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Short chain alkyl imidazolium ionic liquids in enhancing the sensitivity of capillary electrophoresis

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Using of short chain alkyl imidazolium ionic liquids in enhancing the sensitivity of capillary electrophoresis

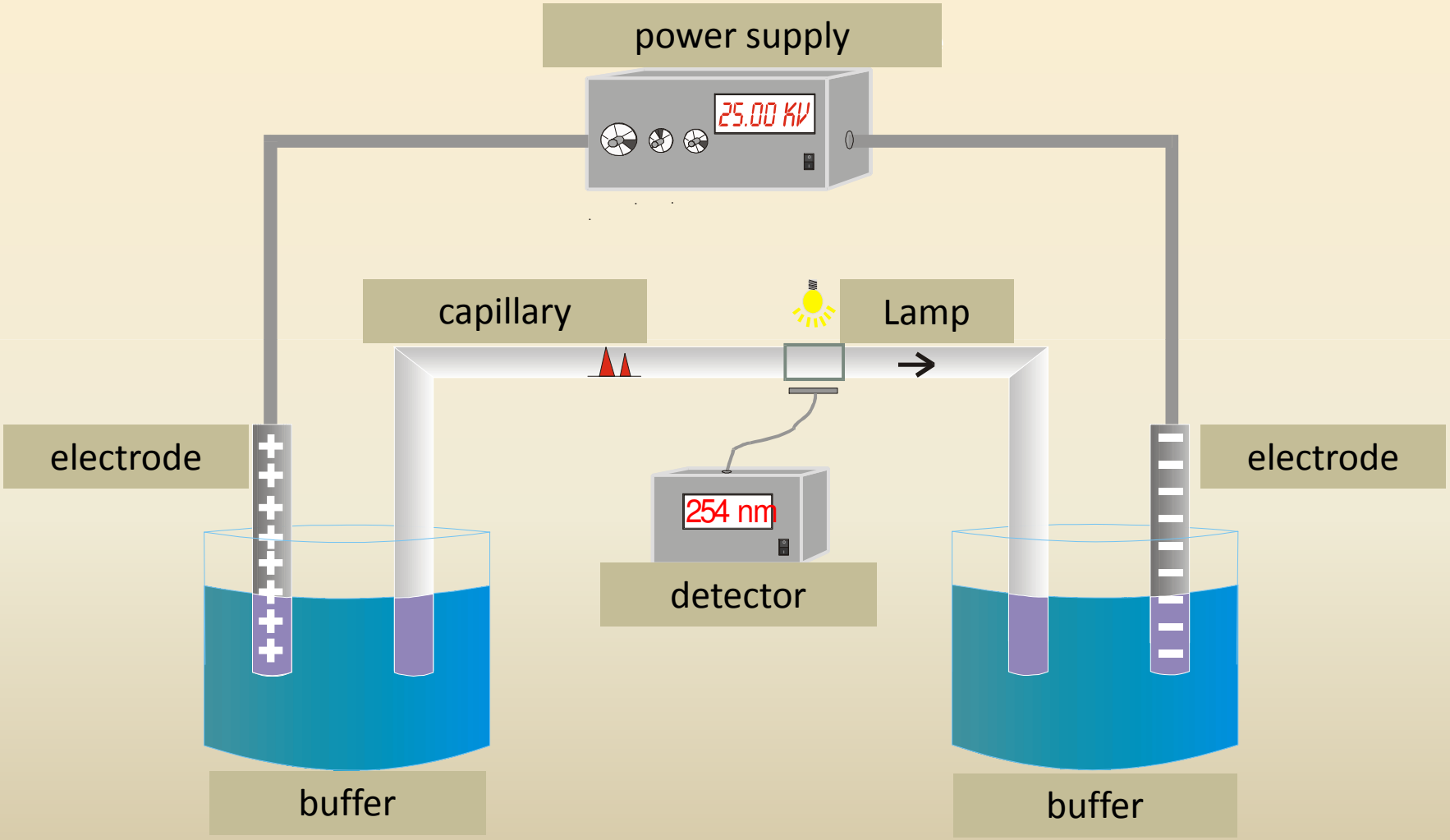
- Capillary Zone electrophoresis
- Micellar electrokinetic Chromatography
- Sweeping-micellar electrokinetic chromatography (sweeping-MEKC)
- Stacking in sweeping-micellar electrokinetic chromatography
- Analyte focusing by micelle collapse (AFMC)
- Short chain ionic liquids (Ils) as micellar forming agents
- Simultaneous determination of methorexate, folinic acid and folic acid by IL based sweeping-MECK and AFMC.

History

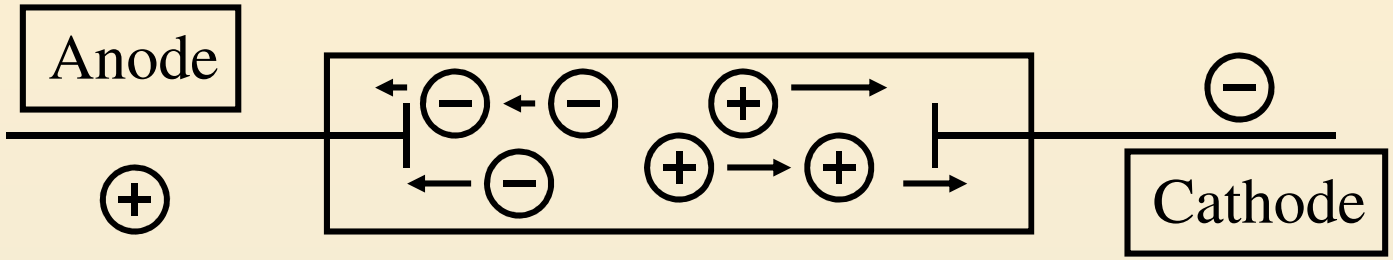
Electrophoresis as an analytical tool was introduced by the Swedish chemist Arne Tiselius, first in his doctoral thesis in 1930. For his pioneer work in this field, Tiselius was awarded the Nobel prize in 1948.

The first appearance of capillary electrophoresis can be found in 1981 with the publication of an art`icle by J.W. Jorgenson and K.D. Lukacs, working at the University of North Carolina, in Analytical Chemistry, 53 (1981) 1298.

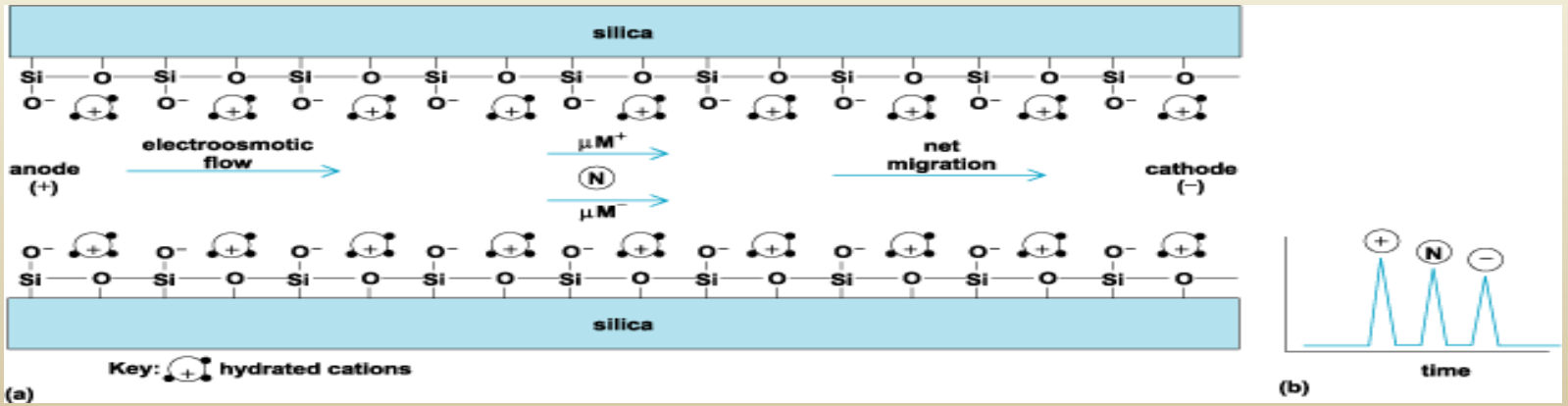
CE Instrumentation



Principles of Capillary Electrophoresis (CE)



