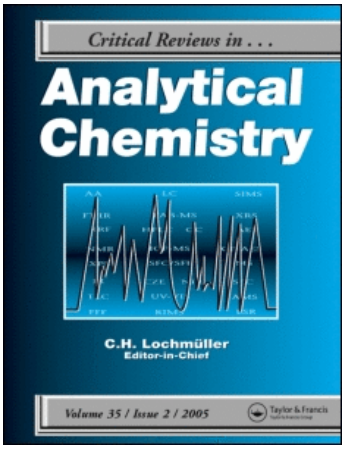

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A Critical Review of Surfactant-Mediated Phase Separations (Cloud-Point Extractions): Theory and Applications

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A Critical Review of Surfactant-Mediated Phase Separations (Cloud-Point Extractions): Theory and Applications

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ABSTRACT: The general concept of using the unique phase separation behavior of some surfactant micelle solutions as a means for extractive separation is outlined and described. Next, the specific micellar parameters and phase separation behavior of nonionic and zwitterionic charge-type surfactant solutions are summarized. In addition, the phase behavior of some derivatized B-cyclodextrin solutions is briefly described. The specific applications of such systems and their phase behavior for the extractive preconcentration, separation, and/or purification of metal chelates, biomaterials, and organic compounds are summarized and discussed. The potential use of affinity ligands in conjunction with the cloud-point approach for selective bioextractions is also mentioned. The experimental protocols, advantages, and limitations of the different cloud-point extraction techniques are outlined. The use of zwitterionic as opposed to nonionic surfactant media in such separations is compared and contrasted. In addition, extractions based on use of aqueous two-phase polymer systems are compared and contrasted to extractions employing the aqueous surfactant systems. Some areas for further work are identified. The use of such phase separation behavior in environmental cleanup applications is described. Last, some general experimental considerations with respect to surfactant purity and analyte recovery in such systems are presented.

KEY WORDS: surfactants, micelles, cyclodextrins, phase behavior, cloud point, cloud-point extractions, surfactant-rich phase, metal chelates, biomaterials, proteins, organic pollutants, preconcentration, affinity ligands, solubilization.

I. INTRODUCTION

Aqueous (normal) surfactant micellar systems[1-5] have been employed with success in almost every facet of analytical chemistry, ranging from applications in spectroscopy, electroanalytical chemistry, and separation science. Overviews of micelles, their properties, and use in such analytical applications are summarized in a recent review article[6] and monograph.[7] Some of the more important and practical applications of micelles seem to lie in the area of separation science.[8-13] For example, aqueous micellar media have been utilized as the mobile phase additive in thin layer and high performance

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liquid chromatography as well as the "active discriminating agent" in the electrolytic medium for electrokinetic capillary electrophoretic separations. However, extractive separation, preconcentration, and purification schemes based upon the unique phase separation behavior of aqueous solutions of surfactant micellar systems appear to have been largely neglected despite the demonstrated success and potential advantages of the technique compared to conventional liquid-liquid extractions.

The basis of the phase separation (or cloud-point) extraction technique, initially reported by Watanabe,[14] stems from the well-known phase phenomenon exhibited by some surfactant micellar solutions. Namely, upon appropriate alteration of the conditions (i.e., temperature or pressure change, addition of salt or other additive, etc.), the separation of an aqueous surfactant micellar solution into a concentrated phase containing most of the surfactant (termed surfactant-rich, micellar, or coacervate phase) and a dilute aqueous phase containing low concentration of surfactant is observed. Any component(s) originally present that binds to the micellar aggregate in solution can thus be extracted from the original solution and concentrated in the small volume element of the surfactant-rich phase.

Figure 1 depicts the usual steps involved in the application of such a surfactant micellar-mediated phase-separation (or cloud-point) extraction process. First, surfactant (or a concentrated surfactant solution) is added to the aqueous solution containing the component(s) to be extracted and/or preconcentrated. The amount of surfactant added must be such to ensure for the formation of micelle aggregates in the solution [i.e., the final surfactant concentration must exceed the critical micelle concentration (CMC) of that surfactant]. Any species that associates and binds to the micellar entity in solution can be subsequently extracted to differing extents depending upon the strength of the micelle-solute binding interaction. Next, the conditions are altered (temperature increase or decrease, addition of salt or another surfactant) to ensure that the micellar solution separates into the surfactant-rich (micellar or coacervate) phase and the dilute aqueous phase. Centrifugation can be employed if required in order to speed up the separation of the two phases. The surfactant-rich phase containing the extracted component(s) can then be subjected to further fractionation or quantitation steps as desired. Extraction of an analyte from a solid sample can be affected in a similar fashion by first merely adding the surfactant micellar-containing solution and allowing adequate time so that the desorption of the analyte(s) from the solid matrix can occur (sonication can sometimes speed up this process) and then following the subsequent steps as outlined.

Although this surfactant-mediated phase separation (cloud-point extraction) as a topic has been briefly mentioned in general reviews concerning micelles in separation science,[8-13] no comprehensive overview of the phase behavior of surfactant micellar solutions nor summary of the different types of applications, advantages, and limitations of separations based upon such phase-separation behavior has been published. The purpose of this paper is to provide a review of the phase behavior of several different types of surfactant micellar and related systems and then summarize the published applications that have utilized such surfactant micellar phase behavior in extraction, preconcentration, and/or purification schemes. Examples will be given for the extraction of inorganic species as well as for organic and biological compounds. In addition, the potential utility of such cloud-point extraction schemes for environmental cleanup procedures will be briefly discussed. The advantages, limitations, and future potential of the technique will also be considered.

II. SURFACTANT AND RELATED SYSTEMS AND THEIR PHASE BEHAVIOR

In this section, a brief discussion of liquid-liquid phase separation in different surfactant micellar systems will be presented. At

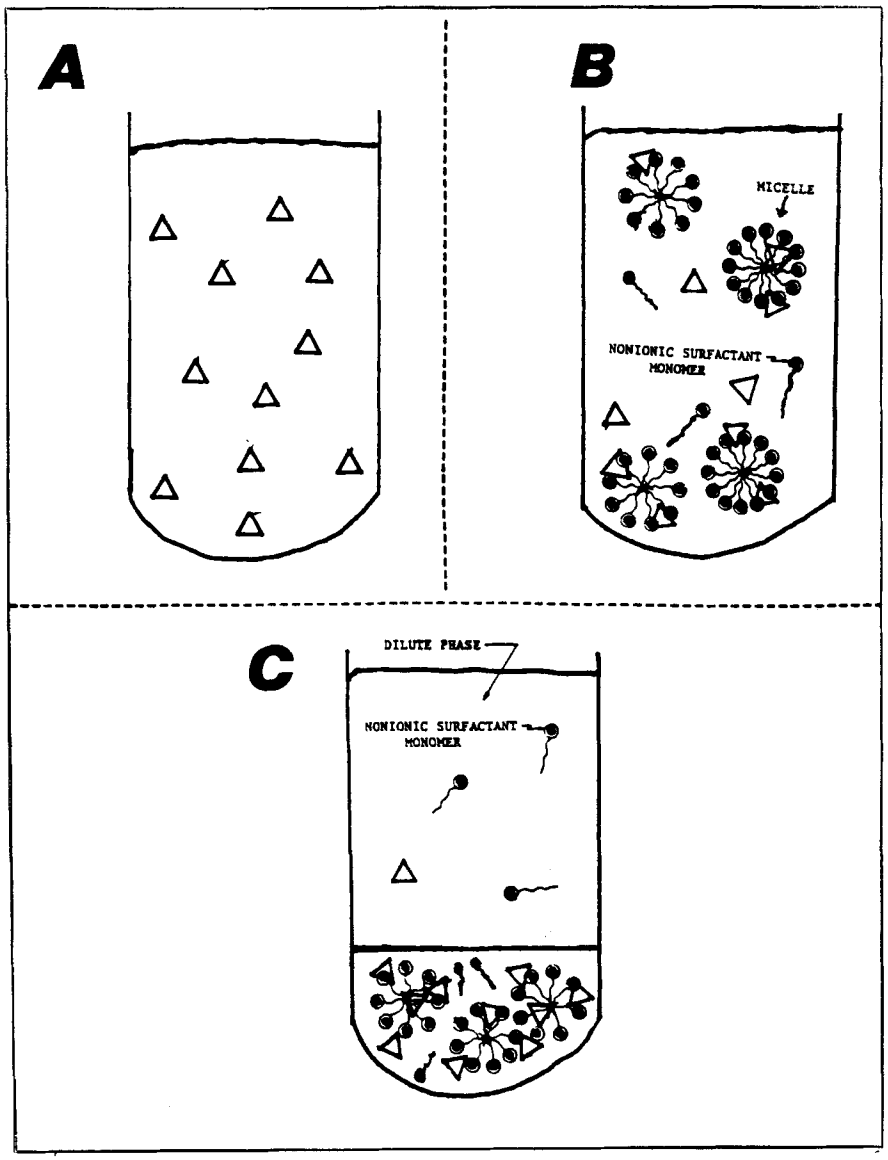


FIGURE 1. Pictorial schematic representation of the micellar-mediated phase separation (cloud-point extraction) technique: (A) the initial solution containing the hydrophobic species (△) to be extracted and/or preconcentrated; (B) the situation after the addition of a micelle-forming surfactant ([surfactant] > CMC) in which the hydrophobic species can become associated with and bind to the micellar aggregates thus formed; (C) the final phase-separated system formed after appropriate alteration of the conditions (temperature change or added salt) and centrifugation in which the hydrophobic species has been concentrated in the small volume element of the surfactant-rich phase and separated from the dilute aqueous phase.

amphiphile surfactant concentrations below ca. 15% (w/w), micellar solutions typically exist as homogeneous isotropic liquid phases.[15] Liquid-liquid phase separation and critical phenomena can be induced in this concentration region by changing the temperature, salt concentration, pressure, and other solution conditions. In many such phase separations, a single isotropic micellar phase (L) separates into two isotropic phases (2L),

both of which contain surfactant and water, but differ in total amphiphile concentration.

There are several situations in which surfactant solutions occur in equilibrium with a separate, predominantly aqueous phase.[16] The best known example is probably the separation of a nonionic micellar phase above the cloud-point temperature of polyoxyethylene surfactant solutions.[17] Second, solutions of zwitterionic micelles have recently been shown to undergo temperature-dependent phase separation.[18] Third, as known for some time, the addition of salt to relatively concentrated ionic (anionic or cationic) surfactant micellar solutions can cause the separation of a surfactant-rich phase.[16,19] This includes the case in which mixtures of both an anionic and cationic surfactant can form two liquid phases at quite low surfactant concentrations.[16] Although separation of such colloidal systems into two liquid phases has been termed *coacervation*,[20] we will follow the terminology proposed by Rubingh and Holland[19] and restrict the use of this term for only one of these three general systems; namely, when the liquid-liquid phase separation is induced in an ionic surfactant micellar system by the addition of a simple electrolyte or another amphiphile, we will use the term coacervate.[19,21] The former two systems, i.e., nonionic and zwitterionic surfactant containing media, have been predominantly utilized in most published applications and thus will be the primary focus of this section.

It is worth noting that phase separation can also occur in some aqueous cyclodextrin-containing solutions. In addition, critical phenomena can be observed in reversed micellar and microemulsion systems. However, there have been few applications reported. Thus, these latter two systems will not be discussed because the focus is on aqueous surfactant micellar (or cyclodextrin) solutions and their phase behavior as applied to separation science. Last, liquid-liquid phase separations are well known to occur in many different types of polymer solution systems. Because such polymer systems and their use in separations have been the subject of two monographs,[22,23] they will not be further discussed in this paper.

A. Nonionic Micellar Media

Table 1 summarizes the structure, nomenclature, CMC, and aggregation number, N, of different nonionic surfactants that form aqueous (normal) micelles.[2,4,17,24-70] It is important to note that these CMC values can be altered by the presence of additives or impurities.[17,47,71,72] Figure 2 depicts the rough two-dimensional shape for a micellar aggregate formed from nonionic surfactants in aqueous solution.[7] A unique feature of aqueous solutions of many such nonionic surfactant micelles is that, when the temperature is raised, the solution becomes turbid in a narrow temperature range, referred to as the cloud point. Above the cloud point, the system separates into two isotropic phases. The phases appear to consist of an almost micelle-free dilute solution of the nonionic surfactant at a concentration equal to its CMC at that temperature and a surfactant-rich phase that appears only when the solution is above its cloud point. The phase separation is reversible and on cooling, the two separated phases merge to form a clear solution once again.

The temperature at which the phase separation occurs is a function of the surfactant concentration, so that one can define in the temperature vs. surfactant concentration plot a consolution curve that separates the one-phase region (L) from the two-phase region (2L). Such consolution (or coexistence) curves exhibit a minimum, referred to as the consolute point (critical point). The temperature and surfactant concentration at which the minimum occurs are referred to as the critical temperature (T_c) and critical concentration (C_c), respectively. A typical consolution curve (phase diagram) in the low-concentration range for a homologous series of C_iE_j surfactant solutions is presented in Figure 3. It is important to emphasize that the phase diagram may become much more complex and exhibit regions of anisotropic phases, liquid crystals, etc. at higher surfactant concentrations.[24,73] Figures 4 and 5 give the phase diagrams for solutions of PONPE-7.5 and Triton X-114, respectively, which have been the two most utilized nonionic surfactant

TABLE 1
Structure and Micellar Characteristics of Common Nonionic Surfactants[a]

Structure, name, and abbreviation	CMC[b], m*M*	*N*[c]
Type I: Polyoxyethylene glycol monoethers, $CH_3(CH_2)_{i-1}O(CH_2CH_2O)_jH$, C_iE_j		
C_4E_1		3[25,d]
C_6E_3	68;[26] 92–97;[27] 100[24]	57[27]
C_6E_4	72[26]	—
C_6E_5	94–109[27]	53–55[27]
C_8E_1	4.9[28]	—
C_8E_3	6.1;[29] 5.6;[e] 6.5;[26] 7.8[12]	—
C_8E_4	6.5;[26] 8.5;[24] 8.0–8.4[27]	82;[24] 147;[27] 85[25]
C_8E_5	6.8;[26] 9.2;[24] 6.0;[30] 9.1;[31] 9.2[12]	80; 130[25]
C_8E_6	9.8;[26] 9.9;[24] 8.6[31]	32;[24] 39[31,32]
C_8E_8	9.3–10.4[27]	41; 72[27]
C_9E_{15}	0.11[33]	80[33]
C_9E_{20}	0.14[33]	140[33]
$C_{10}E_4$	0.64; 0.81;[26] [190][34,f]	[30][34,f]
$C_{10}E_5$	0.86;[e] 0.90;[24] 0.69[31]	—
$C_{10}E_6$	0.83;[31] 0.95;[24] 0.90;[35] [220][34]	73;[30] 76;[32] [30][34]
$C_{10}E_7$	0.90;[35] 0.88[31]	60[35]
$C_{10}E_8$	0.92;[31] 1.00;[24] 1.03;[35] 1.00[36]	70
$C_{11}E_8$	0.30[36]	—
$C_{12}E_2$	0.033[37]	—
$C_{12}E_3$	0.058;[12] 0.052;[37] [44][38]	—
$C_{12}E_4$ (Brig 30)	0.023; 0.04; 0.064;[37] 0.047;[31] 0.08;[26] [40–50][34,38]	[40][34]
$C_{12}E_5$	0.065;[38] 0.045;[39] 0.065;[24] 0.058;[40] 0.049;[31] [47–58][38]	160
$C_{12}E_6$	0.064;[31] 0.069;[38] 0.068;[24] 0.060;[39] 0.087;[26] [39–50][34,38]	105;[32] 110–140; [40][34]
$C_{12}E_7$	0.067;[31] 0.080;[37] 0.069[24,41]	—
$C_{12}E_8$	0.11;[44] 0.067;[31] 0.070;[41] 0.071;[24] 0.11;[32] 0.07–0.08[42]	62–120;[43] 120;[32] 123[32]
$C_{12}E_{10}$	0.09[39]	—
$C_{12}E_{12}$	0.093;[32] 0.14[40]	81[32]
$C_{12}E_{13}$ (Emulgen 120)	0.20[e]	75
$C_{12}E_{23}$ (Brij 35)	0.09–0.10;[45] 0.091;[32] 0.060[46]	40[32,45]
$C_{12}E_{25}$ (Emulgen 147)	0.10[e]	39[e]
$C_{13}E_8$	0.027[36]	—
$C_{14}E_5$	0.009[31]	—
$C_{14}E_6$	—	127[32]
$C_{14}E_8$	0.009;[24,36] 0.0063[41]	—
$C_{15}E_8$	0.0035[36]	—
$C_{16}E_8$	0.00047;[30] 0.00056;[41] [11][38]	2800[25,g]
$C_{16}E_{10}$ (Brij 56)	0.0006[47]	624[47]
$C_{16}E_{18}$	0.00027[47]	372[47]
$C_{16}E_{30}$	0.00012[47]	77[47]
Type II: Polyoxyethylene methyl-*n*-alkyl ethers, $CH_3(OCH_2CH_2)_mO(CH_2)_nH$, $C_iE_mC_n$		
$C_1E_2C_{12}$	−4.12[46,h]	—
$C_1E_{13}C_{12}$	−3.9 to −4.0[48,h]	—
$C_1E_2C_{14}$	−4.46[46,h]	—
$C_1E_{13}C_{14}$	−4.85[48,h]	—
$C_1E_{40}C_{14}$	−4.3[48,h]	—
$C_1E_{53}C_{14}$	−4.3[48,h]	—
$C_1E_2C_{18}$	−5.0[46,h]	—
$C_1E_2C_{23}$	−5.22[46,h]	—
$C_1E_8C_6$	−1.04[49,h]	—
$C_1E_8C_8$	−2.28[49,h]	—
$C_1E_8C_{10}$	−2.87[49,h]	—
$C_1E_8C_{12}$	−3.81[49,h]	—

