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Review

Micelle-mediated extraction

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Abstract

The extraction technique based on phase separation in aqueous micellar solutions is reviewed. The technique has now been utilized for separation and preconcentration of metal chelates, organic compounds, and proteins. Additionally, the phase behavior of the micellar solutions and recent advances in the phase separation technique are also described. In the extraction of metal chelates, distribution equilibria are considered. In contrast to conventional solvent extraction, the distribution of metal chelates into a condensed surfactant phase (surfactant-rich phase) was dependent on metal ions. Proteins were extractable into the surfactant-rich phase according to their hydrophobicity. The recent use of affinity ligands and water-soluble polymers for controlling extractability of proteins are also introduced. © 1997 Elsevier Science B.V.

Keywords: Reviews; Extraction methods; Sample handling; Phase separation; Metal chelates; Proteins

Contents

1. Introduction

Recently, attention is being paid to the use of

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non-ionic surfactant micelles [1–4] for preconcentrations and separations in analytical chemistry and separation sciences. Micelles have been accepted as a microscopic medium which provides a new basis for development of separation techniques. In view of this, important and practical applications are concentrated in separation sciences [5–9].

Micelles have been extensively used for a mobile phase in liquid chromatographic separations such as thin-layer (TLC) and high-performance liquid chromatography (HPLC) [5]. The micellar mobile phase eliminates the use of organic solvents currently used for separation of water-insoluble organic solutes in TLC and HPLC. Micelles in the mobile phase are regarded as being a psuedophase into which water-insoluble organic solutes are distributed, thus providing an effective means for controlling elution of analytes. In contrast, in micellar electrokinetic chromatography (MEKC) [5,10], micelles play a role of a microscopic stationary phase, thus resulting in excellent resolution.

These micellar chromatographic separations are based on solubilization of solutes in micellar solutions, one of the unique properties of micellar solutions. Excellent reviews have already been written on the micellar chromatographic separations [5,6]. However, there are few on micelle-mediated extraction [8], which is based on phase separations in micellar solutions of non-ionic surfactants. Phase behavior is an another unique property of micellar solutions [1,11].

This review is limited to the use of the micelle-mediated extraction with non-ionic surfactants for preconcentration of water-insoluble metal chelates in trace metal analysis and for extraction of hydrophobic proteins. In the latter, recent applications of the micelle-mediated extraction are concentrated.

2. Phase separation in micellar solution

The micelle-mediated extraction is based on the well-known phase phenomenon in micellar solutions of non-ionic surfactants. For example [12,13], a micellar solution of Triton X-100 becomes turbid when heated above about 60°C which is defined as the cloud point. The micellar solution separates into two distinct phases. One phase (surfactant-rich phase) contains most of Triton X-100 and the other an aqueous phase (aqueous phase) in which the concentration of Triton X-100 is equal to or below the critical micelle concentration (CMC). Hydrophobic metal chelates originally present that bind to micelles in solution can thus be extracted from the micellar solution into a small volume element of the surfactant-rich phase. Being based on phase separation at a temperature above cloud point, the micelle-mediated extraction has also been termed as temperature-induced phase separation or cloud point extraction [5,8,9].

For analytical extraction of metal chelates, poly(oxyethylene)-4-nonylphenyl-ether with an average of 7.5 ethylene oxide units (PONPE-7.5) [14,15] has been frequently utilized, because its dilute micellar solution phase separates easily at room temperature. Fig. 1 shows phase diagrams for aqueous solutions of PONPE-7.5 [15] and Triton X-114 [16]. The temperature at which the phase separation occurs is a function of PONPE-7.5 (or Triton X-114) concentrations. We can define a consolution curve, which separates the one-phase region (L) from the two-phase region (2L), by plotting the cloud point temperature as a function of surfactant concentra-

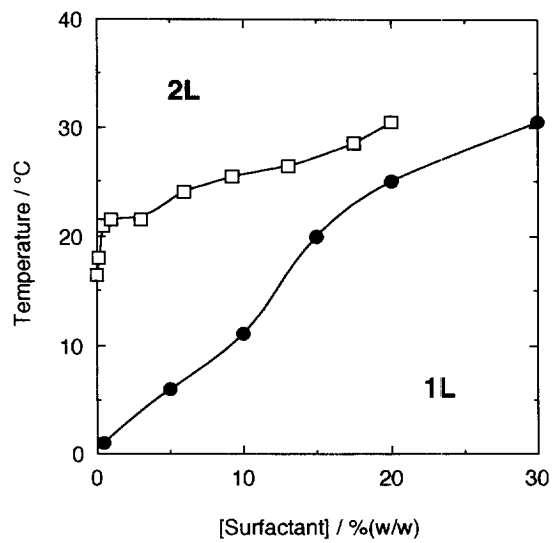


Fig. 1. Cloud point temperature as a function of surfactant concentration for PONPE-7.5 or Triton X-114. (●) PONPE-7.5; (□) Triton X-114. 1L refers single isotropic phase and 2L means the presence of two isotropic phases. (Taken with permission from Ref. [14]).

tions. A dilute micellar solution of PONPE-7.5 has a cloud point far below room temperature, thus the phase separation can be made without heating the micellar solutions. The phase separation is reversible, and on cooling the two phases are mixed again to form an isotropic solution.

