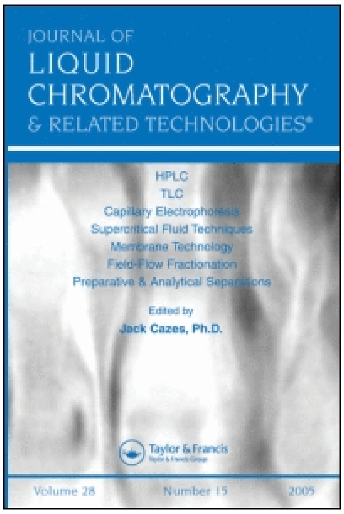

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COMPARISON OF AOT DECANE WATER-IN-OIL AND OIL-IN-WATER MICROEMULSIONS AS MEDIA FOR CAPILLARY ELECTROPHORESIS

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ABSTRACT

This report details the comparison of AOT-decane oil-in-water and water-in-oil microemulsions as media for capillary electrophoresis. The microemulsion composition was varied to produce clear, conductive solutions. Clear microemulsions were obtained for 20% water-in-oil microemulsions and 10% oil-in-water microemulsions. The behavior of the microemulsions under conditions of the capillary electrophoresis experiment showed that the charge carrier Tetraethylammonium salt (TEA^+) was needed to sustain a stable current. It was found that the migration of species in both water-in-oil and oil-in-water microemulsions is based on association with the TEA^+.

Several factors that could affect the migration of solutes in these media were studied. These include the composition of the

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microemulsion, the applied voltage, the temperature, and the pH of the aqueous phase.

INTRODUCTION

The goal of this research was to investigate AOT microemulsion systems with appropriate properties to allow the separation of hydrophobic solutes by CE. Microemulsion systems that are optically transparent in the UV region and have the ability to sustain stable current under conditions of the capillary electrophoresis experiment were sought. These microemulsions were evaluated as media for capillary electrophoresis.

The ability to solvate a very hydrophobic solute while maintaining the ability to sustain stable current is not a trivial one. One way that this can be accomplished is by the use of surfactant systems. Normal micelles are aggregates of surfactants consisting of an oil core surrounded by aqueous solution. These aggregates have been utilized in CE to allow the increased solubility of hydrophobic solutes, as well as acting as a pseudo-stationary phase for the separation of neutral molecules. The micelles migrate in a direction opposite to the general flow. The partitioning of solutes into and out of the micelles has been used to separate uncharged species.[1–4]

It should be noted that while these systems provide increased solubility for hydrophobic species, very hydrophobic compounds tend to be completely solvated by the micelles and co-elute without separation. Organic solvents have also been added to micelle systems to try to extend the elution range,[5–7] but in greater than 20–30% organic solvents the micelles are not stable.[8,9]

Microemulsions are aggregates of surfactants that can be regarded as dispersed swollen micelles. Microemulsions are multi-component fluids with a polar liquid, a hydrophobic phase, one or several surfactants, and a co-surfactant. The polar liquid is usually water or saline, the oil is usually an alkane, and the co-surfactant a medium chain alcohol. These surfactant solutions are relatively stable and have low viscosity. Microemulsions with less than 20–30% water are considered to be water-in-oil microemulsions, microemulsions with less than 20–30% oil are considered to be oil-in-water microemulsions, microemulsions with approximately equal amounts of water and oil are considered bi-continuous.

At certain compositions, a microemulsion can exist in a “percolated” state, where both oil and water nanodroplet networks are continuous. This is characterized by a dramatic increase in the electrical conductivity of the solution. At percolation, it is believed that extensive clustering of the nanodroplets occurs, as there is a large increase in the Kerr constant. There is also an increase in the self-diffusion coefficient of water and an enhancement of dielectric relaxation times.[10–12]

In his groundbreaking series of papers in the early 1980's, Terabe proposed using micelles as media for capillary electrophoresis. He also suggested that microemulsions could be used and would have the advantage of being able to solvate more hydrophobic solutes, and have larger migration windows.[1,2] Terabe and coworkers compared an SDS oil-in-water microemulsion to its corresponding micelle system as a medium for capillary electrophoresis. They concluded, that by increasing the concentration of the surfactant, the migration window could be increased in the microemulsion system. The efficiency for the microemulsion system was slightly lower than the micelle system. The selectivity was also heavily influenced by the kind of oil used as the microemulsion core.[13]

Several researchers have investigated and optimized oil-in-water microemulsions consisting of sodium dodecyl sulfate (SDS), 1-butanol, n-heptane, and aqueous buffers as media for capillary electrophoresis. The separation is based on partitioning of the solutes into the microemulsion droplets. It is believed that the microemulsion droplets migrate more quickly and in the opposite direction to the electro-osmotic flow. The selectivity of the separation could be controlled by manipulation of the pH. The manipulation of the concentration of the surfactant was also believed to be important.[14,15] Boso and co-workers have investigated anionic SDS microemulsions and compared them to cationic TTAB microemulsions for the separation of water and lipid soluble vitamins.[16] Watarai has also compared anionic,cationic and nonionic oil-in-water microemulsions as media for capillary electrophoresis.[17]

Microemulsion capillary electrophoresis is becoming more widely accepted and a review article on the use of microemulsions in separation sciences was recently published by Watari.[18] Applications have included pesticides,[19] sugars,[20] pharmaceuticals,[21–23] natural products,[24] cephalosporins,[25] and fat soluble vitamins.[26]

Ishihama and co-workers explored the use of microemulsion capillary electrophoresis as a way to evaluate the hydrophobicity of test solutes. They developed a migration index scale to allow the comparison of micelle and microemulsion systems. They studied the effect of the structure of the surfactants on the selectivity of the microemulsion capillary electrophoresis. It was found, that for charged solutes the charge and chain length of the surfactant affected the ability of the microemulsion to ion pair with the solutes and thus, affected the separation.[27–29]

Microemulsion capillary electrophoresis has also been used with chiral selectors.[30,31] The surfactant system that has been used most often is the sodium dodecyl sulfate (SDS), 1-butanol, n-heptane, aqueous buffer system.