TABLE 1 (continued)
Structure and Micellar Characteristics of Common Nonionic Surfactants[a]

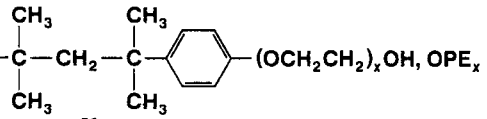
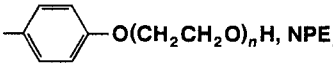
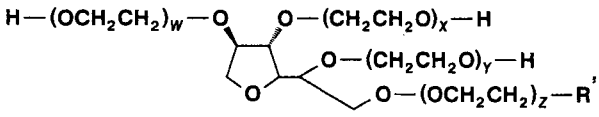
Structure, name, and abbreviation	CMC[b], m*M*	*N*[c]
Type III: *t*-Octylphenoxy polyoxyethylene ethers,  OPE$_x$		
OPE_1	0.0495[50]	—
OPE_2	0.0765[50]	—
OPE_3	0.103;[50] 0.19[51]	—
OPE_5 (Triton X-45)	0.172;[50] 0.11[52]	—
OPE_7	0.268;[50] 0.220[51]	—
OPE_{7-8} (Triton X-114)	0.20;[52] 0.35[53]	—
OPE_8	0.283[50]	—
OPE_{9-10} (Triton X-100; Igepal CA-630)	0.17; 0.30;[e] 0.25;[54] 0.24[31,52] [0.29; 0.39; 0.60; 0.99][54,58,i]	43–66;[55] 100–140;[56] 140[31]
$OPE_{9-10red}$ (reduced Triton X-100)	0.21;[30] 0.25[57]	140[30]
OPE_{10}	0.24;[51] 0.323[50]	—
OPE_{12} (Igepal CA-720)	0.23	—
OPE_{12-13} (Triton X-102)	0.30–0.40;[52] 0.25[54]	121
OPE_{15}	0.418[50]	—
OPE_{30} (Triton X-305)	0.77[54]	26
OPE_{40} (Triton X-405)	0.81[59]	—
Type IV: Polyoxyethylene nonyl phenyl ethers,  NPE$_n$		
NPE_5	0.57[e]	—
NPE_6	[0.00313][64,j]	—
$NPE_{7.5}$ (PONPE-7.5)	0.085[60]	—
NPE_{8-9} (Igepal CO-610)	0.08[12]	—
NPE_{9-10} (Triton N-101)	0.085[59]	100
NPE_{10}	0.085;[61] [0.0055][64,j]	100[24]
$NPE_{10.5}$ (Tergitol NP-10)	0.054	—
NPE_{15} (Igepal CO-730)	0.12;[61] [0.0092][64,j]	52[24]
NPE_{20} (Igepal CO-850)	0.155;[61] [0.0145][64,j]	—
NPE_{20} (brominated)	[0.01027][64,j]	—
Type V: Polyoxyethylene sorbitan esters of fatty acids,  Tweens		
PEG(20) sorbitol monolaurate (Tween 20)	0.04;[44] 0.059[59]	—
PEG(20) sorbitol monopalmitate (Tween 40)	[29][59,k]	—
PEG(20) sorbitol monostearate (Tween 60)	[27][59,k]	—
PEG(20) sorbitol monooleate (Tween 80)	0.012;[59] 0.0145;[62] 0.01–0.02[63]	58[32]
Brominated Tween 80	0.0185[62]	—
PEG(20) sorbitol trioleate (Tween 85)	0.007[62]	—
Brominated Tween 85	0.015[62]	—

TABLE 1 (continued)
Structure and Micellar Characteristics of Common Nonionic Surfactants[a]

Structure, name, and abbreviation	CMC[b], m*M*	*N*[c]
Type VI: Miscellaneous system		
A. R — CHOH — CH_2 — CH_2OH		
1,3-Undecanediol	$-2.64^{28,h}$	—
1,3-Tridecanediol	$-3.85^{28,h}$	—
1,3-Pentadecanediol	$-4.87^{28,h}$	—
B. $CH_3O(CH_2)_6CH(OCH_3)CH(OCH_3)(CH_2)_8(OCH_2CH_2)_xOH$		
$A1E_6$ ($x = 6$)	$-2.10^{65,h}$	—
$A1E_8$ ($x = 8$)	$\geqq -2.0^{65,h}$	—
C. Tergitols		
$C_mH_{(2m+1)}$ / $C_nH_{(2n+1)}$ >— $(OCH_2CH_2)_9OH$ (Tergitol 15-S-9)	$0.021^{66,l}$	—
>— $(OCH_2CH_2)_{10}OH$ (Tergitol TMN-10)	$0.16^{66,l}$	—
D. Dodecanediyl-1,12-*bis*(polyoxyethylene(*n*)monoethers)		
$C_{12}2E_3$	—	40^{67}
$C_{12}2E_4$	—	23^{67}
E. F — C_nF_{2n} — CH_2O — $(CH_2CH_2O)_p$ — Z; C_nE_pZ		
C_6E_3H	$-3.96^{68,h}$	—
C_6E_4H	$-3.78^{68,h}$	—
C_6E_5H	$-3.62^{68,h}$	—
$C_6E_5CH_3$	$-3.89^{68,h}$	—
C_7E_4H	$-4.39^{68,h}$	—
F. Surfactant alkyl crown ethers and cryptands		
2-*n*-Tetradecyl-[2.2.2]-cryptand	0.14^{69}	—
Steroidal Lariat crown ether[m]	0.40^{69}	—
Octyl-15-crown-5	2.5^{70}	—
N-Octyl-monoazo-15-crown-5	0.86^{70}	—

[a] Micellar parameters taken from indicated references are for aqueous solutions at 25°C unless otherwise indicated.
[b] Critical micelle concentration, CMC, values given in millimoles per liter (m*M*) unless otherwise noted.
[c] N = aggregation number, i.e., the number of surfactant monomer molecules per micellar aggregate.
[d] Value at cloud point, 44.5°C.
[e] Authors' unpublished results.
[f] Solvent is formamide rather than water.
[g] Value at cloud point, 65.5°C.
[h] Value given is log CMC (mol / dm^3).
[i] CMC values for Triton X-100 in 10, 20, 30, and 40%. (v / v) ethylene glycol:water solution.
[k] CMC value given in milligrams per liter.
[j] CMC value given in grams per deciliter.
[l] CMC value given as percentage (w / v).
[m] Refers to *N*-(3-cholesteryloxycarbonylmethyl)-aza-18-crown-6 (see Reference 69).

micellar systems employed in cloud-point extractive procedures.

A compilation of cloud-point temperatures for aqueous solutions of nonionic surfactants is summarized in Table 2. (References 24, 25, 74, and 75 give an extensive list of lower critical points.) As can be observed, the temperature at which clouding occurs depends on the structure of the nonionic surfactant. For a homologous series of polyoxyethylated nonionic surfactants, the cloud point increases with decreasing length of the hydrocarbon chain and increasing length of the oxyethylene chain (refer to Table 2 and Figure 3). At a constant oxyethylene content in the surfactant molecule, the cloud point is lowered by the following: decreased molecular mass of the surfactant, branching of the hydrophobic group, replacement of the terminal hydroxyl moiety of the hydrophilic

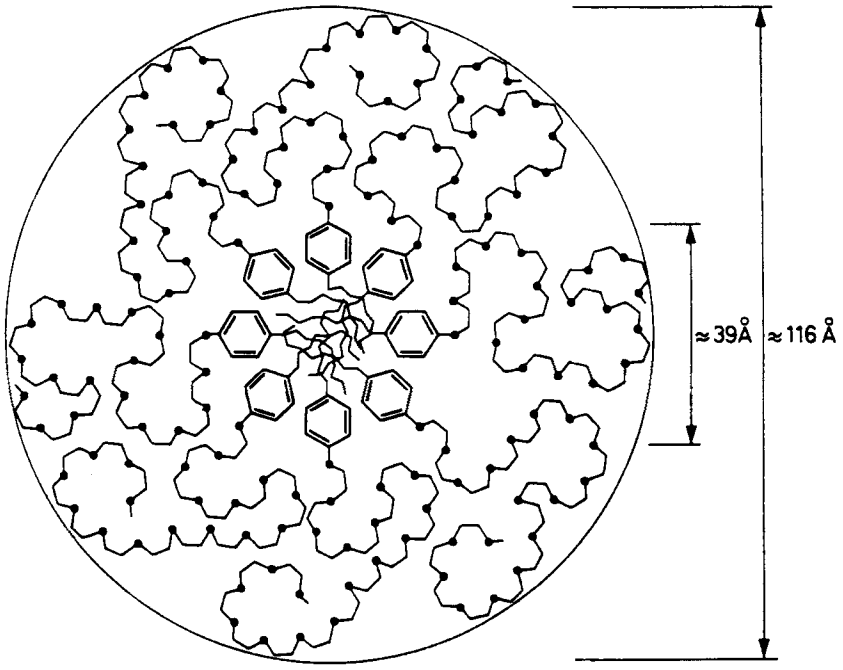


FIGURE 2. Schematic representation of the idealized micelle of polyoxy thylene (16 – 18)-4-nonylphenylether (NPE_{16-18}) showing the rough dimensions of the inner hydrophobic core region formed by the nonylphenyl moiety and the more diffuse, hydrophilic region comprised of the oxyethylene groups which extend into the bulk aqueous solution. (Reproduced with permission from Pfuller, U. *Mizellen, Vesikel, Mikroemulsionen*; VEB Verlag: Berlin, 1986; p. 58, Springer-Verlag.)

group by a methoxyl group, and broader distribution of polyoxyethylene chain length in commercial preparations.[4]

It is important to stress that the cloud point of a given nonionic surfactant can be altered (either increased or decreased) by the presence or addition of other materials (i.e., salts, alcohols, other organic additives, etc.) as can be observed from the data presented in Table 3.[2,69,95-112] For example, the addition of most neutral electrolytes (e.g., chlorides, sulfates, carbonates) typically depresses the cloud point due to their salting-out effect in proportion to their concentration, with the effect of a given salt depending on the hydrated radii of both ions.[4,100,104-109] The lower the lyotropic number of the electrolyte, the greater the effect. On the other hand, salting-in-type electrolytes, such as nitrates, iodides, and thiocyanates, typically increase the cloud point.[104-109] The addition of shorter saturated hydrocarbons generally does not lower the cloud point very much, whereas more nonpolar organic compounds that can be solubilized in the interior of the micelle normally raise the cloud point.[99] The presence of many protein denaturants (such as urea or substituted ureas) also increases the cloud point.[98,115] Polar organic compounds, such as aliphatic alcohols, fatty acids, or phenols[96,97,103] typically depress the cloud point remarkably.[95] Last, studies indicate that an increase in the pressure typically causes a slight increase in the cloud-point temperature of nonionic surfactant solutions.[110,111]

The cloud point of dilute nonionic surfactant solutions increases upon addition of charged ionic surfactants[19,76,80,81,91,101,102] (examples in Table 3). The data presented in Figures 5B and 6 demonstrate that the cloud-point temperatures of the nonionic surfactants Triton X-114 and Triton X-100, respectively, can be dramatically decreased

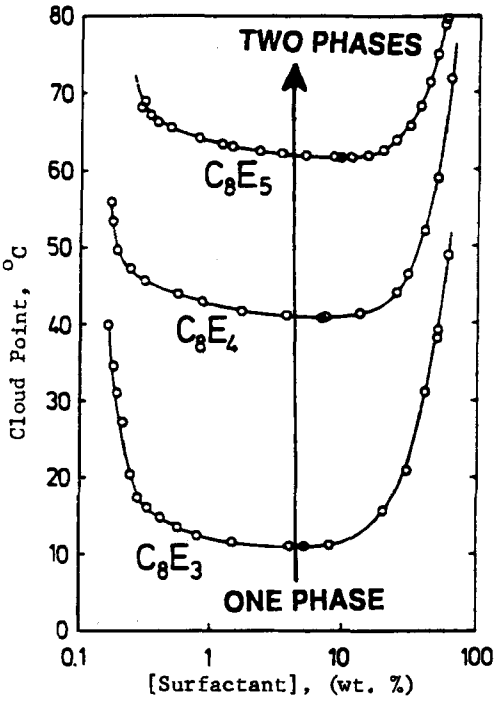


FIGURE 3. Cloud-point temperature (degrees Celsius) vs. surfactant concentration (wt %) for the three binary systems C_8E_3/H_2O, C_8E_4/H_2O, and C_8E_5/H_2O. An arrow is drawn to show the approach from single to two phases taken along the critical concentration at constant weight fraction of the surfactant. (Adapted with permission from Schubert, K.V.; Strey, R.; Kahlweit, M.J. *J. Colloid Interface Sci.* **1991**, *141*, 22, Academic Press, Inc.)

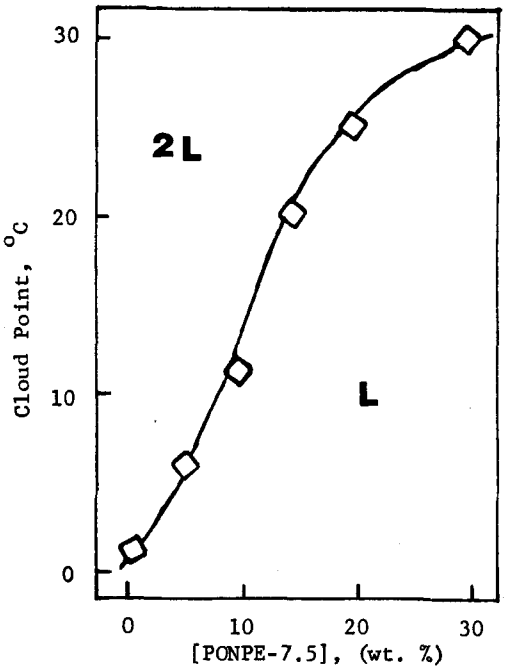


FIGURE 4. Variation of the cloud-point temperature (degrees Celsius) of solutions of the nonionic surfactant PONPE-7.5 ($NPE_{7.5}$) as a function of the surfactant concentration (wt %). L denotes the single isotropic amphiphilic solution phase region and 2L indicates the presence of two coexisting isotropic phases. (Reproduced with permission from Watanabe, H., in *Solution Behavior of Surfactants*; K.L. Mittal and E.J. Fendler, Eds.; Plenum Press: New York, 1982; p. 1308, Plenum Publishing Corp.)

by the addition of another nonionic surfactant, Triton X-45 or the polymer polyethylene glycol (PEG), respectively. In the former case, when a solution contains two different nonionic surfactants, the cloud point of the mixed solution is observed to be intermediate between that of the two pure nonionic surfactants involved. Thus, it is possible to obtain almost any desired cloud-point temperature for a given analytical extraction application via appropriate selection of the nonionic micellar-forming surfactant and/or the proper choice of additive.

