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1-Dodecyl-3-methyl-imidazolium Based Ionic Liquid as Additive for Electrophoretic Separation of Fluoroquinolones

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In present studies, the influences of 1-dodecyl-3-methyl-imidazolium (C_{12}MIM)-based ionic liquid on electrophoretic separation of zwitter ionic fluoroquinolones are presented. The mobilities of electroosmotic flow and fluoroquinolones increased with buffer concentration in the presence of the ionic liquid and variation in separation voltage also influenced the separation. Presence of 0.3 mmol L^{-1} C_{12}MIM-OH in the buffer of 20 mmol L^{-1} tetraborate /10 mmol L^{-1} phosphate significantly improved the resolution of the fluoroquinolones without detectable loss in the UV detection sensitivity. The model fluoroquinolones were separated in 14 min with detection limits of 0.1-0.14 mg L^{-1}. Present experiments suggest that C_{12}MIM-based ionic liquid at sub-millimolar is an effective additive for capillary electrophoresis separation of fluoroquinolones.

Key Words: Capillary electrophoresis, Ionic liquid, Fluoroquinolone, Additive.

INTRODUCTION

Ionic liquids (ILs), a family of materials with melting points at or close to room temperature, possess unique physico-chemical properties of broad liquid range, low to negligible vapour pressures and high thermal stability[1-3]. Their applicabilities as stationary phase in gas chromatography[3-7], stationary phase[8] or modifiers of mobile phase[9-12] in liquid chromatography have been investigated. Recently, ionic liquids were employed in capillary electrophoresis (CE) as additives[13-18], background electrolytes (BGE)[19-21] or pseudo-stationary phases (PSPs)[22,23]. It was found that resolution of flavonoids in capillary electrophoresis was improved by adding 1-butyl-3-methylimidazolium tetrafluoroborate (BMIM-BF_4) to the buffer, mainly due to the hydrogen-bonding interaction of BMIM with the analytes[24]. Recently, Corradini *et al.*[13] reported fast and efficient separation of basic proteins with capillary electrophoresis by employing the 1-alkyl-3-methylimidazolium based ionic liquids as both coatings and additives. The work of Valcarcel *et al.*[25] demonstrated the fast capillary electrophoresis separation of 18 single-walled carbon nanotubes by dissolving/dispersing the nanotubes in ionic liquid and subsequently encapsulating the nanotubes in sodium dodecyl sulfate micelles.

Fluoroquinolones (FQs) are antibiotics used in clinical medicine and food-producing animal husbandry for a variety of infections because of their excellent activity against both pathogenic Gram-negative and Gram-positive bacteria[26,27]. However, the fluoroquinolone residues in animal tissue are toxic to human being upon consumption, potentially causing pathogen resistance and allergic hypersensitivities[28-30]. To ensure human food safety, the European Union (EU) regulates the use of veterinary drugs through Council Regulation No. 2377/90[31], which was amended later by Commission Regulation 1353/2007/EC[32]. Development and improvement of analytical methods for this class of antibiotics are of increasing interest because the vastly different substituents of fluoroquinolone structures greatly affect their individual characteristics[33]. In this context, effective separation of quinolones is an important phase in determination.

The interactions between ionic liquid-cation and the analytes include electrostatic attraction, hydrophobic interaction and hydrogen bonding[34-36]. These interactions exist between ionic liquid-cations and fluoroquinolones because the latter are amphoteric. Contrast to the large amount of works employing ionic liquids in separation of anionic solutes, however, few works have been devoted to the ionic liquid-assisted capillary electrophoresis separation of zwitter ions[13]. When employing ionic liquid as additives in capillary electrophoresis, one should keep in mind that imidazolium cation has strong UV absorbance over 200-230 nm and therefore it might influence the photometric detection. High concentration of short-chain substituted imidazolium cations had to be added to the buffer in order for the strong ionic liquid-analyte interaction required

for the high performance separation[13,18]. But this would severely affect the detection sensitivity[37] and dynamic range[22]. Imidazolium with long alkyl chain is expected to promote the hydrophobic interaction with fluoroquinolone molecules and its concentration needed to achieve the comparable ionic liquid-analyte interaction may be significantly lowered, consequently their influence on detection can be alleviated to a great degree.

