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Alkyltrimethylammonium surfactant-mediated extractions: characterization of surfactant-rich and aqueous layers, and extraction performance

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Abstract

Most surfactants employed for extraction purposes contain strongly absorbing chromophores, and therefore cannot be used with the ultraviolet–visible HPLC detector because of the high background created. Alkyltrimethylammonium surfactants, which do not have strongly absorbing chromophores, have shown promise as an extractant compatible with HPLC–ultraviolet–visible detection. In our extraction procedure, alkyltrimethylammonium surfactants are added to a sample containing organic analytes in distilled water. Sodium chloride is next added, then the entire sample is shaken. Before centrifugation, 1-octanol is added to aid in the two phase formation of surfactant-rich and aqueous phases. In this paper, we present the results of our studies on the extraction behavior of an alkyltrimethylammonium surfactant technique using various organic compounds as test probes. Specifically studied are the extraction behavior of organic bases, isomers of varying polarity and a zwitterionic species that has different charges at various pH values. Results from multiple extractions to obtain quantitative recovery of analytes is also presented. The composition of each phase is elucidated through the interpretation of data obtained from thermogravimetric and carbon, hydrogen and nitrogen (CHN) instrumentation.

Keywords: Surfactant-mediated extraction; Extraction methods; Alkyltrimethylammonium surfactants; Surfactants; Cetrimide

1. Introduction

Analytical scale extractions of organic analytes using surfactants are an attractive alternative to using organic solvents. Advantages of using surfactants versus organic solvents include lower toxicity, lower purchase and disposal costs, direct compatibility with high-performance liquid chromatography (HPLC), and minimal loss of analyte due to evaporation [1]. Non-ionic surfactants are the most commonly used for surfactant-based extractions. Extraction procedures using this class of surfactant are based upon the cloud point phenomenon initially reported by Watanabe [2]. Altering the temperature of a miscible nonionic surfactant aqueous solution will result in a separation of surfactant-rich and aqueous phases. Organic analytes originally present in solution will partition into the surfactant-rich phase. An aliquot of the surfactant-rich phase is then injected into a HPLC. Excellent reviews on cloud-point extractions exist [3–5]. Applications of surfactant-based extrac-

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tions include the analyses of polyaromatic hydrocarbons [6,7], polychlorinated biphenyls [8], pesticides [9,10], vitamins [10,11], and steriodal hormones [11]. Recently, cloud-point extractions have been proven to be compatible with capillary electrophoresis [12].

Unfortunately, most non-ionic surfactants contain the strongly absorbing phenyl chromophore. Method development and trace analysis becomes difficult due to the large background created from the aliquot of injected surfactant-rich phase when using UV–visible detection. Other detectors that do not respond to the surfactant have been used [6–11]; however, UV–visible is the most commonly available detector for HPLC. We have reported the use of a cationic surfactant-mediated extraction technique that is highly compatible with UV–visible detection [13], and incorporates alkyltrimethylammonium surfactants as the extractant. These surfactants do not contain a strong chromophore and the background obtained was minimal. In this previously reported procedure, chlorinated phenols were extracted from a river water matrix into a surfactant-rich phase with the aid of NaCl and a co-surfactant, 1-octanol. These chlorinated phenols, which are weak organic acids, were recovered quantitatively in their neutral or protonated form. Recovery decreased at pH values where the analytes were in their anionic or deprotonated form.

In this paper, we present the results of the extraction behavior of different classes of compounds using alkyltrimethylammonium surfactants as the extractant. These compounds include weak organic bases, a zwitterionic species, and isomers of different polarity. Also, multiple extractions to obtain quantitative recovery of analytes is presented. The composition of the surfactant-rich and aqueous phases is obtained through thermogravimetric and CHN instrumentation.

