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Protein/ionic liquid/glassy carbon sensors following analyte focusing by ionic liquid micelle collapse for simultaneous determination of water soluble vitamins in plasma matrices

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ABSTRACT

Two novel sensors based on human serum albumin (HSA)–ionic liquid (IL) and bovine serum albumin (BSA)–ionic liquid (IL) composites modified glassy carbon electrode (GCE) were produced for simultaneous determination of water soluble vitamins B2, B6 and C in human plasma following analytes focusing by IL micelles collapse (AFILMC). For selective and efficient extraction, vitamins were dissolved in 3.0 mol L^{-1} micellar solution of 1-octyl-3-methyl imidazolium bromide IL. The extracted vitamins were hydrodynamically injected by 25 mbar for 20 s into a running buffer of 12.5 mmol L^{-1} phosphate at pH 6.0 followed by electrochemical detection (ECD) on protein/1-octyl-3-methyl imidazolium hexafluorophosphate IL/GC sensors. The chemical stability of proposed sensors was achieved up to 7 days without any decomposition of PF6-based IL/protein and adsorption of interfering ions. In the current work, the sensitivity enhancement factor (SEF) up to 5000-fold was achieved using the AFILMC/ECD setup compared to conventional CE/UV. Under optimal conditions, linear calibration graphs were obtained from 0.5, 0.5 and 1.0 to 1500.0 μg mL^{-1} of vitamins B2, B6 and C, respectively. Detection limits of analytes were ranged from 180.0 to 520.0 ng mL^{-1}. The proposed AFILMC/ECD setup was successfully applied to the assay of trace level quantification of vitamins in human plasma samples and also their binding constants with HSA and BSA were determined. The concurrent use of IL micelles for the proposed separation and detection processes exhibited some advantages, such as, a reduction of use toxic solvents, an efficient extraction and a direct injection of samples with a short-single run. Furthermore, IL micelles, having variable possibility of interactions, facilitated the successful achievements of AFILMC/ECD setup for the quantification of vitamins in plasma matrices.

1. Introduction

Electrochemical (EC) sensors coupled with capillary electrophoresis (CE) have aroused more attention, from its first reporting by Wallingford and Ewing [1], due to its high sensitivity and good selectivity to electroactive analytes using quite cheaper, simpler and portable equipment. The typical CEEC layout consists of three principal parts: the high voltage CE power supply, the CE capillary, and the EC detection unit [2]. In this case, EC detector must be isolated from the CE separation in order to adapt the EC detection potentials (mV) and currents (nA–pA) with much larger CE voltage (kV) and current (μA) [3]. This can be accomplished by off-column and end-column protocols. End-column is the most popular and the use of relatively small bore capillaries therein, usually 25 μm ID, decreases proportionally the background current associated with the CE separation and the CE voltage drops off more rapidly at the capillary exit [2–4].

Glassy carbon (GC) was commonly used as a substrate for the fabrication of several chemically modified electrodes [5,6] attributed to its extremely stability and robustness. Recently, ionic liquids (ILs) were used for the modification of carbon electrodes because of their properties, including high/moderate conductivity, negligible vapor pressure, good thermal stability and ability to form micelles with the variable possibility of interactions by electrostatic, hydrophobic, π–π and hydrogen bonding [7,8]. The electrode material consisting of IL acquired the same properties of ILs. There is a limited number of ILs that is really suitable for electrochemistry. ILs that have sufficient conductivity may exhibit meager electrochemical windows and/or may have low chemical

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stability toward electrode materials. Therefore, the appropriate choice of IL and working electrode was important to have a stable liquid deposit in contact with aqueous solutions. Recently, short chain imidazolium ILs were successfully used for the modification of GCE surface and the determination of several compounds [7]. IL/GCE modified electrode gave a wide electrochemical range (−2.0 to +2.5 V) and it was insensitive to water content of IL. These ILs are also commercially available or even simply prepared in the lab that made them the first choice for analyst [7–10].

The conjugation between working electrodes and proteins has received considerable attention recently because it can be used in fabricating new generations of sensors. The direct electron transfer between GCE and proteins is limited attributed to the redox centers are deeply buried within the protein molecules [7]. ILs can be easily conjugated with serum albumins (SAs) to facilitate the direct electron transfer between proteins and GCE. Moreover, the presence of protein molecules enhanced significantly the sensitivity and selectivity of IL/GCE for the quantification of compounds in biological matrices. Moreover, these modified electrodes facilitated the study of binding characterization of active compounds with SAs. There are two types of binding sites on SAs including the ionic sites on the SAs surface to interact with an imidazolium ring of ILs and the hydrophobic cavity of SAs to interact with carbon side chains of ILs [7].

The sensitivity of electrochemical detectors (ECDs) coupled with CE could be enhanced by the appropriate wave form. Pulsed voltammetry (PV) could be used [11–14] that a pulse of constant amplitude is modulated on a top of potential scan. Then, the current is sampled just before and at the end of the modulation pulse, as a result, the difference in the pulse is recorded. However, the application of the scanned potential led to non-faradaic current. This shortcoming could overcome by differential pulse voltammetry (DPV) that can distinguish faradaic waves of analytes from the background. This could be attributed to the larger 2nd derivative of the current/potential relation that led markedly to enhance the detection limits [7,12–14].

