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Solubilization of Hydrophilic Compounds in 1,1,1,2-Tetrafluoroethane with a Cationic Surfactant

Jie-Ren Li, Yu-Mei Lee, and Tiing Yu*

Department of Applied Chemistry, National Chiao Tung University, Hsinchu, Taiwan, Republic of China

Solubilization of hydrophilic compounds was examined in liquid 1,1,1,2-tetrafluoroethane (R134a) in the presence of the cationic surfactant trioctylmethylammonium chloride (TOMAC). The absorption spectra of methyl orange in the TOMAC-containing R134a solutions were obtained. Significant blue shifts were observed in comparison with the spectrum of methyl orange in aqueous solution. The shifts decreased as the water-to-surfactant ratio, W_0, increased. In addition, spectral measurements confirmed the dissolution of cytochrome *c* in R134a in the presence of TOMAC. R134a remains as a liquid under mild applied pressure and becomes gas under ambient conditions; it therefore separates from analytes of interest directly without further concentration when used as an extraction solvent. Accordingly, it may be applied to recover valuable hydrophilic substances of low concentration from aqueous solutions.

Supercritical fluid carbon dioxide ($SFCO_2$) has been applied as a useful extraction fluid in many fields. For example, $SFCO_2$ has been extensively applied in extracting chlorinated hydrocarbons[1−3] and polycyclic aromatic hydrocarbons[4−6] from different environmental samples. Organic entrainers, such as methanol, are commonly added to $SFCO_2$ to enhance the solvating power for more polar or high molecular weight solutes.[7,8] In addition, there have been many researches that involve adding surfactants or chelate agents to $SFCO_2$ to solubilize hydrophilic compounds. Biomolecules[9] (such as bovine albumin) and ionic compounds[10] were dissolved in $SFCO_2$ by adding perfluoropolyether (PFPE) surfactants. Solubilization due to micelle formation was verified by a number of spectroscopic techniques. The cloud point for PFPE in $SFCO_2$ was investigated subsequently.[11] In addition, newly synthesized dendritic surfactants were used to transfer ionic compound−methyl orange molecules very efficiently from aqueous solution to liquid carbon dioxide.[12] Many efforts[13−19] were carried out in exploring new chelate compounds to remove toxic metals from wastewater. $SFCO_2$ evaporates under ambient conditions and separates from the extracted components directly after the extraction, therefore requiring no further concentration process. Accordingly, there is a great potential to extract hydrophilic solutes in dilute aqueous solution using $SFCO_2$. However, a relatively high operational pressure is required to maintain suitable solvation power for either $SFCO_2$ or liquid CO_2 as the extraction solvent.

In addition to carbon dioxide, supercritical hydrofluorocarbons (HFCs), fluorocarbons, and hydrochlorofluorocarbons (HCFCs) have been studied as solvents used in supercritical fluid extraction (SFE) and chromatography. These alternative refrigerants were synthesized to replace the ozone-depleting chlorofluorocarbons. Trifluoromethane was successfully employed as an SFE solvent for environmental samples.[20,21] Chlorodifluoromethane (R22) has been used as the mobile phase in supercitical fluid chromatography (SFC) to analyze phenols[22] and used to extract polychlorinated biphenyls and polyaromatic hydrocarbons from environmental matrixes.[23] Recently researchers employed supercritical

* Corresponding author. Tel: (886) 3-573-1673. Fax: (886) 3-572-3764. E-mail: tyu@cc.nctu.edu.tw.

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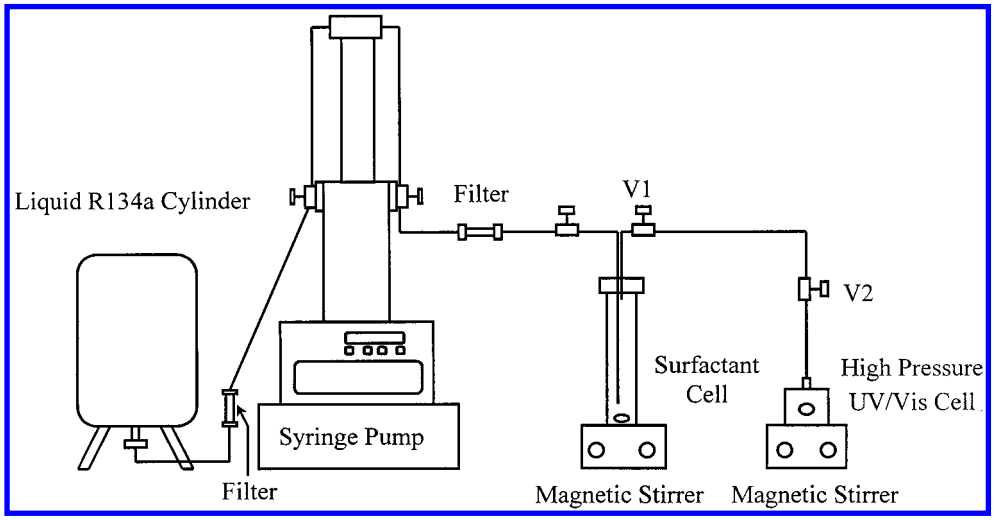

