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Separation of Neutral Compounds in Nonaqueous Solvents by Capillary Zone Electrophoresis

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Capillary electrophoresis (CE) has developed into a versatile and powerful separation technique for charged solutes[1−3] since the pioneering work of Jorgenson.[4] Neutral compounds can be separated in a CE apparatus using the principle that a charged host can carry neutral guests (solutes); commonly, the hosts are micelles or macrocyclic hosts such as cyclodextrins.[5−12] Nonaqueous CE has received the attention of a few laboratories.[13−17] There have been separations of neutral compounds in nonaqueous solvents based on these principles: polycyclic aromatic hydrocarbons were separated in acetonitrile with addition of tetrahexylammonium[18] or tropylium and 2,4,6-triphenylpyrylium ions;[19] polyethers were separated in methanol (with addition of sodium or tetraethylammonium cations,[17] Ag(I) was used in the classical argentation chromatography.[14] But there is not one separation technique that has the capability to separate and quantitate compounds of widely ranging polarity on a picomole and lower scale.

We hypothesized that, as most polar organic compounds have one or more Lewis base centers, a charged Lewis acid should be able to effect their separation. We report here that lanthanide triflates in acetonitrile can effect the separation of a series of alcohols. Acetonitrile is limiting in that the solubilities of many lanthanide triflates are low; thus, we have developed a mixed solvent, acetonitrile/ethylene carbonate in which lanthanide triflate solubility is high. We have also discovered how to control the chemistry of the system so that the most weakly lanthanide-bound solutes have the highest velocity or so that the opposite holds: the most strongly bound have the highest velocity. While there are undoubtedly many analytical applications for this simple system, we also anticipate application in the monitoring of syntheses and in understanding Lewis acid−base interactions relevant to catalysis.[20]

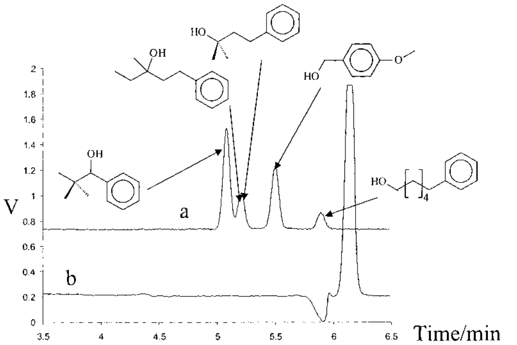

Figure 1. Separation of alcohols with (a) 23 mM $Sm(OTf)_3$ in ACN, −25 kV were applied across the column (detector at ground), the resulting current was 18 μA; (b) 50 mM $Sm(OTf)_3$ in methanol, −20 kV, 34 μA. Peak assignments were made by comparison to peaks generated from injections of single compounds. The earliest eluting peak contains anthracene as well. Concentration of each component before injection is about 0.05%.

Figure 1a shows the partial separation of a series of alcohols (and anthracene, which was used as a marker for the electroosmotic velocity) with an acetonitrile mobile phase containing $Sm(TfO)_3$.[21] So-called "reversed polarity" is used to elute the solutes. Normally, a positive potential at the injector end drives the electroosmotic flow (at velocity v_{eo}) toward the detector because the mobile counterions to the fixed surface charge are positive. In the present case, we believe that Ln^{3+} adsorbs to the silica surface, rendering it positive, so that the mobile counteranions migrate to the detector if the inlet is at a negative potential. The bulk flow is from the inlet to the detector, and the lanthanide ions migrate in the opposite direction. The separation is achieved because the hydroxylic solutes bind to the Ln^{3+} with different formation constants. The more avid the binding, and the higher the $[Ln^{3+}]$, the later is the elution time, because the electrophoretic component of the lanthanide velocity is away from the detector. The acid/base basis for the separation is demonstrated indirectly in Figure 1b, which shows that the separation of the solutes completely fails in the competitive solvent, MeOH.