To simplify phase separation, it is recommended to use a non-ionic surfactant which has a cloud point lower than room temperature. In this respect PONPE-7.5 is superior to Triton X-114. It should be emphasized that the cloud point of micellar solutions can be controlled by addition of salts, alcohols, non-ionic surfactants and some organic compounds [17–26]. For example, micellar solutions of Triton X-100 phase separate above 60°C, however, addition of sodium sulfate considerably lowers the cloud point of Triton X-100, due to a salting-out effect [20]. In general, benzene and phenol are effective for reducing the cloud point [18,21]. As described later, water-soluble polymers such as polyethylene glycol (PEG) and diethylaminoethyl–dextran (DEAE–Dx) are also capable of inducing phase separation in micellar solutions of alkylglucosides which have no definite cloud points [17,27].

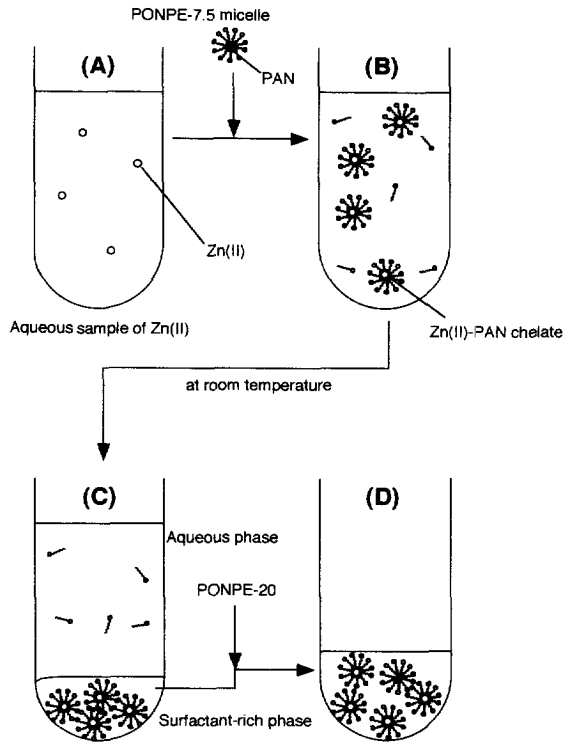


Fig. 2. Experimental schemes of the micelle-mediated extraction: (A) the initial aqueous solution involving a metal ion; (B) the aqueous micellar solution in which water-insoluble metal chelates are solubilized; (C) the phase separation into two phases. The metal chelates are concentrated in a small volume of the surfactant-rich phase and separated from the aqueous phase; (D) the final clear solution having a cloud point higher than room temperature and is submitted to spectrophotometric quantitation.

3. Extraction of metal chelates

3.1. Procedure for extraction of metal chelates

The micelle-mediated extraction with PONPE-7.5 was first exploited in the extraction of a metal ion after complexation as its hydrophobic chelates. As a typical example [14], experimental procedures for extraction of zinc(II) chelate with 1-(2-pyridylazo)-2-naphthol(PAN) are illustrated in Fig. 2. Zinc(II) chelate was quantitatively extracted from 50 g of an aqueous solution into a small volume (~1 ml) of the surfactant-rich phase. In this procedure, a 0.5-g portion of a stock PAN (0.020%) solution containing 20% (w/w) PONPE-7.5 was added to definite amounts (≤50 g) of an aqueous sample. In Fig. 1 the cloud point of 20% (w/w) PONPE-7.5 solution is about 25°C, and hence, the stock solution of PAN is a clear solution at room temperature (20°C). In a mixture of the aqueous sample with stock PAN solution, the concentration of PONPE-7.5 is about 0.2% (w/w). The mixture has a cloud point temperature, about 1–2°C, thus inducing phase separation at room temperature. The mixture was then centrifuged at 8000 rpm to help separate the two phases, and most of supernatant solution was poured off. For convenience in subsequent spectrophotometric measurements, total amounts of solution remaining in a centrifuge tube were adjusted to 2.00 g, by addition of water and a 0.5-g portion of 10% (w/w) PONPE-20, the cloud point of which is >100°C. PONPE-20 was added to increase the cloud point of the final solutions as desired.

Fig. 3 shows the variation of cloud points with compositions of PONPE-7.5 and PONPE-20 in aqueous mixtures [14]. In general, cloud points for aqueous mixtures of two non-ionic surfactants are intermediate between those of the two. Any desired

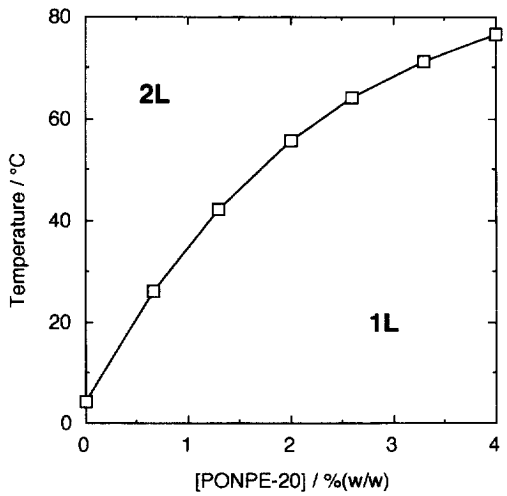


Fig. 3. Cloud point temperature of 0.4% (w/w) PONPE-7.5 as a function of PONPE-20 concentration (1L and 2L have the same meanings as in Fig. 1). (Taken with permission from Ref. [14]).

cloud point temperature can be established by appropriate selection of two non-ionic surfactants. According to the procedure in Fig. 2, the cloud point of the final solutions was 30°C. Finally, whole solutions in the tube were submitted to spectrophotometric quantitation.

