whatever reason, compromise the utility of the ITD as a tool for routine gas chromatography/mass spectrometry (GC/MS) analysis?

We have recently used a Finnigan-MAT ITD-800 (Revision 3.15 software) coupled to a Carlo Erba 4160 gas chromatograph for the routine analysis of natural triglycerides after transesterification to form the fatty acid methyl ester derivatives (*3*). Such long chain fatty acid esters have earlier been used as model compounds to study self-CI effects in Fourier transform mass spectrometers (*4*) where, like the ion trap, ions are held for a given time before analysis. We therefore felt that fatty acid methyl esters would provide a good test of potential interference from pseudo molecular ions on the analytical capabilities offered by the ITD.

For ITD spectra of methyl stearate, a typical long chain fatty acid ester, run in the EI mode, we observed: (a) full scan spectra from as little as 2 pg of analyte entering the ion trap; (b) a steady increase of the pseudo molecular ion with increasing sample quantity for sample levels >10 ng: in a 225-ng spectrum, $(M + 1)^+$ at m/z 229 was the base peak; (c) statistically similar values of library comparison parameters for a given sample size whether ITD spectra of methyl stearate or literature EI spectra from a variety of conventional mass spectrometers were used as library reference spectra; (d) no statistically significant change in values of library comparison parameters, even at sample levels where the pseudo molecular ion became the base peak.

Using an ITD library of fatty acid methyl ester EI reference spectra which we created from pure standards, we have been able to routinely analyze fatty acid methyl ester mixtures from lipids of both animal and vegetable origin, including human milk and blood plasma lipids. Generally, target compounds are identified in rank 1 of the library hit list, and a combination of GC retention data with the mass spectrum systematically gives an unambiguous identification.

We conclude that even in conditions where self-CI or another phenomenon leads to the formation of pseudo molecular ions in ITD EI mass spectra, these still retain enough EI character to be readily recognizable by library algorithms. The ITD offers the sensitivity and selectivity required for routine GC-MS analysis of fatty acid methyl esters over a 10^4–10^5 dynamic range. Although of theoretical interest, pseudo molecular ions in the ITD would appear to be of little practical detriment to the analyst and may even be advantageous in providing "molecular ion" information (*5*).

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Optical Resolution of Enantiomers with Chiral Mixed Micelles by Electrokinetic Chromatography

Sir: Hydrophobic interactions contribute principally to substrate binding in enzymes and to the self-association of surfactants in micelles (*1*, *2*). This paper reports the recognition of molecular chirality based on hydrophobic entanglement of enantiomers with chiral mixed micelles. Many micellar enzyme models, particularly chiral imidazole catalysts in the presence of surfactant micelles for affecting the hydrolysis of enantiomeric substrates (*3*–*8*), have been investigated for greater clarification of the nature of enzyme reactions. Hydrophobic substrate binding to micellar systems is essential for their enantioselective hydrolysis. A comicellar system composed of chiral surfactants with L-amino acid residues and sodium dodecyl sulfate (SDS) was found to vary in its binding affinity to enantiomeric amino acid derivatives, thus becoming a means for their optical resolution. This was confirmed by using the electrokinetic capillary chromatography devised by Terabe et al. (*9*–*15*), which provides a sophisticated means for assessing micellar enantioselectivity without any solid support to hold the stationary liquid phase in place. In capillary zone electrophoresis, metal–chelate complexation using a chiral copper(II)–aspartylphenylalanine methyl ester has been successfully applied to the resolution of enantiomeric dansylated amino acid mixtures (*16*). The chiral recognition discussed here is primarily based on hydrophobic entanglement of amino acid derivatives with the micellar interior core.

There are numerous reports on enantioselective hydrophobic entanglement that accompanies the formation of the inclusion complex, i.e., mutual hydrophobic binding of a solute to a chiral molecular cavity such as that of cyclodextrin (*17*–*21*). In such a case, there may be enantioselectivity by which a solute becomes bound by hydrogen bonds to hydroxy groups at the rim of a cyclodextrin cavity. Chiral recognition of enantiomers is possible when a chiral hydrogen-bonding functionality, such as an amide unit induced from an optically active amino acid, becomes embodied in the micellar hydrophobic core to entangle enantiomers. This was achieved by the comicellization of *N*-dodecanoyl-L-amino acid sodium salts as chiral surfactants and SDS. Enantioselective interactions between the chiral comicelle and solute should thus be incapable of a tight fit, as seems to be the case in the inclusion complex.

