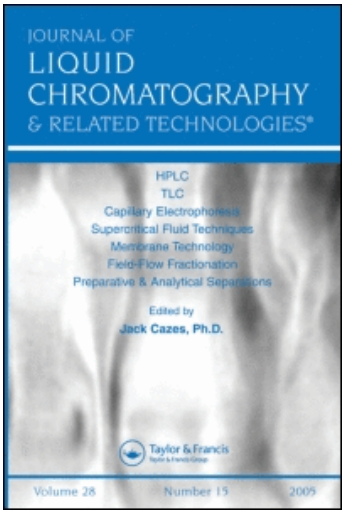

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Separation of Metallated Petroporphyrin Models Using Micellar Electrokinetic Capillary Chromatography

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SEPARATION OF METALLATED PETROPORPHYRIN MODELS USING MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY

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ABSTRACT

Some percentage of the total metals found in crude oils is present as organometallic porphyrin complexes, and petroporphyrin separations are of interest in geochemical sciences, environmental monitoring, and process control. Various chromatographic techniques including GC, SFC, and HPLC have been used to resolve mixtures of petroporphyrins. In this work, a micellar electrokinetic capillary chromatographic method has been developed and applied to the separation of petroporphyrin model compounds, and using the MECC method Ni (II) and V(IV)O Etio I and Octaethyl type porphyrins are completely resolved in 30 to 50 minutes. The MECC technique offers several advantages over a similar HPLC separation including smaller mobile phase and sample volumes required, milder aqueous based solvents, and less expensive columns.

Furthermore, the peak capacity of the MECC separation is expected to be large because less hydrophobic, neutral species can migrate in the 6 to 30 minute separation time window, suggesting that the technique when coupled to an ICP/MS detection system might prove extremely useful for metal speciation studies.

INTRODUCTION

Metals including As, Co, Fe, Mn, Ni, and V are found at concentrations ranging from < 1 to >1000 ppm in crude oils. Some 27 to 100% of the total metals may be present as organometallic porphyrin complexes which are associated with the heavier crude oil fractions.[1] These petroporphyrin complexes, first identified by Treibs in 1934, are derived from heme or chlorophyll, appear red to brown, and are thermally labile. Petroporphyrins usually contain Ni (II) or V(IV)O and are distinguished by various ring types (Etio, DPEP, Rhodo analogs) and different substituents (H, *n*-alkyl).[2] Complex and informative petroporphyrin profiles are used to study the geochemistry of oil formation, maturation, and migration,[3] to identify crude oils for environmental monitoring purposes,[4] and to aid in metal speciation for process control.[5]

Various high resolution chromatographic techniques have been used for multi-component analysis of petroporphyrin mixtures. Capillary GC[6] offers high resolution and efficiency, but the samples must be volatile and mass spectral detection is normally required. Before GC analyses, petroporphyrins are demetallated and derivatized, and this preparation may alter the profiles. SFC[7,8] is less efficient and less common than GC, but it does not require sample demetallation and visible absorbance detection may be used. Unfortunately, however, metalloporphyrins can adsorb onto packed columns sometimes used for SFC separations. Porphyrin adsorption, evidenced by peak tailing and slow bleed off, can foul columns necessitating replacement. HPLC,[9-12] which also uses packed columns, is even less efficient than SFC; furthermore, method development can be time consuming and organic solvents such as hexane, methylene chloride, and tetrahydrofuran may be required.

Capillary electrophoresis,[13] a separation technique used for less volatile and ionic materials, offers rapid analysis times and high efficiencies along with minute sample size requirements and reagent consumption. Capillary zone electrophoresis resembles both HPLC and electrophoresis in that a high voltage potential is applied across the ends of a capillary column filled with an aqueous buffer solution, thereby allowing for migration of charged species and

electroosmotic flow which moves neutral components past the detector. UV-VIS, LIF, and MS detectors are often interfaced to CE systems. In one CE mode called micellar electrokinetic capillary chromatography, a surfactant is added to the separation buffer, and species are separated based upon differential partitioning between the aqueous buffer and psuedo-stationary phase created by the surfactant micelles. Researchers have used CZE and MECC to separate free base[14] and metallated[15] anionic porphyrins, as well as polar and high molecular weight fuel-related materials.[16] In this work, we have examined the applicability of MECC to the separation of metallated petroporphyrin models and compared our results to a similar HPLC analysis.[12]