As a result, the zinc(II) chelate was confirmed to be quantitatively extracted from a 50-g portion of aqueous samples into 2.00 g of the final solution, and hence the concentration factor was 25. Any concentration factors up to several tens are simply obtained by controlling the amount of the aqueous samples and that of the final solution. Under conditions described above, the volume of the surfactant-rich phase was 0.302 ml and that of the aqueous phase 49.11 ml at 20°C. The density of the surfactant-rich phase was 1.016 g cm^{-3} and that of the aqueous phase 1.012 g cm^{-3} [28–30]. The CMC value of PONPE-7.5 was 0.004% (w/w) [14].

The analytical procedure thus established is typical but versatile for any solutes extractable by the micelle-mediated extraction. Solutes that bind to micelles in solution are extracted to differing extent depending upon the micelle-solute binding interaction. Solutes in the surfactant-rich phase can easily be determined by spectroscopy and/or micellar chromatography.

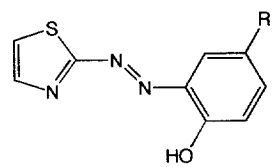
Table 1
Distribution constants for thiazolylazo chelating reagent and their metal chelates

Chelate	log K_d	log K_D			
		Fe(II)	Ni(II)	Cd(II)	Zn(II)
TAC	3.11	4.23	4.03	3.29	3.08
TAMP	2.24	2.48	2.71	3.14	2.95
TAEP	2.50	3.07	3.21	3.25	3.06
TAPP	3.63	2.80	3.77	2.71	3.19

K_d is the distribution for neutral chelating reagent (HA) and K_D for neutral metal chelates (MA_2) at ionic strength 0.10 ($NaClO_4$) and 293 K. (Taken with permission from Ref. [30]).

3.2. Distribution equilibria of chelating reagents and chelates

In the micelle-mediated extraction, distribution ratios for a chelating reagent and its metal chelates are easily measured as a function of pH in the aqueous phase according to a manner similar to that in the conventional solvent extraction. Distribution ratio (D) is defined as a ratio of total concentrations for a solute between the two phases. As a result, the distribution constants (K_d) for neutral species of chelating reagents (HA) and those (K_D) of their neutral metal chelates (MA_2) are obtained [28–31]. These distribution constants for typical chelating reagents and their metal chelates are given in Table 1. The chelating reagents tested were 2-(2-thiazolylazo)-4-methylphenol (TAC), 2-(2-thiazolylazo)-4-methoxyphenol (TAMP), 2-(2-thiazolylazo)-4-ethoxyphenol (TAEP) and 2-(2-thiazolylazo)-4-phenylphenol (TAPP). Their structures are shown in Fig. 4.



R- TAC : CH_3
TAMP : OCH_3
TAEP : OC_2H_5
TAPP : C_6H_5

Fig. 4. Structure of chelating reagent used.

The distribution constants for neutral chelates of iron(II), nickel(II), cadmium(II) and zinc(II) are also included in Table 1. All the chelating reagents are tridentate ligands (A), thus they react with the metal ions (M) to form coordination-saturated MA_2 type chelates [30]. In the conventional solvent extraction with non-polar solvent, the following relation is expected from the regular solution theory [32].

$$\log K_D \approx 2\log K_d$$

In Table 1, the log K_D values for the chelates are considerably smaller than 2 log K_d for the respective chelating reagents. This fact is often seen in conventional solvent extraction with polar organic solvents.

Furthermore, the distribution constants of the chelates in Table 1 are fairly dependent upon metal ions, as is especially apparent in chelates with TAC, where the logarithmic K_D for the iron(II) chelate is one order of magnitude larger than that for the zinc(II) chelate. These facts suggest that distribution mechanism is different from that of conventional solvent extraction where the distribution constants of chelates are, in general, independent of metal ions.

In Fig. 5 the distribution constants of three chelating reagents (TAC, TAMP and 8-hydroxyquinoline (8Q)) in the micelle-mediated extraction with PONPE-7.5 are compared with those in the conventional solvent extraction with octanol. The distribution constant of 8Q between water and octanol was given in the literature [33]. In Fig. 5 there is a good linear relationship between the distribution constants obtained by the two different extraction systems. Such a linear free-energy relationship is observed in conventional solvent extraction with a series of organic solvent having similar properties to each other [34]. Thus, in the micelle-mediated extraction with PONPE-7.5 chelating reagents is extracted probably due to a specific interaction, such as hydrogen bonding, between functional groups on the chelating reagents and ether oxygen or terminal OH group of PONPE-7.5.

In contrast, in Fig. 5 no relationship is seen between the distribution constants of the chelates and TAC, thus indicating that the distribution mechanism for the chelates is different from that of the chelating reagents. An interaction causes the chelates to remain in a hydrophobic part of aggregated micelles having an ordered structure which is different from that of the isotropic octanol phase.