2. Experimental

2.1. Chemicals and materials

Cetrimide (mostly C_{14}, with C_{12} and C_{16} alkyltrimethylammonium surfactants), 2-chloroaniline, 4-chloroaniline, 2,6-dimethylpyridine, 2,4,6-trimethylpyridine, 3-hydroxypyridine, 3-methoxypyridine and the dicyanobenzenes were analytical grade and purchased from Aldrich Chemical Company (Milwaukee, WI). Sodium chloride and 1-octanol were analytical grade and purchased from J.T. Baker (Phillipsburg, NJ). HPLC grade methanol was purchased from Fisher Scientific (Pittsburgh, PA). Water used for solution and mobile phase preparation was deionized (16 megaohms/cm). All chemicals were used as received. 0.1 *M* buffers of NaOAc (pH 4.0), KH_2PO_4 (pH 6.75) Boric Acid–KCl (pH 9), and $NaHCO_3$ (pH 10.0) were made according to Lange's Handbook of Chemistry [14]. Adjusted HCl was used to make solutions of pH 2.0 and pH 3.0, and saturated $Ca(OH)_2$ was used for a pH 12.6 solution.

2.2. Instrumentation

The high-performance liquid chromatograph system utilized consists of a Varian (Walnut Creek, CA) Model 9012 single-piston pump and a Varian Model 9050 single-wavelength UV–visible detector. Separations were performed on an Ultrasphere C-18 4.6× 250 mm, 5 μm particle size (Beckman, Fullerton, CA) column, protected by a guard column of the same phase, 10 μm particle size. Peak integration was performed with a Shimadzu (Columbia, MD) Model CR 601 integrator.

HPLC gradient conditions were used to separate the analytes. All conditions consisted of 30:70 methanol–water to 90:10 methanol–water in 15 min, except for the pyridine analytes which consisted of 30:70 methanol–pH 11 buffer to 90:10 methanol–pH 11 buffer in 15 min. The UV–visible single-wavelength detector was set at 280 nm.

CHN analysis was performed on a Leco (St. Joseph, MI) Model 2000 CHN and thermogravimetric analysis (TGA) was performed on a Leco Model 601 TGA.

Dipole moments in units of Debyes (D) were calculated for the test molecules using Hyperchem 5 (Hypercube, Gainesville, FL).

2.3. Extraction Procedure

A 0.5 ml aliquot of 0.1 g/ml Cetrimide solution was added to 10.0 ml of a buffer solution in a 16×150 mm Teflon-capped test tube. Four grams of

sodium chloride was added next. The mixture was then rotated at 33 rotations/min for 15 min. After this period, 7 μl of the cosurfactant (1-octanol) was added, and the mixture was again rotated for 15 min at the same speed. The mixture was then centrifuged for 5 min at 5000 rpm (2129 *g*), which separated it into surfactant-rich and aqueous phases. The surfactant-rich layer floated on the top of the aqueous layer. A 25 μl aliquot was removed from the surfactant-rich phase (approximately 350 μl) and injected into a 20 μl HPLC sample loop. As an alternative to centrifugation, solutions may be allowed to sit overnight for complete phase separation to occur. All tested compounds in this study were extracted at 1 part-per-million (ppm). Data from all studies was averaged from at least three experimental trials.

3. Results and discussion

3.1. Characterization of surfactant-rich and aqueous layers

Upon the addition of Cetrimide into the aqueous sample, micelles are formed because the surfactants in this mixture are above their critical micelle concentration (3.6×10^{-3} *M*, 1.3×10^{-3} g/ml) [15]. Chloride ions formed upon the addition of the strong electrolyte, NaCl, act to decrease the repulsions between the polar, positively charged head groups of the surfactants in the micelle. Solubilization of water in the micelles decreases because the excess chloride and sodium ions are taken up by water to become hydrated. As the ionic repulsions between the surfactant molecules decrease, strong chain–chain, or hydrophobic interactions occur. The mixture at this stage becomes more viscous. In the procedure, 7 μl of the co-surfactant, 1-octanol, was needed to form separate surfactant-rich and aqueous phases. The co-surfactant aids in the separation of these two phases by absorbing at the surfactant–water interface.