MATERIALS

Apparatus

Porphyrin absorbance measurements were made using either an HP 8452a photodiode array or Cary 3E spectrophotometer. Buffer pH was measured with a Corning Digital 110 meter and combination glass membrane pH electrode, following a 2-point calibration with certified standard pH 7 and 10 reference buffers. MECC separations were accomplished using a Beckman P/ACE 2210 automated capillary electrophoresis system equipped with a fused silica capillary and an absorbance detector which included a deuterium lamp and bandpass filters. MECC data were analyzed using Beckman System Gold™ software.

Reagents

The Ni (II) and V(IV)O derivatives of etioporphyrin I and octaethylporphyrin were obtained from Midcentury Chemicals (Posen, IL). A.C.S. reagent grade sodium tetraborate, boric acid, and other inorganic substances were obtained from Fisher, along with HPLC grade acetone and other organic solvents. ~99% sodium dodecyl sulfate (SDS) was obtained from Sigma. Sudan III and 1-(2-pyridylazo)-2-napthol (PAN) dye markers were obtained from Aldrich.

METHODS

For capillary electrophoresis separations buffers were prepared using 18M Ω water from a Barnstead NANOpure II™ system. Aqueous buffer pH was

measured and adjusted prior to vacuum degassing and filtering through 0.45μm filters. Organic modifiers were added to aqueous buffer solutions immediately prior to use, and modified buffers were sonicated for a few minutes but pH was not readjusted. Samples were prepared by adding aliquots of metalloporphyrin stock solutions in acetone to aqueous buffers. Porphyrin sample solutions could be centrifuged to remove particulate matter, but filtration through Acrodisc membrane filters resulted in porphyrin adsorption and loss.

New fused silica capillaries were validated by rinsing for 5 min. each with 100mM NaOH, water, and 50mM borate buffer (pH 8.35) then separating a Beckman test mixture containing benzoic acid derivatives. Surface activation was completed by rinsing again with base, water, and borate buffer which was allowed to remain in the capillary for at least 8 hours. Before the first porphyrin separations, the capillary was rinsed again with base, water, and run buffer which was allowed to remain in the capillary for at least 30 minutes. 2 min. run buffer pre-rinses and 5 min. water post-rinses were initially programmed into the automated run sequences; however, this rinsing procedure was modified as described later in the text.

Initial separations conditions used were as follows: Run buffer - 40mM SDS, 22.5mM total borate, pH 8.5; Porphyrins - 1 to 4 μM each of metalloporphyrins in 20% acetone, 80% run buffer; Markers - acetone or methanol for electoosmotic flow and Sudan III or PAN for the micelles; Capillary - 57 cm L_t (50 cm L_d) x 75 μm i.d. fused silica, 2518 nL V_t; Injections - pressure, 2 sec, ~12 nL; Voltage - constant, 24 kV, normal polarity (+ injection to - detection side); Temperature - 30°C; Detection - absorbance, 400+5nm. Changes to the initial separation conditions are as described later in the text.

RESULTS AND DISCUSSION

Porphyrin Solubility and Absorbance Data

Structures of the nickelated petroporphyrin model compounds are depicted in Figure 1. Freeman and co-workers[17] have previously discussed nickel and vanadyl petroporphyrin derivatives and suggested that they exhibit maximum solubilities in solvents with Hildebrand solubility parameters, δ, of 9.5. For use in MECC separations, organic solvents must be miscible with aqueous buffers and must also exhibit suitable UV cut-offs if absorbance detection is to be used.

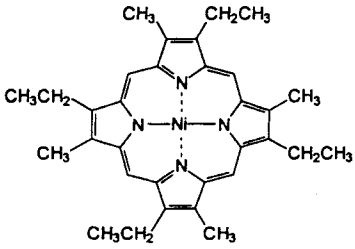

Figure 1. Structures of the Ni (II) derivatives of the petroporphyrin model compounds.