The aim of this report is to study the influence of an imidazolium (1-dodecyl-3-methyl-imidazolium, C_{12}MIM)-based ionic liquid on the separation and detection of fluoroquinolones by capillary electrophoresis-UV. The influences of ionic liquid concentration, buffer concentration and pH and separation voltage were studied by employing four fluoroquinolones, *viz.*, gatifloxacin, lomefloxacin, rufloxacin and pazufloxacin, as model analytes (chemical structures shown in Fig. 1).

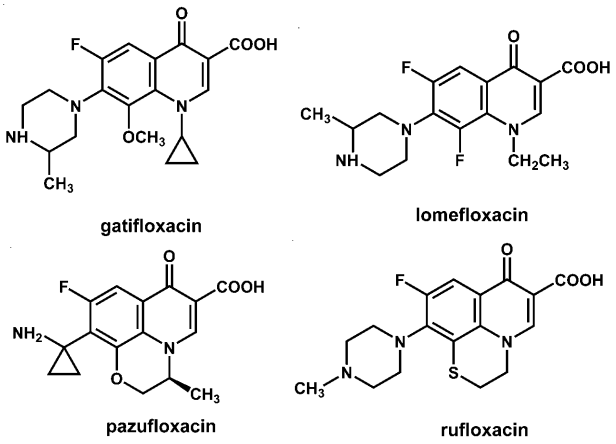

Fig. 1. Structures of the fluoroquinolones studied

EXPERIMENTAL

Gatifloxacin (GAT), lomefloxacin (LOM), rufloxacin (RUF) and pazufloxacin (PAZ) were purchased from the Medicinal and Biological Research Institute (Beijing, China). 1-Methyl-imidazole was product of Sigma (St. Louis, MO, USA). Sodium hydroxide, 1-bromododecane, sodium dihydrogen phosphate, silver oxide and sodium tetraborate were purchased from the Beijing Chemical Plant (Beijing, China). Deionized water (Millipore, Bedford, MA, USA) was used throughout the experiment for preparing solutions.

The 1000 mg L^{-1} individual stock solutions of the fluoroquinolones were prepared in 6 mmol L^{-1} sodium hydroxide and stored under refrigeration. Working standards were prepared by proper dilution of the stock solutions with deionized water.

Analysis was carried out on a capillary electrophoresis system containing a high-voltage power supply (DW-P303-1AC, Sanchuan High Tech, China), a UV detector (operated at 250 nm, capillary electrophoresis-10UV, Johnsson Separation Science, Liaoning, China) and a data acquisition and processing unit (HW2000 chromatography station, Qianpu, Jiangsu, China). A fresh fused-silica capillary of 50 cm (40 cm in effective length) × 75 μm I.D. (Yongnian Photoconductive Fiber Factory, Hebei, China) was employed for separation. It was treated by consecutive rinsing for 0.5 h with 1 M NaOH, 10 min with double-distilled water and 5 min with separation buffer. All electrolyte solutions were passed through a 0.22 μm membrane filter (Jiuding High Tech, Beijing, China) before use. Between two consecutive runs, the capillary was flushed for 2 min with background electrolytes. The NMR spectra were obtained on an Avance DRX-500 NMR spectrometer (Bruker, Fallanden, Switzerland).

Synthesis, characterization and conversion of 1-dodecyl-3-methyl-imidazolium based ionic liquids: The ionic liquid surfactant 1-dodecyl-3-methyl-imidazolium bromide (C_{12}MIM-Br) was synthesized according to the standard method[12]. In brief, equal amounts (0.2 mol) of 1-bromododecane and 1-methylimidazole were added to a 250 mL round-bottom flask fitted with reflux condenser. After reaction at 80 °C for 24 h, the brown viscous liquid was transferred to a separating funnel and washed three times with 50 mL portions of ethyl acetate. The washed ionic liquid was heated under vacuum at 70 °C to remove the organic solvent. The yield was about 65 %. The structure and the purity of the resulting surfactant were confirmed by ^{1}H NMR spectroscopy (500 MHz, DMSO): δ/ppm (relative to TMS) = 9.307 (s, 1H), 7.845 (dd, 1H), 7.776 (dd, 1H), 4.184 (t, 2H, J = 7.2 Hz), 3.877 (s, 3H), 1.227 (m, 20H), 0.842 (t, 3H, J = 7.0 Hz). Bromide is highly conductive and UV-active (190-220 nm) in aqueous solution. In order to decrease Joule heating during capillary electrophoresis as well as to eliminate the potential interference of bromide on the UV detection of quinolones, C_{12}MIM-Br was converted to C_{12}MIM-OH by means of metathesis reaction. Under vigorous stirring, 4-fold molar excess of silver oxide powder was gradually added to 100 mL of 100 mmol L^{-1} aqueous solution of C_{12}MIMBr. The precipitates of silver bromide and the excess silver oxide were removed by centrifugation. The upper solution was filtered with 0.22 μm membrane filters (Jiuding High Tech) before use.