Figure 1. Schematic diagram of the high-pressure cell system for the spectral measurements.

R134a directly or employed it as a modifier of $SFCO_2$ in chromatographic separations.[24−29] It was demonstrated that R134a had different selectivities and solvation power than carbon dioxide and methanol-modified carbon dioxide in SFC. The water−R134a partition coefficients for a number of organic solutes have been measured because R134a may be used as extraction fluid in the pharmaceutical industry and wastewater treatment.[30] In addition, microemulsion formation has been examined in supercritical HCFCs, HFCs, and fluorocarbons.[31] The water-to-surfactant ratio, W_0, was reported to increase as the pressure of supercritical R22 increased. Cytochrome *c* appeared soluble in R22 in the presence of sodium bis(2-ethylhexyl) sulfosuccinate at 102.3 °C and 200 bar.

We investigated the solubilization of hydrophilic components in surfactant-containing liquid R134a in this study. Our spectroscopic results showed evidence of micelle formation in this liquid. Since R134a becomes a gas at room temperatures and pressures, it separates from the analytes of interest after extraction just like $SFCO_2$. In addition, the operational pressures (for example, 25 bar in this study) for liquid R134a are much lower than those for $SFCO_2$ and liquid CO_2, indicating its potential to become a more useful extraction fluid for hydrophilic compounds in a practical manner. For example, recently we successfully separated standard components of *Angelicae radix* in countercurrent chromatography using R134a as the mobile phase.[32] The whole process was done in regular poly(tetrafluoroethylene) (PTFE) tubing without the need of any high-pressure facility. Therefore, liquid R134a may prove to be valuable in the recovery of hydrophilic substances of low concentration from aqueous solutions.

EXPERIMENTAL SECTION

Materials and Sample Preparation. Methyl orange (GR grade) and trioctylmethylammonium chloride (TOMAC, Extra Pure) were purchased from TCI (Tokyo); HPLC/Spectrograde methanol and hexane were obtained from TEDIA (Fairfield, OH). Practical grade cytochrome *c* (bovine heart) was purchased from Sigma (St. Louis, MO) while R134a was from Ausimont (Milano, Italy). Methyl orange solutions of 0.015 M were prepared in methanol and water. A cytochrome *c* solution of 10 mg/L was prepared in a pH 10 buffer solution ($NaHCO_3$) containing 0.1 M KCl.

High-Pressure Spectral Cell System and Procedures. A schematic diagram of the system used for the spectral measurements of microemulsions in liquid R134a is shown in Figure 1. R134a was withdrawn from the cylinder to a syringe pump (model 100DX, Isco, Lincoln, NE) and pressurized to 25 bar. A 0.104 g sample of TOMAC was placed in the stainless steel surfactant cell (∼7 mL). R134a was allowed to flow into the cell and well mixed with the surfactant using a magnetic stirrer for at least 40 min to make a ∼1% (w/w) solution. The weight of R134a was estimated by using a state function derived by Sorner and Strom.[33] No TOMAC was added to the surfactant cell during blank tests. A laboratory-made spectroscopic cell was modified from the design of Betts and Bright[34] to fit the sample chamber of a Hewlett-Packard (Waldronn, Germany) 8453 UV−visible spectrophotometer. The methyl orange or cytochrome *c* sample was transferred into this cell from the top, and the cell was connected to valve V2. Valve V1 was then opened to allow the well-mixed fluid to enter the spectroscopic cell. After a thorough mixing was accomplished using a small magnetic bar in the spectroscopic cell, valve V2 was closed and the cell with valve V2 was detached from the system. The cell containing the sample was then brought to the spectrophotometer for measurement. The cells were cleaned thoroughly after each measurement.