Figures 3, 4, and 5 show the phase diagrams for the aqueous solutions of five nonionic surfactants. The phase diagrams of these nonionic surfactant solutions do not exhibit any anisotropic phases and have a relatively wide region in which a single isotropic phase (L) exists. In the low-concentration region, this isotropic phase is micellar, but clearly, the micellar structure cannot persist up to 100% surfactant concentration.[24] As yet, there is no agreement as to the maximum surfactant concentration at which micelles are still present nor is it clear what the nature of the microscopic structure(s) of such highly concentrated amphiphile solutions might be. In the region above the consolution curves (cloud-point curves) in the figures, it is usually stated in the literature that no micelles are present due to the fact that the surfactant molecules are segregated from the aqueous phase. However, many other reports provide evidence for the existence of micelles or other structured surfactant phases (lamellar, hexagonal, etc.) above the cloud point.[91,93] At

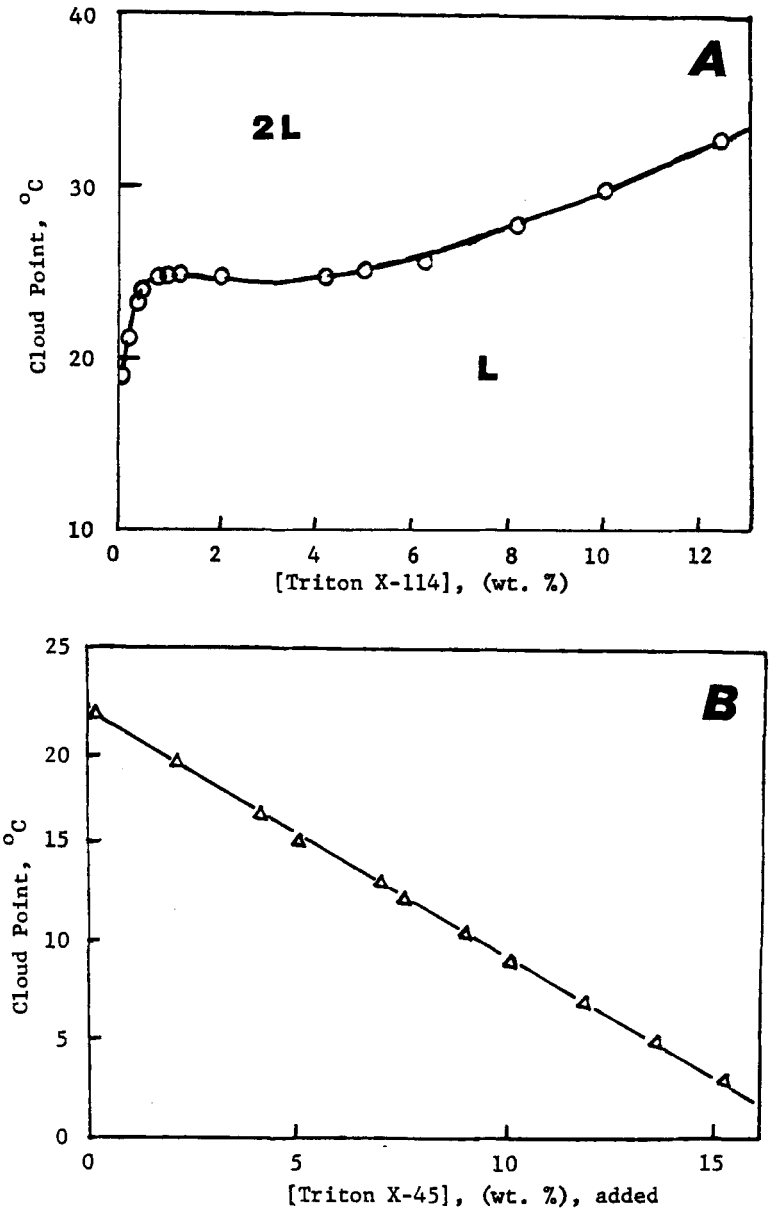


FIGURE 5. (A) Phase diagram of Triton X-114 in aqueous solution (L and 2L as defined in the legend to Figure 4). (Reproduced with permission from Laespada, M.E.F.; Pavon, J.L.P.; Cordero, B.M. *Analyst.* **1993**, *118*, 210. Copyright 1993, The Royal Society of Chemistry.) (B) Plot of the cloud-point temperature (degrees Celsius) of aqueous solutions of Triton X-114 (OPE_{7-8}) as a function of the concentration (wt %) of added nonionic surfactant, Triton X-45 (OPE_5). (Reproduced with permission from Ganong, B.R.; Delmore, J.P. *Anal. Biochem.* **1991**, *193*, 36, Academic Press, Inc.)

TABLE 2
Summary of Cloud-Point Temperatures for Nonionic Surfactant Micellar Solutions

Surfactant system[a]	Cloud point[b], °C
C_4E_1	44.5[25]
C_5E_2	34[25]
C_6E_2	0[25]
C_6E_3	45;[24] 40;[25] 46;[26] 39.6;[67] 44.7 (c = 13%)[77]
C_6E_4	59;[25] 66.1[26]
C_6E_5	75;[24,25] 78.4
C_6E_6	80;[24] 83[25]
C_8E_3	5–8;[24] 8;[25] 11[26]
C_8E_4	39.5;[25] 40.3;[c] 40;[24] 40.8[26]
C_8E_5	59.6;[c] 60;[24,25] 61.7[26]
C_8E_6	71–76;[24] 74;[24] 74.4[26]
C_8E_8	96[24]
$C_{10}E_3$	< 0;[25] 0[24]
$C_{10}E_4$	19.5;[86] 20.4;[25,26] 20.5;[15] 21[24]
$C_{10}E_5$	42.4;[c] 43.6;[15] ca. 44;[78] 45;[24] 46[25]
$C_{10}E_6$	59–63;[24] 58.0;[15] 60;[40] 62.18;[86] 63[25]
$C_{10}E_7$	76[24]
$C_{10}E_8$	84.5;[25] 85[24]
$C_{12}E_3$	< 0;[25] 0;[25] [18][38,d]
$C_{12}E_4$	2;[25] 3.5;[15] 4;[24,78] 6.6;[27] 7.0[c]
$C_{12}E_5$	27;[78] 31;[24] 32;[24,25] 30.5;[77,e] 31.5–32.2[c]
$C_{12}E_6$	50;[24] 50.3;[79] 51.3;[26] 52.5;[25] 53.2;[80] 48.6–47.8;[79,81,e] 51.14[86]
$C_{12}E_7$	61.5;[15] 65;[24,80] 67.2[25]
$C_{12}E_8$	75.5;[77] 77;[24] 78;[25,80] 78.39;[86] 79.2;[67] 71[82,e]
$C_{12}E_{10}$	77[66]
$C_{12}E_{11}$	94.5 (0.12 M)[c]
$C_{12}E_{23}$ (Brig 35)	> 100[45]
$C_{14}E_3$	20[24]
$C_{14}E_5$	20[25]
$C_{14}E_6$	40;[24] 42[25]
$C_{14}E_7$	58;[24] 58.5;[25] 58.6[c]
$C_{14}E_8$	70.5;[25] 72[24]
$C_{16}E_3$	20[24]
$C_{16}E_4$	20[24]
$C_{16}E_6$	31.5;[25] 38[24]
$C_{16}E_7$	53[24,25]
$C_{16}E_8$	65.5;[25] 67[24]
$C_{16}E_{10}$	64–69[c]
$C_{18}E_{10}$	70–72[c]
$C_1E_8C_6$	72, 74[49]
$C_1E_8C_8$	62, 63[49]
$C_1E_8C_{10}$	58[49]
$C_1E_8C_{16}$	45, 47[49]
OPE_6	⩾ 0[168]
Triton X-114	22;[83] 23–25[84,f]
Triton X-100	64;[12] 65[66,83]
PONPE-7.5	5 (5% soln.)[60,g]
$NPE_{8.1}$	6.0[180]
NPE_{8-9} (Igepal CO-610)	26;[12] 30[85]
$NPE_{9.2}$	56[c]
NPE_{10-11} (Igepal CO-710)	70–72[85]
Igepal CO-730	95–100[85]
Igepal CO-850	> 100[85]
NPE_{30} (Igepal CO-880)	> 100[85]
NP-5 / 3 (Nonylphenyl-5-ethoxy-3-propoxylate)	12.5[180]

TABLE 2 (continued)
Summary of Cloud-Point Temperatures for Nonionic Surfactant Micellar Solutions

Surfactant system[a]	Cloud point[b], °C
Tween 80	93[12]
Brominated Tween 80	90[12]
$A1E_6$	31[65]
$A1E_8$	38[65]
$A1E_{18}$	99[65]
Tergitol 15-S-9	59;[c] 60[66]
Tergitol TMN-10	77, 76[66]
$C_{12}2E_3$	50.2[67]
$C_{12}2E_4$	71.1[67]
C_5E_6H	10[c]
C_6E_6H[h]	10.2[40]
AG-8(6)	72.5[113,i]
AG-12(6)	53.5[113,i]
AG-14(6)	51.0[113,i]
AG-12(4)	< 0[113,i]
AGM-8(6)	34.0[114,j]
AGM-12(6)	30.0[114,j]
AGM-14(6)	23.5[114,j]
AGM-14(10)	59.0[114,j]
Pluronic L64[k]	60[86]
Pluronic P75[k]	93[86]
2-*n*-Tetradecyl[2.2.2]-cryptand	12.5[69]
Steroidal Lariat crown ether[l]	64.5[69]
3-Hexyl-2-oxy-18-crown-6	30[112]
Octyl-15-crown-5	13.0[112]
Decyl-15-crown-5	4.5[70]
Dodecyl-15-crown-5	< 0[70]
Octyl-18-crown-6	28.5[112]
N-Octylmonoaza-15-crown-5	23[70]
N-Dodecylmonoaza-15-crown-5	13.0[70]
N-Octylmonoaza-18-crown-6	33.5[112]

[a] Refer to Table 1 for nomenclature and structures. For a more complete listing of the cloud point of some other "nonconventional" nonionic surfactants refer to Reference 117.
[b] Values given are the critical temperature or the cloud point for micellar solutions in which the surfactant concentration is in the 0.1 to 5.0% (w/v) range unless otherwise indicated.
[c] Value obtained in this work for 1.0% aqueous surfactant solution.
[d] Solvent was formamide instead of water.
[e] In deuterated water, D_2O.
[f] Refer to Figure 5A for the cloud point temperature vs. surfactant concentration phase diagram.
[g] Refer to Figure 4 for the dependence of cloud point temperature upon surfactant concentration.
[h] Refers to $C_6F_{13}CH_2(OCH_2CH_2)_6OH$.
[i] Refers to $R - C(H)[O(CH_2CH_2O)_mH]_2$ with $R = C_nH_{2n+1}$, $AG_{(n+1)}2M$.[113]
[j] Refers to $R - C(H)[O(CH_2CH_2O)_mCH_3]_2$ with $R = C_nH_{2n+1}$, $AGM_{(n+1)}2M$.[114]
[k] $HO(C_2H_4O)_a(C_3H_6O)_b(C_2H_4O)_c$; if $a = c = 13$ (or 24) and $a = 30$ (or 35), then we have Pluronic L64 (Poloxamer 184) and Pluronic P75 (Poloxamer 215), respectively.
[l] Refer to Table 1 for exact name.

TABLE 3
Selected Summary of Effect of Alteration of Conditions upon the Cloud-Point Temperature Exhibited by Nonionic Surfactant Micellar Solutions

Nonionic surfactant system	Additive	Cloud point, °C
C_6E_5	None	78.4[76]
	1.3 m*M* DoPyCl[a]	84.5
$C_{12}E_4$	None	0–1[b]
	3 m*M* CTAC[c]	>85
	1 m*M* SDS[d]	78–81
	5 m*M* SB-12[e]	4–7
$C_{12}E_5$	None	32.2[76]
	1.3 m*M* DoPyCl[d]	91
	None	32.5[19]
	CTAC[c] (X = 0.025)	52
$C_{12}E_6$	None	47.8[81]
	0.009 mole ratio SDS[d]	56
	1.4 m*M* Butyl-*p*-hydroxy benzoate	30[96]
	None	52[97]
	4.2 m*M* Methylparaben	40
	8.0 m*M* Methylparaben	30.4
	None	53[108]
	0.025 mol / dm^3 nBu_4 NBr	82
	0.04 mol / dm^3 Na_2CO_3	36
	1.0 mol / dm^3 NaCl	41
	10% (wt) NaCl	31.85[16]
$C_{12}E_8$	None	74[95]
	40% Glycerol	62
	60% Glycerol	43
	None	78.5[42]
	6 *M* Urea	>100
	0.11 mole fraction of *n*-Dodecylmaltoside	85.9
$C_{12}E_{24}$	None	>100[103]
	1.1 *M* NaCl	88
	0.15 *M* Phenol	25
$C_{16}E_{10}$	None	69[98]
	0.10 *M* Tetramethylurea	75.7
	0.30 *M* Tetramethylurea	84.6
Triton X-100	None	63.7[98]
	0.30 *M* Urea	65.7
	0.50 *M* Urea	68.1
	0.30 *M* 1,1-Diethylurea	78.4
	0.50 *M* 1,1-Diethylurea	87.2
	2% Sodium Azide (pH 7)	61[100]
	0.5 *M* Sodium Chloride (pH 7)	56
	1.0 *M* Sodium Chloride (pH 7)	47
	0.29 m*M* NPE_8 + 48 m*M* KCl	40[91]

TABLE 3 (continued)
Selected Summary of Effect of Alteration of Conditions upon the Cloud-Point Temperature Exhibited by Nonionic Surfactant Micellar Solutions

Nonionic surfactant system	Additive	Cloud point, °C
$NPE_{9.2}$	None	56[99]
	Sat'd with 1,2-Dichloroethane	31
	Sat'd with Benzene	< 0
	Sat'd with *n*-Heptane	71.5
	Sat'd with *n*-Hexadecane	80
PONPE-7.5	None	24.8[151]
	0.10 *M* KSCN	35.1
	0.50 *M* KSCN	41.5
	1.0 *M* KSCN	43.8

[a] Refers to dodecylpyridinium chloride.
[b] Determined in this study.
[c] Refers to hexadecyltrimethylammonium chloride.
[d] Refers to sodium dodecylsulfate.
[e] Refers to sulfobetaine-12.

temperatures much larger than T_c, the low-concentration phase becomes more and more dilute, whereas the high-concentration surfactant-rich phase may become nonmicellar.[24]

The origins of such critical phenomena and phase-separation behavior of nonionic amphiphile solutions is still the subject of much debate in the literature, having been explained by either critical-concentration fluctuations or by invoking a large growth of the micellar aggregation number (increase in micelle size) with temperature.[4,24,25,65,74,75,79,82,88-92] More recently, thermodynamic theoretical models have been developed that allow for the accurate prediction of nonionic micellar properties including their phase-separation coexistence curves.[86,87,92] In addition, the kinetic features of such phase-separation processes in micellar solutions have also been reported.[91,94]

B. Zwitterionic Surfactant Micelle Systems

In addition to nonionic surfactants, the zwitterionic micelle-mediated phase-separation behavior has also recently been utilized in extraction and preconcentration schemes.[119] Table 4 summarizes the names as well as micellar and phase properties of some typical zwitterionic surfactant systems.[118-130] Figure 7 presents the phase diagrams for the zwitterionic surfactants C_9APSO_4 and $C_{10}APSO_4$.[18,119] As can be observed, in contrast to the situation just described for nonionic surfactant micelles, phase separations of aqueous solutions of almost all zwitterionic micellar systems are induced by lowering the temperature. Most zwitterionic surfactant solutions display a qualitatively similar liquid-liquid miscibility gap delineated by an upper consolute boundary (rather than the lower consolute boundary seen for nonionics). That is, zwitterionic surfactant systems exhibit their isotropic (L) solution phase at high temperature and upon cooling below the critical temperature (T_c), phase separate.[18,92] Notable exceptions to this general behavior have been reported for the zwitterionic surfactants $C_{12}N_{10}PPh$ and $DC_{12}PO$, whose monophasic solutions at room temperature turned turbid and separated into two phases as the temperature was increased.[121,127] This is the same clouding