Calculation of asymmetric factor and peak capacity: The asymmetric factor (As) was defined as the width ratio of the right part of the peak to the left at 10 % of the peak height. The efficiency (N, theoretical plate number) was estimated by $N = 5.54\ (t/W_{0.5})^2$, where, t is the migration time and $W_{0.5}$ is the peak width at half-height. Peak capacity was defined as the maximum number (n) of peaks that can be resolved between the first and the last peaks. It was calculated by the relation $n = 1 + (N^{1/2}/4)\ln(t_{pazufloxacin}/t_{electroosmotic\ flow})$, where N is the theoretical plate number, $t_{pazufloxacin}$ and $t_{electroosmotic\ flow}$ are migration times of pazufloxacin and electroosmotic flow, respectively.

RESULTS AND DISCUSSION

Influence of ionic liquid: Increased migration time while improved resolution of the fluoroquinolones were observed with addition of ionic liquids to the buffer. The merged peaks of gatifloxacin and lomefloxacin with buffer devoid of ionic liquid (trace A of Fig. 2A) were separated in the presence of 0.3 mmol L^{-1} ionic liquid (Trace D). The electropherograms suggested the feasibility of manipulating resolution of fluoroquinolones by adding ionic liquids into the running buffer. Variation of the neighboring-peak mobility differences

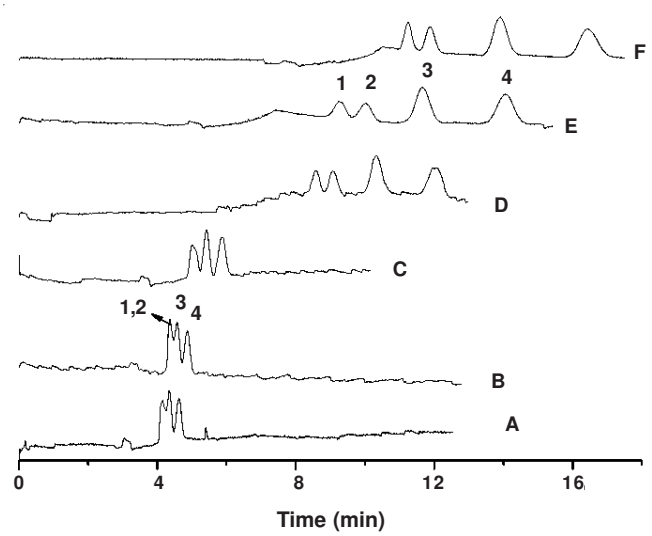

Fig. 2A. Influence of ionic liquid concentration; The buffer: 20 mmol L^{-1} sodium tetraborate and 10 mmol L^{-1} sodium dihydrogen phosphate, which was added C_{12}MIM-OH to concentrations (in mmol L^{-1}) of: A, 0; B, 0.1; C, 0.2; D, 0.3; E, 0.4; F, 0.5. The capillary: 75 μm i.d., with 50 cm in total and 40 cm in effective lengths. Applied voltage: 15 kV; the detection wavelength: 250 nm. Injection: fluoroquinolone mixture of 5 mg L^{-1} each was siphoned into the capillary by 30 cm × 20 s. Peak identities: 1, gatifloxacin (GAT); 2, lomefloxacin (LOM); 3, rufloxacin (RUF); 4, pazufloxacin (PAZ)

