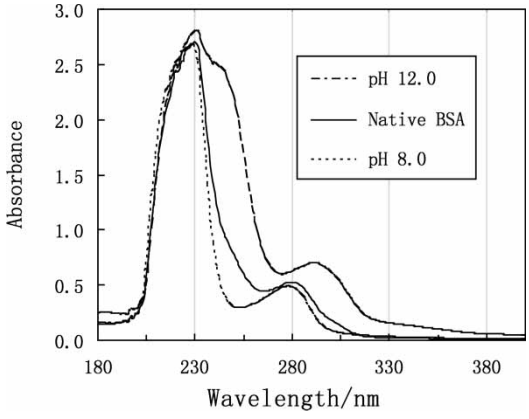


Figure 2. Effect of pH on UV-Spectra of BSA in reversed micelles, CTAB 0.02 mol/L, aqueous feed phase KCl 0.1 mol/L, 25 ± 2°C, 300 rpm.

process when 0.03 mol/L of CTAB was adopted. This may contaminate the products and be unfavorable for the purification process. Consequently, pH 11.0 with 0.02 mol/L of CTAB is considered as the best extraction condition and is adopted in the following experiments.

Effects of Salt Type and Concentration

Figure 3 shows the influence of several salts on BSA extraction ratio at different concentrations. A turbid phenomenon was observed at low salt concentration (<0.05 mol/L) for all of the four salts, and the extraction ratio was lower. The phenomenon may result from the formation of

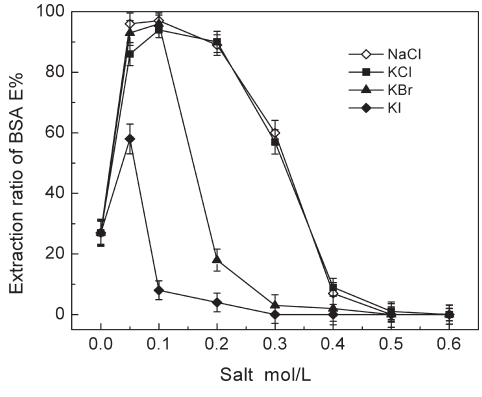


Figure 3. Effects of salt type and concentration on the extraction ratio of BSA, CTAB 0.02 mol/L, aqueous feed phase pH 11.00, 25 ± 2°C, 300 rpm.

water-CTAB-isooctane/1-pentanol O/W emulsion in the aqueous phase because CTAB is a good cationic emulsifier. In addition, the interfacial tension at low salt concentration is lower, which is advantageous to the formation of emulsion. With the varying of salt concentration of KCl or NaCl from 0.05 to 0.1 mol/L, the protein is almost completely extracted into the reversed micelles (>95%). But the curves show a very sharp drop in extraction between 0.2 and 0.6 mol/L of KCl or NaCl, and the drop trend becomes more obvious for KBr or KI. It is probably because the ions formed an electrostatic shield around the wall of micelles polar core with the increase of salt (ion) concentration, and hence decreased the electrostatic attraction between the charged protein and the charged inner core of micelles. The higher the ion concentration is, the stronger the screening effect and the smaller the micelles size are, which is disadvantageous to the extraction of BSA. In general, the smaller ions (Cl^-) produce less screening effect and allow more protein to transfer to the micelle phase, as shown in Fig. 3 at the same salt concentration. Marcozzi et al. (9) found the screening effect of four salts (LiCl, NaCl, $CaCl_2$, KCl) on the extraction of α-chymotrypsin increased with the increase of atomic radii of cations (the atomic radii of Li^+, Na^+, Ca^{2+}, K^+, are 0.68, 0.97, 0.99, 1.33A, respectively) when they studied the extraction of α-chymotrypsin using AOT/isooctane system. However, the cationic surfactant CTAB (with positively charged head groups) was adopted in this study. It was anions Cl^-, Br^-, or I^- instead of cations Na^+ or K^+ that were transferred into the reversed micelles by electrostatic interaction during the BSA extraction process. The anions produce more effect on the extraction than the cations, as shown in Fig. 3, in which the extraction ratio follows the order $I^- > Br^- > Cl^-$ at the same salt concentration, namely the screening effect of the anions on the CTAB micelle system increases with the increase of anions atomic radii (the atomic radius of Cl^-, Br^- and I^- are 1.81, 1.95, and 2.16 Å respectively). Whereas NaCl gives very similar results as KCl, indicating that the cations have almost no influence on the extraction ratio in this study.