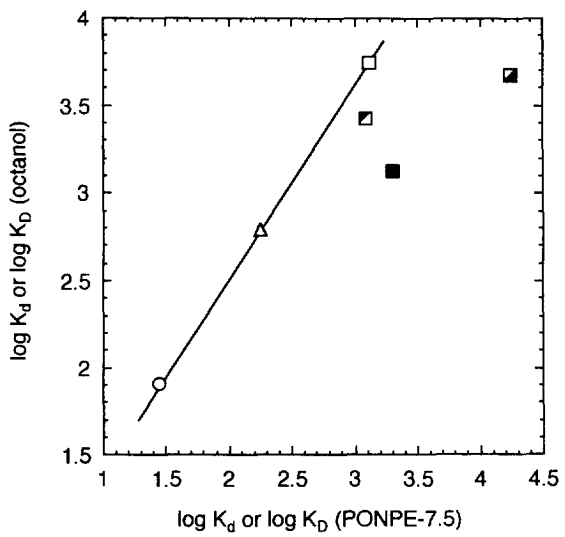


Fig. 5. Correlation between the distribution constants in aqueous-octanol system and those in PONPE-7.5 system. (○) 8Q; (△) TAC; (□) TAMP; (◩) $[Zn(tac)_2]$; (■) $[Cd(tac)_2]$; (◪) $[Fe(tac)_2]$. (Taken with permission from Ref. [30]).

3.3. Application to trace metal analysis

The typical procedure described in Section 3.1 could widely be applicable to extraction and preconcentration of a variety of metal ions in aqueous samples with or without slight modification of conditions for extraction and for subsequent treatment of the surfactant phase. The micelle-mediated extraction has already been widely applied to the spectrophotometric or flow injection analysis of trace metal ions in various samples after their complexation with some chelating reagents [35–40].

Furthermore, high concentration factors and recoveries were also achieved in ion-pair extraction of charged chelates as well as neutral metal chelates. Positively-charged copper(II) chelate with α, β, γ, δ-tetrakis(1-methylpyridinium-4-yl)porphin was extracted, when dodecylbenzene sulfonate was employed as a counter ion [41]. For other ion-pair complexes, the micelle-mediated extraction would be useful [42].

3.4. Feature of micelle-mediated extraction for chelates

There are distinct advantages in the micelle-mediated extraction for metal ions as their chelates [14]. Concentration factors of 10–100 are easily obtained with good recoveries, thus resulting in highly sensitive analyses. The second feature is in the fact that smaller amounts of aqueous samples, typically below 50~100 ml, are only required. In ordinary solvent extraction, handling large volumes up to 1000 ml are required for obtaining the same concentration factor as in the micelle-mediated extraction. The third feature must be in safety. The use of small amounts of non-ionic surfactant eliminates handling large volumes of volatile and flammable organic solvent in conventional solvent extraction. The surfactant-rich phase can be burned in the presence of acetone or ethanol, thus providing a good means for waste disposal.

4. Extraction of organic compounds

The micelle-mediated extraction with Triton X-100 was successfully applied to extraction of several hydrophilic and hydrophobic porphyrins [43,44]. The cloud point of micellar solutions of Triton X-100 was reduced by addition of excess salt. Lipophilic vitamins [45] and pesticides [46,47] were extracted with Triton X-114, prior to HPLC with electrochemical detection. Phenolic, polycyclic aromatic hydrocarbons and pesticides in environmental samples [47–52] were also extractable. In these applications, the organic compounds extracted in the surfactant-rich phase can be submitted to the subsequent quantitation by micellar chromatography.

5. Extraction of hydrophobic proteins

5.1. Procedure for extraction of hydrophobic proteins

The most important utilization of the micelle-mediated extraction has been concerned with the isolation and purification of biological species, typically hydrophobic proteins [9,53–55]. The first application was made by Bordier [56] in 1981. Integral membrane proteins were separated from hydrophilic proteins by the micelle-mediated extraction with Triton X-114 [56]. As is seen in Fig. 1, Triton X-114 is miscible with water at low temperature, whereas two phases are formed above 30°C. Most hydrophilic proteins remain in the aqueous phase, while hydrophobic proteins are extracted into the surfactant-rich phase.

Fig. 6 indicates typical schemes for extraction of brush border membrane proteins [57]. The membrane was first solubilized with an aqueous buffer (pH 7.4) of 1% Triton X-114 at 4°C. After removal of surfactant-insoluble materials by centrifugation, a supernatant solution thus obtained was incubated at 32°C, thus inducing phase separation. Turbid solution thus obtained was then placed onto a cushion of 6% (w/v) sucrose containing 0.06% Triton X-114 (pH 7.4) in a centrifuge tube. After centrifugation, the surfactant-rich phase appeared below the cush-

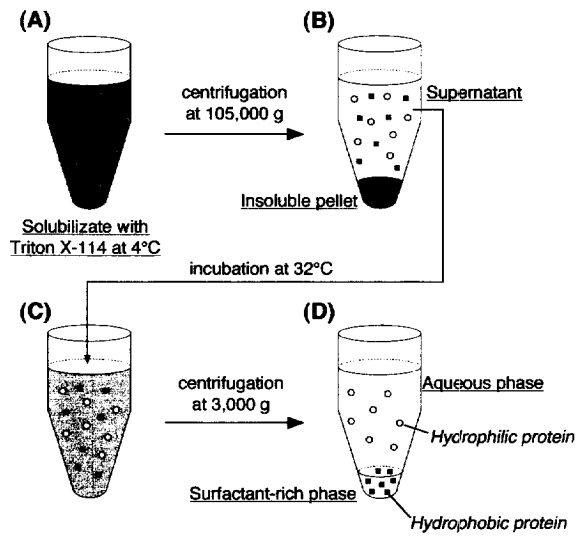


Fig. 6. Experimental schemes for extraction of brush border membrane proteins: (A) the solubilization step for biological membranes in Triton X-114 micellar solution at 4°C; (B) the step for separation of solutes from residual membrane components by centrifugation; (C) the temperature-induced phase separation in micellar solution involving solutes at 30°C; (D) the two phases after centrifugation, where hydrophobic proteins are concentrated in a small volume of the surfactant-rich phase and separated from the aqueous phase.