One microemulsion system that has been studied extensively for its ability to solvate and stabilize hydrophobic solutes is the water-in-oil microemulsion system, which consists of Aerosol-OT (sodium bis-2-ethyl hexyl sulfosuccinate), water and alkane. Aerosol-OT is an anionic surfactant, which forms stable microemulsions without the presence of a cosurfactant. Aerosol-OT systems have been widely studied as models of microemulsion behavior because of this simplicity. It has been shown, that for the Aerosol-OT/water/isooctane microemulsion that the percolation transition does not coincide with phase separation, but occurs in the homogeneous, transparent region of the phase diagram.[12]

The increase in conductance is indicative of the ability of the microemulsion to support the transport of ions. These conditions would also allow stable current to be maintained for CE. It is also important that the microemulsion be transparent to allow UV detection of analytes dissolved in this medium. AOT has also been used as an ion-pairing agent for capillary electrophoresis separation.[32,33] This was performed under conditions (>20% acetonitrile) where surfactant aggregates are not expected to form.

AOT water-in-oil microemulsion systems have also been successfully used as mobile phases in normal phase liquid chromatography. The ability to tightly bind water in the interior of a reversed micelle reduced the solute retention dependence on water in the mobile phase.[34] The effect of the physicochemical structure of AOT water-in-oil microemulsions and SDS oil-in-water microemulsions on chromatographic parameters has been studied by Berthod and co-workers.[35–37] The ability of the microemulsion to offer unique selectivity and solubility was investigated.

In this study, we have manipulated the composition of the Aerosol-OT microemulsions to give a clear, stable, conductive, media. We have compared the capillary electrophoresis separations in water-in-oil and oil-in-water microemulsions. It was found, that for a capillary electrophoresis experiment a charge carrier TEA^+ (tetraethyl ammonium perchlorate) was needed to sustain stable current. We have used this system to investigate the migration behavior of a variety of solutes.

EXPERIMENTAL

Aerosol-OT was purchased from Fisher Scientific (Springfield, NJ), and was purified by stirring for 24 hours in a slurry of methanol and activated carbon, and filtered through a 45 μm nylon membrane filter (Gelman Sciences, Ann Arbor, Mi). The residual solvent is removed by heating gently (Temperatures <45°C).

Water was distilled and then purified with a Barnsted nanopure water purification system (Dubuque, IA) with a resistivity of 18MW-cm. Decane and isooctane were obtained from Fisher Scientific (Springfield, NJ) and used without further purification.

Tetraethyl ammonium perchlorate (TEAP) was obtained from Sigma (St Louis, Mo). Buffer components (TRIS-HCl, sodium borate, sodium phosphate dibasic, potassium biphthlate) were obtained from Fisher Scientific (Springfield, NJ). Buffers were prepared by dissolving the appropriate amount of salt in water and adjusting the pH with HCl.

Intially, water-in-oil microemulsions were prepared by dissolving the appropriate amount of Aerosol-OT in 250 mL of iso-octane or decane. Water (or buffer) was slowly added from a burette with stirring. The conductance and the clarity of the microemulsion were monitored as a function of the amount of aqueous phase added. The aqueous phase was added until the conductance increased, or the microemulsion clouded and phase separated.

Oil-in-water microemulsions were prepared in a similar manner, with the AOT dissolved in 250 mL of water (or buffer) and titrated with decane. Conductance measurements were performed with a YSI Model 35 Conductance meter and Model 3403 conductance probe (Yellow Springs, OH). Once the optimum microemulsion composition had been determined, we found that combining all of the components in the correct proportions with vigorous stirring resulted in clear, conductive microemulsions.

Capillary electrophoresis experiments were performed with a Beckman, PACE 2050 Capillary Electrophoresis Instrument (Fullerton, CA). The fused silica capillary was 40 cm long, with a 0.75 μm i.d. The capillary was initially conditioned with 0.1M NaOH, and then with water for 30 minutes. At the end of each day, the capillary was rinsed with 0.01M NaOH for 15 minutes, water for 15 minutes, and the microemulsion to be used the next day for 30 minutes. The capillary was allowed to equilibrate with the microemulsion overnight. At the beginning of each day, the capillary was rinsed with microemulsion for 30 minutes.

Between each run, the capillary was also rinsed with the microemulsion for 5 mins. This rinsing procedure resulted in more reproducible migration times, especially when working with the oil-in-water microemulsions. Solutes were dissolved in the microemulsion medium and filtered before use. Solute injections were low pressure, the times varying from 2–10 seconds. Detection was at 254 nm.