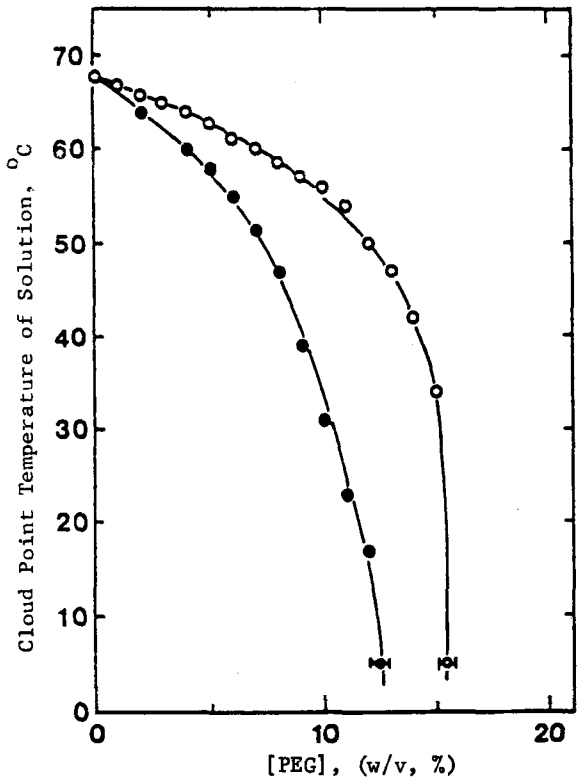


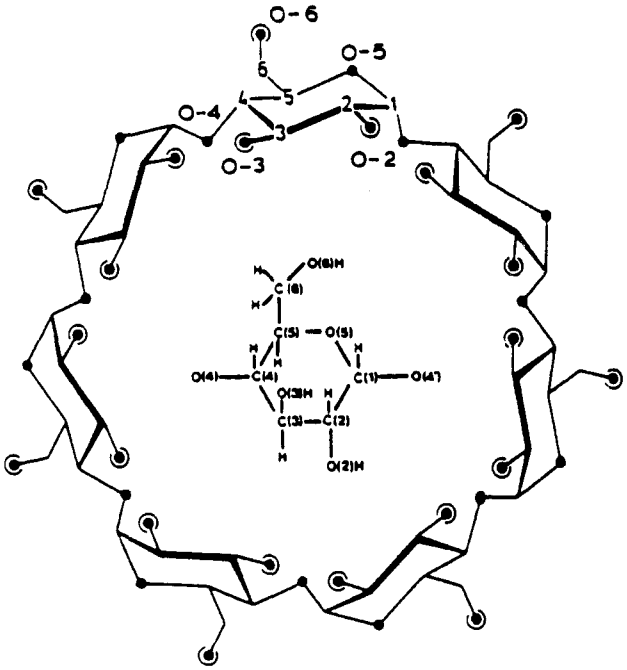
FIGURE 6. Cloud-point temperature (degrees Celsius) of aqueous solutions of Triton X-100 as a function of the concentration of poly(ethylene glycol) (PEG) additives of nominal molecular weight 20,000 (PEG 20K, filled circles) and 7500 (PEG 4K, open circles). (Reproduced with permission from Yamazaki, M.; Ohshika, M.; Ito, T. *Biochim. Biophys. Acta.* **1991**, *1063*, 176, Elsevier Science Publishers.)

phenomenon as observed for nonionic surfactants. The critical temperatures for some zwitterionic surfactant solutions are summarized in Table 4. The dependence of this upper consolute boundary upon the structure of the zwitterionic surfactant has been discussed in the literature.[18,123,130]

Although there are fewer studies reported in the literature, as was the case with the cloud point observed for nonionic surfactant systems, the presence of additives can also alter the upper critical temperature of zwitterionic surfactant solutions.[119,123,126,130] For example, data for the zwitterionic surfactant dimethyloleylamine oxide (C_{18}DAO)[123] in Table 4, serves to illustrate the effect of changes in pH and/or salt concentration upon its critical temperature.[126] Iodide ion and urea lower the cloud-point temperatures of aqueous solutions of zwitterionic surfactants.[128,130] This behavior is exactly opposite to the effect that these species have upon the cloud point of nonionic surfactant solutions. The addition of nonionic surfactants such as octylglucoside or organic solvents such as dichloromethane also dramatically depress the critical temperature of zwitterionic surfactants.[119,131] However, the addition of sulfate ion increases the critical temperature observed for solutions of C_9APSO$_4$.[119] Again, this effect of added sulfate ion is just the opposite of that observed for nonionic surfactants. Thus, just as in the case of nonionic systems, various additives can be utilized in order to manipulate the temperature at which zwitterionic surfactant solutions exhibit their critical behavior. As a general rule of thumb, the effects of most additives examined to date upon the critical temperature of solutions of zwitterionic surfactants are just the opposite of that observed for nonionic surfactant solutions.

C. Cyclodextrin Systems

Cyclodextrins are cyclic oligosaccharides composed of six or more glucopyranose moieties bonded together via 1,4-ether linkages. The three most common cyclodextrins contain six, seven, or eight of these glucopyranose units and are referred to as α-, β-, or γ-cyclodextrin, respectively.[5,9,132,133] The cyclodextrins have a general structure similar to that of a truncated cone with average cavity diameters of 0.57, 0.78, and 0.95 nm for α-, β-, and γ-cyclodextrin, respectively. The structure of native β-cyclodextrin is shown in Structure I. Such molecules can bind a variety of organic and inorganic molecules/species of appropriate size through inclusion complexation.[132] Because cyclodextrins are rigid molecules, there is no minimum critical concentration required as for the formation of nonionic or zwitterionic surfactant micelles.



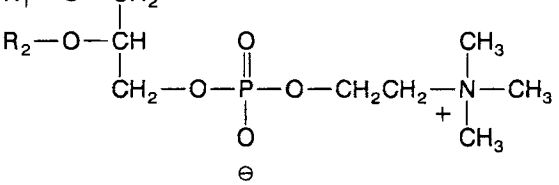
Structure I. Chemical structure and numbering scheme (insert: a glucopyranose moiety) for β-cyclodextrin. The oxygen atoms are designated by solid circules (●) and the hydroxyl groups by◉.

Aqueous solutions of the native cyclodextrins do not exhibit any phase-separation behavior. However, aqueous solutions of some derivatized cyclodextrins, specifically 2,6-di-*O*-methyl-β-cyclodextrin[134] (DOM-B-CD) and permethylhydroxypropyl-β-cyclodextrin[135] (PMHP-B-CD), do exhibit critical phase phenomena. For example, aqueous solutions of DOM-B-CD that are homogeneous at room temperature suddenly cloud on heating due to crystallization. The temperature required for crystallization depends on the DOM-B-CD concentration [i.e., this temperature ranges from 48°C ([DOM-B-CD] = 0.20 *M*) to 93°C ([DOM-B-CD] = 1 m*M*)].[134] On cooling, redissolution occurs abruptly and the entire process is characterized by a hysteresis loop of 7 to 12°C.[134] Such hysteresis upon heating-cooling cycles has also been observed for a few nonionic surfactant systems.[116] Of course, a problem with this DOM-B-CD system as far as its use in extractions is concerned is that the concen-

TABLE 4
Summary of Micellar and Phase Properties of Zwitterionic Surfactant-Water Systems

Zwitterionic surfactant system (name, abbreviation)	CMC, m*M*	*N*	Cloud point,[a] °C
Type I: Ammonioethylsulfates, $R_1(CH_3)_2N^+(CH_2)_2OSO_3^-$, R_1AESO_4			
$C_{10}AESO_4$	—	—	77[18]
$C_{12}AESO_4$	—	—	>120[18]
Type II: Ammoniopropylsulfates, $R_1(CH_3)_2N^+(CH_2)_3OSO_3^-$, R_1APSO_4			
C_8APSO_4	—	—	32[18]
C_9APSO_4	4.5[18,b]	—	65;[119] 69;[18] 74 (D_2O)[18]
$C_{10}APSO_4$	—	—	88;[119] 90[18]
$C_{12}APSO_4$	2.1[30,b]	—	120[18]
Type III: Aminiopropanesulfonates, $R_1(CH_3)_2N^+(CH_2)_3SO_3^-$, R_1APS			
$C_{10}APS$	42.1[118,c]	14[118]	—
$C_{12}APS$	3.4–3.8[118,120,c]	69–71[120]	−0.5[18]
$C_{14}APS$	0.3[118,c]	—	12[18]
$C_{16}APS$	—	—	20[18]
Type IV: *N*,*N*-Dimethylalkylamine N-oxides, $R_1(CH_3)_2N^+O^-$, R_1DAO			
C_8DAO	190;[120,c] 17–223[30]	27[120]	—
$C_{10}DAO$	2100;[120,c] 6–9.1[30]	—	—
$C_{12}DAO$	1.7–2.1;[120,c] 2.2;[24] 0.23–0.48[30]	75;[24] 76[30,120]	—
$C_{18}DAO$[d]	—	—	30[123,e] 46;[f] 52[g]

TABLE 4 (continued)
Summary of Micellar and Phase Properties of Zwitterionic Surfactant-Water Systems

Zwitterionic surfactant system (name, abbreviation)	CMC, m*M*	*N*	Cloud point,[a] °C
Type V: Phosphobetaine surfactants			
$C_{12}PPS$[h]	—	—	9[18]
$C_{12}PBu$[i]	—	—	<0[18]
$C_{12}N_{10}PPh$[j]	—	21[121]	—
Type VI: Dimethylalkylphosphone oxides, $C_nH_{2n+1}P(CH_3)_2O$, DC_nPO			
DC_8PO	0.78[127,k]	—	41[88]
$DC_{10}PO$	0.08–0.10[127,k]	—	38.8;[127,l] 130[88]
$DC_{12}PO$	0.008–0.014[127,k]	—	[m]
Type VII: Lecithins, 			
C_8-lecithin[n]	—	500[130]	47;[92] 46[130] 7;[o] O[p]

[a] Refers to the upper critical temperature unless otherwise noted.[118]
[b] CMC value at 80°C.
[c] CMC value given in millimolars per kilogram.
[d] Refers to dimethyloleylamine oxide, i.e., $CH_3(CH_2)_7CH = CH(CH_2)_8N(CH_3)_2NO$.
[e] In presence of 0.05 *M* NaCl at pH 4.
[f] In presence of 0.05 *M* NaCl at pH 4.75.
[g] In presence of 0.10 *M* NaCl at pH 3.5.
[h] Refers to [(dodecyldimethylammonio)propyl]phosphinate.
[i] Refers to phosphoniobutyrate; see Reference 18.
[j] Refers to [(dodecyldimethylammonio)decyl]phenylphosphinate.
[k] CMC value given in grams per 100 ml solution.
[l] For this system, a lower consolute boundary is observed, similar to that of nonionic surfactant systems and in contrast to the other zwitterionic surfactants.
[m] This surfactant exhibits both an upper and lower consolute boundary with critical temperatures of 177 and 124°C, respectively, at critical concentrations between 10 and 15% (wt) $DC_{10}PO$.[127]
[n] Refers to dioctanoyl phosphatidylocholine (i.e., $R_1 = R_2$ = octanoyl moiety in the given general structure).
[o] In presence of 0.075 *M* urea.
[p] In presence of 1.0 *M* urea.

trated cyclodextrin phase containing the complexed species to be extracted is a solid precipitate rather than liquid. Thus, separation of the crystalline DOM-B-CD (or its complexes) concentrated phase from the bulk aqueous solution phase has to be performed on a heated filter; otherwise, the substance readily redissolves on cooling.[132,135]

Figure 8 gives the phase diagram for permethylhydroxypropyl-β-cyclodextrin.[135] As can be seen, the phase separation occurs at ca. 28°C at [PMHP-B-CD] > ca. 4.5 m*M*. Although the data are limited, the addition of organic solvents and salt are also reported to affect the phase-separation ability of such derivatized cyclodextrins.[135] Although there are limited reports of the utilization of such cyclodextrin derivatives in extraction or preconcentration schemes, they appear to have great potential in this area of chemical analysis. There is a definite need for more work directed toward an examination of the phase

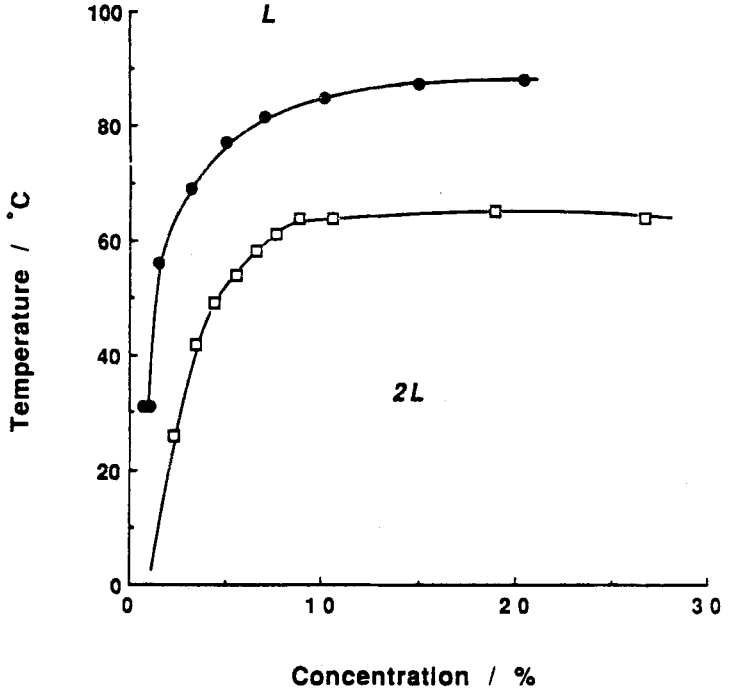


FIGURE 7. Phase diagrams for aqueous solutions of the zwitterionic surfactants C_9APSO_4 (□) and $C_{10}APSO_4$ (●). (Reproduced with permission from Saitoh, T.; Hinze, W.L. *Anal. Chem.* **1991**, *63*, 2521, American Chemical Society.)

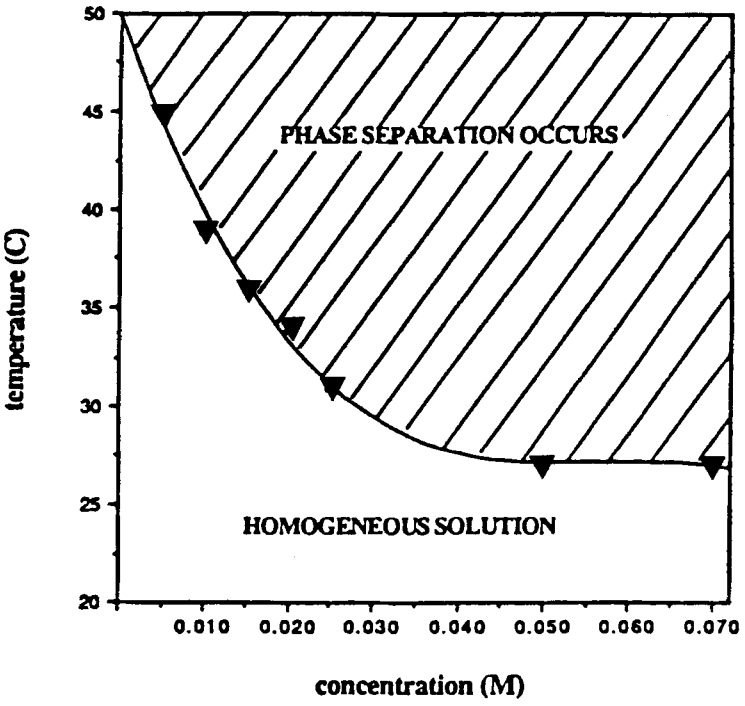


FIGURE 8. Phase diagram for permethyl hydroxypropyl-β-cyclodextrin solutions. (Reproduced with permission from Warner-Schmid, D.; Hoshi, S.; Armstrong, D.W. *Separation Sci. Technol.* **1993**, *28*, 1013, Marcel Dekker, Inc.)

behavior exhibited by other derivatized cyclodextrins and the effect of additives upon their phase behavior.

III. APPLICATIONS AND UTILITY OF PHASE-SEPARATION BEHAVIOR IN CHEMICAL ANALYSIS AND SEPARATION SCIENCE

The phase-separation behavior of neutral (nonionic and zwitterionic) surfactant systems has been successfully utilized in several different analytical applications. For example, the preconcentration of many metal ions, after conversion into their sparingly water-soluble chelates, has been reported using PONPE-7.5, Triton X-100, Triton X-114, or Tween 40 as surfactants; the separation of peripheral membrane proteins using Triton X-114 or C_9APSO_4 has been described; the use of hydrophobic affinity ligands in conjunction with C_9APSO_4 allowed for the extraction of hydrophilic proteins; and most recently, the extraction and/or preconcentration of organic compounds of environmental concern, such as pesticides, polycyclic aromatic hydrocarbons, and phenols, has been reported utilizing the phase behavior of neutral surfactant media as well as a derivatized β-cyclodextrin. In a few cases, the direct extraction of materials from solid matrices has also been described. The next sections will summarize as well as provide more specific details and information on these general analytical applications of the cloud-point extraction technique.

A. Cloud-Point Extraction of Metal Chelates and Related Inorganic Species

The phase separations based on cloud-point phenomena were first exploited to extract metal ions as sparingly water-soluble complexes. The concentration of the complexed analytes takes place in the surfactant-rich low-volume phase, with the efficiency of the process being dependent on a number of parameters, namely, the ligand and complex hydrophobicity, the apparent chemical equilibrium constants of the reactants in the organized media, and the kinetics of complex formation and phase transfer.