Water, or hydroxide, will certainly bind avidly to any lanthanide. We were concerned that small amounts of water would completely suppress the ability of the Ln to form adducts with the hydroxylic solutes; however, this is not the case. In both Figure 2a and b, solutes are well separated in the presence of a significant amount of water. The largest effect of the water is to alter v_{eo}. We have also explored various Ln triflates for their efficacy. Because of the electrostatic nature of the interaction between the solutes and the Ln ion,[22] there is little change in the selectivity from changing ions, but there can be a large change in the retention times. With Yb(III) for example, retention times for the alcohol mixture are longer, but the resolution between the two tertiary alcohols in Figure 2 is much better.

Acetonitrile is not an ideal solvent, as the solubility of many Ln triflates is not good. So as not to be competitive with the solutes (as MeOH) the Lewis base character of the solvent should be low, but the dielectric constant must be high to dissociate salts.[23] Unfortunately, the combination of high dielectric constant

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(21) The CE instrument is an Isco 3850 capillary electropherograph. As circulating air is used in the separation chamber, the front buffer beaker and electrode have been modified to reduce solvent evaporation. The height of front buffer beaker was reduced by 2 mm, the edge is polished, and a Teflon sheet is used as the cover. The modification should not be necessary if the front buffer vessel is designed to be sealed during operation. All of the injections are made by vacuum for 2−4 s, and detection is by UV absorbance at 254 nm. The fused silica column was rinsed with 1.5 M NaOH, H_2O, methanol, and acetonitrile in that order before use.

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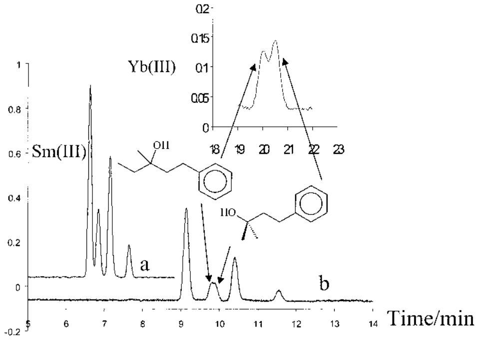

Figure 2. Separation of alcohols (as in Figure 1) with (a) 50 mM Sm(OTf)$_3$, 0.5% H_2O in ACN, −20 kV, 26 μA; (b) 26 mM Sm(OTf)$_3$, 0.3% H_2O in ACN, −20 kV, 26 μA. Inset, portion of the alcohol separation in 50 mM Yb(OTf)$_3$, 0.3% water in AN.

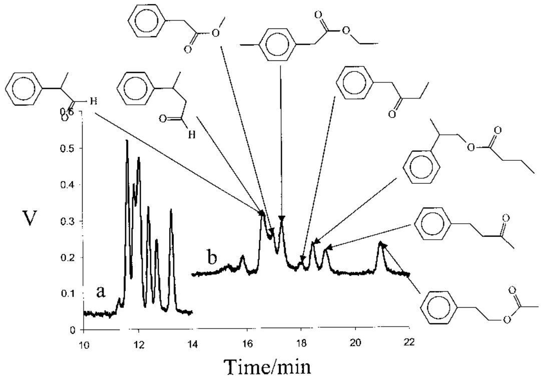

Figure 3. Separation of oxygen-containing compounds with (a) 80 mM La(OTf)$_3$ in EC/AN (16/10 w/v), −28 kV, 40 μA; (b) 100 mM La(OTf)$_3$ in EC/AN (16/10 w/v), −28 kV, 50 μA.

and low electron donor capability (Gutmann's Donicity (DN)[24,25] or Kamlet−Taft's β value[26,27]) is rare. Nitromethane, tetramethylenesulfone, ethylenesulfite, and ethylenecarbonate are candidate solvents. Among them, we selected ethylenecarbonate (EC) for its high dielectric constant (89) and low volatility. Since the melting point of EC is slightly higher than room temperature, the following experiments use a mixed solvent of EC and AN (EC/AV 16/10 w/v).

The mixed solvent EC/AN dissolves the Ln triflates well; solubilities of the triflates tested (La^{3+}, Sm^{3+}, and Yb^{3+}) are over 100 mM. Importantly, the salts are considerably dissociated in this solvent[28] Figure 3 shows a separation of a series of compounds in EC/AN containing a variety of functional groups. At the higher Ln^{3+} concentration, the organic solutes' velocities are more impeded through complexation than at the lower concentration. This result confirms that a complexation equilibrium governs the velocity.