RESULTS

Microemulsion Composition

The initial studies involved the investigation of the conductance and transparency of Aerosol-OT-isooctane solutions as a function of Aerosol-OT concentration and amount of water added. According to Eicke an Aerosol-OT concentration between 0.1 – 0.2 M (~5%) is recommended, because in this range there are a relatively small number of nanodroplets, especially if the water to surfactant ratio ($w_0 = [H_2O]/[S]$) is kept high (>50). The microemulsion is, thus, sufficiently far away from the phase inversion region where the pure microemulsion can form an irregular bi-continuous microemulsion. More importantly, the percolation transition does not coincide with the phase transition, but occurs in the transparent region of the phase diagram.[38]

The Aerosol-OT concentration was varied from about 0.08M to 0.16M. There was a very small increase in conductance (<0.5 μmhos) when more than 12% water was added to the system. Unfortunately, clouding and/or phase separation of the microemulsion occurred before the conductance increased to the required levels.

Robertus et al. showed that for Aerosol-OT microemulsions, the attraction between the microemulsion droplets increases and the microemulsion becomes more stable as the chain length of the oil is increased (decane>iso octane> heptane> hexane).[39] We explored the effect of using decane instead of isooctane and found that the conductance increased without clouding or phase separation of the microemulsion when decane was used as the oil phase. Clear water-in-oil microemulsions with conductance between 100 – 200 μmhos were obtained. This is comparable to the conductance of a 0.01M aqueous borate buffer.

The effect of the phase ratio of the water to decane in these AOT microemulsions was studied across the entire range of water composition. The clarity and conductance of the microemulsions is shown in Figure 1. There are two regions where the microemulsions are clear and conductive, and thus, suitable for use as media for capillary electrophoresis. The first is a water-in-oil microemulsion region with 20–30% water and conductance of 100 to 2000 μmhos. The second is an oil-in-water microemulsion region with 80–90% water and conductance in the 6000–7000 μmhos range. The microemulsions began to cloud at greater than 30% water. At compositions between 50% to 75% water, the microemulsions were phase separated and the conductance of the solution could not be measured accurately. Figure 1 is an aggregate of many titration experiments where the amount of AOT was adjusted so that the final concentration of AOT would be in the 0.15 to 0.20M range.

Water-in-Oil Microemulsions

The clear water-in-oil microemulsions were evaluated for capillary electrophoresis. We observed an initial increase in current, but this immediately decayed to the system current. It was also observed, that the microemulsions were phase separated at the end of the experiment. Aerosol-OT/water/isooctane microemulsions have been used as media for electrochemically mediated catalytic reductions.[40]

In order to maintain stable current, these microemulsions were saturated with TEA^+ (in the form of tetraethyl ammonium perchlorate). TEAP was added to

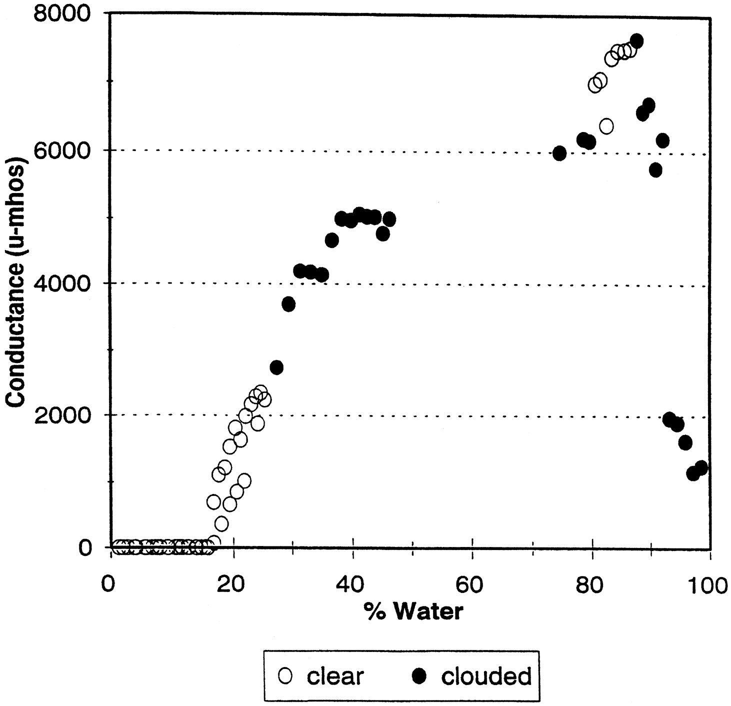

Figure 1. Conductance (μ-mhos) vs % water for AOT (0.15 – 0.20M), decane, and water microemulsions.

our water-in-oil microemulsion system at a concentration of 0.07M. It was noted, that the conductance of the microemulsion started increasing (percolation) at a lower percentage of water added. The final conductance was 200 μmhos compared to 150 μmhos observed for a microemulsion of a similar composition without the TEAP.

When this stabilized microemulsion was evaluated for CE, stable currents were observed, except at 30 kV where the current did not stabilize, but continued to rise sharply. This is probably due to joule heating of the microemulsion, which can cause the microemulsion to phase separate.