Percent water and percent NaCl were obtained from thermogravimetric analysis. Percent moisture and percent ash (NaCl) in the surfactant-rich phase was 69.0±0.7% and 24.1±0.3% respectively. Percent moisture and percent ash (NaCl) in the aqueous phase was 73.5±0.2% and 26.1±0.2% respectively. The CHN instrument was used to determine amount of surfactant and 1-octanol. The density of the aqueous layer was 1.20 g/ml. The amount of surfactant in the surfactant-rich layer was 0.0437±0.0015 g and no surfactant was detected in the aqueous phase below the detection limit of the instrument (approximately 5.8×10^{-4} g). The volume of 1-octanol calculated in the aqueous phase, based on a measured density of 1.20-g/ml, was 1.66±0.13 μl. The volume of 1-octanol calculated in the surfactant-rich phase is 7.66±1.91 μl, based on the density of 1-octanol (0.827 g/ml). Based on this data, the calculated mole ratio of surfactant to 1-octanol in the surfactant-rich phase is 2.27±0.55:1. We feel that this is a critical number because separate phases will not form at added volumes slightly greater than 7 μl; large and less concentrated surfactant-rich layers form at added volumes below 7 μl.

The above-determined composition allows us to classify this surfactant-rich phase as a water in oil microemulsion. Water in oil microemulsions are described as phases having more water than the solubility of water in the co-surfactant and also having high concentrations of NaCl [17].

3.2. Extraction performance using selected organic molecules

Among the various test probes used to study the nature of the surfactant-rich phase were 3-hydroxypyridine and 3-methoxypyridine. 3-hydroxypyridine can possess a positive charge, a net neutral charge [sum of a positive and negative charge (zwitterion)], or a negative charge, depending upon the pH of the solution. Fig. 1 depicts the percent recovery of 3-hydroxypyridine into the surfactant-rich phase from pH 2.0 to pH 12.6 buffered solutions. The acid dissociation constants for these species are pK_{a_1} = 4.80 and pK_{a_2} = 8.72, respectively. In the range of pH 2.0 to pH 3.0, greater than 98% of 3-hydroxypyridine is positively charged because the amine functional group is protonated. Extraction recovery of 3-hydroxypridine in this form is low. From pH 6.8 to 8.0, 3-hydroxypyridine exists mainly in the zwitterionic neutral form. There is a slight increase in recovery in this region as this species loses its net positive charge due to the hydroxy group becoming deprotonated. At pH values greater than 10.0, 3-