The decrease of BSA transfer efficiency with the increase of salt concentration can be explained in terms of electric double layer theory (14). The thermodynamic potential of the inner surface of reversed micelles was Ψ_0. Because of the screening effect caused by the anions extracted into reversed micelles in the extraction process, the Ψ_0 decreased and a new potential (Ψ) was formed, which was called Stern's potential. More anions were transferred to the micelles phase at higher salt concentration, the ion strength (I) and k increased accordingly, as revealed in (Eq. 3). Therefore, Ψ became smaller with the increase of k (Eq. 4), causing the decrease of the electrostatic attraction between the proteins and reversed micelles. Additionally, the thickness of electric double layer (k^{-1}) attenuated and the size of micelles polar core decreased due to the counteracting of positive charge of surfactant head groups by anions, which was disadvantageous to the extraction

of BSA.

$$k = (8\pi e^2 NaI/1000\varepsilon k_B T)^{1/2} \quad (3)$$

$$\Psi = \Psi_0 \exp(-k\ x) \quad (4)$$

Effect of Temperature

The effect of temperature on the extraction efficiency of BSA is shown in Fig. 4. It is seen that the extraction ratio is lower at lower temperature such as 15°C, the reason may be that the movement of BSA molecules and reversed micelles is slower at lower temperature, thus the collision probability between BSA molecules and reversed micelles decreases and so does the extraction ratio. More than 90% of BSA is transferred to the micelles phase in the temperature range from 20 to 35°C. However, the extraction ratio decreases significantly at temperature higher than 35°C this results from the denaturation of the protein as shown in Fig. 5, in which a red shift for the characteristic peak at 280 nm occurs at 35°C, and it disappears at 45°C In addition, a white gel-like substance was observed at the oil-water interface when the experiments were carried out at temperature higher than 35°C. The results show that the experiments are feasible at temperatures from 25 to 35°C.

Effect of Agitating Rate

Figure 6 shows the relation between the extraction ratio and agitating rate of the extraction process. It is found that 90% to 95% of BSA is obtained in the rate range from 200 to 300 rpm, but the transfer efficiency decreases slightly with the increase of the agitating rate and the reasons deserve further discussion.

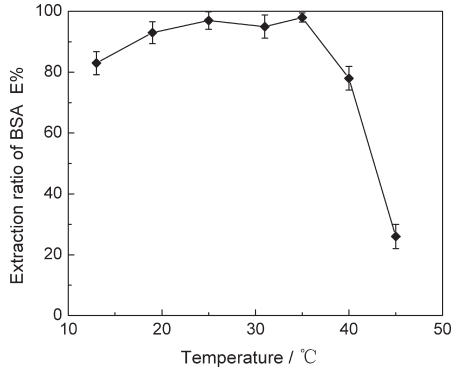


Figure 4. Effect of temperature on extraction ratio of BSA, CTAB 0.02 mol/L, aqueous feed phase: pH 11, KCl 0.05 mol/L, 25 ± 2°C, 300 rpm.

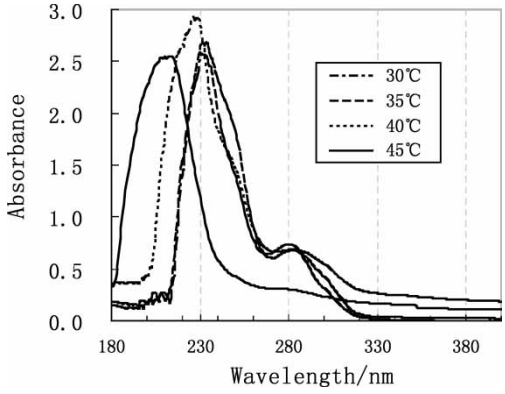


Figure 5. Effect of temperature on the UV-spectra of BSA in reversed micelles at different temperature, CTAB 0.02 mol/L, aqueous feed phase, pH 11.0, KCl 0.05 mol/L, 300 rpm.