EXPERIMENTAL SECTION

Synthesis of Sodium *N*-Dodecanoyl-L-valinate (SDVal). *N*-Dodecanoyl-L-valine was prepared from L-valine by treatment with the dodecanoic acid *N*-hydroxysuccinimide ester (mp 87 °C; lit. (22) mp 75 °C) according to a procedure of the literature (*21*): mp 103–105 °C (recrystallized from ethyl acetate), $[\alpha]^{26.5}_D = -2.6°$ (c = 1.00, methanol). This carboxylic acid was then converted to the corresponding sodium salt by methanolic sodium hydroxide; the slightly residual carboxylic acid was extracted with acetone by the Soxhlet apparatus.

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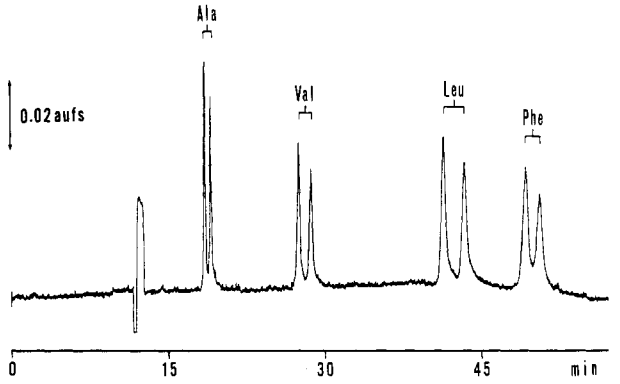

Figure 1. Optical resolution of a mixture containing four enantiomeric pairs of amino acids as their *N*-(3,5-dinitrobenzoyl) O-isopropyl ester derivatives in electrokinetic capillary chromatography. Chromatographic conditions: column, fused silica tubing 40 cm in length (50 μm i.d.) for effecting the separation; micellar solution, 0.0125 M SDVal and SDS each in 0.025 M borate–0.05 M phosphate buffer (pH 7.0) containing 10% (v/v) methanol; samples, 2.5% methanol solution in each solute; total applied voltage, ca. 10.5 kV; current, 26 μA; detection, UV at 254 nm; temperature, ambient (ca. 20 °C). The $\tilde{k}'$ of the first-eluted D enantiomer and α for each derivative are as follows: $\tilde{k}'_D$ 0.79 (α 1.09) for the alanine, $\tilde{k}'_D$ 1.97 (α 1.09) for the valine, $\tilde{k}'_D$ 4.38 (α 1.10) for the leucine, and $\tilde{k}'_D$ 6.24 (α 1.06) for the phenylalanine derivatives.

Chromatographic Procedure. Electrokinetic capillary chromatography was performed according to Terabe et al. (*9*, *10*). The capillary column consisted of a fused silica tubing 64 cm in length (situated 40 cm from the detection cell, 50-μm i.d. and 375-μm o.d.; Gasukuro Kogyo, Inc.). A regulated dc power supply delivering a maximal 25 kV (Model HEL-25R0.1-TYU; Matusada Precision Devices, Otsu, Japan) provided high voltage between the ends of the column filled with a chiral comicellar solution. The chromatography was carried out at a constant electric current of 26 μA. The elution of a solute injected at the positive end of the column was monitored by on-column UV detection (254 nm) through a slit of 0.05 × 3 mm at the negative end. SDS (Tokyo Kasei) was recrystallized from ethanol prior to use. Chiral comicellar solutions were prepared by dissolving equimolar amounts of the chiral surfactant and SDS in the borate–phosphate buffer (pH 7.0) solution consisting of 0.025 M sodium tetraborate and 0.05 M sodium dihydrogen phosphate solution. In all cases, the micellar solution was filtered through a 0.45 μm pore membrane filter and degassed in an ultrasonic bath for 3 min.

The capacity factor ($\tilde{k}'$) for a solute was calculated as follows (*9*, *10*):

$$\tilde{k}' = (T_R - T_0)/[T_0(1 - (T_R/T_{MC}))]$$

where T_R is the retention time of the solute, T_0 that determined with methanol as the solute unsolubilized into a micelle, and T_{MC} that with Sudan III as the solute completely solubilized into a micelle. Sudan III indicates migration of the micellar phase, i.e., a capacity factor of infinity.