The effect of the concentration of TEA$^+$ on the water-in-oil microemulsion was investigated, and it was found that lower concentrations of TEA$^+$ gave more linear current voltage behavior (Figure 2). The joule heating becomes a problem at lower voltages for higher concentration of TEA$^+$. At concentrations of TEA$^+ <$ 0.01M the microemulsion phase separated when voltage was applied. A study of the effect of temperature and voltage on the current stability of several

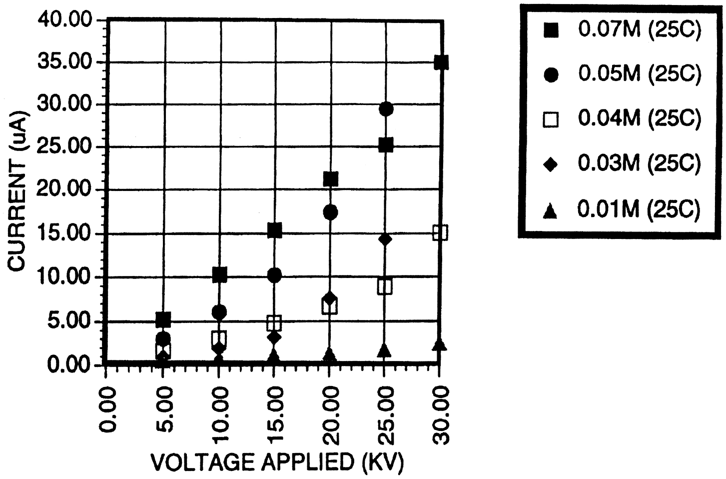

Figure 2. Effect of concetration of TEAP on the current stablilty of AOT (0.18M), 20% water in decane microemulsion.

microemulsions was performed. It was found that as the temperature of the capillary coolant was increased much above 25°C the joule heating, which resulted in phase separation of the microemulsion, increased rapidly above an applied voltage of 15 – 20kV. Thus, care must be taken to maintain the temperature of the microemulsions around 25°C in order to achieve stable currents.

An optimized water-in-oil microemulsion consists of 0.18M AOT, 0.05M TEA^+, with ~ 20% water in decane. This microemulsion has a $W_0 = [H_2O]/[S] = 60$ and a conductance of over 150 μmhos. This gives a current of 10 μA when 15kV is applied. Benzophenone was used as a neutral test solute, and we were able to observe the migration of this species if the polarity of the electrodes was reversed (Figure 3). This indicates that the electro-osmotic flow is occurring from the anode to the cathode. This is similar to reversal of electro-osmotic flow observed when CTA^+ is used to modify the electro-osmotic flow. In the microemulsion system the TEA^+ may be adsorbing onto the walls of the capillary and producing a similar result.[41,42]

The separation of benzophenone and m-amino benzoic acid is shown in Figure 4. It can be seen that decreasing the applied voltage by a factor three increases the analysis time and the resolution of these species. The same holds true for the separation of benzophenone and p-hydroxy benzoic acid is shown in Figure 5.

Attempts to separate all three species have so far met with only limited success (Figure 6). The best separation occurs after an inordinately long migration

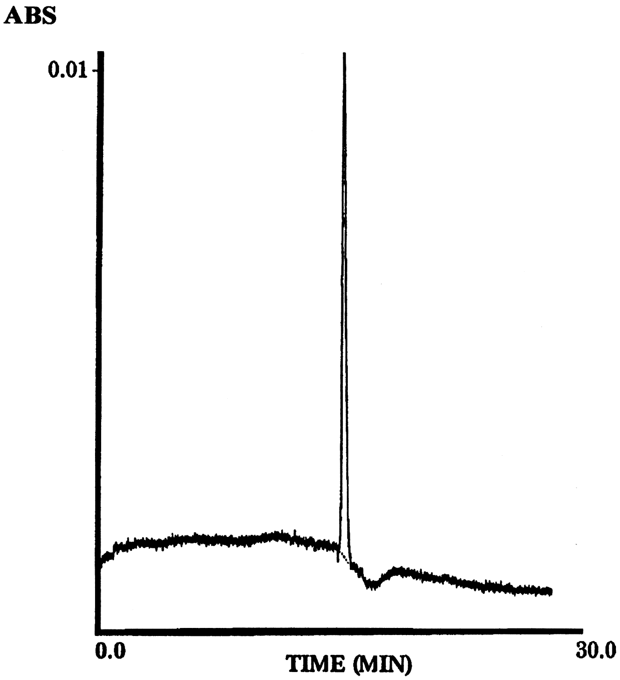

Figure 3. Migration of benzophenone in optimized water-in-oil microemulsion 0.16M AOT, 0.05M TEAP, ~20% water in decane. Benzophenone ~15mM in microemulsion. Injection high pressure for 10s. Separation conditions 15kV with reversed polarity. Detection wavelength 254nm.

time, ~100mins. Attempts to separate other solutes such as amino acids, vitamin A analogs, fat-soluble vitamins, and proteins all show similar behavior. The neutral, very hydrophobic species seem to migrate with the oil phase and the charged species seem to migrate with the water phase. This is consistent with the diffusion behavior of electrochemical systems.[43]

The pH of these microemulsions was estimated roughly to be 4–5 (pH paper), this was the pH of the water used to make the microemulsions. Several buffers were used to replace the water in the microemulsion system to investigate the effect of the pH on the behavior of the AOT water-in-oil microemulsions. Phosphate, phthalate, and borate buffers caused the microemulsions to cloud and phase separate. Tris-hydrochloride buffers were found to give clear conductive microemulsions. Water-in-oil microemulsions were prepared at pH 7.8 and 9. The effect of the pH on the separation of the test solutes, benzophenone and m-amino