Back Extraction

Effect of pH in the Stripping Phase

It is known that the recovery of protein from micelles phase is of great importance. The conditions of back extraction can be selected based on the behaviors of the protein in the forward extraction process. The basic idea is to choose the parameters of back extraction such as pH value, salt type and concentration when the forward extraction ratio is minimal. From Fig. 1 and Fig. 3, we consider that a beneficial back extraction should take place at a pH below 5, with 0.4 mol/L of KCl/NaCl or higher. The back extraction ratio of BSA is almost constant with 0.2 or 0.3 mol/L of KCl at all tested pH values in

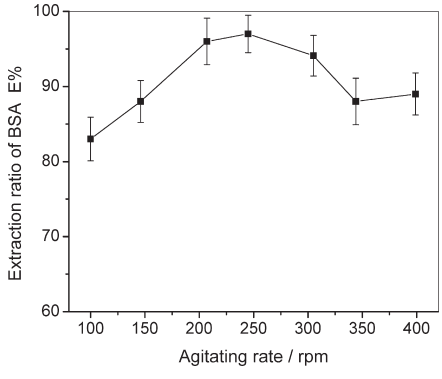


Figure 6. Effect of agitating rate on extraction ratio of BSA, aqueous feed phase: pH 11, KCl 0.05 mol/L, CTAB 0.02 mol/L, 25 $\pm$ 2°C.

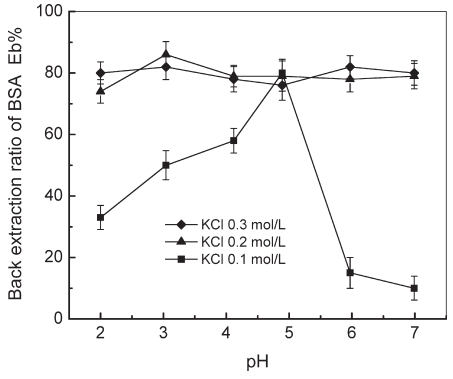


Figure 7. Effects of pH and KCl concentration in stripping phase on the back extraction ratio of BSA, CTAB 0.02 mol/L, aqueous feed phase, pH 11.0, KCl 0.05 mol/L, 25 $\pm$ 2°C, 300 rpm.

Fig. 7, and about 80% of BSA is transferred from the feed phase to the stripping phase. Turbid phenomenon was observed at high salt concentration (KCl $>$ 0.4 mol/L), and this resulted from the precipitation of BSA, CTAB, and 1-pentanol molecules from the aqueous stripping phase in the presence of higher concentration of salt. In addition, the aggregates may be formed at the oil and aqueous phase interface. These induced the turbid phenomenon during the back extraction process.

Figure 7 shows that the maximum back extraction ratio (about 80%) is obtained in the stripping phase with 0.1 mol/L of KCl at pH 5.0. It is because the net charge over the protein surface is counteracted at this pH value, which is almost the same as the pI of the protein (4.9). So the electrostatic attraction between the extracted BSA molecules and micelles decreased and the protein could be easily transferred from the micelle phase to the stripping phase. However, the stripping phase became cloudy for three different KCl concentrations (0.1, 0.2, 0.3 mol/L) at pH below 3, which was disadvantageous for the separation of micelle phase and aqueous stripping phase.

Effects of Salts Type and Concentration in Stripping Phase

The effect of salt concentration in the stripping phase on the recovery of BSA is shown in Fig. 8. It is clear that the E_b value depends on not only the type of salts, but also the salt concentration. In the case of NaCl, the values of E_b initially increase and then fall after passing through the maximum of 82%. A similar tendency is observed for KBr, but lower concentration (0.1 mol/L) is required to attain the maximal E_b. The result is favorable for the purification process with the reversed micelles system, because back extraction of protein using stripping aqueous solution with low salt concentration is advantageous.

