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Imidazolium-based ionic liquid-type surfactant as pseudostationary phase in micellar electrokinetic chromatography of highly hydrophilic urinary nucleosides

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ABSTRACT

Ionic liquid (IL)-type surfactants have been shown to interact more strongly with polar compounds than traditionally used quaternary ammonium cationic surfactants. The aim of this study is to provide an alternative micellar electrokinetic chromatographic method (MEKC) for the analysis of urinary nucleosides in their ionic form at low surfactant concentration. This approach could overcome the use of high surfactant concentrations typically associated with the analysis of these highly hydrophilic metabolites as neutral species, which is frequently accompanied by high electric current, Joule heating and long analysis time. The investigated IL-type surfactant; 1-tetradecyl-3-methylimidazolium bromide (C_{14}MImBr) is similar to the commonly employed cationic surfactant; tetradecyltrimethylammonium bromide (TTAB) but it provides a different separation selectivity. We employed C_{14}MImBr micelles for the MEKC analysis of seven urinary nucleosides. The studied analytes possess a negative charge at pH 9.38 (exceptions are adenosine and cytidine which are neutral at this pH value). Borate imparts an additional negative charge to these compounds after complexation with the cis-diol functionality of the ribose unit, which in turn enables them to interact with the oppositely charged C_{14}MImBr micelles via electrostatic (Coulomb) forces. The effect of the concentration of borate (the complexing, competing and buffering ion) on the effective electrophoretic mobilities and on the retention factors was investigated. The effective electrophoretic mobility data show that complexation between these nucleosides and borate occurs with high degree of complexation even at very low borate concentration (2.5 mmol L^{-1} disodium tetraborate). In addition, we found that the retention factors are strongly dependent on the borate concentration being the highest when using the lowest borate concentration and they can be regulated by variation of either tetraborate concentration or the pH of the background electrolyte using only 20 mmol L^{-1} C_{14}MImBr. We confirmed also that the main mode of interaction between these analytes and the C_{14}MImBr micelles is electrostatic interaction. Our experimental results reveal that the cationic surfactant C_{14}MImBr exhibits superior selectivity and higher reproducibility relative to that of TTAB, which makes this surfactant a promising cationic surfactant for the MEKC separation of other hydrophilic polar analytes.

1. Introduction

Ionic liquids (ILs) are known as molten salts with melting points below 100 °C. They are a class of ionic non-molecular solvents with a multitude of physicochemical properties. The most remarkable properties include their high thermal stability, a low vapour pressure, the miscibility with water and a variety of organic solvents, notable catalytic properties, as well as good extraction coefficients for various organic compounds [1]. They are environmentally benign and their non-flammability and low volatility allow them to gain an increasing interest in the field of green chemistry. The low symmetry and the relatively large size of the ions constituting the ILs lead to a lowering of the lattice energy, and hence the melting point of the resulting ionic liquid. ILs were a subject of many recent review articles, which highlight a variety of applications in many areas of separation science [1–4].

It was reported that ILs possessing long hydrophobic alkyl tails with cationic polar headgroups can form micelles when they are dissolved in water in a concentration above their CMC (critical micelle concentration). This property enables ILs to emerge as a new class of surfactants, especially because they possess the properties of cationic surfactants in addition to the restricted number of the classic cationic surfactants and the growing interest in applications that involve cationic surfactants. As recently pointed out in the review article of Pino et al. [1], it is more adequate to employ the term ionic liquid-based surfactants rather than IL surfactants as

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the cation/anion pair forming the ionic liquid does no longer constitute a true ionic liquid once the ionic liquid is dissolved in water or any organic solvent [1].

Dong et al. [5] and Vanyur et al. [6] reported that micelles are formed in an aqueous solution of long-chain ILs; namely 1-tetradecyl-3-methylimidazolium bromide (C_{14}MImBr) and 1-hexadecyl-3-methylimidazolium bromide (C_{16}MImBr). They found that imidazolium-based ILs are superior to the traditional cationic alkyltrimethyl ammonium bromides in their ability to form micelles, i.e. their CMC values are significantly lower than those of the classic cationic surfactants with comparable alkyl chain length. Alkylimidazolium-based ILs are the most widely used class of IL-type surfactants in capillary electrophoresis [3]. In addition to their role as background electrolyte (BGE) modifier in capillary electromigration separation techniques [7–10], few reports have described the MEKC utility of long-chain imidazolium-based ILs as pseudostationary phase (PSP) [11–13] in MEKC. Interest in IL-type surfactants stems from the fact that they offer a high versatility of interaction types (electrostatic, π–π, ion-dipole or hydrogen bonding interactions with the imidazolium cation head group and hydrophobic interaction due to the long alkyl tail) [14].

Metabolomics has acquired a special focus and increased popularity in life science over the last decade [15,16]. The change in the concentration of specific metabolites in urine reflects a certain physiological or pathological state in the human body, therefore the identification of metabolite profiles is one of the main goals of metabolomics [17]. Nucleosides are metabolites of either RNA's turnover or oxidative damage of DNA. Normal or unmodified nucleosides, e.g. uridine and guanosine, can be either reutilized to form nucleotide triphosphates or further degraded to form uric acid and β-alanine. Modified nucleosides, which mostly exist in the transfer RNA (tRNA) and are formed during posttranscriptional modification by numerous modification enzymes [18], cannot be reutilized or degraded, but are circulated unchanged in the blood stream and are excreted intact in the urine. Abnormal levels of modified nucleosides can indicate degradation of RNA. Therefore, urinary nucleosides, especially modified ones, can be used as a useful biologic marker of cancer. Such a marker reflects the presence of cancer or indicates changes in the tumour mass and can be useful in following up chemotherapy [19].