Experimental observations with pyridine-*N*-oxides known to bind strongly to Ln(III) lead us to believe that, under the conditions that we have used, the magnitude of the velocity of the Ln(III) is greater than v_{eo}. This means that weakly bound solutes would migrate in one direction, that of v_{eo}, while strongly bound solutes would migrate in the opposite direction. This has one really superb advantage, in that it is a countercurrent system; therefore, a solute with a net velocity of zero can be separated from closely migrating compounds based on very small differences in velocity (this is equivalent to having an infinitely long chromatographic column). However, in routine applications it would usually be better if all solutes migrated from the injector to the detector. It would be preferable to have a low v_{eo} in the same direction as the Ln^{3+}, then both weakly bound and strongly bound solutes would elute, with strongly bound solutes eluting first. Having a small, negative charge on the capillary wall would solve the problem. Analogous difficulties have been solved in aqueous systems through the use of surfactants. Unfortunately, typical surfactants do not adsorb to surfaces from solutions in organic solvents as they do from solutions in water.

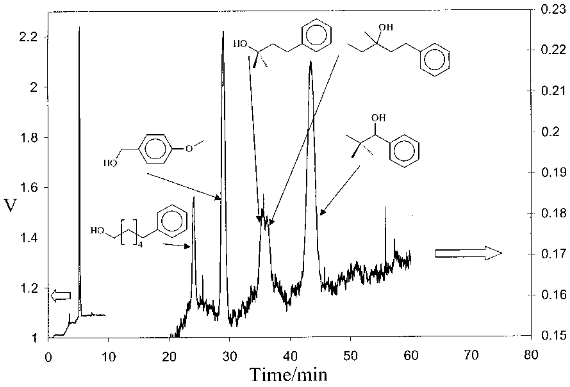

Figure 4. Electropherogram of (left) Reichardt's betaine with 50 mM Sm(OTf)$_3$, 7.5% EC in ACN, FC column, +20 kV, 23 μA; (right) Separation of mixed compounds with 50 mM Sm(OTf)$_3$, 0.3% H_2O, 25 mM K^+ perfluorooctane sulfonate in AN, FC column, +25 kV, 21 μA.

We therefore turned to a fluorocarbon-based system. In a fluorocarbon-modified capillary, we expect very low v_{eo}. Figure 4 shows the elution of the betaine when using a fluorocarbon capillary and a positive voltage at the injection. Figure 4 also shows the separation of the alcohol mixture. The mobile phase contains potassium perfluorooctane sulfonate, added to create a small net negative charge on the wall. The noncomplexing sulfonate does not interact strongly with Ln^{3+}, and the K^+ counterions induce a v_{eo} in the same direction as Ln^{3+}. The weakly complexed alcohols now elute slowly, and the more strongly bound alcohols elute rapidly. We have carried out other separations successfully, for example of the oligooxyethylene-based surfactant Triton X-114.

The potential for broad application is evidenced by two pairs of peaks. In Figure 2 the two tertiary alcohols are separated. They differ by a methylene group. In Figure 3, there is a very good separation of the isomeric ketones phenethylmethyl ketone and benzylethyl ketone. Reversed phase liquid chromatography and gas liquid chromatography are effective at separating compounds differing by a methylene group, and adsorption chromatography is effective at separating geometrical isomers, but it is uncommon to find a single separation system that is adept at both.

The system as presented is not optimized at all. For one thing, the number of theoretical plates is from about 10−30 000, which is low for CE. We suspect that the solute exchange with the Ln(III) is responsible and that increasing the capillary temperature (not straightforward with our current instrumentation) will improve the situation. On the positive side, the selectivity is based on simple, measurable, and understandable chemistry. The potential for selectivity control, through solvent, metal ion, and the ability to have countercurrent separation or not lend the technique great operational flexibility. Extensions to the determination of binding energies of substrates for lanthanide catalysts, to higher molecular weight species, to better detection, and to other metals for softer bases, are easily envisioned.

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