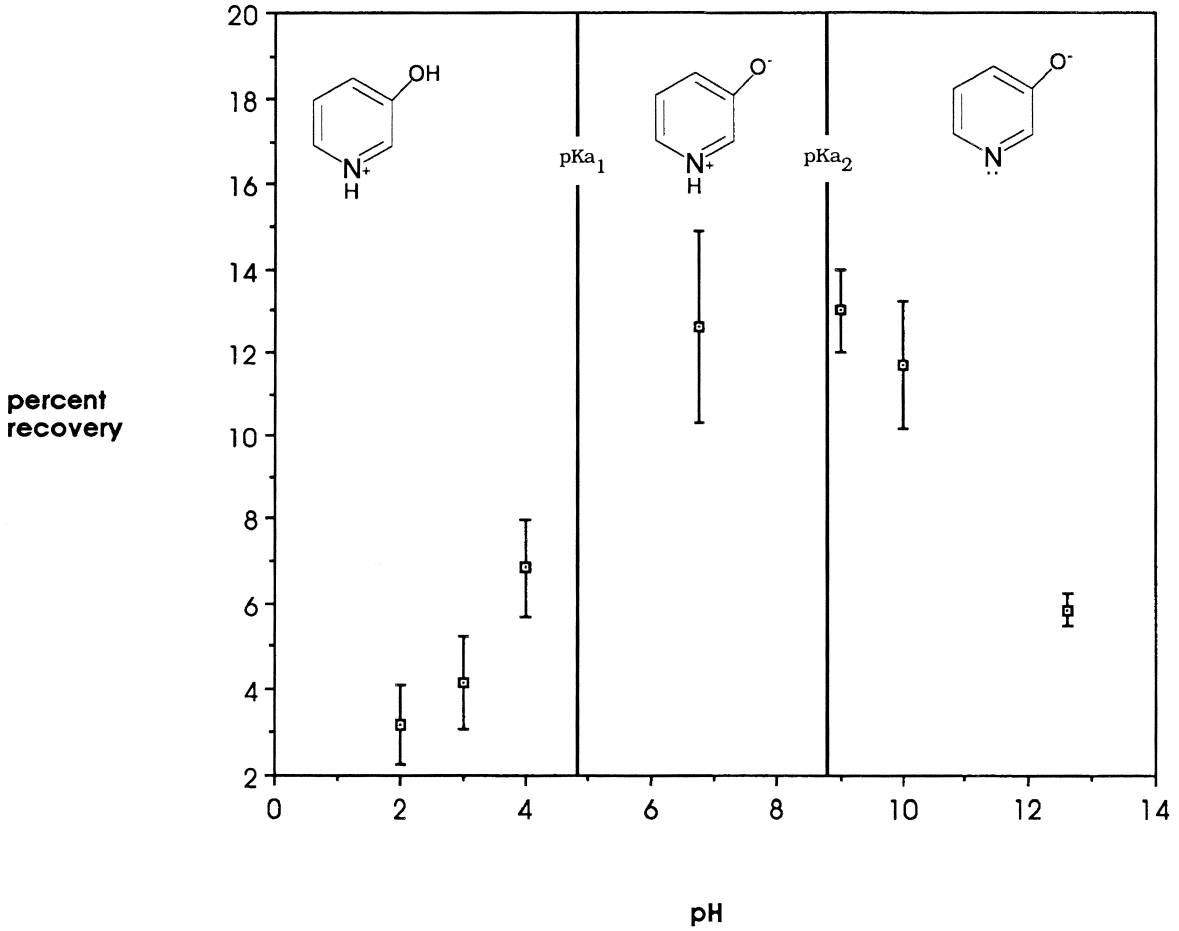

Fig. 1. Percent recovery of 3-hydroxypyridine from pH 2.0 to 12.

hydroxypyridine exists mostly in a negatively charged form because the amine group is now deprotonated. In this region percent recovery falls low once again. Significant increases or decreases in recoveries between the net charged forms of 3-hydroxypyridine are not observed. Therefore, electrostatic interactions are not likely occurring between the different forms of 3-hydroxypyridine and the surfactant-rich phase. The net neutral form has a slightly greater affinity for the surfactant-rich phase than the positive or negative form. However, this zwitterionic form is charged and therefore very hydrophilic; hence recovery is low. Lower recoveries for the charged forms are due to these forms being slightly more hydrophilic than the net neutral form. Although the recovery of the zwitterionic form is greater than the individually charged forms, the absolute recovery (approximately 12.5%) is still low.

In our previous work, we studied the extraction behavior of organic acids (chlorinated phenols) [13]. Our results indicated that extraction efficiency was high (greater than 95%) at low pH values, where the phenols were in their neutral form. At high pH values, where the phenols acquired a negative charge from the deprotonation of the hydroxy group, recovery declined (approximately 70%). The extraction behavior of the organic base, 3-methoxypyridine, was studied at pH values similar to 3-hydroxypyridine. 3-methoxypyridine exists in two forms, cationic and neutral, in solution. Unlike the proton on the hydroxy group of 3-hydroxypyridine, protons on the methoxy group are not acidic. Only the amine

functionality in 3-methoxypyridine is pH dependent. As pH values increase, the positively charged 3-methoxypyridine becomes neutral because the amine functionality is now deprotonated. The acid dissociation constant for 3-methoxypyridine is $pK_a = 4.20$. Fig. 2 depicts the percent extraction of 3-methoxypyridine at various pH values. An increase in recovery is observed as the pH increases. The recovery increase coincides with 3-methoxypyridine acquiring a predominately neutral form. Because the recovery of 3-methoxypyridine is low even in the neutral form, we tested other organic amines above and below their respective pK_a values to see if recovery of other bases is better. The percent extraction values are listed in Table 1 for some pyridine and aniline compounds. There is a clear observation in recoveries for these organic amines above and below their pK_a values. At pH values below the pK_a values of the amines, the recoveries are lower than when the pH values are above the pK_a values. The surfactant-rich phase clearly prefers to extract the less hydrophilic or neutral form of these organic bases. This Table also reveals that higher recoveries are obtained with organic amines (anilines) other than pyridines. Never the less, we were unable to obtain quantitative recovery of these amines for a single extraction. To test whether a quantitative recovery is possible, we attempted repetitive extractions of the anilines at pH 6.75. The surfactant-rich phase from the first extraction was removed and placed into a separate container. Another 0.5 ml, 1.0 g/ml aliquot of surfactant