ion, while the aqueous phase was retained above the cushion. If necessary, after removal of the surfactant-rich phase, additional Triton X-114 was added to the aqueous phase, and then the extraction was repeated to ensure efficiency. Then, respective phases were washed repeatedly for decontamination.

Because of the simple and rapid procedure, the micelle-mediated extraction has now been employed for processing a variety of membrane proteins, enzymes and receptors [56–66]. Some recent applications are listed in Table 2. Cytochrome *b* in the inner membranes of bacterial mitochondria was purified from a fraction containing cytochrome bc_1 complex [61]. Pyruvate oxidase has been isolated from crude extracts from *E. coli* cells [62]. Pyruvate oxidase was highly extractable into the surfactant-rich phase, so that, the micelle-mediated extraction gave a simple and highly efficient procedure in comparison with chromatographic separations currently employed.

In these examples, additional proteins are generally co-extracted in the surfactant-rich phase. In the procedure in Fig. 6, eleven proteins were reported to be present in the surfactant-rich phase [57]. Thus, fine purification steps by column chromatography or electrophoresis are required. However, it is important to point out that the micelle-mediated extraction has been effective for reducing some tedious and time-consuming steps in purifications based on column chromatographic separations and precipitation with ammonium sulfate.

5.2. Theoretical aspects for protein partitioning

There are few investigations on the mechanism of protein distribution in the micelle-mediated extraction. Recently, Nikas et al. [67–70] proposed a model for the distribution of proteins theoretically, based on the excluded-volume interaction between globular hydrophilic proteins and non-charged micelles. In this model, micelles grow into an elongated-cylindrical structure and thus form entangled-network configuration upon phase separation in aqueous micellar solutions. As a result, hydrophilic proteins are excluded from the network depending on their size and thus are mostly distributed into the aqueous phase. The model was experimentally confirmed to apply to the partitioning behavior of hydrophilic proteins in two-phase separation in tetraethyleneoxide decyl ether and dioctanoyl phosphatidylcholine micellar solutions [69,70].

5.3. Modification of micelle-mediated extraction

In the micelle-mediated extraction with Triton X-114, its micellar solution must be warmed above

Table 2
Some recent examples of micelle-mediated extraction of proteins

Proteins	Surfactant used and conditions	Ref.
Bacteriorhodopsin, hemoglobin	Triton X-114, 0.15 *M* NaCl (pH 7.4), 30°C	[56]
Thyrakoid polypeptides in cyanobacterium *Anacystis nidulans R2*	Triton X-114, 0.15 *M* NaCl (pH 7.5), 37°C	[58]
Glycoproteins, phosphoproteins in human blood platelets	Triton X-114, 0.154 *M* NaCl, 37°C	[59]
NADH-cytochrome b_5 reductase in human neutrophil	Triton X-114, 32°C	[60]
Alkaline phosphatase, leucine aminopeptidase, γ-glutamyl transpeptidase, Ca^{2+}–Mg^{2+} ATPase, glucoamylase, sucrose–isomaltase, lactase, trehalase, intrinsic factor-cobalamin receptor in rat intestinal brush border membrane	Triton X-114, 0.14 *M* NaCl (pH 7.4), 32°C	[57]
Cytochrome *b*, cytochrome c_1, iron–sulfur protein in bacteria	Triton X-114, 0.05~0.15 *M* NaCl (pH 7.5), 30°C	[61]
Pyruvate oxidase in *Escherichia coli*	Triton X-114, 0.15 *M* NaCl (pH 6.0), 30°C	[62]
Polyphenol oxidase in grape berries	Triton X-114 (pH 7.3), 35°C	[63]
Cholesterol oxidase in *Nocardia rhodochrous*	C12E5, 30°C	[64,65]
Thyrosinase in mushroom	Triton X-114 (pH 7.3), 37°C	[66]

30°C. Phase separation at higher temperatures is not favorable for handling thermolabile enzymes. It is desirable to conduct phase separation at a temperature close to 0~4°C. Several investigators have been successful in lowering the cloud point of non-ionic surfactants for protein extraction [27,71,73–75].

The combined use of Triton X-114 with Triton X-45 which has a cloud point considerably lower than Triton X-114 was tested [71]. As a result, the cloud point could freely be controlled, in a temperature range from 0–23°C.

Salts facilitated lowering of cloud points of non-ionic surfactants. Ammonium sulfate was used not only for lowering cloud points of non-ionic surfactant but also for inducing phase separation in micellar solutions of anionic and zwitterionic surfactants [72,73]. Meanwhile, for fractionation of hydrophobic proteins in solutes from cells, the micelle-mediated extraction was repeated in a stepwise manner at a fixed and various levels of ammonium sulfate in a batch. This method is analogous to the classical precipitation methods by ammonium sulfate [72].