This nonconventional extraction approach was first described by Watanabe et al.,[60,136] who reported the concentration of nickel(II) using 1-(2-thiazolylazo)-2-naphthol (TAN) as the chelate-forming ligand and Triton X-100 [OPE_{9-10}, polyoxyethylene(9.5)-4-*tert*-octylphenol] as the amphiphilic extractant system. Since this initial work, several extractions of other metal ions using similar heterocyclic azo ligands and the surfactant PONPE-7.5 [$NPE_{7.5}$, polyoxyethylene(7.5)-4-nonylphenol] have been reported. In addition, two publications concerned the extraction of metals as their chloro or thiocyanato complex.[143]

This new cloud-point extraction procedure has been applied successfully to the extraction of metal chelates for the spectrophotometric[136-148,150] or flow injection[149] analysis of trace metals in a variety of different samples (tap water, coastal water, soils, alloys, etc.). The extraction conditions and data for some selected examples of such metal ion separations are summarized in Table 5. As can be observed, very high concentration factors and recoveries were achieved.

Despite the potential interest of micelle-based extraction techniques, relatively few systematic studies have been devoted to the investigation of complex formation equilibria and to the kinetic analysis of the extraction mechanism(s). In particular, there is a lack of information concerning the influence of the ligand and the ligand and micelle structures upon the process efficiency. Such fundamental information is required in order to help with the design and optimization of the technique for extraction of metal ions, particularly those involving process level hydrometallurgical applications.

I. General Experimental Protocols

Most of the reported laboratory experiments were performed by adding a small

TABLE 5
Summary of Cloud-Point Extractions of Metal Chelates using Nonionic Surfactant Micelles

Metal ion	Ligand	Nonionic surfactant	Experimental conditions (pH; C_F,[a] %E[b])	Ref.
Ni(II)	TAN[c]	Triton X-100	pH 7.0 (phosphate); $C_F = 30$	136
	PAN	Triton X-100	pH 5–6	137
Zn(II)	PAN	PONPE-7.5	pH 10 (carbonate); $C_F = 40$	60, 138
	QADI	PONPE-7.5	pH 9; NH_3; $C_F = 40$	139
Ni(II), Zn(II), Cd(II)	PAMP	PONPE-7.5		140–142
Au(III)	HCl	PONPE-7.5	HCl; %E > 95	143
Ni(II), Cd(II), Cu(II)	PAMP	PONPE-7.5	pH 5.6 (or 7.5)	144
Ni(II)	PAN	OP[d]	pH 6.0; $C_F = 15–25$	145
Transition metal ions	TAN	PONPE-7.5		146
Fe(III), Ni(II)	TAC	Triton X-100		147, 148
U(VI)	PAN	Triton X-114	pH 9.2; %E = 98	149
U(VI), Zn(IV)	Arsenazo	Tween 40[e]	pH 3; %E > 96	150
Cu(II), Zn(II), Fe(III)	Thiocyanate	PONPE-7.5	%E = 72.5–96.8	151

[a] Concentration factor.
[b] Percent extracted.
[c] Abbreviations for ligands: PAN, 1-(2-pyridylazo)-2-naphthol; QADI, 2-(8-quinolylazo)-4,5-diphenylbenzimidazole; PAMP, 2-(2-pyridylazo)-5-methyphenol; PAP, 2-(2-pyridylazo)-phenol; TAC, 2-(2-thiazoylazo)-4-methylphenol; TAN, 1-(2-thiazolylazo)-2-naphthol.
[d] OP surfactants refer to (polyethyleneglycol octylphenyl ethers).
[e] In this extraction, the concentrated, surfactant-rich phase was a solid rather than a liquid.

volume (typically a few milliliters) of a concentrated nonionic surfactant solution to a buffered aqueous sample (50 to 100 ml) containing the metal ion to be extracted and suitable masking agents. The ligand (chelating agent) may be dissolved in the aqueous solution or in the added surfactant solution, depending upon its solubility in water. The solution is then heated until a desired fixed temperature, above the cloud point, is achieved in a thermostated bath. Because the densities of both phases are not very different, their spontaneous separation is slow and hence centrifugation (between 3000 and 10,000 rpm) is recommended in order to speed up the separation of the two phases.

2. Concentration Factor in Cloud-Point Extractions

Only a few nonionic polyoxyethylene-type amphiphiles (or their mixtures), possessing cloud points below room temperature, have been selected to conduct such extractions up until now. Although the use of dilute aqueous solutions of Triton X-100 (cloud point ca. 70°C) has been reported,[136,137] some difficulties arise when these hot solutions are centrifuged due to the fact that the temperature goes down and the system becomes monophasic. That is, leakage of surfactant and metal chelates back into the aqueous medium occurred when the temperature of the system fell during the centrifugation step, resulting in a loss of extraction efficiency (and recovery). Moreover, a partial decomposition of the amphiphile may occur under these conditions.

Because the cloud-point temperature depends on the polyoxyethylene chain length (refer to Table 2), being lower for amphiphiles with shorter chains, suitable mixtures of surfactants can be used in order to achieve cloud-point values slightly above room temperature. Figure 5B shows the dependence of cloud point on surfactant composition for solutions containing two different nonionic surfactants. By appropriate manipulation of such composition, cloud

points near or slightly above room temperature can be achieved for such metal ion extractions.[161]

The volume of the extraction layer (and, thus, the concentration factor) depends on the nature of surfactant or mixture used, and on the total amount of added amphiphile. In most extraction experiments, the surfactant concentration was kept in the range of 0.2 to 2.0% w/v, with a corresponding volume of the final extraction layer being between 2 and 10% of the initial solution volume.

3. Partitioning of Reagents and Products between the Phases

Above the cloud point, the surfactant-rich phase acts as an organic solvent with the analytes being partitioned between this phase and the aqueous solution containing only very small amounts of the dissolved surfactant. Thus, an evaluation of the pertinent partition coefficients of ligands and complexes is essential in order to quantitatively describe the performance of the cloud-point extraction process. The pertinent distribution coefficients and pKs of the following ligand and metal ligand chelate systems have been reported in the literature: Zn(II), Ni(II), Cd(II), and Cu(II) complexes of PAMP;[140,141,144] Ni(II), Zn(II), and Cd(II) complexes of PAP;[142,152] Fe(III), Ni(II), Cd(II), and Zn(II) complexes of a variety of thiazolylazo dyes;[146,153] and the Fe(III) and Ni(II) complexes with TAC.[147] One feature noted with the surfactant phase-mediated extractions was that the partition coefficients of the metal chelates varied with the type of metal being extracted, whereas such partition coefficients were essentially independent of the nature of the metal ion if a conventional liquid-liquid extraction was performed using octanol as the organic solvent.[146,152]

Due to the hydrated nature of the extracting surfactant-rich phase, the partition coefficients of neutral solutes (including the usual ligand molecules) are generally somewhat to much lower than those measured in nonmiscible organic solvents. For comparison purposes, Table 6 reports the partition data of 8-hydroxyquinoline (oxine) in different solvent and micellar systems.[148,151] For comparison purposes, data are also presented for a more hydrophobic quinoline derivative.[155]

The partition coefficients of many neutral chelates bearing more than one ligand molecule are usually greater than those of the corresponding free ligands. For example, oxinates obtained from bivalent metal ions, having the general formula $M(Ox)_2$, exhibit K_D values in the range 10^3 to 10^4 (chloroform–water). Thus, acceptable extraction efficiencies for these complexes are also ex-

TABLE 6
Partition Coefficients of Oxine (8-Hydroxyquinoline or 8-Quinolinol) between Water and Other Solvent Systems (at 20°C; ionic strength = 0.10 *M*)

	Partition coefficient, K_D[a]						
Ligand form	$CHCl_3$	1-Octanol	PONPE-7.5	Triton X-100	Triton X-305[b]	Tween 20	Brij 35
HOx	436	105	28	51.3 (124)[c]	63[c]	34[c]	4365[d]
H_2Ox^+	0.54[e]	—	1.95	1.1	—	—	—
Ox^-	2.80[f]	—	7.08	2.6	—	—	588[d]

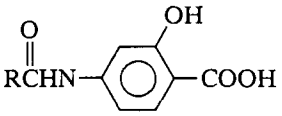
[a] Data taken from References 147 and 148 unless otherwise specified.
[b] Triton X-305 or OPE_{30}.
[c] Value determined at 25°C, pH 6.9 to 7.8. Data taken from Reference 154.
[d] Data for a more hydrophobic quinoline derivative, e.g., 5,7-dichloro-2-methyl-8-hydroxyquinoline. Data taken from Reference 155.
[e] Values of the distribution ratio (D) at pH 2.0. Taken from References 156 and 157.
[f] Values of the distribution ratio (D) at pH 12.0. Taken from References 156 and 157.

pected when working with the cloud-point technique.

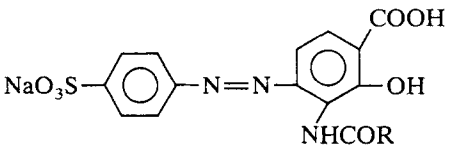
4. Use of Amphiphilic Ligands in Cloud-Point Extractions

Amphiphilic ligands possessing a chelating group and a tunable hydrophobic moiety (usually a linear alkyl chain) have been exploited in several separation techniques, both in monomeric or aggregate form, either in water or water-organic systems.[158] In particular, these compounds can form complexes of differing overall hydrophobicity with selected metal ions, which can be readily dissolved in ionic and nonionic micelles.

Because the extent of ligand and complex binding to the host aggregates can be varied by simply changing the alkyl chain length, some of these ligands have been investigated as potential candidates for selective cloud-point extractions of metal ions. For example, two series of lipophilic derivatives of 4-aminosalicylic acid have been employed to extract Fe(III) from aqueous samples. These compounds are capable of either self-micellizing under certain conditions[159,160] or forming mixed aggregates with other surfactants. The formulas of these two ligands are shown in Structures II and III.



Structure II. Amphiphilic derivative of 4-aminosalicylic acid: PAS-C_n series where C_n refers to the moiety $C_{(n-1)}H_{(2n-1)}CO$.



Structure III. Amphiphilic derivative of 4-aminosalicylic acid: Y-PAS-C_n series where C_n refers to the moiety $C_{(n-1)}H_{(2n-1)}CO$.

The first reported investigation concerned the extraction of Fe(III)-PAS-C_n chelate complexes using mixed micelles of Triton X-100 and $C_{12}E_{4.2}$.[161] Working in the pH range 2.0 to 4.0, in the presence of an excess of ligand and surfactant, the stoichiometry of these chelates was found to be 1:1. Because positively charged complexes are formed, only the more hydrophobic members of the series exhibit binding constants to the aggregates high enough to ensure for a quantitative extraction in the micellar-rich phase.

In order to explain the experimental results, the partition equilibria of ligands and complexes between the micelles and the bulk phases have to be considered. The binding constants of the PAS-C_n ligands to Brij 35 [$C_{12}E_{23}$, polyoxyethylene(23)dodecylether] aggregates have been determined using the micellar chromatographic technique[162] and from the variation of the apparent pK_a,[163] according to the equations

$$V_s/(V_e - V_m) = 1/P_{sw} + K_{B(HL)}\, C_m/P_{sw} \quad (1)$$

$$K_{a(w)}/K_{a(app)} = K_{B(HL)}\, C_m/K_{a(w)} \quad (2)$$

In Eq. (1), V_s and V_m are the volumes of stationary and mobile phases, respectively, V_e is the elution volume of the ligand measured at a given surfactant concentration, P_{sw} represents the partition coefficient of the solute between the stationary and the aqueous phase, $K_{B(HL)}$ is the binding constant of the ligand, and C_m is the conentration of micellized surfactant. In Eq. (2), $K_{a(w)}$ and $K_{a(app)}$ are the dissociation constants of the carboxylic group of the ligand in water and in the presence of micelles, respectively. Because most of the surfactants used in extractions show a cloud point below or near room temperature, the partitioning studies were performed using related micellar systems. For example, Brij 35 has been chosen due to the favorable cloud point ($> 100°C$) and because its UV spectrum does not interfere with those of the investigated analytes.

The determination of the complex formation constants at different surfactant concentrations allows the estimation of the binding constants of the FeL^+ species, $K_{B(FeL)}$, according to the equation

$$K_{C(app)} = K_{C(w)}\left[1 + K_{B(FeL)}\, C_m\right] \quad (3)$$

where $K_{C(app)}$ is the complex formation constant measured in the presence of micelles and $K_{C(w)}$ is the corresponding value in water. This expression accounts for an appreciable binding of the ionic complexes to neutral micelles and a negligibly small micellar association of the ligand, salicylate anion. The pertinent partition data of some PAS-C_n ligands determined in this manner are reported in Table 7.[161,165] In addition, the binding constants for the interaction of the neutral and protonated forms of 6-(alkylamino)-methyl-2-hydroxymethylpyridine (C_nNHMePyr) ligands with a nonionic surfactant micelle system are also presented in the table.[194]

As is evident from the data in Table 7, even for quite hydrophobic ligands as PAS-C_4, the corresponding iron complexes are not completely bound to the micellar aggregates. Only for the PAS-C_{10} ligand (or higher analogues) are the metal chelates very strongly associated to the micelles, thus allowing for the quantitative recovery of the metal ion in the extracting phase.

More recently, extractions performed with the Y-PAS-C_n ligands, under the same experimental conditions, showed higher efficiencies.[164,165] The stoichiometry of the corresponding metal chelates is also 1:1 and the binding constants of the ligands to nonionic micelles are not significantly different from those of PAS-C_n having the same alkyl chain length.[165] Thus, the improvement in extraction efficiency can be attributed to the formation of zero-charge complexes, which are more tightly bound to the nonionic micelles.

The dependence of extraction efficiency on hydrophobicity of the PAS-C_n and Y-PAS-C_n series of ligands is shown in Figure 9. It has to be underscored that the desired extraction performance can be obtained, within a series of compounds, by simply adjusting the hydrophobic chain length of the ligand. The influence of other experimental parameters, including pH and ligand concentration, is similar to that found in conventional liquid-liquid extractions.

The development of new amphiphilic ligands bearing different chelating functional groups and tunable hydrophobic moieties could open up many new and interesting perspectives, increasing the selectivity of these surfactant-based cloud-point separations. For instance, the utilization of

TABLE 7
Binding Constants of Functional Ligands and Complexes in Nonionic Micelles

Ligand	$K_{B(HL)}$,[a] M^{-1}	$K_{B(FeL)}$,[b] M^{-1}
PAS-C_2	170 (120)[c]	65
PAS-C_4	500 (350)[c]	72
PAS-C_7	1500	[d]
	$K_{B(protonated)}$[e]	$K_{B(neutral)}$[f]
C_4NHMePyr	—	6.3
C_8NHMePyr	12.6	316
C_{10}NHMePyr	79.4	6310
C_{12}NHMePyr	631	—

[a] Data in Brij 35 micelles taken from Reference 161.
[b] Data in Brij 35 micelles taken from Reference 166.
[c] Data for binding to $C_{12}E_8$ micelles taken from Reference 165.
[d] Not determined.
[e] Value at pH 3.0 taken from Reference 194. Units are moles per cubic decimeter.
[f] Value in units of moles per cubic decimeter at pH 11.0 taken from Reference 194.

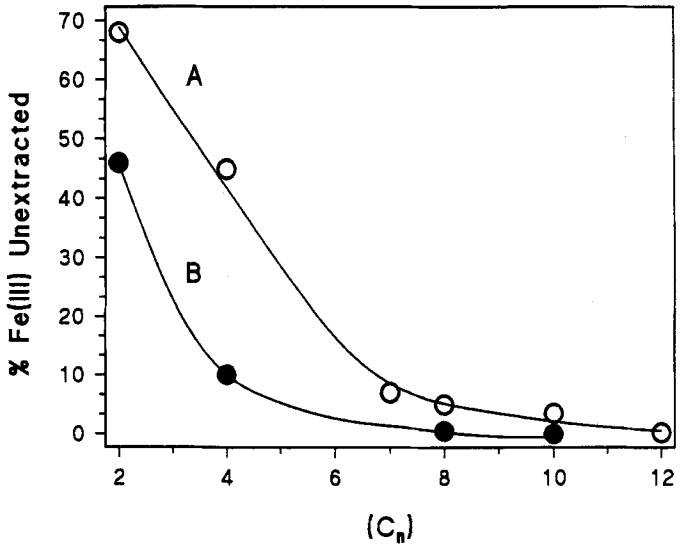


FIGURE 9. Extraction performances (amount of ferric ion not extracted) as a function of the ligand hydrophobicity (increasing *n* value) for: A (○), PAS-C_n and B (●), Y-PAS-C_n ligand systems. Conditions: [ligand] = 2.0 m*M*; [Fe^{3+}] = 0.10 m*M*; 1.0% (w/v) Triton X-100; 1.0% (w/v) $C_{12}E_{4.2}$; pH 3.5; 5% (w/v) added salt, $NaNO_3$; temperature 35°C.

surface-active ligands derived from ethylenediamine [e.g., $(CH_3)_2NCH_2CH_2N(CH_3)(C_8H_{17})$, CMC = 1.12×10^{-4} mol dm^{-3}][167] might prove beneficial. The use of this ligand in the presence of nonionic micelles allowed for the extraction and preconcentration of Cu(II) ion.[164] In addition, the use of zwitterionic surfactant media in all of these types of applications instead of the nonionics employed to date should be examined because they might prove to offer possible advantages. Last, the use of the nonionic alkyl crown ether surfactants[69,70] as chelating ligands in cloud-point extraction work, which has not yet been reported, might allow for the selective extraction of alkali and alkaline earth metals if the appropriate crowns are employed.