- Electrophoresis is the differential movement of ions in an electric field
- Detection occurs as resolved components move past a detector, typically UV, with output shown as peaks on a baseline



Factors affecting electrophoretic mobilities

1. Nature of the charged particles:

net charge, size, relative mass,
charge-to-size ratio or charge-to-mass ratio

2. Nature of the electrophoretic system

1) The ionic composition of the electrophoresis buffer

2) The temperature

3) The pH of the electrophoresis buffer

4) The applied voltage

Types of electrophoretic separations

1) Capillary zone electrophoresis (CZE)

2) Capillary isotachophoresis (CITP)

3) Capillary gel electrophoresis (CGE)

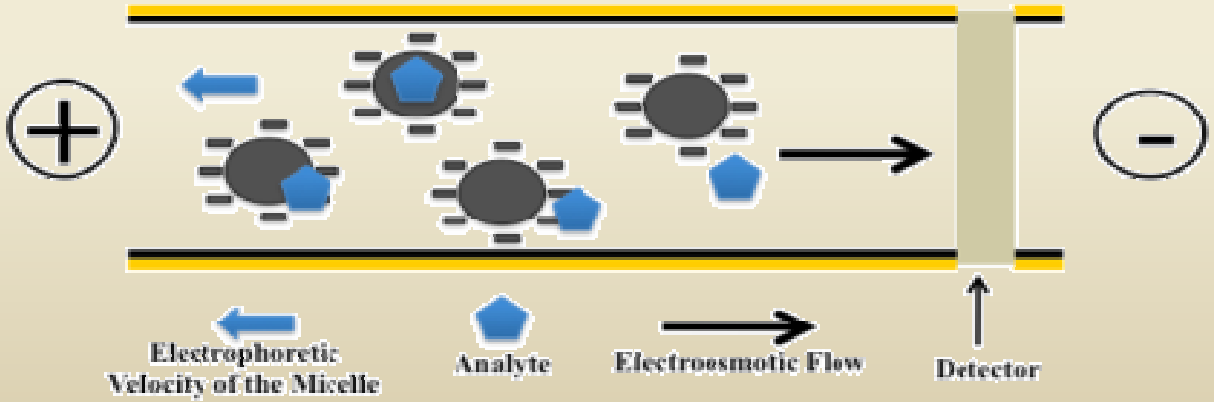
4) Capillary isoelectric focusing (CIEF)

5) Micellar electrokinetic chromatography (MEKC)

6) Capillary electrochromatography (CEC)

Micellar electrokinetic chromatography (MEKC)

- It is a useful branch of CE that utilizes surfactant above critical micelle concentration (CMC) as pseudo-stationary phase.
- MEKC can be employed to separate both charged and neutral molecules.
- MEKC benefits from high peak efficiency

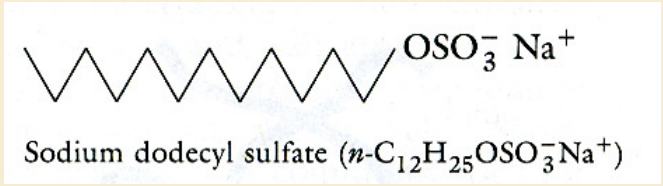


Terabe et al. Anal. Chem. 56, 111-113, 1984

Micellar electrokinetic chromatography (MEKC)

Separation of neutral solute

Psuedo-stationary phase



Sodium dodecyl sulfate (n-$C_{12}H_{25}OSO_3^-Na^+$)

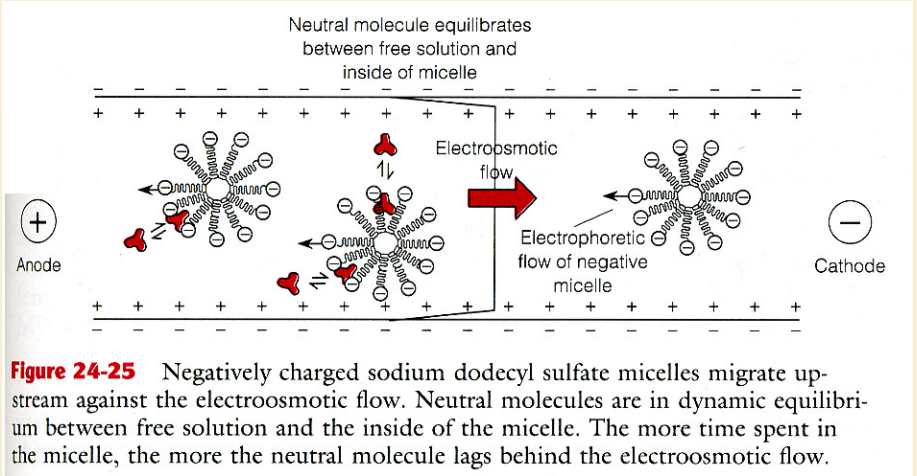
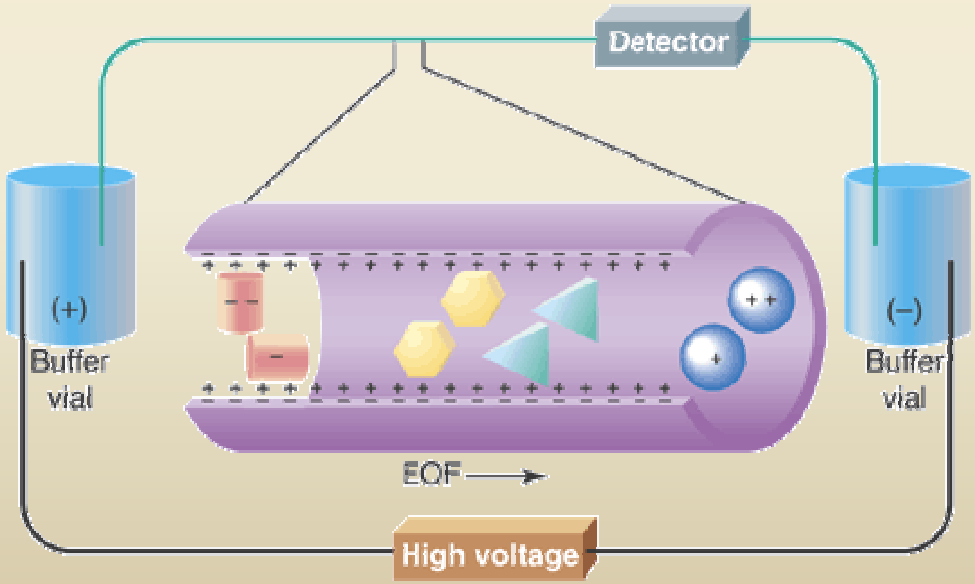


Figure 24-25 Negatively charged sodium dodecyl sulfate micelles migrate upstream against the electroosmotic flow. Neutral molecules are in dynamic equilibrium between free solution and the inside of the micelle. The more time spent in the micelle, the more the neutral molecule lags behind the electroosmotic flow.