Polyols such as sugars [76] and glycerol [74], and water-soluble polymers [76] are very efficient additives for allowing low-temperature phase separation of Triton X-114. Werck-Reichhart et al. [74,75] used glycerol for reducing cloud point in the extraction of cytochrome b_5 and *P*450 from plant microsomes. Glycerol and sugars have frequently been used for stabilizing proteins, thus they may be a good choice with a view to preventing denaturation of labile proteins. As described later, water-soluble polymers could be employed not only for lowering cloud points but also for inducing phase separation of alkylglucosides and for controlling extractability of proteins [27].

Even in the presence of sugars or glycerol, a considerable loss in enzyme activity was often observed during solubilization or phase separation, when Triton X-114 was used [74,75]. The decrease in the enzyme activity is probably due to strong hydrophobic nature of Triton X-114 and to the presence of highly concentrated surfactant aggregates in the surfactant-rich phase. Enzyme inactivation is also ascribable to the presence of some oxidized impurities in reagent Triton X-114.

An additional problem is the small numbers of non-ionic surfactants employable. They are exclusively limited to polyoxyethylene alkyl ether type, such as Triton X-114. In the micelle-mediated extraction, the most appropriate non-ionic surfactant should be chosen for a specific protein with respect to extraction yield, selectivity, denaturing character for proteins, and so on. Other non-ionic surfactants must be explored for use in the micelle-mediated extraction, and our recent studies [27] on the polymer-induced phase separation with alkylglucosides which are accepted to be mild solubilizing reagents will be discussed in the next section.

5.4. Phase separation induced by water-soluble polymer

It is accepted that polyethylene glycol (PEG) causes separation into two phases when mixed with a micellar solution of a non-ionic surfactant [17,26,27]. As is seen in phase diagrams in Figs. 7 and 8 for micellar solutions of octyl-β-D-glucoside (OG) and octyl-β-D-thioglucoside (OTG), PEG, Dx and DEAE–Dx are capable of inducing phase separation in a wide temperature range at any levels of the water-soluble polymer concentrations [27]. In Fig. 7, micellar solutions of OG, as an example, are present in the region above the curve, and the two

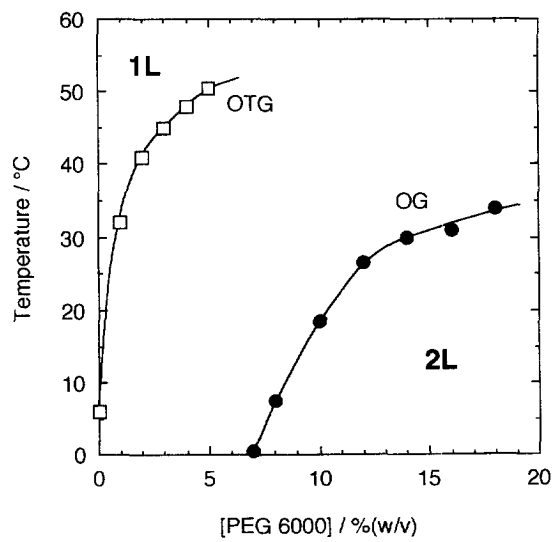


Fig. 7. Phase diagram for 2% (w/v) OG and OTG as a function of PEG 6000 concentration (1L and 2L as in Fig. 1). (●) OG; octylglucoside; (□) OTG; octylthioglucoside. (Taken with permission from Ref. [27]).

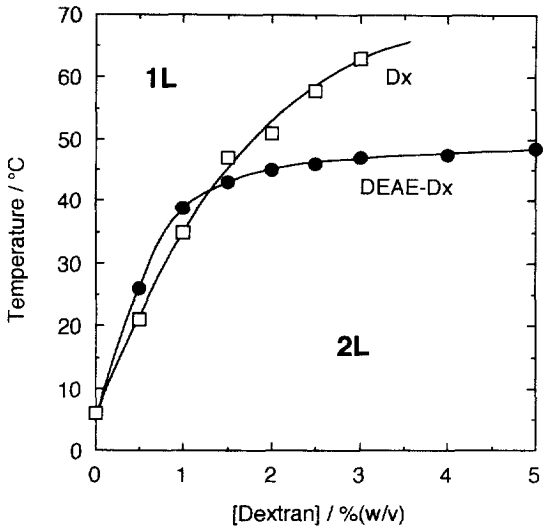


Fig. 8. Phase diagram for OTG as a function of Dx and DEAE–Dx concentrations (1L and 2L as in Fig. 1). (□) Dx; dextran; (●) DEAE–Dx; diethylaminoethyl–dextran. (Taken with permission from Ref. [27]).

phases are present in the region below the curve (upper consolute boundary). In the latter, one is an aqueous phase in which PEG is almost retained and the other an OG-rich phase. In contrast, a lower consolute boundary is present in the phase diagram for Triton X-114 in Fig. 1.

Hence, the phase separation can be made at any desired temperatures in an appropriate combination of a non-ionic surfactant and a water-soluble polymer. The polymer-induced phase separation makes it possible to conduct whole process from solubilization to phase separation at 0~4°C, a temperature of the least possibility of denaturation. Other water-soluble polymers, such as methylcellulose, polyvinylpyrrolidone and Ficoll, also induce the phase separation in micellar solutions of a variety of non-ionic surfactants [27].