versus the ionic liquid concentration follows the order of gatifloxacin/lomefloxacin > gatifloxacin/rufloxacin > rufloxacin/pazufloxacin (Fig. 2B). The charge-to-size ratios of the analytes in the buffer without ionic liquid, as inferred from the trace A of Fig. 2A, are in the order of gatifloxacin = lomefloxacin < rufloxacin < pazufloxacin. It is presumed that the hydrophobic interaction and conjugation may be the dominant factors accountable for the mobility change. Nevertheless, the complementary electrostatic interactions between the fluoroquinolones and the ionic liquid-cation also exist as can be inferred from the increasing asymmetric factor of pazufloxacin, for example, with the ionic liquid concentration (Table-1), which is the consequence of increasing electrostatically-driven adsorption of pazufloxacin to the wall-immobilized ionic liquid-cation[38]. The enhanced peak capacity with addition of ionic liquids (Table-1) facilitates high performance separation. Due to the different maximal absorbance wavelengths of fluoroquinolones and $C_{12}MIM^+$ and to the very low concentration of ionic liquid added, the detection sensitivity of the fluoroquinolones virtually did not change in the presence of ionic liquids, as depicted in Fig. 2A.

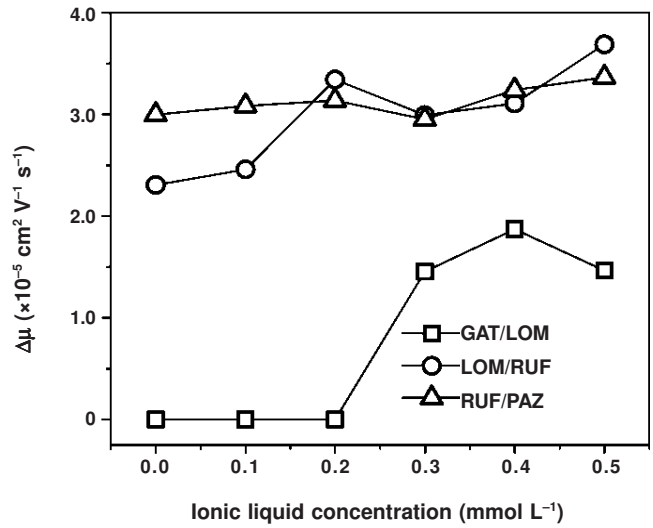

Fig. 2B. Variation of mobility difference of neighboring peaks as a function of ionic liquid concentrations; The experimental conditions are the same as Fig. 2A

TABLE-1
ASYMMETRIC FACTOR AND PEAK CAPACITY UNDER DIFFERENT CONCENTRATIONS OF IONIC LIQUIDS

Ionic liquid concentration (mM)	0	0.30	0.50
Asymmetric factor of pazufloxacin	0.90	0.95	1.14
n	8.00	8.60	12.50

Effect of buffer pH: Variation in buffer pH will affect the surface charge density of the zwitter ionic fluoroquinolones, therefore bringing about changes of the fluoroquinolone mobilities and the resolution as well. The positively charged fluoroquinolones did not reach the detection window in 25 min at pH 2.1 (Fig. 3) due to the reversed electroosmotic flow. The analysis time with buffer at pH 6.8 is short, suggesting the co-electroosmotic flow migration of the fluoroquinolones. However, the analytes were not resolved due to their similar charge-to-size ratios. At pH 8.6, the fluoroquinolones were well resolved because the pH value was close to but slightly higher than their pIs[39,40]. Further increase of buffer alkalinity resulted in shorter analysis time; however, the resolutions among the fluoroquinolone peaks were deteriorated. Taking into account the resolution, we used buffers at pH 8.6 for further studies.