Advantage: easy to apply

Disadvantage: less sensitivity



Deia et al. Electrophoresis 25, 2830-2837, 2004

Factors Contributing to Low Sensitivity

- Small amount of analyte injected
- Tiny peak volumes (Separation suffers if injection volume exceeds 2% of the column volume)
- UV-Vis detection is the most common detector

- Beers Law
  - $A = \varepsilon * L * C$
  - A: absorbance
  - ε: epsilon (L/mol*cm)
  - L: path length (cm)
  - C: concentration (mol/L)

Limit of Detection in Capillary Electrophoresis

- The path length of the cell is the internal diameter of the tube
  ~ 10-100 µm
- Leads to LOD of 10(-8) M
- Detector portion of the tube must be bare
  - Could lead to breakage of the tube



On-line sample preconcentration

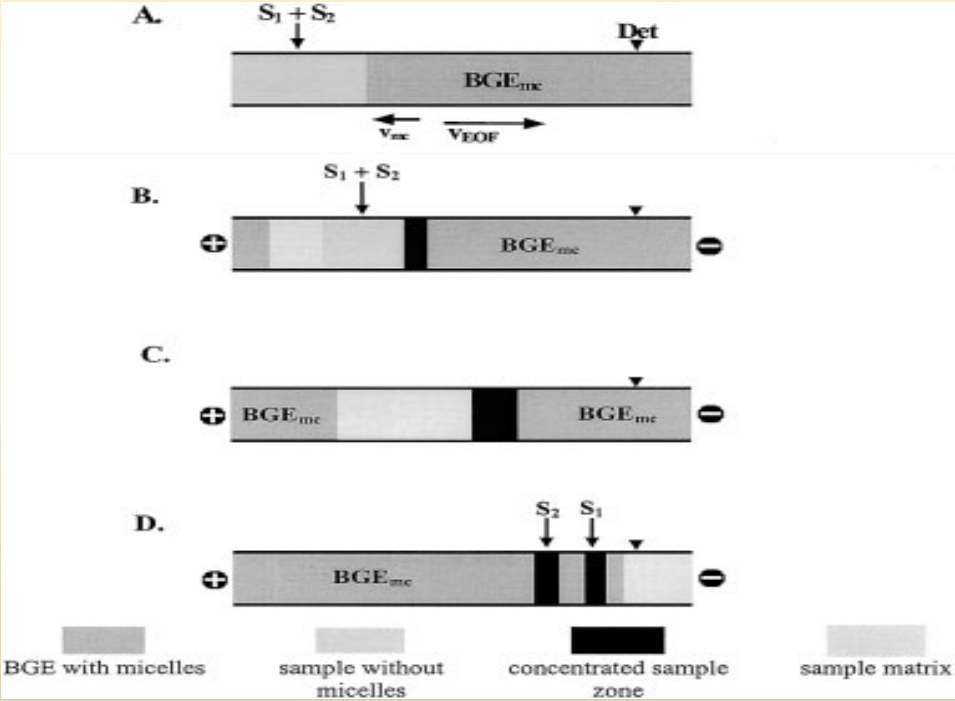
Various on-line techniques are used to enhance the sensitivity of capillary electrophoretic analyses including:

- Field-amplified sample stacking,
- Large-volume sample stacking,
- Field-amplified sample injection,
- Dynamic pH junction,
- Isotachophoretic sample stacking,
- Micelle to solvent stacking,
- Sweeping-micellar electrokinetic chromatography (sweeping-MEKC) and

- Analyte focusing by micelle collapse (AFMC)

Sweeping-MEKC

- It is considered a versatile enrichment technique that both charged and neutral analytes can be preconcentrated.
- In sweeping-MEKC, the sample, which is void of the micelles, is injected into a capillary pre-filled with BGE containing micelles.



Petr Bocek et al. *Electrophoresis* 2003, *24,* 466–485

Sweeping-MEKC

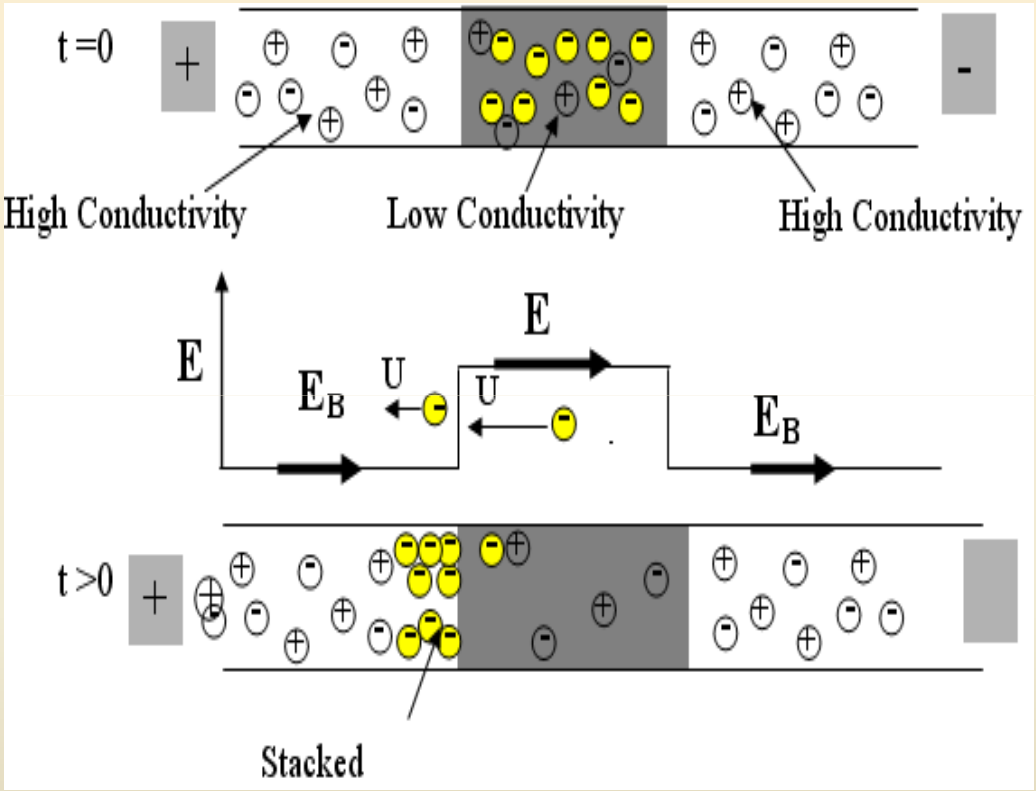
- The extent of sweeping is dictated by the strength of the chromatographic interaction (retention factor, *k*) during sweeping and the initial sample plug length.

- The sweeping can occur in a homogeneous or enhanced or reduced electric field in the sample region.

- In the case of enhanced electric field, the sample is prepared in a matrix having a conductance lower than the BGE and forms a stacking boundary (SB) in the interface between sample and BGE.