5. Kinetic Studies

Chemical reactions occurring in the presence of micellar aggregates usually show strong kinetic effects, which can be explained in terms of reagent(s) and product(s) distribution between the aggregates and the bulk phase.[2,5,7] Because complex formation is the rate-determining step in several chelate extractions, the detailed study of reaction kinetics before the cloud-point separation may be of remarkable interest. For the model reaction system, Fe(III)-PAS-C_n, investigated in the presence of Brij 35 micelles, the kinetic results indicated that the complex formation takes place in the aqueous bulk phase for the less hydrophobic ligands (such as PAS-C_2), whereas it occurs at the micelle-water interface for the more hydrophobic PAS-C_n ligands.[166] The reaction is still very fast (in the seconds time scale) under the reported experimental conditions, being complete before the phase transfer occurs.

In addition, the kinetics of the complexation reaction between Ni(II) ion and 8-quinolinol in the presence of the nonionic surfactants, Triton X-100, Triton X-305, or Tween 20 has been reported[154] as has the kinetics of complex formation and extraction of Cu(II) ion by micelle-solubilized ligands derived from pyridines.[234,235] Most recently, a novel metallochromic indicator method has been developed that yields simultaneous ki-

netic and extraction equilibrium data for extractive systems using nonionic micelles.[195]

6. Advantages and Problems in Cloud-Point Extractions of Metal Chelates

The advantages of these surfactant-based procedures for the extraction of metal ions include the following:

1. Excellent preconcentration of the metal analytes (by factors of 10 to 100) with good extraction efficiences.[14,60]
2. In particular, the method requires the addition of small amounts of nonionic surfactant and, correspondingly, smaller amounts of sample solutions (typically less than 100 g) in order to obtain the same concentration factor typically achieved in ordinary liquid-liquid extractions, which often require the handling of much larger volumes (0.50 to 1.0 l) of the aqueous solution.[14]
3. Safety and cost benefits (i.e., the use of small amounts of the nonionic surfactant extractant solvent obviates the need to handle the usually large volumes of organic solvent required for traditional liquid-liquid extractions; thus volatility, flammability, and cost are greatly reduced).
4. Easy disposal of the nonionic solvent (can be easily burned in the presence of waste acetone or ethanol[60]).
5. Convenience and simplicity of the extraction procedure.[151]
6. The surfactant-rich extractant phase is compatible with micellar mobile phases employed in thin-layer chromatography (TLC) or high-performance liquid chromatography (HPLC),[6,7] or pseudostationary phase in capillary zone electrophoresis (CZE).
7. Enhanced detection of the metal complexes (using spectrophotometric or fluorimetric techniques) is possible in this surfactant-rich extractant phase, exploiting the surfactant-sensitization effects.[6,8,12]

The main disadvantage of these cloud-point extractions arises from the relatively low partition coefficients of many neutral metal chelate species, which can be increased only via the use of highly hydrophobic ligands. Because long chain derivatives are less soluble in aqueous surfactant solutions, this imposes a limit on the amount of ligand excess available in such systems. Moreover, due to the presence of the extractant surfactant(s), the recovery and purification of the separated metals may be more difficult compared to that when employing the classic liquid-liquid extraction (this aspect will be discussed in a later section).

B. Cloud-Point Extractive Procedures for Biological and Clinical Species

Perhaps the most popular and frequent utilization of the cloud-point extraction technique to date has been for the separation and purification of biological species, e.g., mostly proteins.[168-199] Specifically, the technique offers a simple means for separating hydrophobic and hydrophilic materials.[187] The first application in this area was by Bordier, who in 1981 reported the separation of integral membrane proteins (acetylcholinesterase, bacteriorhodopsin, and cytochrome *c* oxidase) from hydrophilic proteins (catalase, ovalbumin, concanavalin A, and serum albumin) via use of the phase behavior of Triton X-114.[168] This surfactant is miscible with water at low temperature, whereas two phases are formed at temperatures above ca. 30°C (refer to Figure 5A), with the hydrophilic proteins present in the aqueous phase while the amphiphilic and more hydrophobic proteins reside in the surfactant-rich phase.

This approach allowed for the separation of integral membrane proteins from cytoplasmic and peripheral membrane proteins in animal cell as well as plant sources. Since then, this cloud-point extraction system has been successfully utilized to extract and purify (either partially or completely) a variety of proteins and related biochemicals[169-192] (see Table 8). More recently, zwitterionic

TABLE 8
Summary of Cloud-Point Extractions of Biochemical and Clinical Species using Nonionic Surfactant Micelles

Biological material(s) separated / extracted	Surfactant employed and conditions	Comments	Ref.
Hemoglobin from bacteriorhodopsin (among others)	Triton X-114; pH 7.4; 150 m*M* NaCl; 30°C	96% Bacteriorhodopsin extracted into Triton X-114 phase while 98% of hemoglobin remained in aqueous phase	168
Phosphatidylinositol kinase (PI kinase)	Triton X-114; 25°C	84% PI kinase recovered in the surfactant-rich phase	169
	Triton X-114: Triton X-45 mixed surfactant system (9:1 w / w); 10°C	79% PI kinase recovered in mixed surfactant-rich phase	169
Polypeptides from rat intestinal microvillus membrane	Triton X-114; pH 7.4; 140 m*M* NaCl; 1 m*M* PMSF; 32°C	Of 24 polypeptides solubilized, 11 proteins separated nearly exclusively in the surfactant-rich phase while 9 proteins were exclusively in the aqueous phase	170
Adenovirus type 2(Ad_2)	Triton X-114; pH $\leq$ 5.0; 34°C	60% Extracted into surfactant-rich phase[a]	171
	Triton X-114; pH = 7.0; 34°C	Only 5% extraction achieved	171
Proteins of the wall-less procaryote *Mycoplasma hyorhinis*	Triton X-114; 37°C	The Triton X-114 extractant phase contained ca. 30 identifiable proteins	172
Integral membrane proteins of *Treponema pallidum* subsp. pallidum	Triton X-114; 100 m*M* KCl; 37°C	7 Antigens were concentrated and identified in the surfactant phase	173
Subunits of the receptor for immunoglobulin E (IgE)	Triton X-114; 150 m*M* NaCl; pH 8; 25°C	Unliganded receptors for IgE extracted in Triton X-114 phase; 80% recovery	174
Colicin E3	Triton X-114; citrate buffer; 37°C	At pH 7, no colicin E3 was extracted into the surfactant-rich phase while at pH 3, it was almost completely extracted	175
Hormone-sensitive lipase and ATP-citrate lyase	Triton X-114; pH 7.2; 50 m*M* NaCl; 37°C	Over 80% of the hormone lipase was extracted into the surfactant phase (enzymatic activity was 78%) while the hydrophilic ATP-citrate lyase was almost exclusively (ca. 95%) in bulk aqueuos phase	176
Large-scale purification of pyruvate oxidase from crude enzyme preparations	Triton X-114; pH 6; 150 m*M* NaCl; 30°C	Reduced form of pyruvate oxidase extracted into the surfactant-rich phase (95% recovery, specific activity 70 units / mg); method much more convenient and rapid compared to the DEAE-Sephadex chromatographic procedure	177
Separation of platelet membrane glycoproteins from normal subjects and a patient with Type I thrombasthenia	Triton X-114; pH 6.9; 30°C	Extracted and enriched the glycoproteins, GPs IV, VI, VII, VIII, and GP_{3a} in the surfactant-rich phase while the GPs Ib, V, and IX remained essentially in the aqueous phase	178

TABLE 8 (continued)
Summary of Cloud-Point Extractions of Biochemical and Clinical Species using Nonionic Surfactant Micelles

Biological material(s) separated / extracted	Surfactant employed and conditions	Comments	Ref.
Separation of proteins associated with preparations of membranes of adrenal-medulary secretory granules (chromaffin granules)	Triton X-114; 30°C	Triton X-114 allows for the separation of up to four distinct families of proteins from purified membrane preparations	179
Extraction of cycloheximide from fermentation media	NP-5 / 3;[b] 28°C	The antibiotic cycloheximide was *in situ* extracted into the NP-5 / 3 surfactant phase (distribution coefficient 10.9); the presence of NP-5 / 3 did not affect the biosynthesis of the antibiotic	180
Extraction of clathrin	Triton X-114; 30°C	At pH < 5, all the clathrin extracted into the surfactant phase; at pH ⩾ 6, no extraction of chlathrin into the surfactant phase occurs	181
Partial purification of cytochrome b_{558} by extracton from (bovine) granulocytes	Triton X-114; 25°C	Cytochrome b_{558} partitions exclusively into the surfactant phase; this can be used for the first stage of the overall purification scheme; obtained an ca. five-fold purification with this cloud-point procedure; yield 52%	182
Purification of plant cytochrome P-450 and b_5	Triton X-114; ⩾20% (v / v) glycerol; 4°C	⩾ 90% of the detectable cytochromes P-450 and b_5 were recovered from the surfactant-rich phase; however, a 50% loss of enzyme activity was observed	183
Purification of cytochrome *b* and bc_1 complexes of bacteria	Triton X-114; pH 7.5; 150 m*M* NaCl; 30°C	Cytochrome *b* partitions into the surfactant-rich phase while cyto-c_1 remains in the aqueous phase	184, 185
Isolation of proteins from peroxisomal membranes	Triton X-114; 30°C	Extracted into the Triton X-114 surfactant-rich phase were 90% of the phospholipid, over 90% of acyl-CoA ligase and alkyl-DHAP synthase, and 92% urate oxidase; an advantage was that most enzymes retained their enzymatic activity	188
Separation of platelet glycoproteins and phosphoproteins	Triton X-114; 37°C	Most glycoproteins partitioned into the surfactant-rich phase while the hydrophilic proteins, fibrinogen, albumin, and actin remained in the aqueous phase; cloud-point extraction proposed as first step in purifying many platelet components	189
Partial purification of polyphenol oxidase (PPO)	Triton X-114; pH 7.3; 35°C	Chlorophylls (58%) and phenols (98%) were extracted into the surfactant-rich phase leaving the desired PPO in the aqueous phase; 43% recovery of PPO achieved with purification factors of 4.4 to 12.4 attained	190

TABLE 8 (continued)
Summary of Cloud-Point Extractions of Biochemical and Clinical Species using Nonionic Surfactant Micelles

Biological material(s) separated / extracted	Surfactant employed and conditions	Comments	Ref.
Pseudomonas exotoxin A	Triton X-114; 37°C	At pH = 3 (no salt present), %E = 70%;[c] at pH = 4 (with 0.14 *M* added salt as NaCl, KCl, or $NaNO_3$), %E = 80–90%; full biological activity observed at 37°C for 30 months	191
Purification of 5′-nucleotidase from human or bull seminal plasma	Triton X-114; pH 7.4; 30°C	HSP 5′-N was recovered in the aqueous phase (90%) whereas BSP 5′-*N* (ca. 80%) partitioned into the surfactant-rich phase; upon treatment with PI-PLC, the BSP 5′-*N* was also found in the aqueous phase[d]	192
Extraction of porphyrins and metalloporphyrins	Triton X-100; 5–15 *M* KOH or NaOH (or 1.5–5.0 *M* K_3PO_4); centrifugation at 4000 rpm[e]	Hemato-, proto-, copro-, and uroporphyrins effectively extracted into Triton X-100 surfactant-rich phase; %E = 97.3–99.8 with preconcentration factors of 10 to > 100	200, 201
Lipophilic vitamins	Triton X-114; 40°C	Vitamin A and E extracted into the surfactant-rich phase with recoveries of 100 and 67%, respectively and preconcentration factors of 20 and 30, respectively	202

[a] Upon subsequent incubation at pH 7.0, Ad_2 is released back into the aqueous phase.[171]
[b] Refers to nonylphenol-5-ethoxy-3-propoxylate.
[c] %E refers to percent extraction.
[d] HSP 5′-*N* and BSP 5′-*N* refer to human and bull seminal plasma 5′-nucleotidase, respectively.
[e] The surfactant-rich phase was the top layer under the experimental conditions.

surfactants and their phase separation behavior have also been used for the extraction and/or preconcentration of such materials.[119,131] In addition, the use of affinity surfactants in conjunction with zwitterionic surfactants has allowed for the selective cloud-point extraction and concentration of some hydrophilic materials.[193]

1. General Experimental Protocol Employed with Nonionic Surfactants

Briefly, the method is based upon solubilizing the hydrophobic material(s) in the Triton X-114 micelles formed upon the addition of the nonionic surfactant, Triton X-114 ($[\text{Triton X-114}]_{\text{final}}$ = 0.5 to 5.0%) at 0 to 4°C, followed by incubation of the solution at an elevated temperature, usually 30 to 37°C, for a specified time period. The solution then separates into two phases: a Triton X-114 surfactant-rich phase (present as fine, oily droplets) that contains the hydrophobic biomaterial(s) and an aqueous phase (representing ca. 95% of the total volume) containing the hydrophilic material(s).[168] After centrifugation [typically at from 300 to 13,000*g* (or at 500 rpm)], the surfactant-rich phase along with the hydrophobic biomaterial(s) can be recovered in the small volume element at the bottom of the test tube. In a few cases, depending upon the specific sample being extracted and the experimental conditions, the surfactant-rich layer separated out above, rather than below, the aqueous phase.[173] After removal of the surfactant-rich phase, additional surfactant can be added and the

extraction cycle repeated until the desired extraction efficiency (or percent recovery) is achieved.[168,170,172,173]

In some reported work using this extraction technique, a cushion of 6% (w/v) sucrose (plus appropriate buffer and/or salt) and 0.06% Triton X-114 was first placed in the sample container with the analyte-Triton X-114 solution referred to previously, and then overlaid on this cushion.[168,170,181,184,185,189] The remaining procedure was the same as just outlined. Figure 10 gives a pictorial representation of this modified cloud-point extraction approach.[185] During centrifugation, the surfactant-rich phase typically sedimented through the sucrose cushion while the aqueous phase remained above the cushion as shown in the figure.

Last, it should be noted that in a few instances, mixed nonionic surfactant[169] (or polymer[186]) systems were utilized in order to manipulate and control the cloud-point temperature required for the desired extraction. For example, when Triton X-45 is mixed with Triton X-114 in various proportions, the cloud point can be adjusted anywhere between the temperature range 0 to 22°C (refer to Figure 5B).[169] In this latter system, the cloud point (CP) of the mixed surfactant micelle solution system depends upon the weight percentage (W) of Triton X-45 added according to

$$CP = 21.4 - 1.23W \quad (4)$$

In addition, glycerol has been added to the Triton X-114 solutions in order to lower the temperature required for the phase separation in extractive applications. The presence of 20% glycerol lowers the cloud point by about 10°C.[183] One could also employ other additives as shown in Table 3 in order to adjust the clouding temperature.

A report has discussed the nature of the aggregate structures (micellar and mesomorphic) of the Triton X-114 surfactant-rich phase that are present under the conditions typically employed during the cloud-point extraction of biological molecules.[229] There is a definite need for more such studies because they would aid in the mechanistic studies as well as lead to better design of such extractive systems.