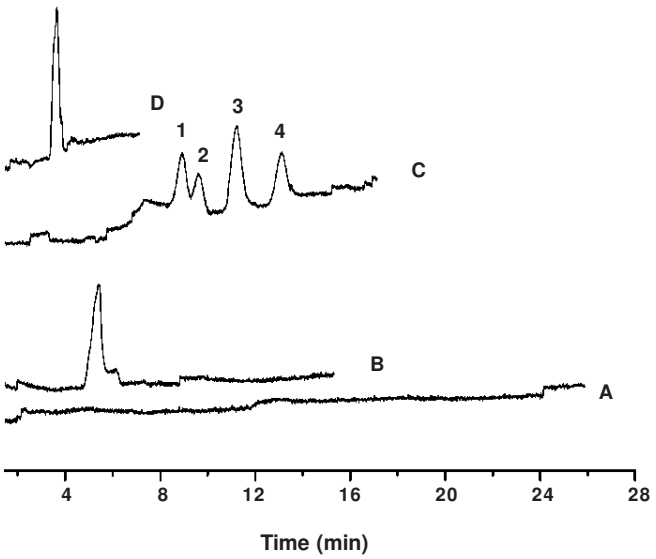

Fig. 3. Influence of pH; C_{12}MIM-OH was added to 0.2 mmol L^{-1} to the buffers of: 17 mmol L^{-1} phosphoric acid at pH 2.1 (A); 13 mmol L^{-1} sodium dihydrogen phosphate and 2 mmol L^{-1} sodium tetraborate at pH 6.8 (B); 13 mmol L^{-1} sodium tetraborate and 7 mmol L^{-1} sodium dihydrogen phosphate at pH 8.6 (C); 13 mmol L^{-1} sodium hydrogen phosphate and 6 mmol L^{-1} sodium hydroxide at pH 10.4 (D). Other conditions were the same as those in Fig. 2A

Influence of buffer concentration: Effects of buffer concentration was investigated at fixed concentration ratio of 2:1 for tetraborate/phosphate at pH 8.6, in the presence of 0.2 mmol L^{-1} ionic liquid. No peak was detected in 25 min with buffer of 6 mmol L^{-1} (the sum of tetraborate and phosphate). Fluoroquinolones could be detected in the buffers beyond 12 mmol L^{-1} with decreasing analysis time, from more than 0.5 h at 12 mmol L^{-1} to less than 5 min at 45 mmol L^{-1} (figures are not shown). The phenomenon is interesting because high buffer concentration normally prolongs analysis time due to the increasing buffer viscosity and the decreasing double layer thickness of both the analytes and the capillary surface[41]. As illustrated in Fig. 4, the mobilities of fluoroquinolones increase with buffer concentration until reaching maximum at 36 mmol L^{-1}; whereas the electroosmotic flow mobility increases nearly linearly with buffer concentration. In buffer of low ionic strength, more ionic liquid-cations were immobilized at the silica surface driven by the electrostatic attraction, reducing the cathodic electroosmotic flow and retarding migration of the fluoroquinolone anions. The high ionic strength buffer not only suppressed the wall-adsorption of ionic liquid-cations but also enhanced mobilities of fluoroquinolones through because the buffer co-ions such as phosphate can interact with quinolones[42]. Unfortunately, such interaction mask the resolvability of the ionic liquid additives, the resolution between gatifloxacin and lomefloxacin deteriorate with the increasing buffer concentration (Fig. 4).

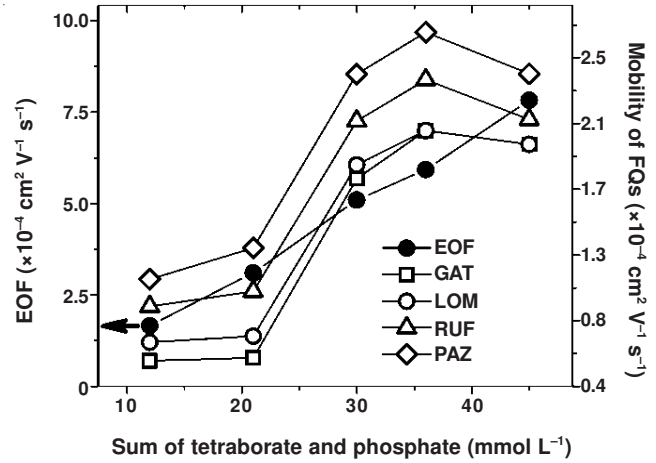

Fig. 4. Buffer-concentration dependence of the mobilities of fluoroquinolones and electroosmotic flow (EOF); The concentration ratios of sodium tetraborate to sodium dihydrogen phosphate in the buffers were kept at 2:1. The sum of concentrations of tetraborate and phosphate were depicted in the figure. The concentration of ionic liquid was 0.2 mmol L^{-1}. Other conditions were the same as those in Fig. 2A.

Influence of separation voltage: The mobilities of electroosmotic flow and the fluoroquinolones in the buffer void of ionic liquid were almost unchanged as a function of the applied voltage (Fig. 5A), obviously different from the increasing trends in the presence of 0.3 mmol L^{-1} C_{12}MIM-OH (Fig. 5B). The negative charge density of the capillary surface increased in the electric field because part of the ionic liquid -cations desorbed from the wall and migrated to the cathode, both were accelerated by the increasing field strength. Similarly, the voltage weaken the quinolones-$C_{12}MIM^+$ interaction and improve the anodic mobility of fluoroquinolones. High separation voltage leads to the enhanced resolution between rufloxacin and pazufloxacin, but the resolution of gatifloxacin/rufloxacin is reduced (Fig. 5B). The results reveal that the applied voltage also possesses selectivity towards the analytes by affecting the background electrolytes and ionic liquid-fluoroquinolone interactions.