Stacking in sweeping-MEKC

- Sample stacking can be done to increase the concentration of the sample within the column

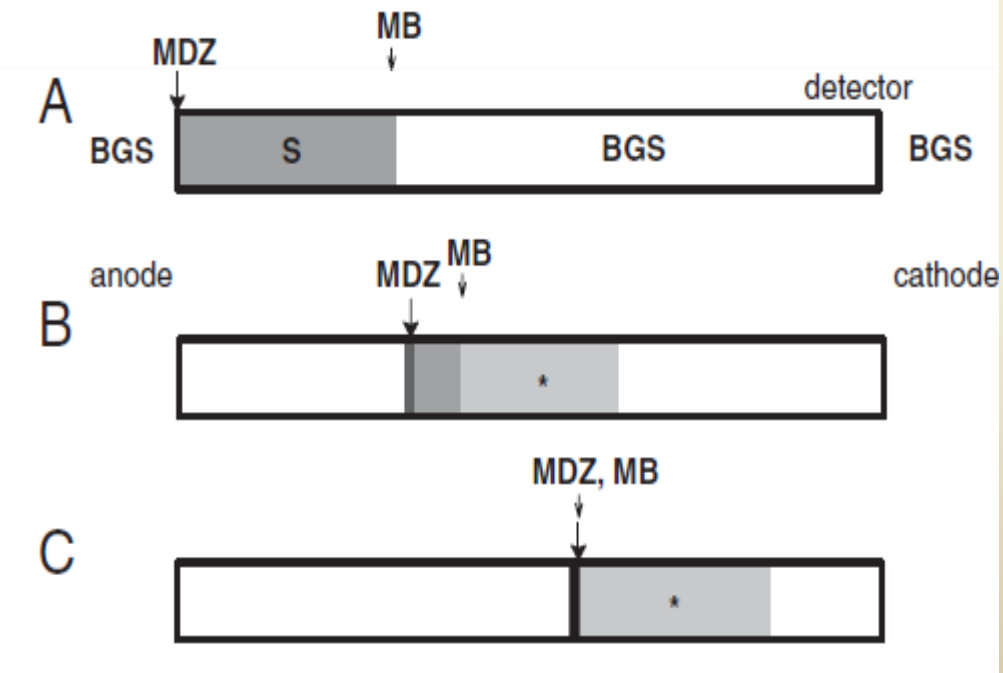


The ratio of the electric conductivities of the BGE and the sample (BGE/S) is known as (gamma) factor.

Not only the *k* factor of the analytes (through sweeping) but also the gamma factor (through stacking) and the length of sample plug can impact on the enrichment sensitivity.

Analyte focusing by micelle collapse (AFMC)

- AFMC is based on the conductivity of sample zone, which contains the micelles, is higher than that of BGE.
- The steps of AFMC includes the transport, release, and accumulation of analytes with the aid of micelles.



- The amount of focused analyte molecules depends on the affinity of the analytes to the micellar carrier phase (*k* factor) and conductivity ratio of BGE to sample (gamma factor)

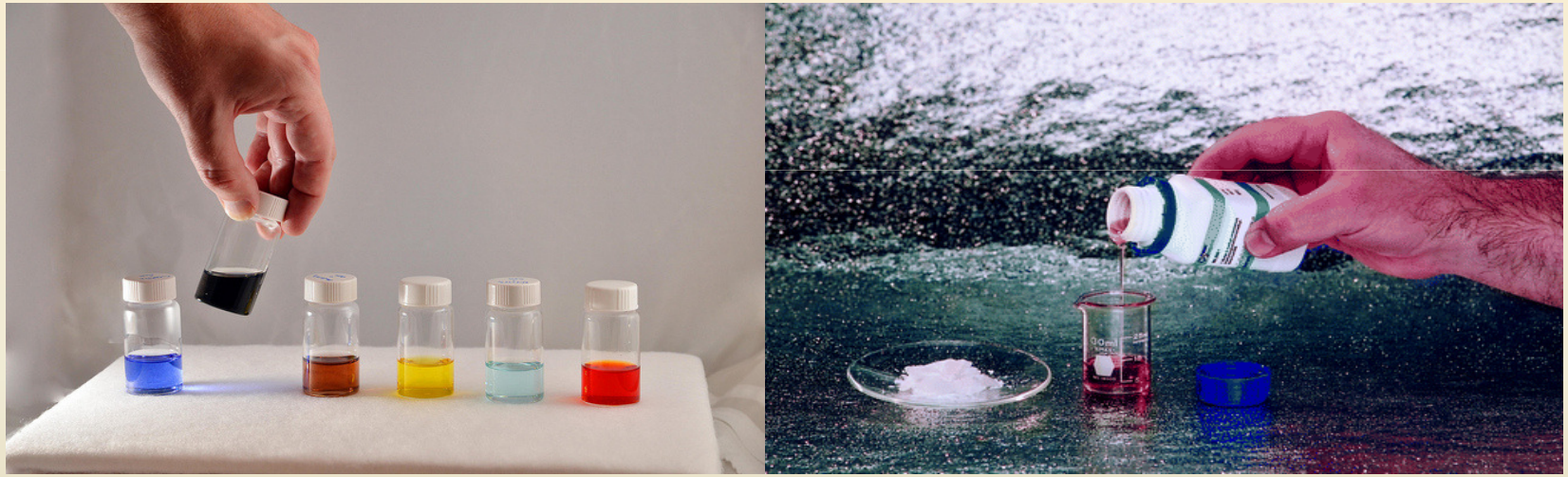
Quirino, J. P., Electrophoresis 2009, 30, 875–882

What are Ionic Liquids?



Definition of ILs

- The term “ionic liquid” (IL) is currently used to describe a broad class of salts which have appreciable liquid ranges.(ILs melt at or below 100 ◦C)



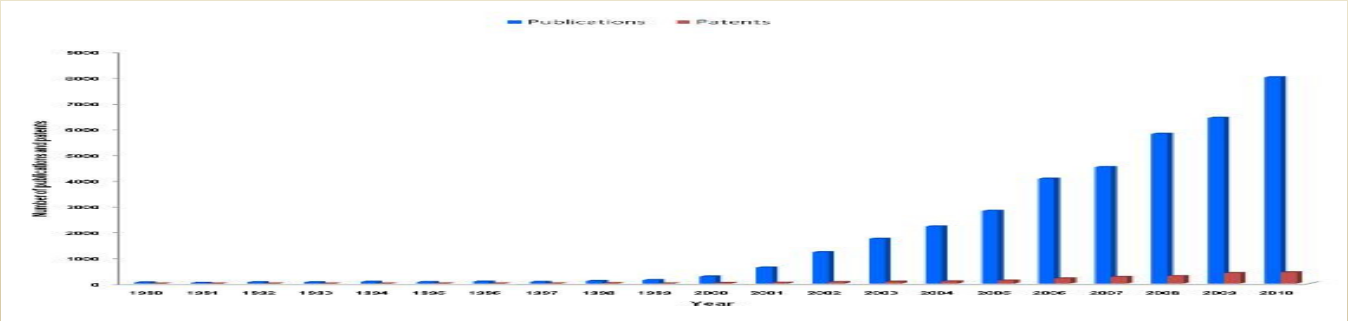
- Room temperature Ionic liquids (RTIL) are salts which are already liquid below room temperature