2. Reported Applications Using Nonionic Surfactants

A summary of some of the biological and clinical materials that have been extracted

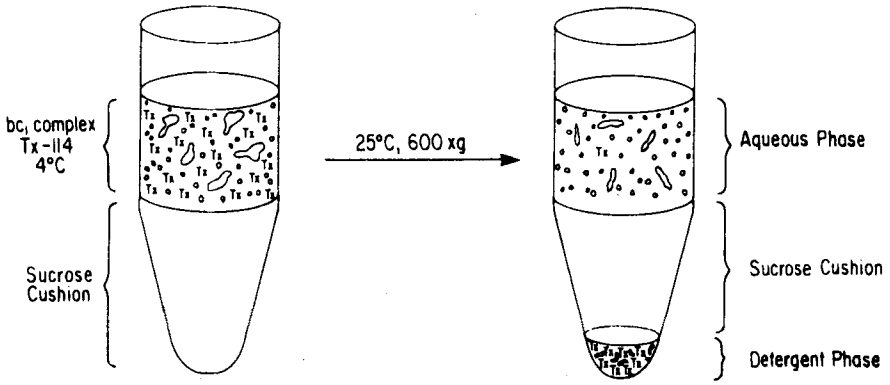


FIGURE 10. Schematic pictorial representation of the purification of biomaterials by phase separation with nonionic surfactants using a sucrose cushion. The specific system concerned the extraction of cytochrome *b* from bacterial bc_1 complexes with Triton X-114 as surfactant. The cytochrome *b* partitions to the surfactant- (also termed detergent-) rich phase while the hydrophilic bc_1 complex remains in the aqueous phase. (Reproduced by permission from Payne, W.E.; Trumpower, B.L. *FEBS Lett.* **1987**, *213*, 108, Elsevier Science Publishing Co., Inc.)

and isolated or partially purified using the cloud-point extraction technique based upon the phase-separation behavior of nonionic surfactants (mostly Triton X-114) is presented in Table 8. As can be seen, since the initial report in 1981, many useful applications have been reported. One interesting example is the partial purification of the thylakoid-bound enzyme, polyphenol oxidase (PPO) from broad bean leaves.[190] In this procedure, the usual interferents, chlorophylls and phenols, are effectively concentrated in the Triton X-114 surfactant-rich phase while the PPO remains in the aqueous phase. The PPO obtained by this method was latent and could be reactivated by trypsin. The purification achieved was 4.4 to 12.4 and the recovery was 43%. This method of purification was faster compared with others used for the isolation of PPO, such as the ammonium sulfate fractionation. The phenolic compounds and chlorophylls were removed in a single step, thus avoiding the need to use other purification methods, which could activate the latent enzyme. These features of the cloud-point extraction approach were thought to make the Triton X-114 method potentially very useful for the extraction of other plant enzymes.[190]

In another application, the phase-separation extraction and distribution of the receptor for immunoglobulin E (IgE) and its subunits in the Triton X-114 system was examined.[174] It was found that the beta and gamma chains, once dissociated from the alpha chain, are readily separated from the latter, provided that the alpha chains remain attached to IgE. This technique was subsequently employed for the preparative isolation of these biomaterials.[174] In addition, the large-scale purification of pyruvate oxidase and protein kinase C using the Triton X-114 cloud-point extraction has been reported.[177]

A very interesting application involves using nonylphenol-5-ethoxy-3-propoxylate (NP-5/3) as the nonionic surfactant for the cloud-point extraction of the antibiotic, cycloheximide, from its fermentation broth.[180] Whereas the product yield in fermentation processes is often limited by product inhibition or degradation, the use of an *in situ* cloud-point extraction can eliminate this difficulty. In addition, the nonionic surfactant used in this extractive fermentation is selective for this hydrophobic antibiotic and yet is nontoxic for the microorganism involved in the fermentation process, *Streptomyces griseus*. The potential problem of foaming is prevented by selecting an appropriate nonionic surfactant for the process (i.e., one that does not foam very much), such as the NP-5/3.[180] A similar approach should be possible for the extractive fermentation of many other biological and pharmaceutical value added products. Thus, the low-energy cloud-point separation technique has demonstrated potential in concentrating and enriching in a single step chemicals obtained from biotechnological processes.

Most recently, use of Triton X-100 (in the presence of excess salt to facilitate lowering of its cloud-point temperature) has been reported to be very effective for the extraction of several hydrophilic and hydrophobic metal-free porphyrins and one metalloporphyrin (hemin).[200] Very high extraction efficiencies and preconcentration factors were achieved at room temperature with fluorescence detection. This same cloud-point extraction system, when coupled with the peroxyoxalate chemiluminescence detection reaction, reportedly provides a highly sensitive and selective method for the detection of urinary coproporphyrin.[201] In addition, the preconcentration of lipophilic vitamins and dansyl amino acids prior to HPLC analysis with electrochemical, UV-visible absorption, and/or fluorescence detection has been reported using Triton X-114 and the cloud-point technique.[202,236]

3. Use of Zwitterionic Surfactants in the Cloud-Point Extraction of Biological Constituents

Recently, it has been demonstrated that zwitterionic surfactants (e.g., C_9 or $C_{10}APSO_4$) could also be employed for the separation of hydrophilic from hydrophobic biological materials.[119] For example, ca. 90% of bacteriorhodopsin could be recovered in

the surfactant-rich phase of C_9APSO_4, whereas the more hydrophilic cytochrome *c* remained in the aqueous phase. In addition, some steroids and vitamin E were effectively extracted with very good preconcentration factors achieved (Table 9).[119] As can be observed from the data, the concentration factors achieved for this aqueous two-phase extraction technique using C_{10}-$APSO_4$ ranged from 26 to 35 with recoveries in the range of 88 to > 96%. Thus, it appears that the use of zwitterionic surfactants should also be considered in such extractive techniques for biological or clinical materials.

As was described in an earlier section, aqueous solutions of zwitterionic surfactants exhibit an upper (rather than lower, in the case of nonionic surfactants) consolute boundary. Thus, their two-phase region exists at low temperature with the one-phase region obtained only upon heating the solution above the critical temperature. The typical experimental procedure employed when utilizing zwitterionic surfactants in the cloud-point technique consists of adding either an appropriate amount of the solid surfactant (or an aliquot of the warmed, concentrated surfactant solution) to the sample-containing solution and mixing for a specified time period. The amount of surfactant added must be such that the final surfactant concentration in the solution exceeds the CMC value to ensure the formation of micelles. Next, the solution (if not already at a low temperature) is cooled below its critical temperature and centrifuged (typically at 1500 rpm for 10 min) in order to promote the phase separation. The two phases can then be analyzed for the desired constituent(s).[119,193]

Comparative data suggest that zwitterionic surfactants might have several advantages over nonionic surfactants in such cloud-point extractive procedures.[119] First, the zwitterionic surfactant materials are homogeneous preparations, whereas many, if not most, nonionic surfactants are still marketed as mixtures (i.e., as a mixture of homologs that differ in the distribution of the

TABLE 9
Summary of Concentration Factors and % Recoveries Possible for the Surfactant-Mediated Phase Separations using Zwitterionic Surfactant Systems (and Nonionic Surfactant for Comparison Purposes)

Surfactant system	Component(s) extracted	Recovery achieved, %	Concentration factor achieved
PONPE-7.5, 2 g / dl at 35°C	Estriol	67	12
	β-Estradiol	80	19
	Estrone	82	19
	Progesterone	—	11
C_{10}-$APSO_4$, 2 g / dl at 35°C	Estriol	90	26
	β-Estradiol	> 96	35
	Estrone	> 96	35
	Progesterone	88	29
PONPE-7.5, 2 g / dl at 30°C[a]	Vitamin E	—	dec.[b]
C_{10}-$APSO_4$, 2 g / dl at 30°C[a]	Vitamin E	—	45[c]

[a] 0.001 *M* 2-mercaptoethanol present.
[b] Vitamin E decomposed.
[c] Some decomposition of vitamin E occurred.
Source: All data taken from Reference 119.

ethyleneoxide moieties present in the surfactant molecule). The fact that aqueous solutions of nonionic surfactants exhibit an upper consolute curve means that they are already phase separated at normal temperatures and do not require any heating. Thus, this is very advantageous when working with thermally liabile biomaterials, such as vitamin E.[119] Most important, as gleaned from the comparative data presented in Table 9, greater extraction efficiencies and preconcentration factors appear to be possible for some analytes when they are extracted with the zwitterionic surfactant media compared to that possible using nonionic surfactants. The zwitterionic-mediated phase-separation step thus offers an ideal prechromatographic approach for extraction and volume reduction of organic and biochemicals.

Another important advantage when using zwitterionic surfactants in preparative or process level applications is that it is easier to subsequently recover the biomaterial from the surfactant-rich concentrated phase via dialysis. This stems from the fact that dialysis is most suitable for removal of the surfactant from biomolecules in situations where the surfactant has a relatively high CMC value (i.e., greater than ca. 1 m*M*), which is the case for many zwitterionic surfactant micelle systems (refer to CMC values in Table 4). In contrast, the CMCs (Table 1) for most nonionic surfactants employed in cloud-point extractive applications are much less and their solutions are not amenable to dialysis for the recovery of the biological material.

4. Affinity Cloud-Point Extraction of Biomaterials

Until recently, there were no reports in the literature concerning the use of appropriate affinity ligands in conjunction with the cloud-point approach for the selective extraction and preconcentration of hydrophilic biomaterials in the surfactant-rich phase. The idea of course would be to select a relatively hydrophobic affinity ligand that would be specific for and strongly interact with the desired hydrophilic biomaterial so that the resulting affinity ligand:biomaterial complex would partition to and bind the surfactant micellar aggregate and thus become concentrated in the surfactant-rich phase following the phase-separation step. Preliminary data indicate that this approach is feasible. Namely, avidin and hexokinase were extracted employing alkyl-biotin and octyl-β-D-glucoside, respectively, as the hydrophobic affinity ligands in cloud-point extractions with the zwitterionic surfactant, C_9APSO_4.[193] A comparative study indicated that the use of the nonionic surfactant, Triton X-114, was not successful in extracting these two hydrophilic proteins using such an affinity cloud-point extraction approach. Although much more work needs to be conducted in this field, the work suggests that the selective extraction of hydrophilic materials using affinity ligands and the cloud-point approach are possible.

5. Mechanism of Protein Partitioning in Two-Phase Aqueous Nonionic Micellar Solutions with Comparison to that in Aqueous Two-Phase Polymer Systems

There have been very few studies aimed at examining the factors that influence the extent of extraction in the surfactant phase-separated systems nor studies aimed at delineating the mechanism involved in such separations. However, recently an eloquent theoretical formulation has been developed that describes and accurately predicts the partitioning of hydrophilic proteins in phase-separated aqueous nonionic micellar solutions.[198] In this model, prior to the phase separation, in the dilute regime, it is thought that the micelles are isolated from each other in the aqueous solution, whereas under conditions of phase separation, the micelles are entangled in a netlike configuration. Using the model developed with this view of the micellar configurations, the theoretically predicted protein partitioning agreed nicely with the experimental values obtained for the partitioning of the hydrophilic protein, ovalbumin, in the two-phase aqueous micellar system of the nonionic surfactant, $C_{10}E_4$. The

basis of the theory revolves around proposed steric excluded-volume interactions between the protein and the elongated, cylindrical, nonionic micelle under the extraction conditions that play a dominant role in determining the extent of partitioning of hydrophilic proteins into the surfactant-rich micellar phase.[198]

In addition, this same group has compared and contrasted the surfactant-mediated with the polymer-mediated aqueous two-phase system with respect to protein separations.[198,199] Based upon the theoretical formulations developed, it is thought that in the partitioning of hydrophobic proteins, surfactant solutions should be much more effective than polymer solutions. "The unique self-associating nature of surfactants enables them to incorporate hydrophobic proteins into micelles and thus to shield the hydrophobic residues of a protein from the bulk aqueous environment." The dual nature of surfactants, that they appear hydrophobic to hydrophobic proteins yet hydrophilic to hydrophilic ones, "is remarkable, in that it allows, through the use of two-phase aqueous micellar solutions, the separation of proteins based on their relative hydrophobic characteristics."[198]

In contrast, polymer systems appear to be superior to surfactant micelles in their ability to separate proteins based on their size.[199] In addition, it is thought that cloud-point extractions with surfactants require much less material compared to polymer systems (i.e., the amount of surfactant required is much less than the amount of polymer required in aqueous polymer two-phase systems for extractions).[203] Last, the surfactant micellar systems appear to be more versatile in that one can *in situ* alter their size and shape merely by altering the temperature or nature of additives in the system.

6. Advantages and Limitations of Cloud-Point Extraction of Biomaterials

Many of the advantages alluded to under the section on extraction of metal chelates also apply to the use of the cloud-point technique in the biological arena. Advantages include experimental convenience, lower cost, and enhanced analytical sensitivity due to the fact that the hydrophobic biomaterials can be easily extracted from the bulk aqueous solution into the much smaller volume element of the surfactant-rich phase following phase separation. As was mentioned in the applications section, the cloud-point extraction technique for many of the bioanalytes mentioned was superior to other techniques used for their isolation and purification. In addition, use of the cloud-point technique provided the purest product possible with maximum recovery of biological activity in many cases. In many instances, the presence of the surfactant micelle in solution aids in the preservation of the biological sample during storage prior to any extraction by preventing its loss due to adsorption to the container walls. For example, the presence of nonionic Tween 20 prevented the loss of DNA due to its adhesion to the surface of its container.[233] The surfactant-rich extractant phase is also very compatible with the carrier solutions utilized in hydrodynamic systems [such as flow injection analysis (FIA) or HPLC] in which case large enhancements in the analytical detector signal are often observed.[236]

Nonionic and zwitterionic surfactants have proven to be very important tools in the initial solubilization, fractionation, and manipulation in aqueous solution of biomaterials as well as for the reconstitution of components of biological membranes.[204] Thus, because these surfactants are already present in such separation schemes means that it is very easy to also try to utilize their cloud-point behavior in order to further facilitate the isolation of the desired biomaterial. In these cases no other extractant material is required, only initial selection of a surfactant that exhibits phase-separation behavior and slight manipulation of the surfactant concentration (so micelles form) and of the other conditions (to achieve the phase separation). Thus, an advantage of the cloud-point technique stems from the fact that it is compatible with the first step in many existing biomaterial separation and purification schemes.[170] Also, the surfactants commonly employed in

the cloud-point technique, i.e., nonionic and zwitterionic surfactants, are very mild and thus normally do not alter the bioactivity of the extracted material(s).

Last, the simplicity of the cloud-point extraction approach and the mildness of the conditions required to effect it have proven to be especially useful for studies in which one wishes to test the function of receptors reformed from isolated units,[174] studies of proteins in hydrophobic domains[171,181,190] as well as studies of the physical properties of proteins,[178,182] and hydrophobic interactions in such systems, among others.[175,176,192] It has proved useful for characterizing differences between integral membrane proteins as well.[173] Its value in the diagnosis of some patient conditions (as those with type I thrombasthenia) has also been discussed.[178]

C. Extraction and/or Preconcentration of Organic Species and Applications in Environmental Cleanup Procedures

1. Extraction and Preconcentration of Organics from Aqueous and Solid Samples

Solubilization of water-insoluble or sparingly soluble organic materials in micelles is a well-known fact.[2,5] Generally, the order of solubilization capacity, i.e., the moles of hydrocarbons and polar compounds solubilized per mole of surfactant, appears to be as follows for the different charge-type surfactant micelles: nonionics > cationics > anionics for surfactants with the same hydrocarbon chain length. In addition, for the nonionic surfactant micelles, their solubilization capacity appears to rapidly increase as the temperature is raised to near the cloud point.[209] This has been attributed to an increase in the micelle size. In addition, solubilization kinetic studies indicate that the rate of solubilization also increases as the lower consolute (cloud) temperature of the nonionic surfactant is approached.[230] As a general class, nonionic surfactants are usually the most effective in solubilizing and binding organic solutes. Table 10 summarizes some of the binding constant (or partition coefficient) data from the literature for the association of organic solutes with micelles (or for the distribution of organics between the micellar and aqueous phases). As can be seen, even polar organic materials, such as hexanol and benzyl alcohol, can partition to nonionic surfactants to an appreciable extent. Thus, the cloud-point technique should be applicable for the extraction and preconcentration of organic compounds, particularly those that need to be monitored in water or soil samples.