RESULTS AND DISCUSSION

Spectral Measurements for Methyl Orange. Approximately 1 mg of methyl orange was placed in the cell. Neat R134a (without

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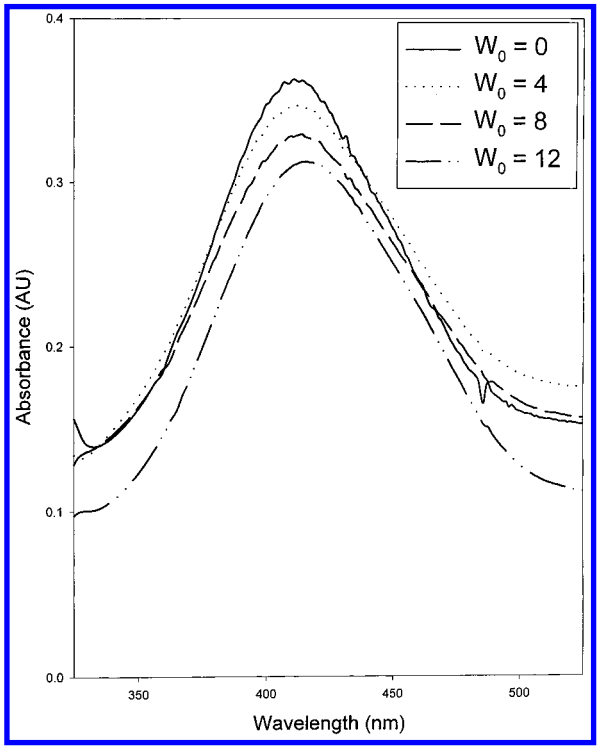

Figure 2. Absorption spectra of methyl orange in TOMAC-containing R134a (0.3 mM) solutions with various W_0 values.

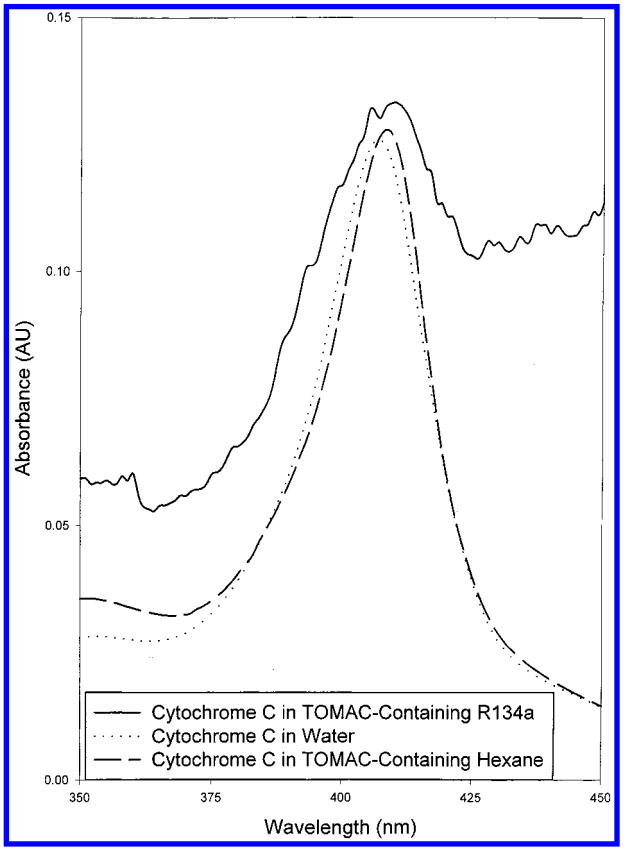

Figure 3. Absorption spectra of cytochrome *c* in TOMAC-containing R134a, in water (50 mg/L) and in TOMAC-containing hexane (45 mg/L) solutions. Each spectrum was normalized to the maximum of cytochrome *c* in TOMAC-containing R134a solution.

TOMAC) was then added to fill the cell, and the contents were mixed with a magnetic stirrer. Methyl orange particles were observed on the cell bottom from the windows after stirring for 2 h. No detectable absorption signals were observed in the 350−500 nm wavelength range.

To examine the solubilization of methyl orange in R134a in the presence of water, a 200 μL aqueous solution (0.05 M) of methyl orange was pipetted into the cell. We purposely allowed less R134a to flow into the cell to improve the visibility of the water/R134a two-phase interface at the windows for direct observations. After 2 h of stirring, the R134a/water interface remained the same and no color was observed in the R134a layer. More R134a liquid was added to the cell in order to ensure only R134a appearing along the optical path of the cell. Again, no detectable signals were observed. Apparently, neither dry nor aqueous methyl orange was soluble in R134a without the addition of TOMAC.