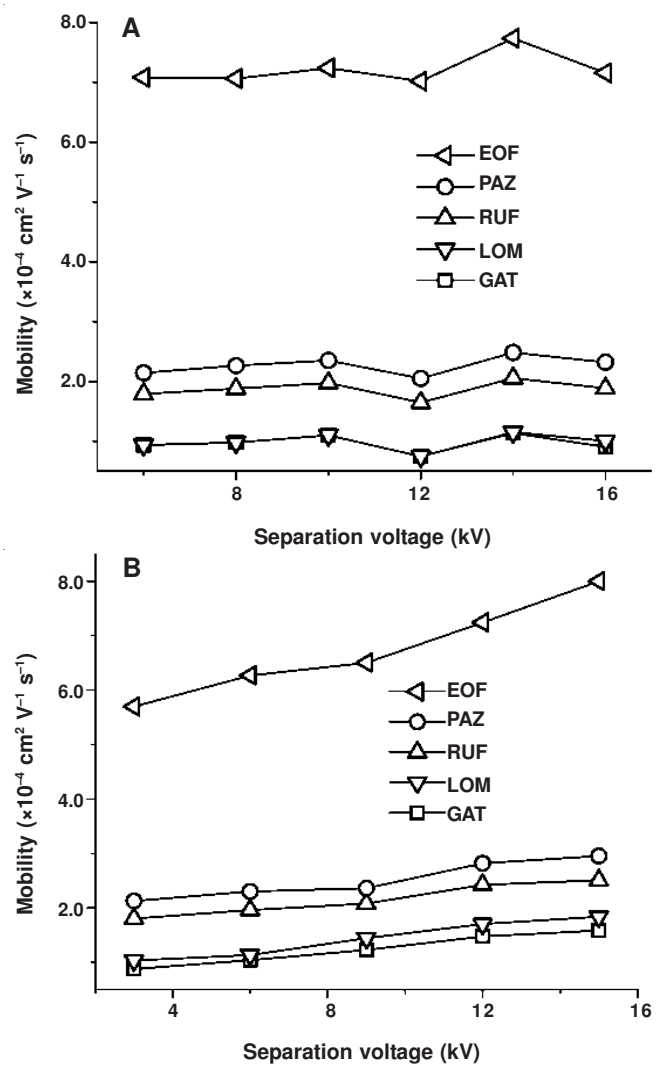

Fig. 5. Influence of separation voltage; The buffer: 20 mmol L^{-1} tetraborate/10 mmol L^{-1} phosphate A), was added to 0.3 mmol L^{-1} C_{12}MIM-OH B). Other conditions are the same as that in Fig. 3

Performances of the method: In the buffer of 20 mmol L^{-1} sodium tetraborate, 10 mmol L^{-1} sodium dihydrogen phosphate and 0.3 mmol L^{-1} C^{12}MIM-OH, all the four fluoroquinolones were baseline separated in 14 min by applying a separation voltage of 12 kV. The detection limits of the analytes, defined as S/N = 3, were 0.10, 0.10, 0.14 and 0.14 mg L^{-1} for gatifloxacin, gatifloxacin, rufloxacin and pazufloxacin, respectively.

Conclusion

We investigated the influence of 1-dodecyl-3-methyl-imidazolium-based ionic liquid on capillary electrophoresis separation of the zwitter ionic fluoroquinolones. The cathodic

electroosmotic flow velocity was reduced in presence of ionic liquid due to the dynamic coating of the silica wall by the C_{12}MIM-cations. However, it increased with buffer concentration or applied voltage. The electrostatic interaction and hydrophobic conjugation between the $C_{12}MIM^+$ and fluoroquinolones ultimately rendered baseline separation of the model analytes. In the presence of C_{12}MIM-based ionic liquid, applied voltage influences the migration of the fluoroquinolones, therefore might provides an additional approach to optimize separation. Present results suggest that the C_{12}MIM-based ionic liquid at sub-millimolar is an effective additive for capillary electrophoresis separation of fluoroquinolones without loss in detection sensitivity.

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