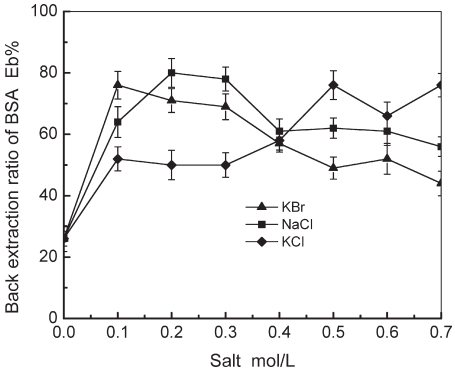


Figure 8. Effects of type and salt concentration in the stripping phase on the back extraction ratio of BSA, CTAB 0.02 mol/L, aqueous feed phase pH 11.0, KCl 0.05 mol/L, stripping phase pH 3.75, 25 $\pm$ 2°C, 300 rpm.

The E_b increases in the presence of KCl in the stripping solution. Some insoluble gel like substance (aggregates of denatured protein and CTAB) was found at the interface for three different kinds of salts in the concentration range from 0.4 to 0.7 mol/L.

It is apparent that the back transfer efficiency is also strongly influenced by salt concentration in the aqueous feed phase. The E_b is very low (10–30%) whatever the KCl concentration (0.1 or 0.3 mol/L) is in the stripping phase throughout the range of pH used as shown in Fig. 9, although the E of BSA is between 50% and 80% with low concentration of NaCl (0.01–0.03 mol/L) in the aqueous feed phase as shown in Fig. 3. It can be explained by the screening effect of the salt. It is known that more anions (Cl^-) in aqueous feed phase were transferred to the reversed micelles along with the extraction of BSA at higher salt concentration (0.05–0.2 mol/L), and the anions (Cl^-) counteracted the net positive charge of the surfactant head groups as shown in Fig. 10(a). Therefore, the electrostatic repulsion among CTAB head groups decreased and the size of the reversed micelles became smaller. In addition, the electrostatic attraction between the protein and the charged inner core of reversed micelles became weaker. These two aspects were beneficial to the back extraction of BSA. Conversely, the yield of BSA was low in stripping phase with lower concentration of salts (0.01–0.03 mol/L) in the aqueous feed phase, as indicated in Fig. 10(b).

Effect of Volume Ratio of Organic Phase to Stripping Phase

The effect of the volume ratio of the protein–loaded micelle phase to the stripping phase on the back extraction ratio of BSA is shown in Fig. 11. At the lower volume ratio, nearly all the BSA can be transferred from the organic solution to the aqueous stripping solutions. It indicates that the

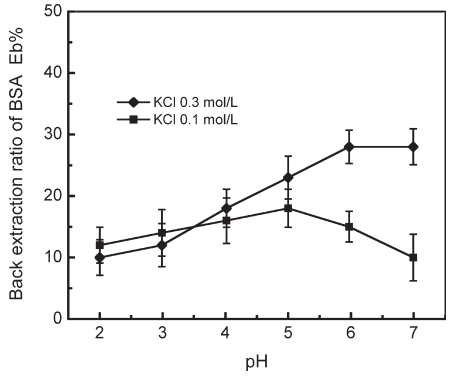
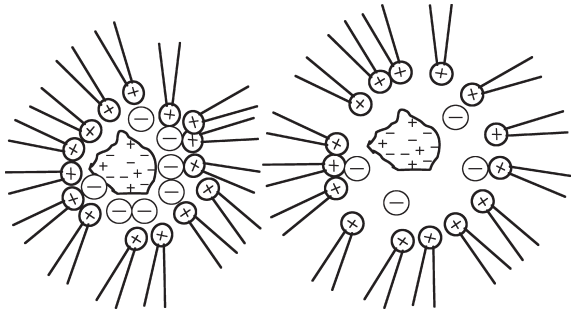


Figure 9. Effects of pH and salt concentration in the stripping phase on the back extraction ratio of BSA, CTAB 0.02 mol/L, aqueous feed phase pH 11.0, KCl 0.02 mol/L, 25 $\pm$ 2°C, 300 rpm.