Table1

Solvent Parameters and Porphyrin Solubilities[a]

Solvent	δ[b]	UV Cut Off (nm)[c]	Porphyrin Solubilities (μg/mL) VO-EtioI	VO-Octa-ethyl	N-EtioI	Ni-Octa-ethyl
CH_2Cl_2	9.70	235			≥1500	
$(CH_3)_2CO$	9.90	330	≥220	≥220	≥270	≥150
CH_3CN	11.7	190	≥46		≥2	
CH_3OH	14.4	210	≥3		≥1	

[a] All porphyrin solubility values taken from Reference 17 except those in $(CH_3)_2CO$ which were measured in this work using methods described in that literature.

[b] Taken from Reference 18.

[c] Taken from Reference 19.

Various solvent parameters and sample solubility information is listed in Table 1. Absorbance maxima and extinction coefficients measured for the petroporphyrins in acetone, a common solvent which met the criteria discussed above, are listed in Table 2. For MECC separations, stock solutions of petroporphyrins in acetone were prepared and mixed in varying proportions with the run buffers.

Table 2

Porphyrin Absorbance Data in Acetone at Ambient Temperatures

	Soret Band		α Band	
Porphyrin	**λmax (nm)**	**ε(L/mol-cm)**	**λmax (nm)**	**ε(L/mol-cm)**
VO-Etio I	404	3.34E5±0.01	568	2.98E4±0.02
VO-Octaethyl	404	3.25E5±0.01	568	2.87E4±0.02
Ni-Etio I	388	1.95E5±0.02	550	3.33E4±0.01
Ni-Octaethyl	390	2.05E5±0.03	550	2.70E4±0.07

Initial MECC Separations

Shigeru Terabe[20] has suggested a scheme for optimization of MECC separations which includes running under a standard set of conditions and calculating capacity factors, k'. If k' < 0.5, the surfactant concentration is increased; if k' > 10, buffer additives such as organic modifiers, cyclodextrins, or bile salts are utilized. Initial trials using conditions listed in the experimental section resulted in average migration times of 2.5±0.1 and 6.2±0.2 min. for the electroosmotic flow (t_0) and micellar (t_{mc}) markers; respectively. The analyte mixture was not resolved and also passed the detector window in 6.2 min., indicating complete retention of the porphyrins within the micelles such that k' >> 10 and buffer additives would be required.

Before buffer additives were attempted, however, the surfactant concentration was varied from 20 to 100 mM with the same net results - no resolution of the mixture and porphyrin co-migration with the micelles. As buffer SDS concentration and conductivity increased, the t_0 changed from 2.5 to 3.1 min., while t_{mc} lengthened from 6.2 to 10 min. Power levels were also increased from 1.2 to 3.8 W/m over this range of SDS concentrations, making Joule heating of the buffer a concern.

Interestingly, the average peak area increased by a factor of 29 going from 20 to 100 mM SDS indicating that more porphyrin was being solubilized in the aqueous buffer. At 20 mM SDS the peak area %RSD was extremely high due to low signal-to-noise resulting from low porphyrin solubility and low deuterium lamp energy at 400 nm; at ≥ 40 mM SDS peak area %RSD was ≤ 10. Migration time precision was typically ≤ 1.3 %RSD in these early separations.

Table 3

Separation as a Function of Acetone Fraction in the Buffer

Acetone (% v/v)	SDS (mM)	Borate Avg. (mM)	M_t (min.)	%RSD (n=3)	Signal Identification
0	40	22.5	2.45	0.35	EOF
			5.81	1.04	Micelles/Porphyrins
5	38	21.4	2.55	0.06	EOF
			6.30	0.04	Micelles/Porphyrins
10	36	20.3	3.12	0.22	EOF
			9.32	0.90	Micelles
			9.54	0.85	Porphyrins
15	34	19.1	3.49	0.35	EOF
			11.04	1.66	Micelles
			12.07	1.51	Porphyrins
20	32	18	4.04	0.14	EOF
			11.38	1.59	Micelles
			15.79	0.59	VO-Porphyrins
			16.07	0.65	Ni-Porphyrins
30	28	15.8	6.09	0.37	EOF
			22.02	0.05	VO Etio I
			24.95	0.30	VO Octaethyl
			27.32	0.33	Ni Etio I
			30.63	0.25	Ni Octaethyl