Bacteriorhodopsin, cytochrome b_5 (b_5), peroxidase, and cytochrome *c* were used for testing the effectiveness of the polymer-induced phase separation of alkylglucosides [27]. Table 3 lists the volume fractions of the two phases, extraction yields (%) of the proteins, and their concentration factors. Two hydrophobic proteins, bacteriorhodopsin and b_5, are extractable, indicating their high concentration factors.

One of the advantages of the polymer-induced phase separation is a relatively higher CMC value of alkylglucoside than that of Triton X-114. The CMC values of OG and OTG are 25 [77] and 9 m*M* [78], respectively, while that of Triton X-114 is 0.2 m*M* [79]. In this respect, OG and OTG can by dialyzed more readily than Triton X-114. A simple expedient of dialyzing a micellar solution will be effective for isolation of surfactant-free proteins.

In the polymer-induced phase separation, extraction can be conducted at 0°C, thus reducing the possibility of denaturation of thermolabile proteins even in a highly concentrated solution of OG or OTG. The denaturation is, in general, dependent on the nature of surfactants and on their concentrations.

Fig. 9 shows the stability of bacteriorhodopsin in the Triton X-114-rich phase at 30°C and in the OTG-rich phase at 0°C by monitoring absorbance at 540 nm [27]. Decreasing absorbance indicates the denaturation of the protein. The absorbance in Triton X-114 decreased faster than in OTG, thus indicating stability of bacteriorhodopsin in the latter. This fact is attributable to differences in the hydrophobic nature of the two surfactants and in the two temperatures for phase separation.

Table 3
Extractability of hydrophilic and hydrophobic proteins in polymer-induced phase separation

	Volume fraction (%)		Extraction (%)				Concentration factor	
	AP	SRP	BR	b_5	POD	*C*	BR	b_5
2% (w/v) OTG 2% (w/v) PEG 6000	95.6	4.4	64	94	4	3	13.1	21.3
2% (w/v) OTG 2% (w/v) Dx	92.7	7.3	52	87	1	3	7.1	11.9

Solutions contain potassium phosphate (0.025 *M*, pH 6.90). AP: aqueous phase; SRP: surfactant-rich phase; BR: bacteriorhodopsin; b_5: cytochrome b_5; POD: peroxidase; *C*: cytochrome *c*. (Taken with permission from Ref. [27]).

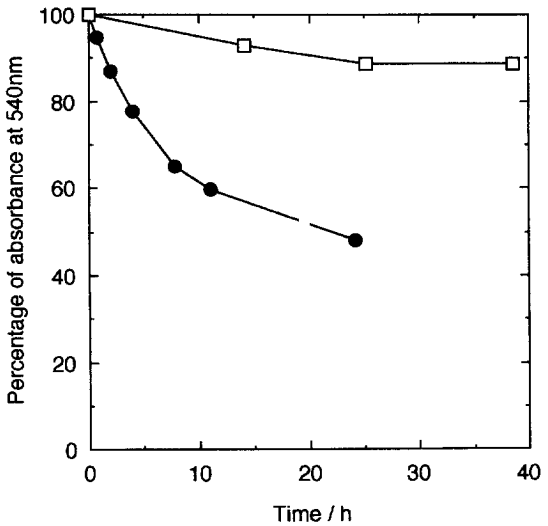


Fig. 9. Denaturation of bacteriorhodopsin in surfactant-rich phase. (●): change in relative absorbance in Triton X-114-rich phase at 30°C; (□): change in relative absorbance in OTG-rich phase at 0°C. The absorbance was measured after complete solubilization of bacteriorhodopsin is 0.83 mg ml^{-1}. (Taken with permission from Ref. [27]).

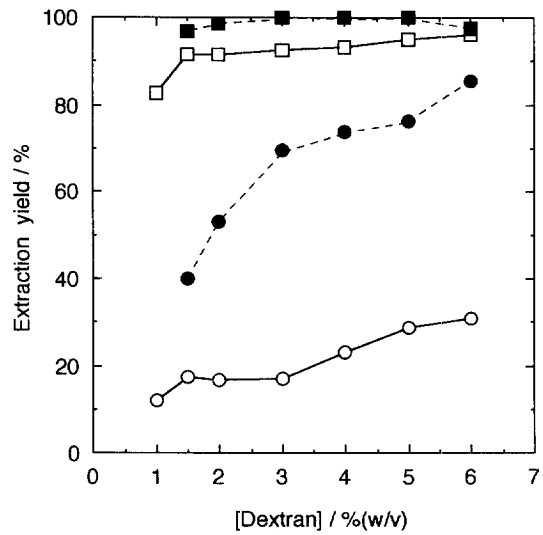


Fig. 10. Extraction yields of *P*450 and b_5 as a function of concentration (%, w/v) of DEAE–Dx. (□) and (■): *P*450; (○) and (●): b_5. Dotted lines and filled symbols represent extraction yields when Dx was used. (Taken with permission from H. Tani, T. Saitoh, T. Kamidate, T. Kamataki and H. Watanabe, unpublished data submitted for publication to Biotechnol. Bioeng., 1996)

Thus, the use of a water-soluble polymer will extend the range of choice of a non-ionic surfactant, independently of its cloud point, which is desirable in view of the denaturation.