BSA in the stripping phase is enriched by V_{org}/V_{aqu} times. While the concentrations of the BSA in the stripping phase rises slowly with the increase of volume ratio, it reveals that the recovering capacity of the stripping phase is limited. We obtained similar results for the recovery of the protein upon decreasing the concentration in feed phase from 1.0 to 0.5 mg/ml in Fig. 11.

No conformational change of BSA is observed by varying V_{org}/V_{aqu} in Fig. 12, demonstrating that the activity of BSA recovered is well kept. The reversed micelles system is a suitable method for protein extraction if the volume ratio is optimized. Figure 13 shows that both the values of E and E_b at BSA concentration of 0.5 mg/ml are higher than those at 1.0 mg/ml. It can be seen that the variation of E_b is independent of V_{org}/V_{aqu} at 0.5 mg/ml. About



(a) (b)

Figure 10. Interaction between anions solubilized in the reversed micelles and the inner surface of micelle system, (a) 0.02 mol/L of NaCl in aqueous feed phase (b) 0.1 mol/L of NaCl in aqueous feed phase, CTAB 0.02 mol/L, aqueous feed phase pH 11.0, 25 $\pm$ 2°C, 300 rpm.

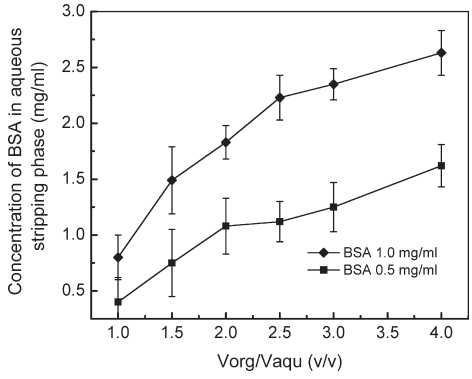


Figure 11. Effect of V_{org}/V_{aqu} on concentration of BSA in stripping phase, CTAB 0.02 mol/L, aqueous feed phase pH 11.0, KCl 0.05 mol/L, stripping phase pH 3.75, KCl 0.1 mol/L, 25 ± 2°C, 300 rpm.

95% of BSA is transferred from the feed phase to the stripping phase for every V_{org}/V_{aqu} value. This is a desirable feature for concentrating target proteins with low concentration in feed solutions using the reversed micelles.

UV-Spectra of BSA in Each Phase

Figure 14 illustrates the UV-visible absorption of BSA in aqueous feed solution (the native BSA), in micelle phase and in the stripping phase, respectively. The pH and salt concentration in the aqueous feed phase are 11.0 and 0.05 mol/L of KCl, respectively. The back extraction of BSA

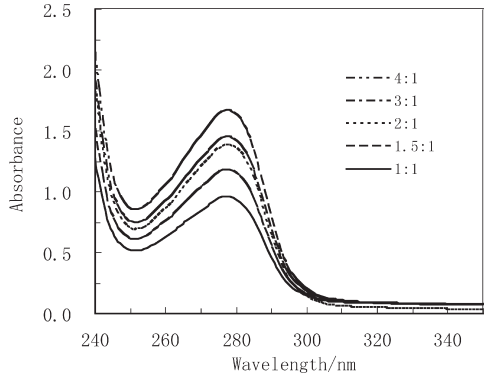


Figure 12. Effect of volume ratio (V_{org}/V_{aqu}) on UV-spectra of BSA in stripping phase, CTAB 0.02 mol/L, aqueous feed phase pH 11.0, KCl 0.05 mol/L, stripping phase pH 3.75, KCl 0.1 mol/L, 25 ± 2°C, 300 rpm.