Organic Modifier Studies

Various organic modifiers including acetone, acetonitrile, dimethyl sulfoxide, ethanol, ethylene glycol, methanol, and 2-propanol have been used in CZE[21] and MECC[22] separations; although, the two most common modifiers are probably acetonitrile and methanol. These buffer additives effect MECC resolution and efficiency by altering capacity factors and selectivities of the analytes, and the electroosmotic flow and electrophoretic mobilities are effected

Table 4

Separation as a Function of Organic Modifier at 30% v/v in the Buffer[a]

Organic Modifier	M_t (min)	%RSD	n	Signal
Methyl ethyl ketone	4.95	1.68	6	EOF
	16.20	3.87		Porphyrin 1
	16.83	3.56		Porphyrin 2
	17.10	4.12		Porphyrin 3
	17.46	4.00		Porphyrin 4
Acetonitrile	5.03	2.05	6	EOF
	16.59	9.85		Porphyrins
Methanol	6.21	0.37	2	EOF

[a] Final concentrations of 28mM SDS - 16 mM Borate in the Buffer.

by changes in the dielectric constant and viscosity of the buffer medium, as well as in the zeta potential. In addition, the surfactant critical micelle concentration (CMC) and aggregation number may be modified by ions and organic solvents present in aqueous solutions.[23]

Organic modifier studies were conducted using the same initial conditions listed in the experimental section except that samples were 1 to 2 μM of each porphyrin in 30% acetone / 70% 40 mM SDS- 22.5 mM Borate-pH 8.5 buffer and sample injections were increased to 5 sec (~29 nL or 1% V_t).

In the first study, the fraction of acetone in the separation buffer was increased from 0 to 30%, and in the second study, acetone was compared to other organic modifiers each at 30% (v/v) added. Results are given in Tables 3 and 4; representative electropherograms are shown in Figures 2 and 3.

With acetone as the organic modifier in the run buffer, below 10% (v/v) added, no porphyrin separation occurred and species eluted with the micellar marker. At acetone concentrations $\geq$ 10% the micellar marker actually eluted before the petroporphyrins, indicating that migration of the metallated porphyrins was related to factors other than micellar partitioning alone. At 20% acetone added, vanadyl porphyrins were separated from nickel complexes

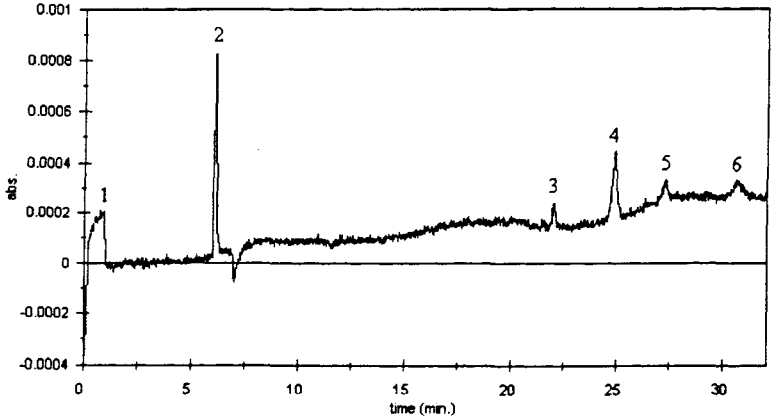

Figure 2. MECC separation of a mixture of vanadyl and nickel petroporphyrins using run buffer which contained 28 mM SDS - 16 mM Borate - 30 % v/v Acetone. A 5 sec sample injection was used and other conditions are as listed in the experimental section. Events or peaks are identified as: 1) baseline zero, 2) EOF, 3) VO Etio I, 4) VO Octaethyl, 5) Ni Etio I, and 6) Ni Octaethyl porphyrin.