The solubilization of organic materials in micelles and subsequent cloud-point extraction technique thus offers a convenient alternative to conventional liquid-liquid extractions that use organic solvents. In addition to cost, time, sensitivity, and safety hazard considerations, the use of micelles in this extraction technique allows direct analysis of small samples of low concentration.[197,211,212] The small energies involved in the cloud-point technique (heating step if required) make this separation process appealing from an economic viewpoint also.[197] The surfactant-rich phase is also compatible with micellar or conventional hydroorganic mobile phases, which allows for the further fractionation and/or analysis of the extracted material using HPLC.[45,212]

Another attractive feature is that the use of surfactant micellar media also aids in the storage of aqueous environmental samples prior to the extraction step by preventing the loss of the organic material due to its sorption on the surface of the container walls.[212,231] For example, the presence of nonionic Brij 35 micellar media in the sample proved to be as effective as acetonitrile at a concentration of 40% (v/v) in preventing loss of polycyclic aromatic hydrocarbons (PAHs) due to adsorption on the surface of PTFE or borosilicate glass containers.[231]

The use of micelles and their phase-separation behavior for the extraction of organics is relatively recent.[212,232] Some of the published data on such extractions are summarized in Table 11. As can be seen, the cloud-point extraction of organic pollutants,

TABLE 10
Summary of Some Organic Solute-Micelle Binding Constants or Partition Coefficients for Nonionic Surfactant Micellar Systems

Organic compound	Surfactant micelle system	K_B[a] (pr P[b])
Benzyl alcohol	Brij 35 ($C_{12}E_{23}$)	(401)[45]
Benzaldehyde		(644)
Acetophenone		(815)
Methyl benzoate		(1376)
Benzene		(1985)
Dimethyl terephthalate		(3222)
Benzene	Witconol SN70 ($C_{10-14}E_7$)	(165)[226]
Toluene		(355)
o-Xylene		(1100)
Phenol	$C_{12}E_{4.2}$ / $C_{12}E_8$	55[205]
4-Chlorophenol		260
3,5-Dichlorophenol		1050
2,4-D acid		700
2,4, 5-T acid		1600
p,p'-DDT[c]	Brij 35	(log P = 5.75)[206]
	Triton X-100	(6.15)
	Triton X-114	(6.18)
1,2,3-TCB[d]	Brij 35	(3.31)
	Triton X-100	(3.82)
	Triton X-114	(3.95)
Naphthalene	Brij 30 ($E_{12}E_4$)	(log P = 4.59)[207,e]
	Igepal CA-720 (OPE_{12})	(4.63)
	Triton X-100	(4.64)
Phenanthrene	Brij 30	(5.57)
	Igepal CA-720	(5.68)
	Triton X-100	(5.70)
Pyrene	Brij 30	(6.53)
	Igepal CA-720	(6.01)
	Triton X-100	(6.03)
cis-DE-I[f]	Tween 80	5320[228]
trans-DE-2[f]	Tween 80	12600
Ferrocene	$C_{12}E_5$	730[208,g]
1,1'-Dimethylferrocene	$C_{12}E_5$	4000
n-Butylferrocene	$C_{12}E_5$	6500
4-Nitrophenol	Triton X-100	(log P = 1.98)[147]
2,4-Dinitrophenol	Triton X-100	(1.34)
2-Nitrophenol	Triton X-100	(1.62)
4,6-Dinitro-*o*-cresol	Triton X-100	(2.24)
4-Nitrobenzoic acid	Triton X-100	(1.45)
Fuberidazole (neutral)[h]	$C_{12}E_{10}$	(190)[214]
Fuberidazole (protonated)	$C_{12}E_{10}$	(3.8)

[a] K_B refers to the solute-micelle association (or binding constant) per molar.
[b] P refers to the partition coefficient of the solute between the micellar and aqueous phase. These values are given in parentheses.
[c] Refers to 1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethane.
[d] Refers to 1,2,3-trichlorobenzene.
[e] Value refers to the mole fraction micelle phase / aqueous phase partition coefficients.
[f] Refers to *cis*- and *trans*-7,8-diol-9,10-epoxides of benzo[*a*]pyrene, respectively.
[g] Value of binding constant in units of cubic decimeters per mole.
[h] Refers to the fungicide, [2-(2'-furanyl)-1H-benzimidazole].

TABLE 11
Summary of Extraction Parameters for the Extraction and Preconcentration of Organic Compounds from Water using the Cloud-Point Extraction Technique

Organic compound	Surfactant micelle system	Extraction parameters[a] %
Phenol	$C_{12}E_{4.2}$ / $C_{12}E_8$	72[205]
4-Chlorophenol		88
2-Nitrophenol	PMHP-B-CD	73[135]
3-Nitrophenol		34
4-Nitrophenol		70
3,5-Dichlorophenol	$C_{12}E_{4.2}$ / $C_{12}E_8$	98.5[205]
2,4,5-Tricholorophenol		>99.9
2,3,4,5-Tetrachlorophenol		>99.9
Pentachlorophenol		>99.9
4-Chlorophenol	C_8E_3	87.1[213]
2,4-Dichlorophenol		95.7
2,4,5-Trichlorophenol		97.1
Pentachlorophenol		>99.9
4-*t*-Butylphenol	Igepal CA-520 (OPE_5)	97 (19.4)[210]
2-Naphthol	PMHP-B-CD	99[135]
n-Hexanol	Igepal CA-620 ($OPE_{7.2}$)	39–60[b]
n-Octanol	Igepal CA-620	93
Parathion	Triton X-114	94 (47)[202]
2,4-D	$C_{12}E_{4.2}$ / $C_{12}E_8$	84.5[205]
2,4,5-T		98
DDT		>99.9
3-Chlorobiphenyl		>99.9
3,3′-Dichlorobiphenyl		>99.9
2,2′-Dihydroxybiphenyl	PMHP-B-CD	71[135]
Decane	$C_{12}E_5$	c[196]
Benzene	C_8E_3	47.3[213]
Toluene		77.1
p-Xylene		99.0
o-Xylene		99.5
Fluoranthene	Triton X-100	80[164,d]
	C_8E_3	90
	GX-80[e]	79[211]
Benzo[*k*]fluoranthene	GX-80	95[211]
Benzo[*a*]pyrene	GX-80	104
Aniline	PMHP-B-CD	45[135]
N-Methylaniline		74
2-Nitroaniline		73
3-Nitroaniline		34
4-Nitroaniline		70

[a] Refers to the percent of the organic material extracted into the surfactant-rich (or cyclodextrin-rich) phase. Numbers in parentheses give the preconcentration factors (i.e., the concentration of the organic solute in the surfactant- (or cyclodextrin-) rich phase compared to that originally present in the bulk aqueous solution.

[b] % Extraction depends upon the temperature at which the cloud-point extraction was conducted.

[c] Double cloud-point procedure employed, i.e., conditions were such that a microemulsion formed. The extraction kinetics of this system have also been examined.[197]

[d] The low extraction percent resulted in part due to the fact that the cloud point of Triton X-100 is quite high and the solution cooled somewhat during the centrifugation step.

[e] Refers to the surfactant Genapol X80, a C_iE_j type surfactant in which the alkyl moiety is *i*-tridecyl and which has 8 oxyethylene units (CMC = 0.05 m*M*; cloud point = 42°C).

including phenolic, polycyclic aromatic hydrocarbons, and pesticides using nonionic surfactant micelles has been reported. In addition, derivatized cyclodextrins have also recently been utilized to affect the extraction of organics from aqueous solutions (Table 11).[135] The percent extraction and the concentration factors achieved with the cloud-point extraction technique using surfactants or cyclodextrins are in most cases comparable to those obtained using conventional extraction procedures with organic solvents or supercritical fluids.[232]

The cloud-point technique also offers the possibility for extraction of organics from solid sample matrices. In such an application, one first contacts the solid sample with an aqueous nonionic surfactant micellar-containing solution below (or above for zwitterionic surfactant systems) its critical temperature for some time period to allow for desorption of the organic from the solid matrix and solubilization in the micellar aggregate. Next, the solution is filtered to remove the soil or solid sample. Following these steps, the regular cloud-point approach is followed as previously outlined. Using this approach, anthracene has been extracted from a coal sample using the zwitterionic surfactant C_9APSO_4.[119] Additionally, polychlorinated biphenyls (PCBs), anthracene, pyrene, phenanthrene, and benzopyrenes have been extracted from spiked soil samples with the recoveries being in the range of 65 to 90%.[213] In addition, chrysene and benzo[*a*]pyrene removal from coal tar contaminated soil was in excess of 98% using C_8E_3 as the surfactant and the mentioned cloud-point technique (with sonication). Recently, ca. 67% of benzo[*a*]pyrene and benzo[*k*]fluoranthene were reportedly solubilized and recovered from soil suspension using the nonionic surfactant Genapol X-80.[211]

2. Utilization of Phase Separations in Environmental Cleanup Procedures

In addition to the extraction and preconcentration of organic materials, the cloud-point approach has been proposed as a means with which to treat organic contaminated water.[210] In this suggested approach, the basic cloud-point technique can be utilized in order to concentrate the organics in the surfactant-rich phase. This surfactant-rich phase would be separated from the aqueous phase via use of a phase splitter, completing the separation. If the dilute aqueous phase after such process contains organic solute and/or surfactant in low enough concentrations, it could then be recycled to the process or returned to the environment. The organic solute and/or surfactant could be separated from the surfactant-rich phase (e.g., by a foam separation step) and sold, reused, or disposed.[210]

As previously noted, the cloud-point technique can be utilized for the direct extraction of organics from solid matrices. That nonionic surfactant solutions should be effective in desorbing and extracting organic compounds from soil samples or solid matrices should not be surprising in view of the fact that such solutions have been proposed to wash and clean up organic contaminated soils.[215-224] The preliminary data on such a soil remediation approach are encouraging, with recoveries of the organics being in the range of 20 to 93% depending upon the type and nature (porosity, organic content, etc.) of both the soil involved and the nonionic surfactant utilized (i.e., surfactant hydrophobicity, dose concentration employed, etc.). Table 12 summarizes the published literature on the utilization of nonionic surfactant solutions to remove organic materials from either actual field contaminated or spiked soil samples. Note that these extractions were all conducted below the cloud-point temperature and merely relied on the solubilization and/or interfacial tension lowering effect(s) of the surfactant system.

In such soil remediation applications, one problem that arises is what to do with the subsequent large volumes of aqueous surfactant wash solutions obtained, which contain the organics desorbed from the contaminated soil. Well, by proper selection of the initial nonionic surfactant employed, one can merely apply the phase-separation (cloud-point) extraction technique that would further con-

TABLE 12
Summary of Organic Compounds / Material Extracted from Contaminated or Spiked Soil Samples using Aqueous Nonionic Surfactant Solutions[a]

Nonionic surfactant system	Contaminant recovered from the soil (% removed)	Ref.
0.75% Solution of Adsee 799 and Hyonic NP-90	PCBs (92%)	215
2.0% Solution of Adsee 799 and Hyonic NP-90	Petroleum hydrocarbons (93%)	215
2% Makon 10 or 1% Triton DF16[b]	11 TOXs[c]	216
0.5–2.0% Witconol SN 70[d]	Automatic transmission oil (73–82%)	217, 221
Surfynol 485	Total petroleum hydrocarbons (est. 83%)	218
1.0–1.1% Brij 30 ($C_{12}E_4$)	Anthracene (60%); pyrene (65%)	219
1.5% Igepal CA-720	Pyrene (83%)	219
1.5% Triton X-100	Pyrene (78%)	219
Witconol SN 70[d]	Aroclor 1248 (PCBs) (56–86%)	220
0.5% Triton X-100	Trichloroethylene (80–95%)[e]	222
1.5% Brij 30	Phenanthrene (82%)	223
1.5% Triton X-100	Phenanthrene (88%)	223
100 ppm Alfonic 810-60[f]	Phenanthrene (20%); biphenyl (18%)	224

[a] These extractions did not involve any cloud-point procedure.
[b] Polyoxyethylene alcohol and ethyoxylated nonylphenol, respectively.
[c] Specific compounds extracted from soil included hexachlorobenzene, three isomeric dichlorobenzenes, two isomeric trichlorobenzenes, hexachlorobutadiene, endrin, dieldrin, aldrin, and heptachlor. 135–150 mg TOX per kilogram of soil were removed in the procedure.
[d] Refers to $C_{10-12}E_7$.
[e] Depends upon the exact surfactant concentration in the aqueous extracting solution.
[f] Refers to $C_{8-14}E_{4.5}$.

centrate the organics and surfactant into the small volume element of the surfactant-rich phase. The bulk aqueous phase could be returned to the environment and/or subsequently subjected to another cloud-point extraction step in order to remove any residual organics. The approach previously mentioned for the treatment of wastewater[210] would be applicable to this situation and could be configured to provide a continuous, on-line system as proposed.

A survey of the literature reveals only two articles that made mention of such proposed application of the cloud-point technique to soil cleanup protocols. The GHEA process for the decontamination of soil and water alludes to the phase-separation behavior of surfactant solutions as one step in an overall process.[225] Namely, this process specifies the separation of the surfactant and organic contaminant species from the bulk wash water by phase separation under controlled temperature and pH conditions. However, no details as to the specific surfactants employed nor actual conditions involved appear in the open literature. More recently, the direct cloud-point extraction of engine oil spiked soil samples using aqueous solutions of the nonionic surfactant Triton X-114 was reported.[227] Roughly 85 to 98% of the oil originally present in the soil was extracted and recovered in the surfactant-rich phase. Thus, it appears that the use of the cloud-point technique for the further concentration and volume reduction of surfactant and solubilized organic contaminants in soil remediation procedures should be very attractive. In addition, the use of appropriate chelating

agents and the cloud-point technique should allow for the recovery of metal ions from spills and/or waste site soils.

D. Some General Experimental Considerations

1. Surfactant Preparations and Purity

Industrial and commercial preparations of nonionic surfactants are, as a general rule, chemically impure.[204,237-239] In many instances, they may contain varying amounts of additives such as catalysts, one batch may differ from the next, and, after prolonged storage, liquid nonionic surfactants often exhibit different compositions at the bottom of the container compared to that on the top. In addition, storage can lead to aging effects of nonionic micellar surfactant solutions[245] as well as the occurrence of degradation reactions.[26] The surfactant purity is probably most critical in those applications involving biological materials, such as proteins.[237,238] For example, sulfhydryl oxidizing and peroxide contaminants, among others, have been reported to be present in commercial nonionic surfactant preparations and to cause difficulty in protein solubilization studies.[237,238] In many applications, in order to obtain accurate and reproducible results, it is advisable to purify such industrial or general commercial grade surfactants prior to use. Several purification schemes have been published for nonionic surfactants,[237,240,241] including a recently reported three-phase extraction procedure.[26] Alternatively, the use of highly purified analytical nonionic surfactant preparations that have recently become available is recommended.

Another potential problem with nonionic surfactants is their heterogeneity.[239] That is, unless they are fractionated, nonionic surfactants will have polydisperse polyoxyethylene head groups due to the statistical polymerization of the ethylene oxide moiety (i.e., the surfactant preparation is an oligomeric mixture of the monosubstituted nonionic surfactants, which differ in the distribution of their ethoxy groups). The number of ethylene oxide units per molecule given by the manufacturer is only a mean value. This value can be easily determined using fast atom bombardment mass spectrometry.[242] A TLC method with flame ionization detection has also been reported[243] as has a gas chromatographic procedure involving prior silylation.[239] An excellent monograph on the chemical analysis of nonionic surfactants is available.[244]

In addition, there can also be some heterogeneity in the hydrophobic portion of synthetic nonionic surfactants because inhomogeneous alcohols and fatty acids are typically utilized in their synthesis. Derivatized cyclodextrins also suffer from the same problem because a number of different substituted isomers are typically formed upon derivatization of the native cyclodextrins. In this regard, the zwitterionic surfactants are typically superior in the sense that they can be easily crystallized and purified so as to yield pure and homogeneous surfactant preparations. It should be noted that several companies now do offer highly purified, homogeneous, nonionic polyoxyethyleneglycol alkylether surfactant preparations. These surfactants have no distribution of ethylene oxide. However, as yet, no corresponding materials are available for the aromatic-containing nonionic surfactants, i.e., the OPE or NPE series.

As observed in Tables 1 and 2, a range of values for the properties (i.e., CMC, N, cloud-point temperature) for the same nonionic surfactant solution has been reported. The origin of these observed discrepancies stems from the fact that most nonionic surfactant preparations often contain impurities and are polydisperse, which alters their physical properties.[26]

2. Removal of the Surfactant from the Extracted Material

In many applications involving the cloud-point extraction, it is necessary to separate the extractant surfactant from the extracted material. Many different possible schemes have been developed for this purpose depending upon the objective and/or

subsequent utilization of the material to be recovered. Because these procedures are nicely summarized in the chemical literature,[239,246,247] only a few will be briefly described here. Large quantities of nonionic surfactants are probably best extracted with polar solvents (such as dichloromethane) or solvent mixtures.[248] Probably the most common and easiest method for surfactant removal is dialysis.[246,249] This approach is simple and effective for those surfactants that have a relatively high CMC value (> 1 mM). Thus, dialysis can be employed for the removal of most zwitterionic but not nonionic surfactants due to the low CMC values of the nonionics. The most useful alternative method for the removal of nonionic surfactants, either by batch or column, is via use of appropriate absorbent resins or chromatographic supports.[247] A variety of chromatographic supports have been developed for the removal of nonionic and other charge-type surfactants from biological samples.[247,252] The absorption capacity of such materials is in the range of ca. 60 to 170 mg surfactant per milliliter of resin solution.[250] Density centrifugation, ultrafiltration, electrodialysis, and comicellization procedures have also been reported for the removal of surfactants in the literature.[247] Furth has published a comparative survey of the various possibilities for separating surfactants from proteins along with their advantages and limitations.[246,251]

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Note Added in Proof: Very recently, a new nonionic alkyldimethylphosphine oxide-phospholipid surfactant-based two phase system for the separation of proteins has been described [G. C. Kresheck, Abstract of Papers from 206th ACS National Meeting, American Chemical Society (Div. of Colloid & Surface Chem.): Washington, D.C., 1993, Abstr. #160].

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