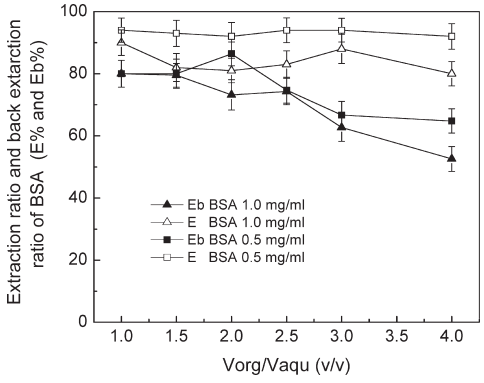


Figure 13. Effect of V_{org}/V_{aqu} on E% and E_b% of BSA, CTAB 0.02 mol/L, aqueous feed phase pH 11.0, KCl 0.05 mol/L, stripping phase pH 3.75, KCl 0.1 mol/L, 25 $\pm$ 2°C, 300 rpm.

was carried out using an aqueous stripping phase with pH 3.75 and 0.1 mol/L of KCl. The forward and the backward extraction were performed at room temperature (25 $\pm$ 2°C) with an agitating rate of 300 rpm. The conformational change of recovered BSA can be inferred from the absorption spectra. It is found that the peaks of the absorption spectra at 280 nm and 230 nm are almost consistent in the three solutions, which is a good indication that no conformation change has taken place, namely that the conformation of the recovered BSA in the stripping phase was not damaged throughout the entire process.

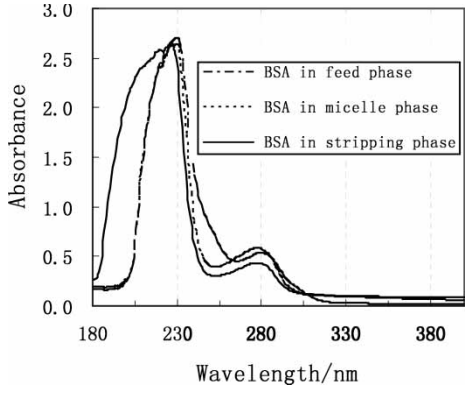


Figure 14. The UV-spectra of BSA in three different phases, micelles phase CTAB 0.02 mol/L, aqueous feed phase pH 11.0, KCl 0.05 mol/L, stripping phase pH 3.75, KCl 0.1 mol/L, 25 $\pm$ 2°C, 300 rpm.

THE SELECTIVITY OF REVERSED MICELLES TO BSA

We obtained feasible conditions for extraction and back extraction of BSA through experiments. In order to examine the selectivity of the reversed micelles to BSA, α-amylase solution, lysozyme solution, and bovine blood serum solution were selected as three kinds of feed phase. The methodology used for BSA separation from the bovine blood serum solution was similar to other proteins. First, the pH value and salt concentration in the bovine blood serum solution were adjusted to be 11.0 and 0.05 mol/L by the addition of NaOH and KCl respectively, and then the extraction process was carried out using reversed micelles with 0.02 mol/L of CTAB, and finally, the protein was back extracted by mixing the reversed micelles contained BSA and the aqueous stripping phase.

From Table 1, it can be seen that the maximal E and Eb of α-amylase which has similar MW and pI to BSA are 60% and 50% respectively. While the E and the Eb of BSA at the same conditions is 98% and 80%, respectively. The reasons deserve to be further studied. Table 1 shows that only 2% of lysozyme is obtained in the stripping phase, and this is due to the weak electrostatic interaction between the micelle phase and the lysozyme molecules. Furthermore, separation of the BSA from the bovine blood serum solution was carried out. The micelle system in this experiment was repeatedly used three times, and the average extraction ratio and back extraction ratio were 85% and 69%, respectively. The UV-spectra of BSA in the stripping phase is shown in Fig. 15. It is found that the position of the peak at 280 nm accords with that of the native BSA (aqueous feed phase). The extraction of the BSA from a mixture solution proves that CTAB/isooctane/1-pentanol has certain extent selectivity to BSA, and the target protein can be recovered by adjusting the pH and the salt concentration in the feed and the stripping phases. The recycling of the micelles phase is valuable to save materials and to minimize the operating cost, which leads

Table 1. The extraction and back extraction ratio of several proteins

Protein	BSA	α-Amylase solution	Lysozyme solution	Bovine serum solution
MW	67,000	55,000	13,600	67,000 (BSA)
pI	4.9	5.3	10.9	4.9 (BSA)
C_0 (mg/ml)	1.0	0.5	0.5	0.78
E (Max%)	98	60.0	2.0	85
E_b (Max%)	80	50.0	2.0	69

Reversed micelles phase: isooctane and 1-pentanol (4:1 v/v), CTAB 0.02 mol/L.
Aqueous feed phase: pH 11.0, KCl 0.05 mol/L.
Stripping aqueous phase: pH 3.75, KCl 0.3 mol/L.
Condition: 25 $\pm$ 2°C, 300 rpm.