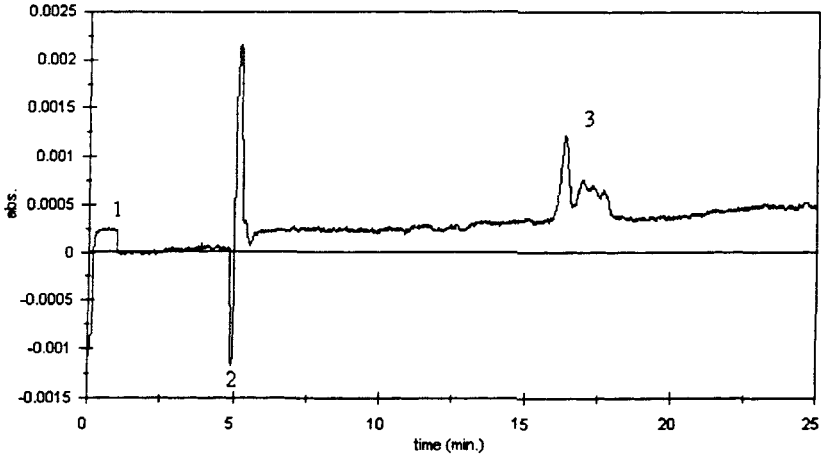

Figure 3. MECC separation of a mixture of vanadyl and nickel petroporphyrins using run buffer which contained 30 % v/v Methyl Ethyl Ketone as the organic modifier. Other conditions are as listed for Figure 2. Events or peaks are identified as: 1) baseline zero, 2) EOF, and 3) incompletely resolved porphyrins.

and, at 30%, all four porphyrins resolved in under 31 minutes. Porphyrins migrated in the order VO Etio I, VO Octaethyl, Ni Etio I, and Ni Octaethyl, and some porphyrin interaction occured with the fused silica capillary surface.[24,25]

In comparing porphyrin separations using different organic modifiers at 30% (v/v), it was concluded that acetone was better than methylethyl ketone, acetonitrile, or methanol in resolving the mixture. Although methylethyl ketone demonstrated some promise in separating the analytes, porphyrins could not be resolved with acetonitrile, and absolutely no porphyrin peaks were observed when methanol was used in the buffer. The final concentrations of buffer components were 28 mM SDS-16 mM Borate-30% Organic Modifier for this comparison.

Reproducibility and Treatment of the Capillary Surface

The porphyrin separation results, obtained with 30% acetone added to the run buffer, appeared very promising at first, but subsequent runs were not very reproducible as evidenced by increases in migration times and decreases in peak areas. Sample degradation, changes in buffer composition, and porphyrin adsorption to the capillary surface were thought likely causes of irreproducibility.

In an attempt to alleviate these problems, porphyrin stock solutions were deoxygenated and stored in amber bottles, and porphyrin samples were prepared immediately prior to use. Organic modifiers were added to the aqueous buffers fresh each day, and buffer solutions in the instrument vials were replaced frequently (after 3 runs). The capillary post-run rinsing procedure was modified to include a 15 sec 1M HF rinse followed by washing with water, and this proved useful in creating a reproducible capillary surface and EOF, as discussed by Schwer and Kenndler.[21] The effect of this capillary treatment was examined by including the HF rinsing procedure in the separation of the Beckman test mixture of benzoic acid derivatives; after 31 runs peak migration time reproducibilities were less than 2.5% RSD.

Although migration times were more consistent, porphyrin peak area reproducibility was still a challenge. Sample degradation and/or precipitation appeared to transpire as evidenced by loss of solution color. de Waal and co-workers[11] noted that poor separations are obtained when precipitation of vanadyl and nickel porphyrins in oil extracts occurs in the starting mobile phase. For the MECC separations, porphyrin solubilization might be effected if

surfactant aggregation in the modified run buffer was significantly different than that in aqueous solution; therefore, the critical micelle concentration of SDS was determined in a solution containing 16 mM Borate-30% Acetone using a fluorescence technique.[26] However, at ambient temperature a CMC value of 7 mM, well below the 28 mM SDS present in the modified run buffer, was indicated by a curve fit of the data.

Increased SDS Concentrations

At constant 30% (v/v) acetone and reduced total borate (12mM) concentrations, the amount of SDS in the run buffer was varied from 15 to 42 mM. Over this range, the EOF and power level increased from 5.2 to 6.1 min. and 0.83 to 2.1 W/m; respectively. At 15 mM SDS, two small porphyrin peaks were resolved and migration times were near 10 minutes. With 42 mM SDS present, peak areas were substantially larger and all four porphyrins were resolved in a migration time window of 36 to 50 min. Representative data are shown in Figure 4. It should be noted that injection times were increased to 10 sec (~59 nL, 2% V_t) for the sample and a secondary buffer plug added to push the porphyrins further up into the capillary before application of the separation voltage.