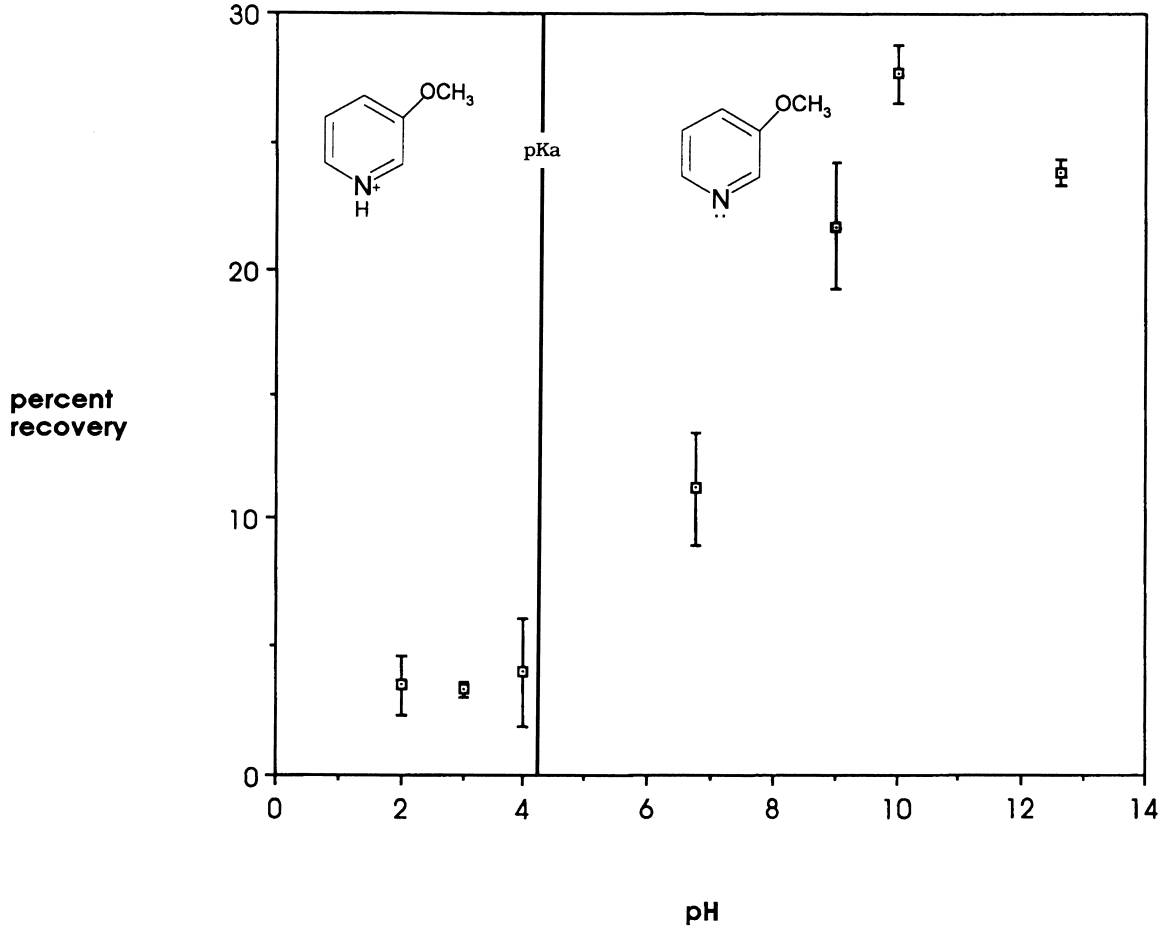

Fig. 2. Percent recovery of 3-methoxypyridine from pH 2.0 to 12.