Armstrong et al. Annu. Rev. Anal. Chem. 2, 145–168, 2009

History

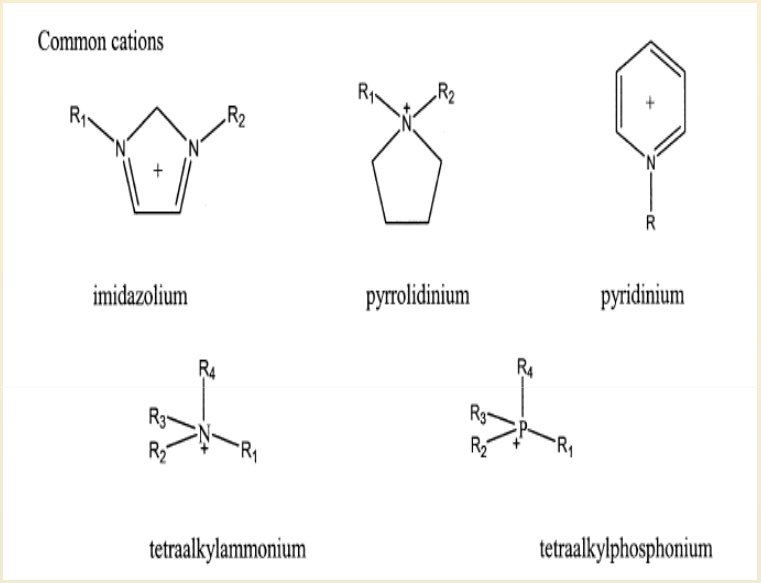
- 1914 The “first” RTIL ethylammonium nitrate [EtNH3][NO3] with melting point12 ◦C
- 1982 reported by Wilkes , A new class of RTILs that consist of dialkylimidazolium chloroaluminate
- 1992 Development of air- and water-stable imidazolium based ILs by Wilkes et al.

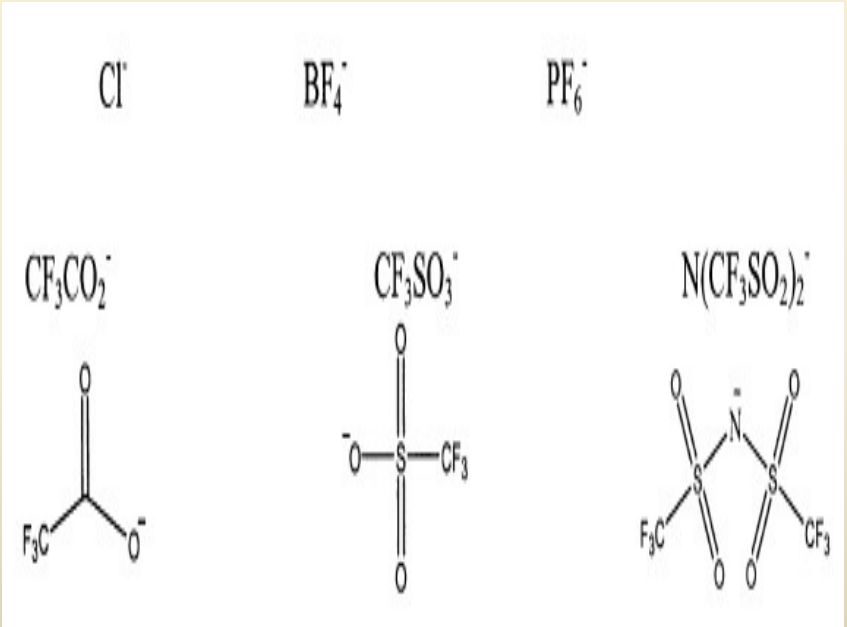
❖The number of publications based on ionic liquids raised from 14 in 1990 to over 8000 in 2010



Principle of ionic liquid

❖ Most RTILs have organic cations.





❖Anions could be inorganic, and more and more current RTILs consist of organic anions.

Armstrong et al. Anal. Chem. Acta 661, 1-47, 2010

Advantages of IL

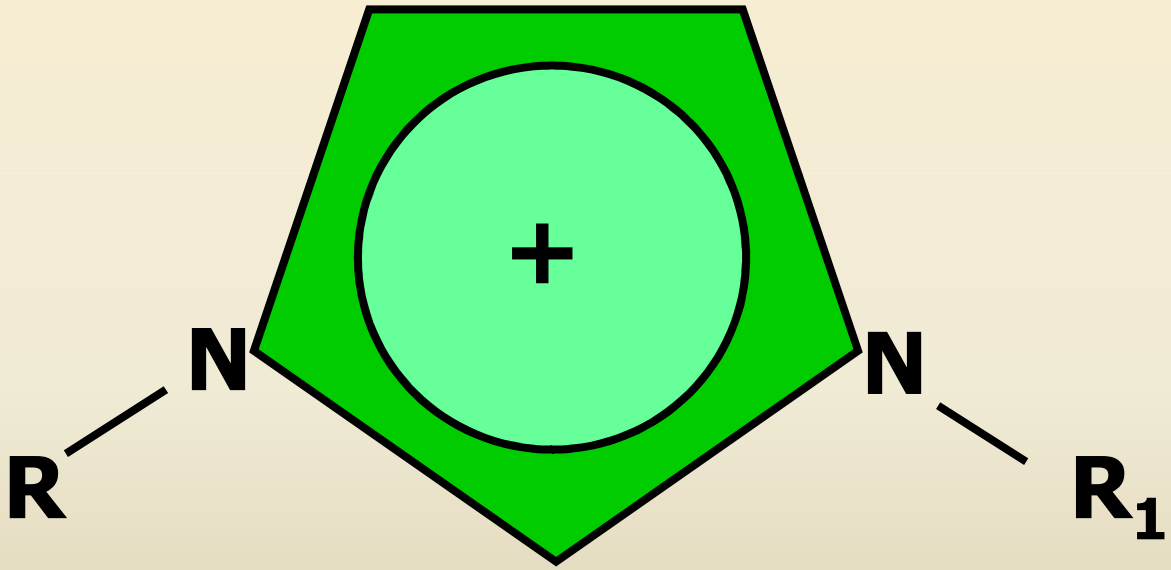
- wide liquid ranges
- wide range of viscosities
- Very low vapor pressure
- Non-flammable substance
- good thermal stabilities
- High mechanically stable
- electrolytic conductivity
- Low toxicity
- Non-volatility
- adjustable miscibility



P.Wasserscheid & T.Welton, **Ionic Liquids in Synthesis**, **Volume 1**, 2008 WILEY-VCH

Short Chain imidazolium IL

1-alkyl-3-methylimidazolium is the most common cation



With R = C_2 to $C_{8.}$, R_1 = methyl

Most advantages of short chain imidazolium IL

- Different types of interactions by electrostatic, hydrophobic, hydrogen bonding and bi–bi interactions
- Cheap price
- Simple syntheses have made them the first choice

Therefore, the aim of the current work is to develop new sweeping-MEKC and AFMC methods using BMIMBr as short chain alkyl imidazolium IL micelles. Both sweeping and stacking modes were compared to emphasize their effectiveness for the simultaneous determination of trace levels of methotrexate (MTX), Folinic acid (FNA) and folic acid (FA) in human plasma and urine samples.

Deia et al. Electrophoresis, 35, 1956–1964, 2014

Operating conditions

- Bare fused-silica capillaries with 50 microm ID, 8.5 cm outlet and 56 cm effective length.

- The analytes were hydrodynamically injected at 75.0 mbar for 150.0 s (in case of sweeping-MEKC) or 25.0 mbar for 10.0 s (in case of AFMC).

- BGE: 67.0 mmol/L phosphate buffer at pH 7.4 (in case of AFMC) or contained 3.0 mol/L BMIMBr (in case of sweeping-MEKC).