At constant SDS and total borate concentrations of 42 and 12 mM; respectively, the acetone fraction was varied from 20 to 30% by volume. Over this range, the EOF increased from 4.6 to 6.3 min. At 20% acetone, two incompletely resolved porphyrin signals migrated with times of 29 and 31 min.; while, at 30% acetone, all four porphyrin signals were completely resolved in a migration time window of 32 to 47 min. as shown in Figure 5. Reproducibility data for the petroporphyrin separation in 42 mM SDS-12mM Borate-30% Acetone over different days and with different capillaries is summarized in Table 5.

Results obtained by varying the organic modifier composition are in agreement with studies[21] which demonstrate that the aprotic solvent acetone causes decreases in the electroosmotic flow velocity due to decreases in the dielectric constant and increases in the solution viscosity coefficient over the 20 to 30% v/v range. The zeta potential depends on the apparent pH of the buffer solution, and, in low ionic strength phosphate buffer containing 50% v/v acetone, it is approximately constant above pH' ~9.[21] For the 42 mM SDS-12 mM Borate-30% v/v Acetone buffer used in this study, the measured pH' was 9.6; however, the effect of this higher ionic strength SDS buffer on the deprotonation equilibria of silanol groups on the capillary surface is not known.

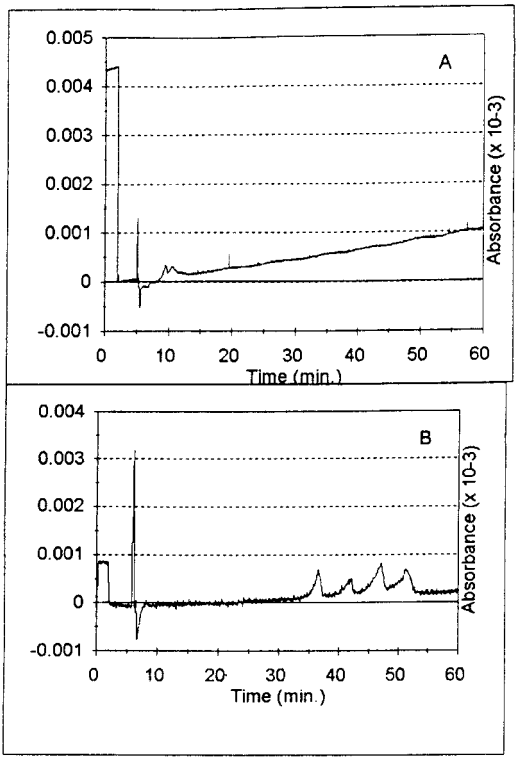

Figure 4. Petroporphyrin separation as a function of SDS concentration in the run buffer which contained 12 mM Borate - 30 % v/v Acetone. 10 sec primary and secondary injections of sample and buffer were used along with an HF rinse. Other conditions are as listed in the experimental section. A) 15 mM SDS, and B) 42 mM SDS.

Slight differences in buffer composition may lead to pronounced effects on solution properties and surface charge which combine to alter observed electrophoretic mobilities. This may partially explain variations in migration time behavior, in addition to other effects previously discussed.

Quantitative irreproducibility is related to low D_2 lamp energy at 400 nm and variable porphyrin concentrations which combine to make absorbance detection less than ideal in this case. Weinberger and co-workers[14] have demonstrated that a tungsten lamp provides better sensitivity than a deuterium lamp (LOD = 0.7-1.7 x 10^{-12}M) for absorbance detection of urinary porphyrins in CE separations. The petroporphyrin samples are estimated to be in the 1 to