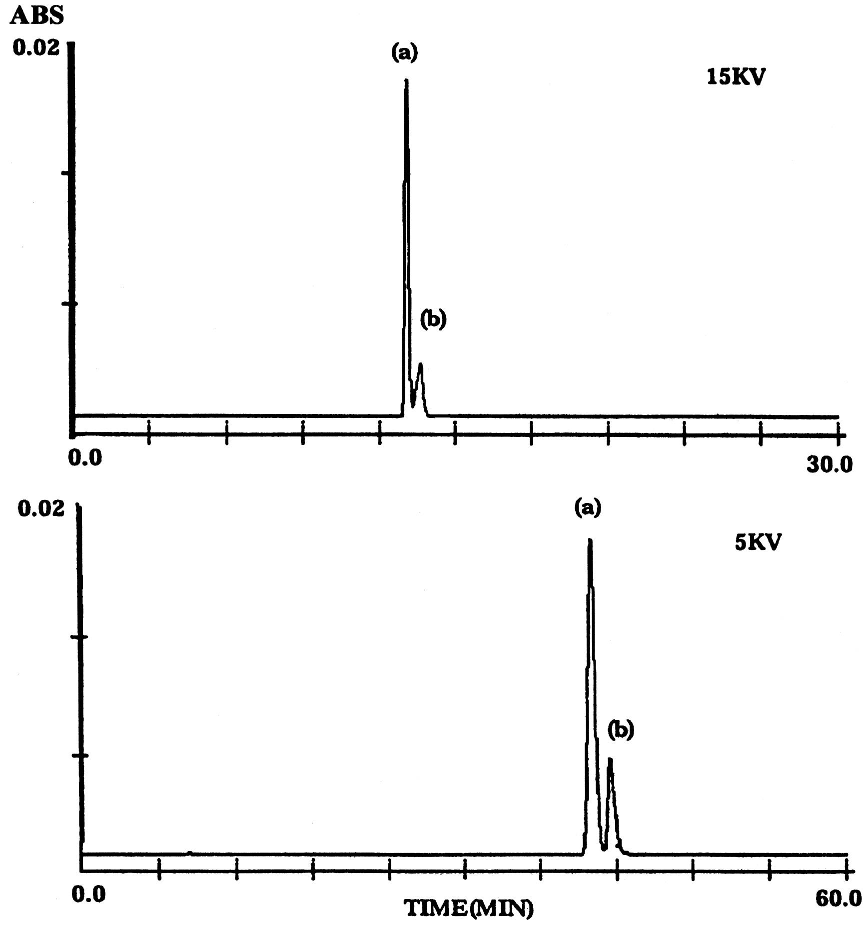

Figure 4. Effect of the voltage on the separation of (a)benzophenone (~15mM) and (b)m-amino benzoic acid (~7mM). All other experimental conditions the same as Figure 3.

benzoic acid (pKa = 4.6) and p-hydroxybenzoic acid (pKa = 2.97), is shown in Figure 7.

At pH 7, two peaks are observed at 26.4minutes and 28.1 minutes, with a resolution of 1.4. The second peak is very broad which may be indicative of co-eluting species. At pH 8, the two peaks have merged into one peak with a shoulder at 27.7 minutes. At pH 9 two peaks are again observed at 29.9, and 31.7minutes with a resolution of 1.7. The later eluting peak is very broad with a distinct shoulder on the higher migration side.

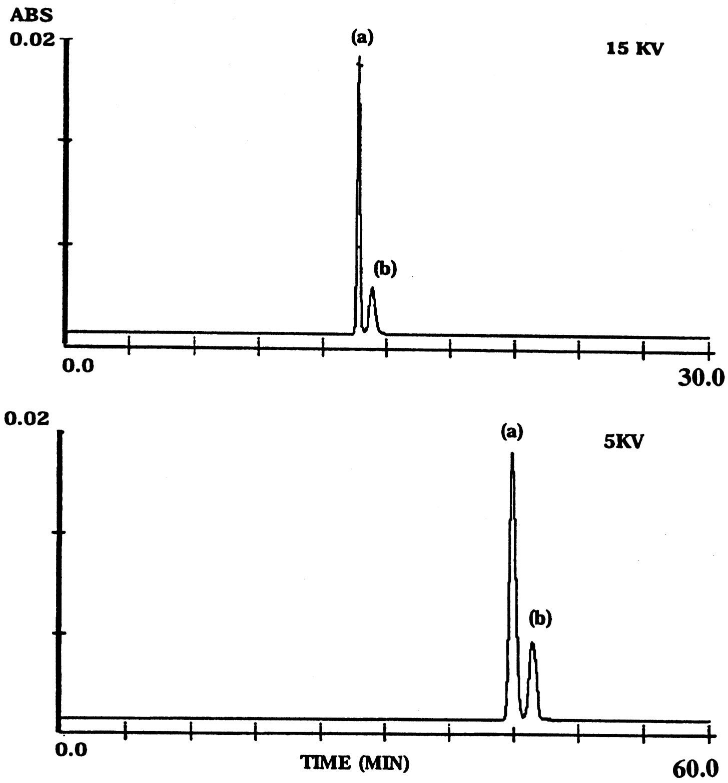

Figure 5. Effect of the voltage on the separation of (a) benzophenone (~15mM) and (b) p-hydroxy benzoic acid (~7mM). Other other experimental conditions the same as Figure 3.