- The temperature of the capillary was kept at 30°C.

Deia et al. Electrophoresis, 35, 1956–1964, 2014

Operating conditions

- Voltage of 20.0 kV with positive polarity.
- The wavelength selected for the electropherograms was 250 nm.
- Rinsing procedure: At the beginning of each day, the capillary was rinsed with 0.1 mol/L hydrochloric acid (2.0 min) and water (5.0 min).

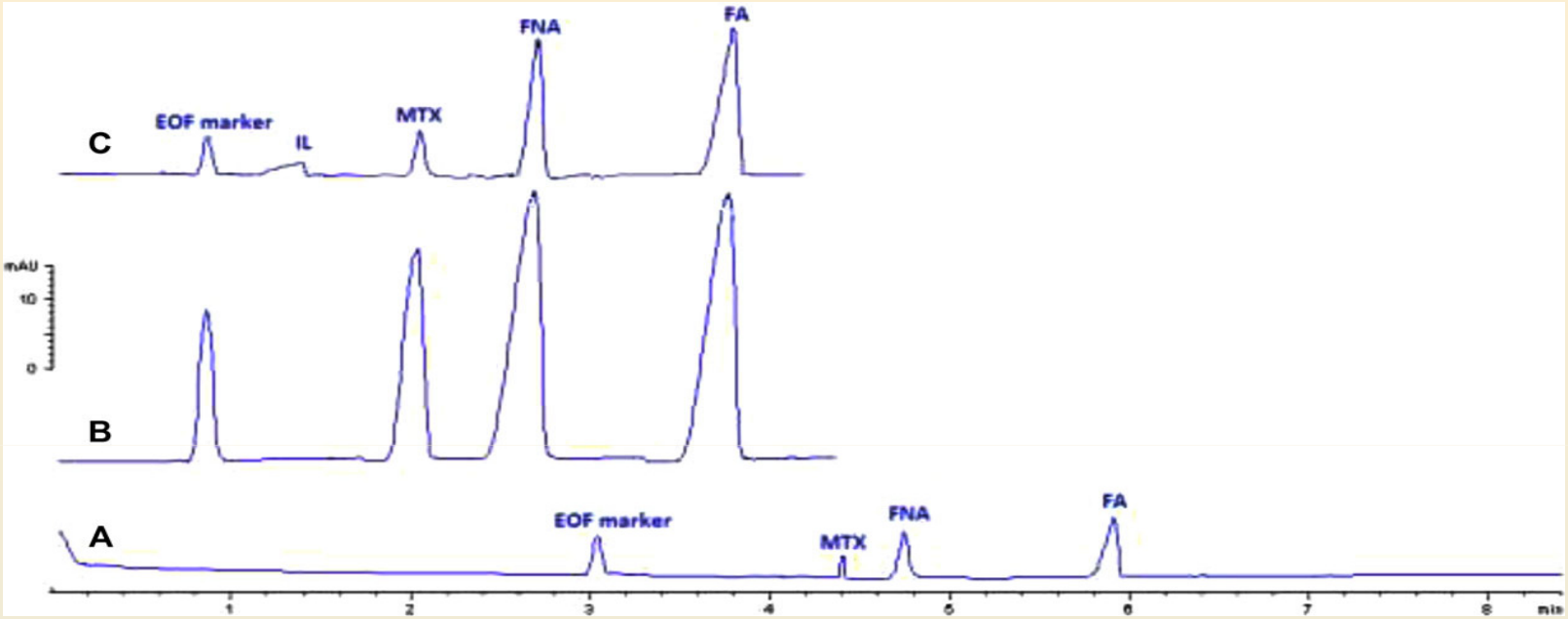
  Between runs, the capillary was rinsed with 0.1 mol/L hydrochloric acid (60.0 s) followed by water (30.0 s) and running buffer (60.0 s).

  At the end of each day, the capillary is washed with 0.1 mol/L hydrochloric acid (5.0 min) and water (10.0 min).

  All washing steps were performed by applying 940.0 mbar of pressure.

Deia et al. Electrophoresis, 35, 1956–1964, 2014

Results



A comparison between (A) CZE, (B) sweeping-MEKC and (C) AFMC under the following experimental conditions: Injected sample: mixture of MTX (4.0 ng/mL), FNA (6.0 ng/mL) and FA (4.5 ng/mL) in phosphate buffer by CZE and sweeping-MEKC or in 3.0 mol/L BMIMBr by AFMC

Deia et al. Electrophoresis, 35, 1956–1964, 2014

Results

Table 1. Calibration graphs: $Y = bX + a$ with concentration (Y, ng/mL), CPA (X), slope (b) and intercept (a), $n = 7$, LODs and LOQs of MTX, FNA and FA using sweeping-MEKC

Analyte	Concentration range(ng/mL)	b	a	$r^{2a)}$	LOD (ng/mL)	LOQ (ng/mL)
Methotrexate (MTX)	0.1–50.0	0.07	2.61	0.998	0.03	0.10
Folinic acid (FNA)	0.05–50.0	0.10	2.69	0.995	0.02	0.05
Folic acid (FA)	0.05–50.0	0.03	2.39	0.996	0.02	0.05

a) Coefficient of determination.

Table 2. Calibration graphs: $Y = bX + a$ with concentration (Y, ng/mL), CPA (X), slope (b) and intercept (a), $n = 7$, LODs and LOQs of MTX, FNA and FA using AFMC

Analyte	Concentration range (ng/mL)	b	a	$r^{2a)}$	LOD (ng/mL)	LOQ (ng/mL)
Methotrexate (MTX)	0.5–100.0	0.11	2.71	0.995	0.20	0.50
Folinic acid (FNA)	0.3–100.0	0.25	1.93	0.996	0.09	0.30
Folic acid (FA)	0.3–100.0	0.15	1.32	0.997	0.09	0.30

a) Coefficient of determination.

Deia et al. Electrophoresis, 35, 1956–1964, 2014

Applications

Table 3. Replicate analysis and accuracy of MTX, FNA and FA in human plasma and urine samples by sweeping-MEKC method

Sample	Analyte	Spiked concentration (ng/mL)	Found concentration (ng/mL)	Intra-day precision, RSD of CPA (%, $n = 10$)	Inter-day precision, RSD of CPA (%, $n = 24$)	Recovery (%)
Plasma	MTX	1.0; 20.0; 40.0	0.9; 20.5; 40.3	0.5; 0.3; 0.1	1.2; 0.9; 0.8	90.0; 102.5; 100.8
	FNA	0.5; 15.0; 30.0	0.5; 14.8; 30.1	0.6; 0.4; 0.1	1.8; 1.3; 0.9	100.0; 98.7; 100.3
	FA	0.5; 15.0; 30.0	0.5; 15.1; 30.1	0.6; 0.3; 0.2	1.4; 0.8; 0.9	100.0; 100.7; 100.3
Urine	MTX	1.0; 20.0; 40.0	1.0; 20.1; 40.0	0.4; 0.2; 0.2	2.4; 1.5; 1.1	100.0; 100.5; 100.0
	FNA	0.5; 15.0; 30.0	0.5; 14.9; 30.0	0.4; 0.3; 0.2	1.9; 1.2; 0.7	100.0; 99.3; 100.0
	FA	0.5; 15.0; 30.0	0.5; 15.1; 30.0	0.5; 0.4; 0.1	2.1; 1.6; 1.2	100.0; 100.7; 100.0