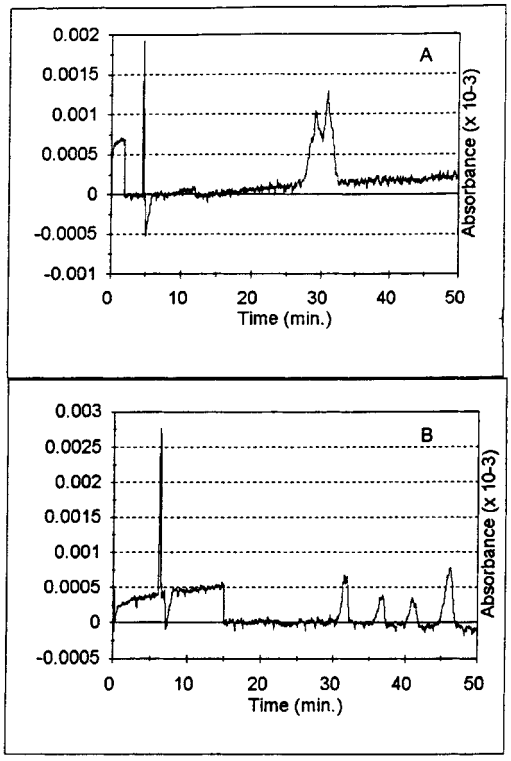

Figure 5. Petroporphyrin separation as a function of Acetone fraction in the run buffer which contained 42 mM SDS - 12 mM Borate. Other conditions are as listed for Figure 4. A) 20 % v/v Acetone, and B) 30 % v/v Acetone.

2 x 10^{-6}M range when first prepared; however, some sample precipitation, decomposition, and/or wall adsorption occurs before analytes reach the detector window. Average peak base widths of 2.5 min. in the MECC method are indicative of zone spreading which reduces the peak height and detectability. Various attempts to focus sample zones by field amplification methods did not improve detection, and porphyrins could not be separated on a linear polyacrylamide coated capillary. Thus, future plans include utilization of a tungsten radiation source or a different type of detector. Sample solubility and stability will be improved by using less polar organic modifiers and/or more surfactant in the buffer, and methyl ethyl ketone is currently being re-examined at higher SDS concentrations.

Table 5

Separation with 42mM SDS -12mM Borate - 30% Acetone Buffer[a]

Signal	M_t (min.)	%RSD	Peak Area	%RSD
EOF	6.26	0.80		
VO Etio I	32.32	3.30	758	6.33
VO Octaethyl	37.84	3.80	473	4.86
Ni Etio I	42.60	4.82	508	28.2
Ni Octaethyl	47.72	4.80	1092	9.71

[a] Data are from Day 1, Capillary 1; other migration time results are summarized below:

	Day 2, Capillary 1	Day 1, Capillary 2	Day 2, Capillary 2
EOF	6.14	6.04	6.05 [min]
Porphyrins	29.86 - 42.62	28.31 - 46.55	34.09 - 51.16 [min.]

Provided that detection can be improved, the MECC method presented here compares favorably to the HPLC separation of petroporphyrin compounds by Xu and Lesage.[12] The MECC method utilizes fused silica capillaries which are more cost effective (~$10) than the aminopropyl HPLC column (~$350). Aqueous based buffers are used in the MECC separation as opposed to hexane, toluene, and methylene chloride HPLC mobile phases. 60 nL MECC sample injections are two orders of magnitude smaller than those used in the HPLC work. MECC run times of 60 min. are comparable to 50 min. HPLC separation times plus column reconditioning. The MECC migration order was VO Etio I, VO Octaethyl, Ni Etio I, and Ni Octaethyl porphyrins, the reverse of that observed in the HPLC separation. The MECC method gave an average peak resolution of 2.2; whereas, the HPLC method appeared to range from 2.8 (two vanadyl porphyrins) to 36 (Ni Etio I and VO Octaethyl porphyrins). Average efficiency for the MECC method was 4450 theoretical plates compared to 6545 calculated from the HPLC chromatogram. Column lifetime and reproducibility were not discussed in the HPLC work.

Due to different separation mechanisms being operative, the elution order for the petroporphyrin model compounds in MECC is opposite to that in the HPLC work. These differences are important in terms of the total peak capacity. Although Xu and Lesage could separate similar porphyrins in a 19 minute window, it is doubtful that very polar compounds can be separated using

the gradient elution scheme they described. The MECC separation, on the other hand, offers a "window of opportunity" which stretches from the EOF time (~ 6 min.) to the porphyrin migration time (~ 30 min.). During this period, compounds which are much less hydrophobic than the petroporphyrins may elute, allowing the separation mechanism to be exploited for metal speciation studies by MECC. Researchers have sucessfully interfaced micellar LC to ICP/MS detection systems for the analysis of various organometallic compounds,[27,28] and efforts are ongoing to develop CE systems for speciation of metalloporphyrins and metalloproteins.[29]

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