Oil-in-Water Microemulsions

For the oil-in-water microemulsions, it was found that TEA^+ is also necessary for the maintenance of stable current. The optimized oil-in-water microemulsion consists of 0.16M AOT, 0.05M TEA^+, 20% decane in water. It was found that stable currents and reproducible migration times could be achieved only if the capillary was allowed to equilibrate overnight with the oil-in-water microemulsion. This is consistent with the behavior of capillaries where the adsorption of a cation on the walls of the capillary results in reversal of the electro-osmotic flow.

A comparison of the separation of benzophenone, m-aminobenzoic acid, and p-hydroxy benzoic acid in the oil-in-water and water-in-oil microemulsions is shown in Figure 8. In the oil-in-water microemulsion, the benzophenone elutes

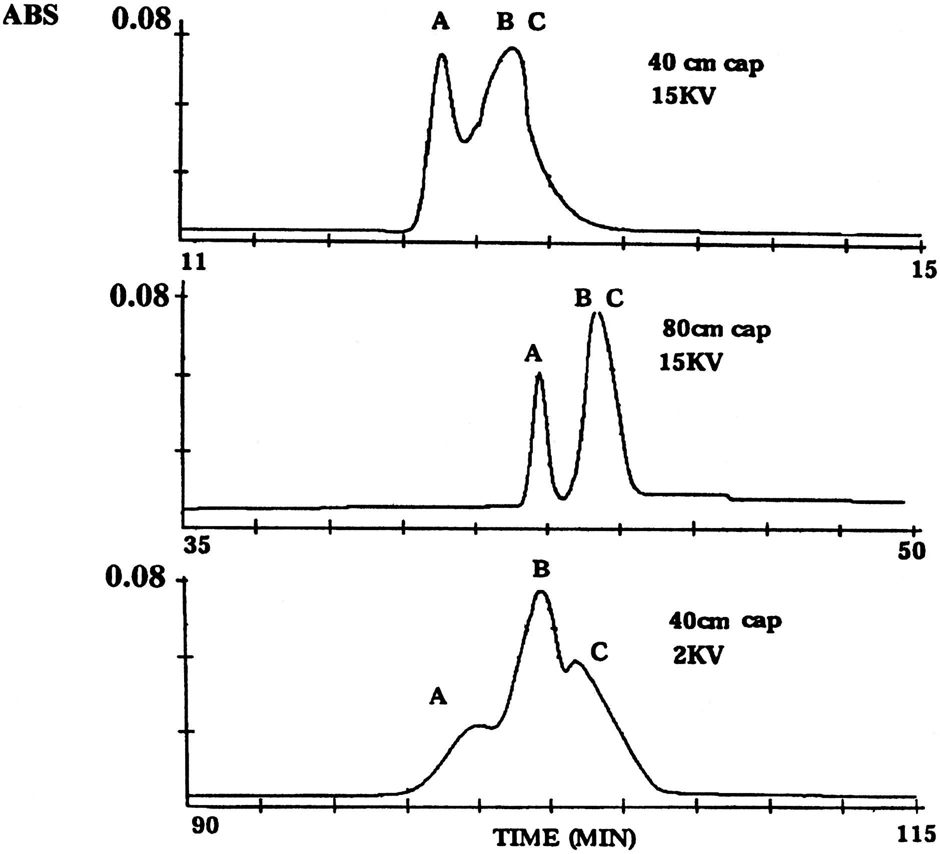

Figure 6. Effect of varying both the capillary length and voltage on the separation of A = benzophenone (~15mM), B = p-hydroxybenzoic acid (~7mM) and C = m-aminobenzoic acid (~7mM). All other experimental conditions the same as Figure 3.

at 16 minutes, the m-amino benzoic acid elutes at 30 minutes, and the p-hydroxy benzoic acid elutes at 45 mins. The separation is much improved as compared to that observed for the water-in-oil microemulsion. The small peak next to the MABA peak may be the ionized form separated from the neutral species. The effect of the pH on the separation of the test solutes in the oil-in-water microemulsion is shown in Figure 9. The aqueous phase was replaced with TRIS buffer of appropriate pH. The resolution seems to decrease at pH 8 and increase at pH 7 and pH 9.

The migration order and the effect of the pH on the separation can give some insight into the mechanism of separation in these microemulsions. In a typ-