Table 1
Percent recovery of selected organic bases

Organic base	pK_a	pH 3.0	Percent pH 11	Recovery pH 1.0	pH 6.75
2,6-dimethylpyridine	6.71	5.1±0.9	21.0±3.2	–	–
2,4,6-trimethylpyridine	7.43	3.3±1.0	28.3±1.8	–	–
2-chloroaniline	2.64	–	–	33.5±1.6	75.3±3.7
4-chloroaniline	3.99	–	–	11.5±4.7	76.9±7.6

solution was delivered to the test tube containing the remaining anilines. The mixture was shaken, then 6 μl of 1-octanol was added. The mixture was rotated in the same manner as the procedure and after allowing the mixture settle, a surfactant-rich layer of similar volume to the original volume was obtained. After three repetitive extractions, a quantitative recovery of the amines was obtained. Table 2 lists the percent recoveries for these amines after the respective extractions. A concentration factor of 11.5 was obtained after three extractions for both analytes at 1 ppm.

We also studied the extraction behavior of the three isomers of dicyanobenzene. These isomers have great differences in dipole moments and thus, wide polarity differences. The dipole moments are 0 for 1,4-dicyanobenzene, 3.86 for 1,3-dicyanobenzene, and 6.57 for 1,2-dicyanobenzene [16]. The percent recoveries for these species were 39.5±2.2 for 1,4-dicyanobenzene, 30.7±5.2 for 1,3-dicyanobenzene, and 27.6±3.1 for 1,2-dicyanobenzene. The percent recovery values for the 1,4 and 1,3 isomers differ significantly at the 95% confidence level. The isomer having the lowest polarity, 1,4-dicyanobenzene, gave the greatest recovery, while the more polar isomers had lower, but similar recoveries. The same inverse trend is observed when comparing the percent recovery versus solubility in water. The percent recovery of these individual isomers increases as their solubility in water decreases. However, this trend is not observed between the different test molecules used in this study (Table 3). For example, we were unable to explain the recovery differences of the pyridines versus the

Table 2
Percent recovery of repetitive extractions

Organic base	Percent recovery		
	1st extraction	2nd extraction	3rd extraction
2-chloroaniline	75.3±3.7	87.8±2.8	102±10
4-chloroaniline	76.9±7.6	89.4±3.3	101±16

Table 3
Percent recovery, dipole moments and solubilities of selected organic bases

Organic base	Percent recovery	Dipole moment[a] (D)	Solubility in water[b] (parts per 100)
2,6-dimethylpyridine (neutral)	21.0±3.2	1.48	43
2,6-dimethylpyridine (protonated, H^+)	5.1±0.9	1.21	–
2,4,6-trimethylpyridine (neutral)	28.3±1.8	1.81	3.5
2,4,6-trimethylpyridine (protonated, H^+)	3.3±1.0	1.99	–
2-chloroaniline (neutral)	75.3±3.7	1.67	0.88
2-chloroaniline (protonated, H^+)	33.5±1.6	7.01	–

[a] Calculated using HyperChem.
[b] Ref. [14] (only neutral forms available).

anilines based upon dipole moments or solubilities in water. Phenomena other than molecule polarity and solubility would have to be taken into account. These phenomena may include solubility of the test molecules in the saturated NaCl matrix and other possible interactions between the test molecules and surfactant and co-surfactant. We suggest experimentally testing possible analytes for percent recovery before further method development. If the analyte is an organic acid or base, a pH adjustment should be made to put the analyte in the neutral form.

4. Conclusion

The physical extraction behavior of this alkyltrimethylammonium surfactant-mediated extractant has been elucidated. This water in oil microemulsion phase extracts organic analytes based solely on hydrophobic interactions. Charged and other hydrophilic species do not extract well into this surfactant-rich phase. To obtain the best recovery for organic amines, the pH should be adjusted for this species to be predominately in the neutral form. No evidence for electrostatic interactions were found between charged analytes and the surfactant-rich phase. Also, analytes in aqueous solutions not being recovered quantitatively for the first extraction may be extracted repetitively.

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