After the above blank tests, spectral measurements were performed in the presence of TOMAC in the surfactant cell. A 10 μL aliquot of methyl orange in methanol solution (0.015 M) was transferred to the high-pressure spectral cell. The cell was then heated in an oven to remove methanol. The well-mixed TOMAC in R134a (1% w/w) liquid was transferred to the spectral cell. After 15−20 min of mixing, a pale orange color developed in the R134a liquid visible through the cell windows. Three more experiments were conducted to examine the solubilization of methyl orange in the presence of water. Again, we transferred 10 μL of the methyl orange in methanol solution (0.015 M) to the cell for each measurement. The cell was then heated in an oven to remove methanol. Subsequently, three aliquots (4.4, 8.8, and 13.2 μL, respectively) of water were added to the cell for the three experiments to make the water-to-surfactant ratio, W_0, equal 4, 8, and 12 for the individual micellar solutions. A suitable amount of the TOMAC-containing R134a fluid was allowed in the cell in order to observe the water/R134a interface. After 40 min of mixing, the water layer disappeared, and a homogeneous pale orange liquid was observed through the windows. The absorption spectra of the dry and three water-containing methyl orange samples are shown in Figure 2. To make comparisons, the absorption spectra of methyl orange in water (0.3 mM) and in TOMAC (1% w/w)-containing hexane (0.3 mM) were also measured. The absorption maximum for the aqueous solution appeared at 464 nm, while the absorbance peaks for methyl orange in hexane micellar solution at $W_0 = 0$ and $W_0 = 12$ were 408 and 410 nm, respectively. Large shifts were observed for methyl orange in micellar solutions (for both of R134a and hexane) compared with methyl orange in aqueous solution, while the absorption maxima of methyl orange in the R134a micellar solutions were located at wavelengths comparable to that observed for the hexane micellar solution. A small (6 nm) red shift for R134a micellar solutions was observed when the W_0 value increased from 0 to 12. This shift may indicate that methyl orange molecules resided in an environment of increasing polarity due to the increasing water content,[9] although the shift was not very pronounced.

Spectral Measurements for Cytochrome *c*. We followed the same procedures as for the methyl orange measurements. No detectable absorbance of cytochrome *c* was observed in the 375−450 nm wavelength range for either a dry or an aqueous sample without the addition of TOMAC to liquid R134a. In the presence of TOMAC, a suitable amount of R134a solution was added to

100 μL of cytochrome *c* aqueous solution in the cell to observe the water/R134a interface. The interface remained after 1 h of mixing. However, the pale red color of cytochrome *c* in the water layer became lighter while a very light red color was observed in the R134a layer—an indication of the successful solubilization of cytochrome *c*. More R134a liquid was added to the cell to move the water layer from the area of the viewing windows. A spectral measurement was made subsequently. The spectra of cytochrome *c* in water (50 mg/L) and in TOMAC (1% w/w)-containing hexane solution (45 mg/L) were also recorded for comparison. The micellar solution was made by adding 3.5 μL of water and 7.3 μL of cytochrome *c* aqueous solution (50 mg/L) to 5 mL of hexane to make $W_0 = 8$. A 4 μL aliquot of isopropyl alcohol was added to the above liquid to obtain a clear solution for spectral measurements. All three cytochrome *c* absorption spectra are shown in Figure 3.

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Although the spectra of methyl orange in the reverse micelles obtained above were recorded without controlling the pH, it would be valuable to examine the spectra at different pH values. While pH determinations within the water pool in the nonaqueous reverse micelle systems may pose a difficult technical problem,[34−40] further investigations should help to clarify the pH effect on the absorption spectra. While a number of papers have reported studies of ionic solute (such as methyl orange and methylene blue)−solute, solute−surfactant, and solute−solvent interactions[41−48] below and above the critical micelle concentration to reveal the local microenvironments in aqueous solvents, reports of research in the similar area of ionic or polar solutes in reverse micelle systems are still very rare.[49] Future studies in this area should provide a better understanding of hydrophilic compounds in this reverse micelle system.

CONCLUSION

We demonstrate in this study that methyl orange and cytochrome *c* can be dissolved in liquid R134a in the presence of the surfactant TOMAC, although the experimental conditions employed in this study are yet to be optimized to increase the solubilities of the components of interest. Practical applications of extracting hydrophilic substances of low concentration from aqueous solutions are currently being investigated in our laboratory.

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