5.5. Enhanced selectivity in micelle-mediated extraction

In the micelle-mediated extraction of proteins, the extractability is mainly determined by the hydrophobicity of proteins [80]. Thus, applications have been exclusively concentrated to separation of hydrophobic membrane proteins from cytoplasmic proteins. There are very few studies for controlling extractability by introducing other functions into the micelle-mediated extraction [81–83].

Saitoh and Hinze [81] were the first to introduce a hydrophobic affinity ligand for extraction of hydrophilic proteins. In combination of octyl-β-D-glucoside as an affinity ligand with a zwitterionic surfactant, 3-(nonyldimethylammonio)propylsulfate, hexokinase, hydrophilic enzyme, was successfully extracted into the surfactant-rich phase.

In the polymer-induced phase separation with alkylglucosides, extractability of hydrophobic proteins was controlled by the use of charged polymer [83]. In Fig. 10 the extraction yields of cytochrome *P*450 (*P*450) and b_5 as a function of Dx or DEAE–Dx concentrations are presented in the polymer-induced phase separation with OTG. Dx is effective for both extractions of *P*450 and b_5. In contrast to Dx, DEAE–Dx markedly reduced the extraction yields of b_5, indicating the selective DEAE–Dx interactions with b_5 under aqueous conditions (pH 7.4) in Fig. 10. On the other hand, DEAE–Dx gave only slight effect on the extraction of *P*450.

Fig. 11 shows pH dependence of the valences of *P*450 and b_5 which are calculated from acid dissociation constants of amino acid residues. At pH 7.4, *P*450 is almost neutral, whereas b_5 is negatively charged. Thus, a decrease in the extraction yield of b_5 is indicative of the electrostatic interactions between positively charged DEAE–Dx and negatively charged b_5 in the aqueous phase. Meanwhile, a slight decrease in the extraction yield of *P*450 is ascribed to the limited presence of charged species of *P*450 at pH 7.4.

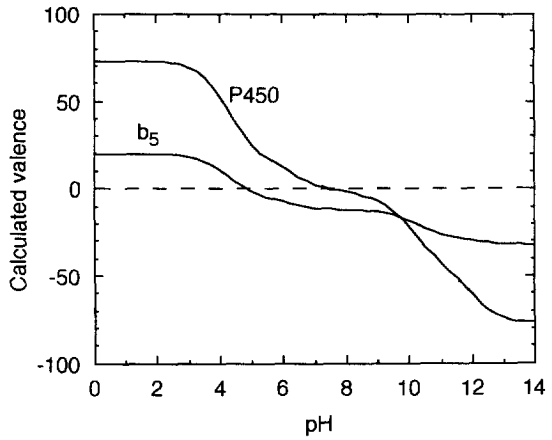


Fig. 11. Valency of *P*450 and that of b_5 calculated from amino acid sequences as a function of pH. The amino acid sequences are rat liver *P*450 (CYP2B1) [84] and b_5 [85]. (Taken with permission from H. Tani, T. Saitoh, T. Kamidate, T. Kamataki and H. Watanabe, unpublished data submitted for publication to Biotechnol. Bioeng., 1996)

DEAE–Dx could serve dual functions of inducing the phase separation and of preventing the entry of b_5 to the OTG-rich phase. Thus the charged polymer-induced phase separation provides a good means for highly-efficient separation of membrane proteins under mild conditions which would be physiologically acceptable for thermolabile membrane proteins.

More recently, extraction of hydrophilic proteins was investigated on the basis of the polymer-induced phase separation in combination with a charged surfactant or a charged polymer [82]. Phase separation in micellar solutions of pentaethyleneoxide dodecyl ether was made by addition of dextran. The charged surfactant used is sodium dodecyl sulfate or dodecyl trimethyl ammonium chloride, and the charged polymer dextran sulfate. In this system, hydrophilic proteins were distributed into the surfactant-rich phase according to the electrostatic repulsion for the charged polymer in the aqueous phase or the attraction to the charged surfactant in the surfactant-rich phase.

6. Conclusion

In the present review, we have covered several important advances in the micelle-mediated extraction that have been employed mainly in inorganic trace analysis and in protein separations to which applications are being concentrated.

In inorganic analyses, the micelle-mediated extraction has mainly been used for extraction of metal chelates in combination with spectroscopic quantitation. The surfactant-rich phase is converted to aqueous micellar mixtures having a cloud point higher than room temperature, and thus the mixtures are submitted to further spectroscopic measurements and micellar chromatographic separations. Hence, the micelle-mediated extraction seems to be useful also for trace analysis of organic species of environmental concern as well as inorganic species.

Recent progress on the micelle-mediated extraction is in the use of a water-soluble polymer for phase-separation in micellar solutions of non-ionic surfactant. This polymer-induced phase separation would provide a new route for isolation of hydrophobic proteins. This is because the water-soluble polymer plays dual roles of inducing phase separation in the micellar solutions at a lower temperatures and of controlling the extractability of the proteins. Hence, solubilization and phase separation in processing thermolabile proteins can be made successively at 0°C with the least possibility of denaturation. Water-soluble polymers are also very efficient for extending the range on the choice of non-ionic surfactants, independent of their cloud point. Combined use of a charged polymer or a charged surfactant must be fruitful for further developments of the micelle-mediated extraction for hydrophilic proteins as well as hydrophobic proteins.

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