Nucleosides have been separated using different analytical techniques such as reversed phase high-performance liquid chromatography (RP-HPLC) [20–22], hydrophilic interaction chromatography (HILIC) (due to their highly hydrophilic nature) [23–25], immunoassay [26] and capillary electromigration separation techniques including capillary zone electrophoresis (CZE) [27], capillary electrochromatography (CEC) [28,29] and MEKC [17,21,30–32]. Capillary electromigration separation techniques for nucleoside analysis compared to HPLC offer high resolution and separation efficiency, short analysis time, low sample volume (nL), environmental compatibility, relatively low costs and suitability for the analysis of large series of urine samples which is highly desirable in the clinical laboratory [15]. Nucleoside analysis using capillary electromigration separation techniques is usually performed in two subsequent steps. The first step involves the extraction of the nucleosides from urine using phenylboronate affinity gel as solid phase extraction (SPE) stationary phase [19]. This extraction medium provides a unique means for the selective enrichment of cis-diol containing metabolites. It forms cyclic esters with the vicinal hydroxyl groups of the ribose unit of nucleosides under basic pH conditions. While maintaining the basic conditions, the analytes can be purified from interferences without any loss. When the pH is switched to acidic conditions, the analytes are eluted and after evaporating the extract to dryness, the extract is dissolved in water and analyzed further. The second step is the analysis step, which is commonly performed by MEKC with the nucleosides in their neutral form and to a lesser extent when they are charged by using either MEKC or CZE.

Terabe and co-workers [33] were the first who reported the MEKC analysis of nucleosides. They used a BGE composed of 200 mmol L^{-1} SDS, 25 mmol L^{-1} sodium tetraborate, 50 mmol L^{-1} sodium dihydrogen phosphate, pH 7 for the separation of 4 nucleosides and one deoxynucleoside. This buffer composition was widely adopted by many authors for the MEKC analysis of urinary nucleosides with slight modifications in the concentrations of SDS, phosphate and borate buffers [17,21,30–32]. The use of a high concentration of the PSP reflects the highly hydrophilic nature of the nucleosides which results in low retention factors. These high concentrations of SDS are typically associated with bubble formation, migration time irreproducibility, noisy baseline [34], high electric current and Joule heating. Working under low applied voltage (to avoid such problems) results in long analysis times [18,31]. Hence, the employment of a new pseudostationary phase that interacts more strongly with these polar anaytes and can be used at a lower concentration compared to the concentration needed for SDS is highly desirable.

Jiang and Ma [34] reported a fast MEKC method for the analysis of urinary nucleosides (as charged species) in which they used a borate-phosphate buffer, pH 9.5 containing 25 mmol L^{-1} cetyltrimethylammonium bromide (CTAB) as BGE. Unfortunately, there is no emphasis in this work about the role of borate complexation in enhancing the interaction between the nucleosides and the PSP. In addition, there is no explanation for the effect of the buffer ionic strength on the retention factor of these analytes and no clarification why most of the investigated analytes are eluted even before the EOF (electro-osmotic flow) marker, which according to our assumption may be attributed to the low interaction between the nucleosides and the CTAB micelles. This low interaction confirms the need for new surfactants that are capable of interacting more strongly with these highly hydrophilic analytes, which may enhance their resolution while maintaining a short run time. Poor reproducibility of EOF and migration times was also reported for the use of tetraalkylammonium ions as a PSP [35]. Therefore, it is also required to employ new cationic surfactants that provide a stable dynamic coating and a reproducible EOF velocity resulting in reproducible migration times.

According to the best of our knowledge, the MEKC analysis of urinary nucleosides employing IL-type surfactants has not been reported so far. In this work, we employ C_{14}MImBr as PSP for the MEKC separation of six unmodified nucleosides and one methylated nucleoside, based on the assumption that the main mode of interaction between the nucleosides and the micelles formed by C_{14}MImBr is electrostatic interaction, while regarding the C_{14}MImBr micelles to be a pseudostationary ion-exchanger. In addition, we investigate the optimum borate concentration that enhances the interaction of urinary nucleosides (as charged analytes) with the positively charged pseudostationary ion-exchanger. The effect of the buffer type, the buffer pH and the buffer ionic strength on the retention factors of the investigated analytes is studied. The performance parameters for C_{14}MImBr are compared to those exhibited by a conventional cationic surfactant (TTAB) in terms of resolution, selectivity, and migration time reproducibility. Moreover, the selectivity of the developed method regarding the separation of nucleosides from nucleic bases and uric acid is compared to that reported for HILIC.

2. Experimental

2.1. Chemicals and background electrolytes

Nucleoside standards cytidine (Cyd), adenosine (Ado), 5-methyluridine (5MeUrd), uridine (Urd), guanosine (Guo), inosine