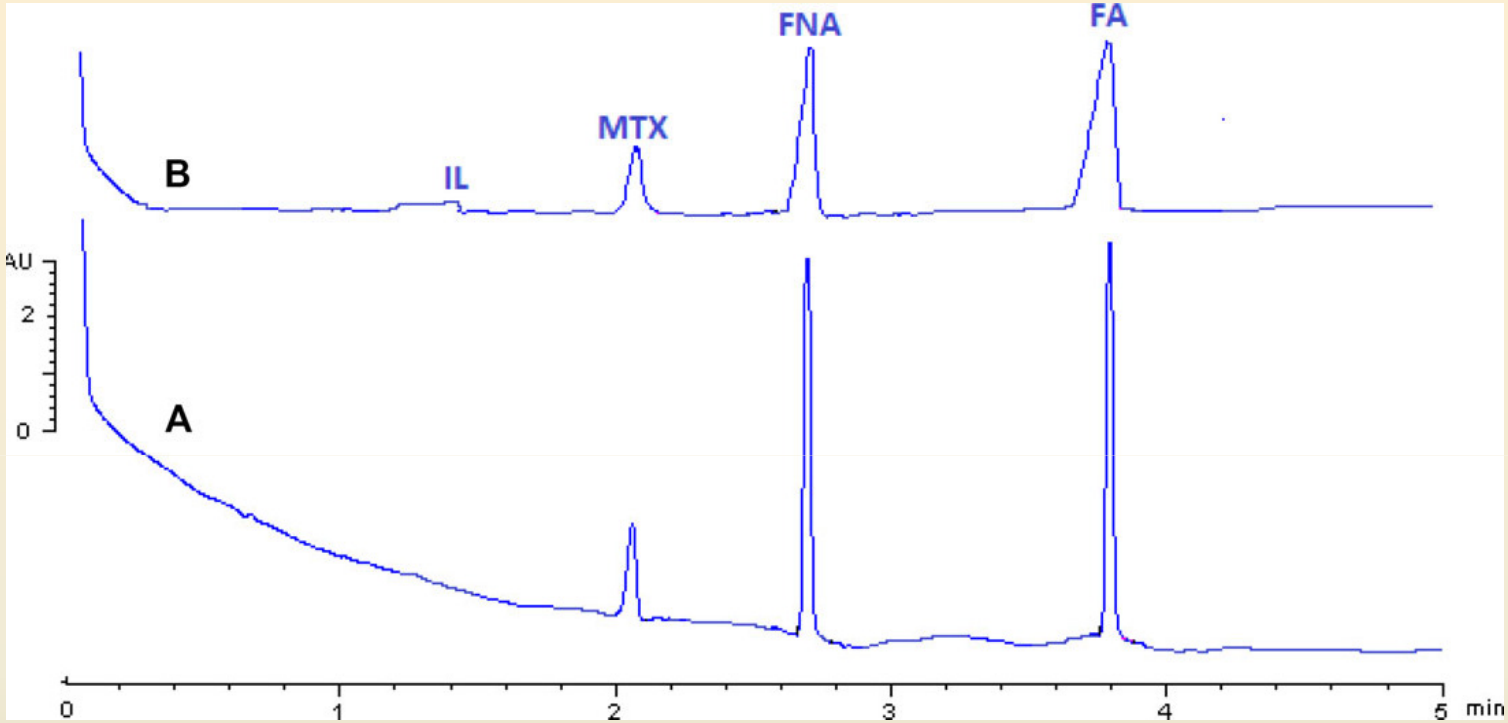
Table 4. Replicate analysis and accuracy of MTX, FNA and FA in human plasma and urine samples by AFMC method

Sample	Analyte	Spiked concentration (ng/mL)	Found concentration (ng/mL)	Intra-day precision, RSD of CPA (%, $n = 10$)	Inter-day precision, RSD of CPA (%, $n = 24$)	Recovery (%)
Plasma	MTX	10.0; 40.0; 80.0	9.8; 40.5; 80.2	0.7; 0.4; 0.2	2.2; 1.9; 0.8	98.0; 101.3; 100.3
	FNA	5.0; 30.0; 60.0	5.3; 30.4; 60.1	0.6; 0.2; 0.3	3.5; 2.3; 1.9	106.0; 101.3; 100.2
	FA	5.0; 30.0; 60.0	4.7; 30.1; 60.0	0.9; 0.6; 0.2	2.4; 1.8; 0.9	94.0; 100.3; 100.0
Urine	MTX	10.0; 40.0; 80.0	9.6; 40.5; 80.2	0.4; 0.2; 0.2	3.4; 2.2; 1.1	96.0; 101.3; 100.3
	FNA	5.0; 30.0; 60.0	5.5; 30.6; 60.0	0.4; 0.3; 0.2	1.7; 1.5; 0.6	110.0; 102.0; 100.0
	FA	5.0; 30.0; 60.0	5.2; 30.1; 59.8	0.5; 0.4; 0.1	2.5; 1.3; 1.5	104.0; 100.3; 99.6