The sensitivity of ECDs could be further improved by coupling with on-line preconcentration CE. Various on-line focusing CE methods such as field amplified sample injection, sweeping, transient isotachophoresis (tITP) and dynamic pH junction (DypH) were developed [8,15–18]. Analyte focusing by micelle collapse (AFMC) is recently formulated in CE which is based on the presence of sample zone contained the micelles-forming agent with higher conductivity than that of background electrolyte (BGE) zone voided of micelles [7,8]. Due to concentration difference at the boundary that separates the sample and BGE inside the capillary, the micelles dilution zone is formed [8,17,18]. Subsequently, micelles were collapsed and the analyte was accumulated. The amount of focused analyte was influenced by the magnitude of the affinity factor of analyte to the micellar phase (k factor) and the conductivity ratio of BGE to sample (γ factor) [8]. Finally, the focused analyte should be separated by CE principles. Recently, short chain imidazolium ILs were successfully used as micellar agents in AFILMC for the determination of drugs in biological samples [8].

Water soluble vitamins including B group vitamins and ascorbic acid (vitamin C) are micronutrients (antioxidants) that are essential to life and many of them are playing important roles in regulating brain functions. Humans cannot make or store these vitamins and must obtain them through the diets or supplements. Analytical determinations of vitamins in foods, formulations or cancer cells [6,19–25] were successfully achieved. The determination of these vitamins in human plasma matrices faces some challenges attributed to their low concentrations and the presence of other electroactive species that have the same potentials [6,26,27]. The used methods till now are often based on tedious and time consuming microbiological assays that may lack specificity and stability of measurements.

Therefore, the aim of the current work is to establish and evaluate the utility of two sensors composed of OMIMPF6 IL with HSA and BSA on GCE following analyte focusing by IL micelle collapse (AFILMC) platform for the simultaneous quantification of vitamins (B2, B6 and C) in human plasma. Furthermore, IL micelles were also used for an efficient extraction of vitamins from plasma matrices with a long term stability of their solutions under lab environmental conditions. The amounts of unchanged vitamins in plasma after interval times from their administration were measured. In addition, the binding characterizations of vitamins with HSA and BSA were investigated. To the best of our knowledge, this work was reported for the first time on the proposed HSA or BSA/IL/GCE sensors coupled with AFILMC for high sensitive and selective measurements of vitamins B2, B6 and C in human plasma and their binding characterizations with HSA and BSA.

2. Material and methods

2.1. Chemicals and reagents

Vitamin B2 (riboflavin, 99.8%), vitamin B6 (pyridoxine hydrochloride, 99.7%) and vitamin C (ascorbic acid, 99.9%) reference materials were purchased from Fluka (Chemie AG, Buchs, Germany). Human serum albumin (HSA, ≥99%), bovine serum albumin (BSA, ≥98%), L-ibuprofen, sodium dodecyl sulfate, hydroxypropyl-β-cyclodextrin, cetyltrimethylammonium bromide, acetanilide (AC), sodium dihydrogen phosphate, hydrochloric acid and sodium hydroxide were obtained from Sigma (St. Louis, MO, USA). Short chain ILs such as 1-ethyl-3-methylimidazolium bromide (EMIMBr), 1-butyl-3-methylimidazolium bromide (BMIMBr), 1-hexyl-3-methylimidazolium bromide (HMIMBr), 1-octyl-3-methylimidazolium bromide (OMIMBr) and 1-octyl-3-methylimidazolium hexafluorophosphate (OMIMPF6) were prepared as described elsewhere [28]. High purity nitrogen was used to obtain a controlled atmosphere in the electrochemical cell. Deionized water (type 2) was purified by a Millipore apparatus (Milford, USA).

The phosphate buffer (12.5 mmol L^{-1}) was prepared by dissolving the appropriate weight of the phosphate salt in water and then adjusted the pH to 6.0. The phosphate buffer was stable for 1 week at 4 °C. Stock solutions of vitamins B2, B6 and C were prepared by dissolving 10.0 mg of each in 5.0 mL solution of 3.0 mol L^{-1} OMIMBr dissolved in water. The 1.0 mg mL^{-1} stock solutions of HSA and BSA were prepared in phosphate buffer. The BGE and injected sample solutions were filtered through 0.22 μm membrane syringes prior to their input into the CE system.

2.2. Instruments

CE experiments were carried out by a system composed of ±30 kV high-voltage dc power supply to give the required separation voltage between the two ends of capillary. The inlet of the capillary was held at a positive potential (anode). The cathode and anode are short length electrodes provided by Agilent (Germany). A bare fused-silica capillary (Agilent, Germany; 25.0 μm ID, 360.0 μm OD) of 64.5 cm total length and 56.0 cm effective length was employed. Electropherograms were monitored using the ChemStation software. EC detections were performed in the outlet buffer vial using 3-electrode configuration comprising a protein/IL/GC working electrode, a Ag/AgCl (3.0 mol L^{-1} KCl) reference electrode and a coiled Pt wire placed at the bottom of the EC cell as the counter electrode. The 3-electrodes were fixed by polymer insulators to prevent any leakage. The EC detection cell was shielded in a metal box to reduce external disturbance. A IME-663 module with PGSTAT