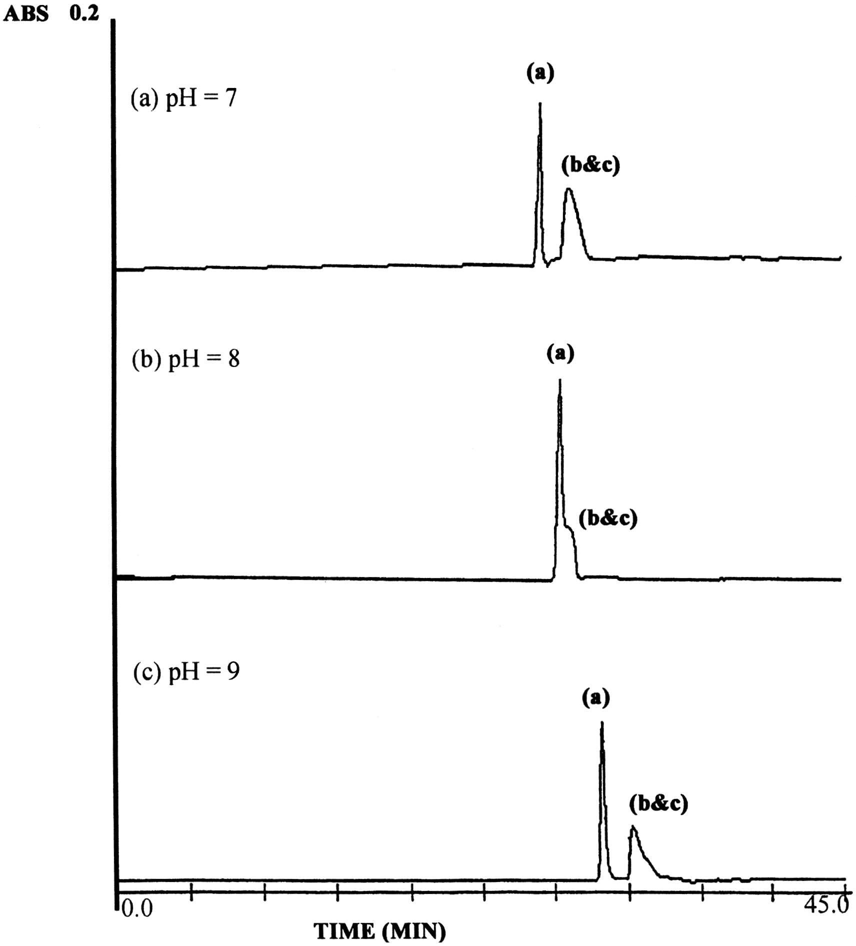

Figure 7. Effect of the pH on the separation of (a) benzophenone (~15mM), (b) m-amino benzoic acid (~7mM) and (c) p-hydroxybenzoic acid (~7mM) in an AOT water-in oil microemulsion consisiting of 0.16M AOT, 0.05M TEAP, 20% 0.01M tris hydrochloride buffer in decane. Separation conditions are given in Figure 3.

ical reversed polarity system, the expected order of migration would be to have the negative ions elute first, then the neutral molecules, followed by the positively charged species. What is observed to occur is that the neutral molecules migrate first, followed by the negatively charged species. It is possible that the neutral molecules are associated with AOT microemulsion aggregates that would tend to migrate the fastest based on the negative charge on the surfactant.

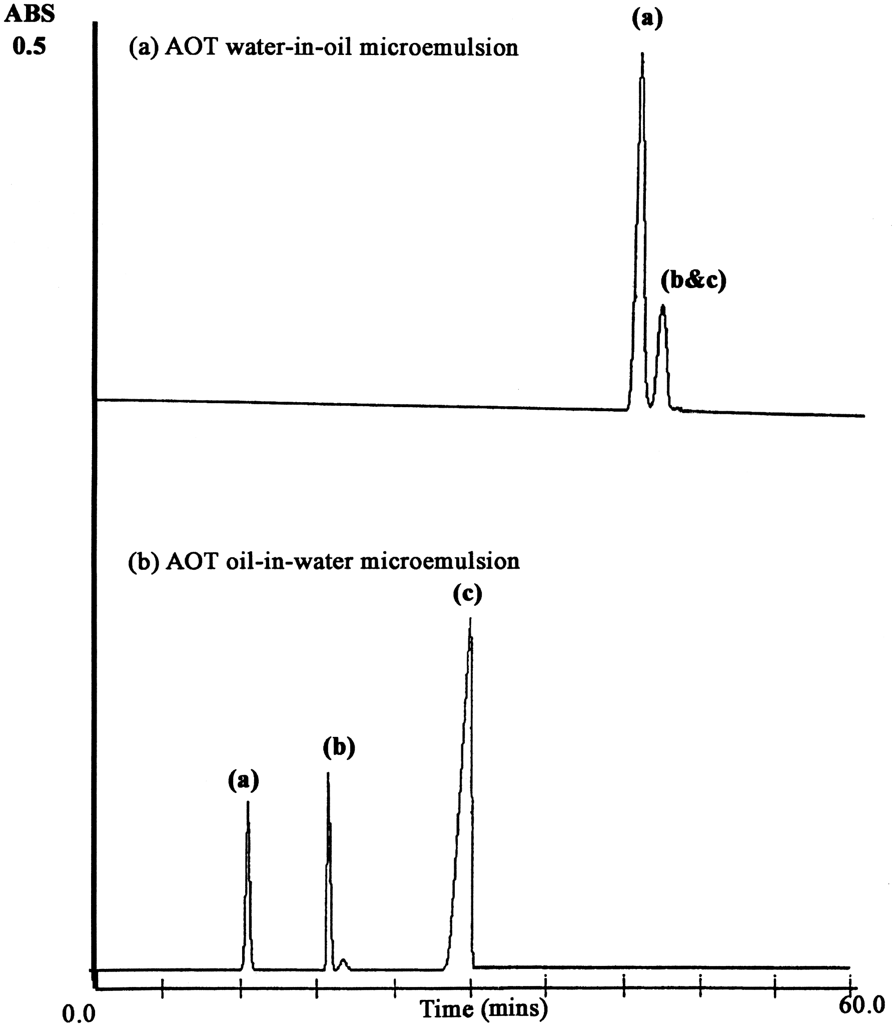

Figure 8. Comparison of separation in AOT water-in-oil and oil-in-water miccroemulsions 0f (a) benzophenone (~15mM), (b) m-amino benzoic acid (~7mM) and (c) p-hydroxybenzoic acid (~7mM). Water-in-oil microemulsion is 0.16M AOT, 0.05M TEAP, 20% water in decane. Oil-in-water microemulsion is 0.16M AOT, 0.05M TEAP, 15% decane in water. Separation conditions are given in Figure 3.