Fluorescence Measurements in Microenvironmental Polarity. Steady-state fluorescence spectra were obtained with a Hitachi 650-60 spectrometer, using an excitation and emission slit width of 5 nm. Emission intensity was measured during excitation at 337 nm and at both 373 and 383 nm, using pyrene (Tokyo Kasei; recrystallized from ethyl acetate) as the probe in SDVal–SDS mixtures dissolved in the borate–phosphate buffer solution.

RESULTS AND DISCUSSION

It was possible by use of a comicellar system consisting of equimolar amounts of sodium *N*-dodecanoyl-L-valinate (SDVal) and SDS to effectively bring about the enantiomeric resolution of *N*-3,5-dinitrobenzoylated amino acid isopropyl esters, as shown in Figure 1. The negatively charged micellar phase migrates at a velocity slower than that of the aqueous phase toward the negative end of the column, since the electroosmotic velocity of the aqueous phase is much greater than the electrophoretic velocity of the micelle in the opposite direction. The separation is thus based on distribution processes between two phases. The 3,5-dinitrobenzoyl derivatives provided the most effective resolution for all examined solutes containing the corresponding 4-nitrobenzoyl and benzoyl derivatives. Among the different amino acid derivatives used, the alanine derivatives were the least hydrophobic and thus eluted first. When a comicellar solution of 0.1 M (total concentration) was used, the capacity factor of the first-eluted D enantiomer ($\tilde{k}'_D$) and separation factor (α) between enantiomers for each derivative were as follows: $\tilde{k}'_D$ 7.42 (α 1.12) for the 3,5-dinitrobenzoyl, $\tilde{k}'_D$ 5.76 (α 1.05) for the 4-nitrobenzoyl, and $\tilde{k}'_D$ 3.59 (α 1.05) for the benzoyl derivative. Decreasing the micellar total concentration from 0.1 to 0.025 M led to a smaller $\tilde{k}'$ and virtually constant α values for these derivatives. The $\tilde{k}'_D$ and α values observed for the 3,5-dinitrobenzoylalanine derivative were 1.19 and 1.11, respectively, in 0.025 M solution. In contrast, the corresponding phenylalanine derivative was the most hydrophobic and thus had the largest $\tilde{k}'_D$ value of 13.07, but its resolution even in the 0.025 M solution was poor. In electrokinetic chromatography, the resolution (R_s) is very much dependent on $\tilde{k}'$ of the solute, as has been reported by Terabe et al. (*10*), and a large $\tilde{k}'$ value is unfavorable for obtaining good resolution. Resolution of the enantiomeric mixtures was improved by adding methanol to the comicellar solution at 5–10% (v/v) (*15*), leading to a greater total elution range, as is evident from the values of T_0/T_{MC} (*10*). With the 0.025 M comicellar solution, an increase in the organic modifier concentration decreased T_0/T_{MC} from 0.20 in the absence of methanol to 0.10 in 10% (v/v) methanol. This corresponded to a decrease in $\tilde{k}'$ of the enantiomers and an increase in resolution (R_s), as was noted in particular for the phenylalanine derivative with the largest retention.

In the elution order of the amino acid derivatives resolved, the D enantiomer eluted faster than the corresponding L enantiomer in all cases, indicating that the chiral comicelle binds to the L enantiomer having the same configuration as its chiral component to a greater extent than the D enantiomer. Such a difference in binding capacity has been reported in kinetic measurements in the enantioselective hydrolysis of enantiomeric *N*-acyl amino acid esters using a cationic comicellar system containing *N*-acyl-L-histidine; the binding constant for the L enantiomer was less than that of the corresponding D enantiomer (*3*). This finding is at variance with our observations.