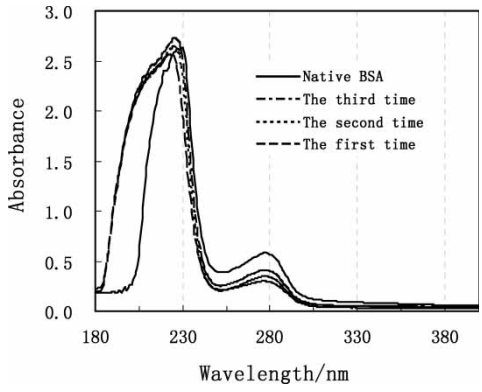


Figure 15. The UV-spectra of BSA in stripping phase, CTAB 0.02 mol/L, aqueous feed phase pH 11.0, KCl 0.05 mol/L, stripping phase pH 3.75, KCl 0.1 mol/L, 25 ± 2°C, 300 rpm.

to a possibility of continuous operation of the protein extraction using the CTAB micelles solutions.

CONCLUSION

BSA, which is difficult to be extracted by conventional AOT reversed micelles, can be solubilized easily in the CTAB reversed micelles. Several parameters were optimized in order to promote the transfer efficiency of the protein into the micelles solution and then into the stripping phase. Presently, under the following conditions with CTab 0.02–0.03 mol/L, pH 11.0, salt concentration KCl/NaCl 0.05–0.2 mol/L, temperature 20–35°C, agitating rate 200–300 rpm (for extraction), pH 3.5–5.0, salt concentration KCl/NaCl 0.1–0.3 mol/L(for back extraction), the extraction and the back extraction ratio of BSA reached about 98% and 82%, respectively. UV-spectra of the BSA enriched in the stripping phase demonstrate that no significant change takes place in the structure of main chain of BSA. The extraction ratio was enhanced rapidly with the increase of the pH value, but denatured BSA, which looked like a gel substance, was observed at high pH values (>11.0). The salt type and concentration in the feed phase affect protein extraction by causing electrostatic screening. It is found that large ions such as Br^-, I^- caused larger screening effect than the smaller such as Cl^-. Extraction of the BSA from a mixture solution of proteins proves that the micelles system has a certain extent selectivity to BSA. Micelles phase could be repeatedly used without addition of the surfactant and the cosurfactant in the organic solvent. The experimental results show that the micelle system has a promising future for the extraction of large molecular proteins.

NOMENCLATURE

E	Extraction ratio of BSA, $100[\text{protein}]_{RM}/[\text{protein}]_0$ (%)
E_b	Back (total) extraction ratio of BSA, $100[\text{protein}]_{BE}/[\text{protein}]_0$ (%)
C_0	Protein concentration in aqueous feed phase, mg/ml
C_{RM}	Protein concentration in aqueous feed phase, mg/ml
C_{BE}	Protein concentration in stripping phase, mg/ml
V_0	The volume of aqueous feed phase, ml
V_{RM}	The volume of organic phase, ml
V_{BE}	The volume of stripping phase, ml
k^{-1}	The thickness of electric double layer, m^{-1}
k_B	Boltzmann's constant, $1.38 \times 10^{-23} J \cdot K$
E	Unit of electrostatic, 1.602×10^{-19} C
Na	Avagadro'sconstant, Entries $\cdot$ mol^{-1}
I	Ionic strength
Ψ	Stern's potential, v
Ψ_0	Thermodynamic potential of the inner surface of reversed micelles, v
V_{org}/V_{aqu}	Volume ratio of protein-loaded reversed micelles to feed phase

Subscripts

b	Back extraction
0	Initial
RM	Reversed micelles
BE	Stripping phase

ACKNOWLEDGMENT

We appreciate the financial support of the National Nature Science Foundation of China (No. 20006001).

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