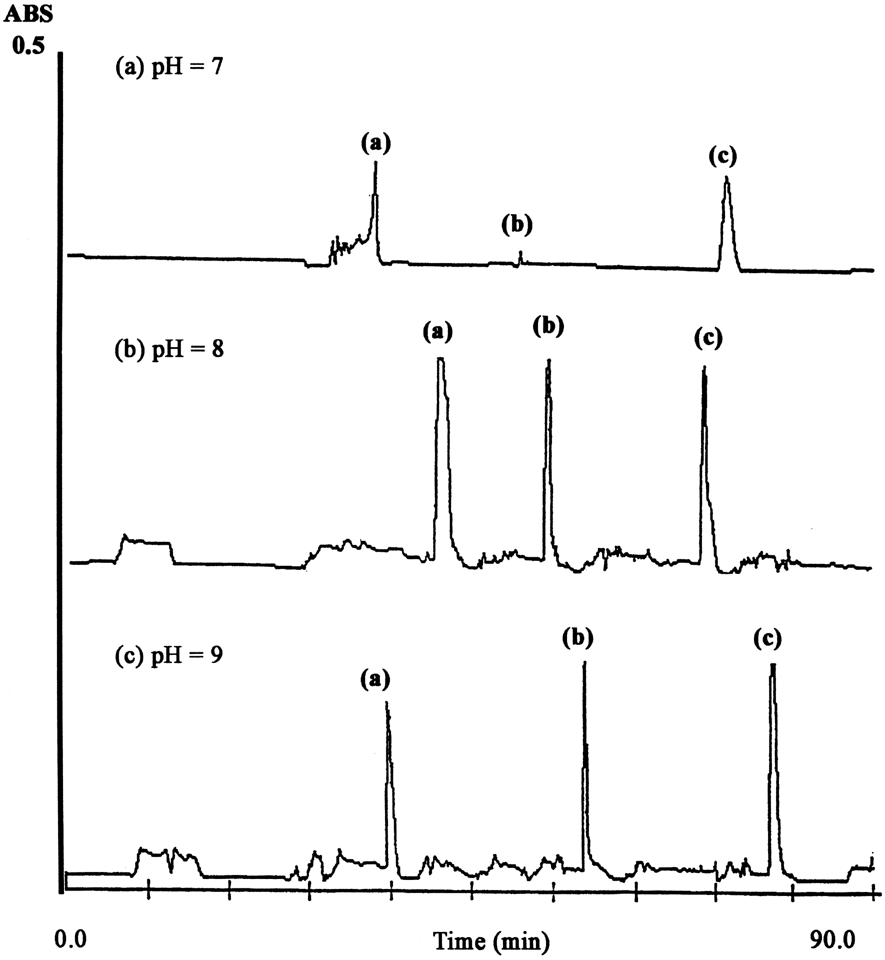

Figure 9. Effect of pH on the separation of (a) benzophenone (~15mM), (b) m-amino benzoic acid (~7mM) and (c) p-hydroxybenzoic acid (~7mM) in an AOT oil-in-water microemulsion consisting of 0.16M AOT, 0.05M TEAP, 15 % decane in 0.01M tris hydrochloride buffer . Separation conditions are given in Figure 3.

It is interesting to note, that in both oil-in-water and water-in-oil microemulsions the resolution seems to undergo a minimum at pH = 8. The electroosmotic flow has been observed to plateau at pH = 8. This shows that the resolution and separation of analytes in the microemulsion systems can be optimized by careful adjustment of the pH and the electroosmotic flow.

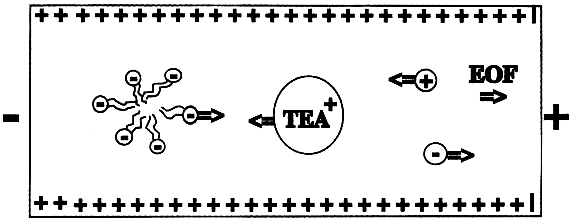

Figure 10. Schematic model of the mechanism of separation in AOT microemulsion capillary electrophoresis. The AOT aggregates migrate towards the detector, the TEA$^+$ migrates away from the detector.

The other mechanism of migration may be the ion-pair association of the negatively charged species with the TEA$^+$.[33] This possibility has been preliminarily investigated by observing the effect of the chain length of the alkyl amine on the migration of benzophenone. The migration time increases as the chain length of the alkyl amine increases. The separations are not affected by the counterion (perchlorate, bromide, chloride) of the alkyl ammonium ion. The manipulation of the alkyl ammonium ion is another promising factor that can be optimized to improve the separations in AOT microemulsions.

A tentative schematic model of the separation mechanism in AOT microemulsion capillary electrophoresis based on our investigations is shown in Figure 10. Neutral molecules and positive ions are associated with the AOT aggregate which migrates most quickly towards the detector based on the large negative charge. Separations of negatively charged species can be achieved by ion-pairing with the TEA$^+$.

CONCLUSIONS

We have obtained clear conductive water-in-oil and oil-in-water AOT microemulsions. The conditions that will sustain stable current under the conditions of the capillary electrophoresis experiment have been optimized for these systems. The water-in-oil microemulsions can dissolve very hydrophobic species, and we have separated the oil soluble from the water-soluble species. Improved separations were obtained for the oil-in-water microemulsion systems. Investigation of the effect of the pH and the composition of the microemulsion has led to a tentative model of the migration of species in these media.

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