The critical micelle concentration (cmc) of the comicellar system was determined at 2×10^{-3} M by using the intensity ratio of pyrene fluorescence peaks at 383 nm (I_{383}) relative to that at 373 nm (I_{373}), which reflected the micropolarity around pyrene as a probe (*23*). The intensity ratio (I_{383}/I_{373}) increased from 0.69–0.70 (in the borate–phosphate buffer solution, 0.68) to 1.10–1.11 at cmc and remained virtually constant above cmc owing to the solubilization of pyrene in the interior core of the comicelle. This comicellar micropolarity was lower than that observed for the SDS micelle in the borate–phosphate buffer solution (1.00–1.03). The smaller extent of water penetration into the chiral comicelle than into the SDS micelle (*22*) may be the explanation for this. Thus possibly, the comicellar system may provide a favorably ordered medium for hydrogen bonding with the solute enantiomers since chiral recognition of the enantiomers should be induced, at least to some degree, by hydrogen bonding between the chiral surfactant and the enantiomers in the shallow hydrophobic region, near the Stern layer, in which hydrogen bonding sites exist. The addition of methanol to the comicellar solutions resulted in no change, as expected, in the micropolarity of the interior core. The intensity ratio of pyrene fluorescence peaks appeared essentially constant (I_{383}/I_{373} =

1.10–1.12) at 0–20% (v/v) methanol. This appears to support the notion that water soluble alcohols predominantly dissolve in the water phase, causing the aggregation number of a surfactant to change according to the alcohol concentration (*24*). Thus, the main effect of an organic modifier may be to change micellar size, as well as electroosmotic flow, as suggested by Gorse et al. (*15*).

We are now examining better chiral surfactants containing SDVal congeners and *N*-acyldipeptide sodium salt. The results will be presented in detail in the near future.

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Quantitative Supercritical Fluid Extraction/Supercritical Fluid Chromatography of a Phosphonate from Aqueous Media

Sir: There are several problems associated with supercritical fluid extraction (SFE) of analytes from aqueous samples. Water is soluble in CO_2 to approximately 0.3% at supercritical conditions (*1*). For a dynamic type of extraction where fresh fluid is continually passed over the sample to be extracted, the removal of 0.3% water over time can cause problems such as restrictor plugging and activation of the trapping or chromatographic phase. Aside from these problems should the resulting stream be collected into a nonpolar solvent for further chromatographic analysis, a two-phase system would result. Clearly very little constructive extraction can be realized in this manner since the sample and matrix would simply have been moved from one container to another. Equilibrium static extractions on the other hand, wherein the vessel is pressurized for a time and then analyzed, may be inefficient and slow.

The vast majority of the work that has been reported for SFE of aqueous systems has been performed on large scales such as for wastewater treatment purposes (*1–4*). Many applications for SFE of aqueous samples at the analytical scale can be envisioned, such as the analysis of pesticides and herbicides from field drainage, municipal wastewater, and drugs/drug metabolites from biological fluids. We, therefore, offer here a preliminary description of an on-line extraction system for performing trace analysis of phosphonates in an aqueous matrix. To our knowledge, this constitutes the first study of this type reported.

EXPERIMENTAL SECTION

The extraction vessel currently in use was acquired from Suprex Corp. (Pittsburgh, PA) and is 1 cm i.d. × 10 cm in length (8 mL volume). The supercritical CO_2 (Scott Specialty Gas, Plumsteadville, PA) was delivered by and subsequent chromatography was done with a Suprex SFC 200 (Suprex Corp., Pittsburgh, PA). The system shown in Figure 1 in the recirculation mode consists of three six-port valves (Rheodyne, Inc., Cotati, CA), a recirculating pump (Micropump, Inc., Concord, CA), extraction vessel, and associated plumbing in a temperature-controlled oven. A three-port switching valve (VICI, Houston, TX) was used to allow for easy conversion of the instrument for conventional SFC analysis. A 1-m length of 100 μm deactivated fused silica was used to interface the extraction apparatus to either a 1.0 mm or 4.6 mm (i.d.) × 250 mm DELTABOND cyanopropyl packed column (Keystone Scientific, Inc., Bellefonte, PA). The results stated herein were obtained with a 20-μL sample loop. The volume of extracted phase was determined to be 6 mL when using 3-mL aqueous samples. Each 20-μL injection represents only 0.3% of the total extracted phase, so that multiple injections could be run on the sample without significantly depleting the extracted phase of analyte. Fourier transform infrared (FT-IR) data were acquired from a Nicolet (Madison, WI) supercritical fluid chromatography/infrared (SFC/IR) interface.