PROOFS

Deia et al. Electrophoresis, 35, 1956–1964, 2014

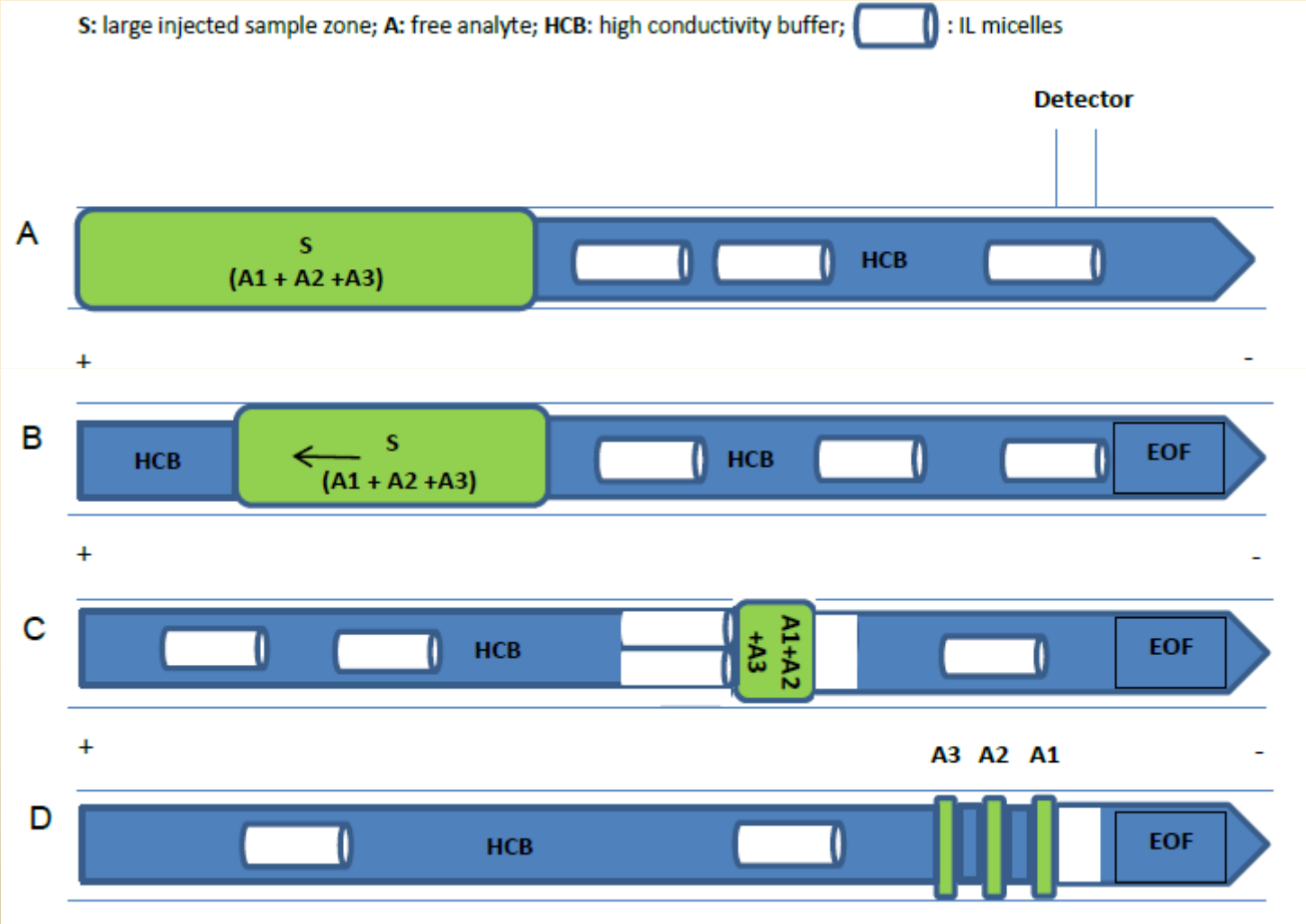
Applications



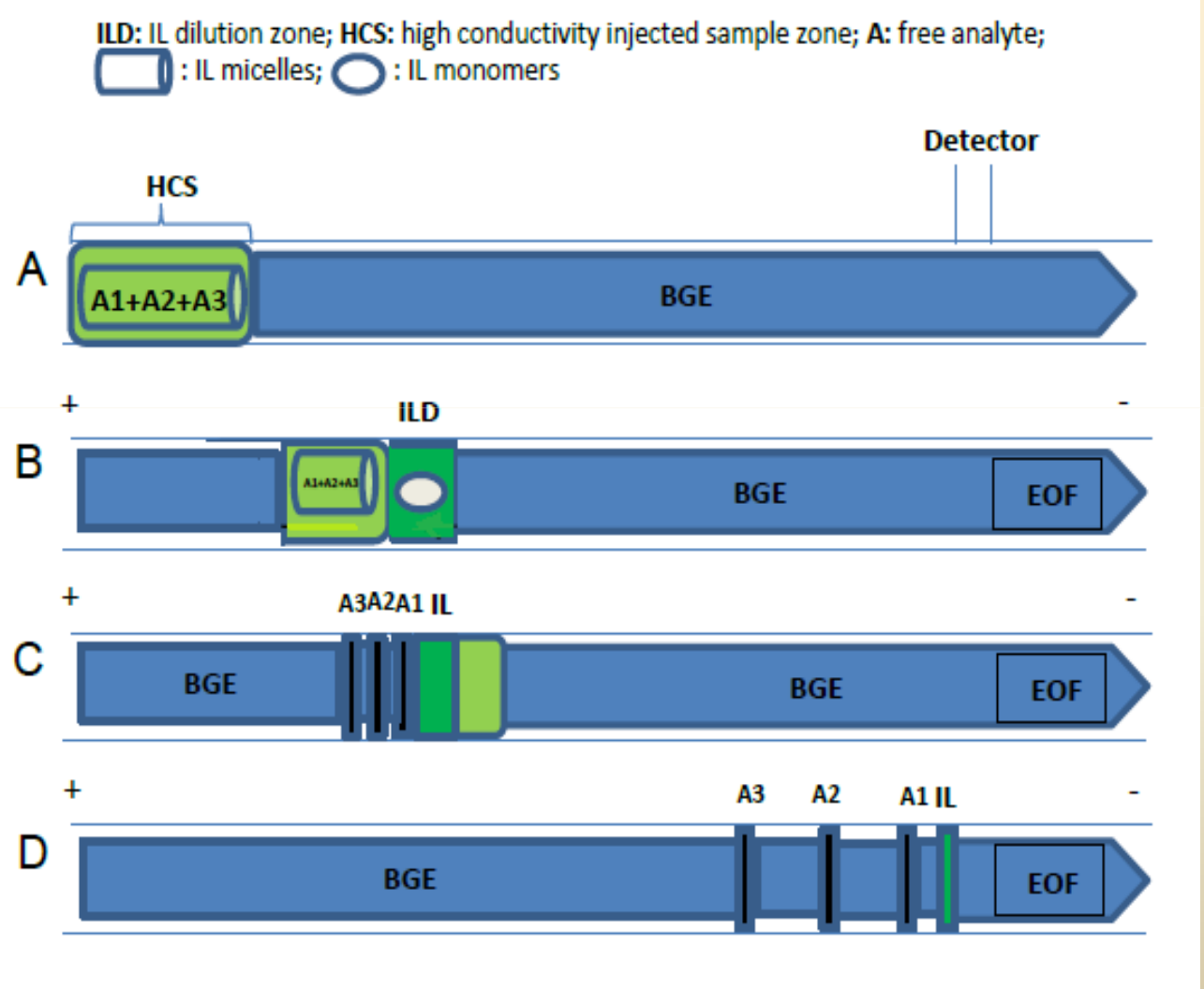
Electropherograms of analysis of MTX (0.6 ng/mL), FNA (1.3 ng/mL) and FA (1.0 ng/mL) in a urine sample by sweeping-MEKC (A) and AFMC (B).

Deia et al. Electrophoresis, 35, 1956–1964, 2014

Separation by IL based sweeping-MEKC



Separation by IL based AFMC



Conclusion

- The fascinating properties of the short chain BMIMBr ionic liquid regarding its high conductivity, high availability, easy preparation and formation of micelles with versatile possibility of interactions add significantly improved possibilities for on-line stacking and sweeping.

- The conductivity ratio (gamma, BGE/S) was optimized to be 3 in sweeping-MEKC and 0.33 in AFMC with a run time of 4.0 min for analyses.

Deia et al. Electrophoresis, 35, 1956–1964, 2014

Conclusion

- The rinsing procedure using hydrochloric acid significantly reduced the possibility of BMIMBr adsorption on the interior surface of capillary.

- the Sensitivity enhancement factor (SEFs) achieved by sweeping-MEKC is about 6–22 and 2–5 fold greater than CZE and AFMC, respectively. Due to the possibility to inject a high volume (approximately 3 × 10(−4) mL) of sample as well as the improvement of *k* and gamma factors.

Deia et al. Electrophoresis, 35, 1956–1964, 2014

Publication

Electrophoresis 2014, *35*, 1956–1964

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Research Article

Use of short chain alkyl imidazolium ionic liquids for on-line stacking and sweeping of methotrexate, flinic acid and folic acid: Their application to biological fluids

Methotrexate (MTX) is widely used for the treatment of many types of cancer. Folinic acid (FNA) and folic acid (FA) were usually simultaneously supplemented with MTX to reduce the side effects of a folate deficiency. This study, for the first time, included on-line sample preconcentration by stacking and sweeping techniques under reduced or enhanced electric conductivity in the sample region using short chain alkyl imidazolium ionic liquids (ILs) as micelle forming agents for analyte focusing. Both analyte focusing by micelle collapse (AFMC) and sweeping-MEKC had been investigated for the comparison of

Acknowledgment

We are greatly thankful for the financial support of our work by the funding of King AbdulAziz University, Jeddah, Saudi Arabia.









Thank You