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Alkyl imidazolium ionic liquid based sweeping-micellar electrokinetic chromatography for simultaneous determination of seven tea catechins in human plasma

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ABSTRACT

Determination of tea catechins in human plasma might provide a means of better evaluation of their benefits. The main difficulty in their analysis is the low catechins concentrations in plasma and their susceptible to oxidation during sample pretreatment. In the current work, a sweeping-micellar electrokinetic chromatography (sweeping-MEKC) by long alkyl chain ionic liquid was investigated for the simultaneous determination of seven principal naturally-occurring tea catechins in human plasma under acidic conditions after the intake of green tea beverage. The effects of type and concentration of three 1-tetradecyl-3-methylimidazolium ionic liquids, namely bromide, acetate and hydrogen sulfate salts were studied. The seven catechins were successfully separated within 5 min by micellar running buffer of 5 mmol L^{-1} 1-tetradecyl-3-methylimidazolium bromide and 15 mmol L^{-1} phosphate buffer at pH 4.5 under optimal parameters of 50 mbar injection for 150 s, 10 kV, 25 °C and 200 nm. To prevent the possibility of IL adsorption, an appropriate rinsing protocol was established. The method has analytical ranges from 0.5, 1, 0.5, 1, 2, 1 and 1 to 500 ng mL^{-1} for GC, C, EC, EGCG, GCG, ECG and EGC, respectively (r ranged from 0.995 to 0.999). The intraday precision and accuracy were 0.1–0.9% RSD (n = 10) and 97–106% recovery, respectively. Limits of detections of analytes were ranged from 0.2 to 1.2 ng mL^{-1}. The current sweeping-MEKC achieved sensitivity enhancement factor (SEF) up to 183-fold of analytes concentrations compared to other hitherto published CE reports that is suitable to find out the trace amounts of catechins in plasma.

1. Introduction

Catechins are flavanols that exist in a variety of foods such as tea, fruits and chocolate. They have radical scavenging activity that is about ten times higher than that of L-ascorbate and beta-carotene [1]. The principal naturally-occurring tea catechins are epigallocatechingalate (EGCG), epigallocatechin (EGC), epicatechingallate (ECG), epicatechin (EC), gallocatechin (GC) and catechin (C). During the tea manufacturing processes, some of catechins undergo isomerization at the C-2 position of flavan-3-ol, and they are turned to gallocatechin gallate (GCG) [2]. Green tea has attracted much attention for its beneficial health effects, particularly with respect to its potential for preventing cancer and cardiovascular diseases [1,2]. These health benefits were mostly attributed to the presence of catechins. Further knowledge on the absorption and bioavailability of tea catechins after dietary intake could be considered essential for understanding of their action mechanism.

Several published reports for the determination of tea catechins in plasma were performed by HPLC with fluorescence [3], mass spectrometry [4–6], coulometric electrode array [7], electrochemical [8] and chemiluminescence [9] detectors. However, these methods were suffered from cost effective, enzymatic sample pretreatment, toxic organic solvents and easy contamination of mass detectors. Furthermore, LC-UV detection is not very sensitive. Fluorescence detection required pre-column or post-column oxidation to convert analytes into strongly fluorescent compounds. Capillary electrophoresis (CE) has become one of the most convenient analytical techniques. The use of CE-UV for the determination of catechins suffered from difficultly when they applied to human plasma [10] due to the low concentrations of catechins. The sensitivity of CE-UV is limited to short optical length (25–100 μm) and small sample

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volume injection (2–10 nL). Furthermore, catechins are susceptible to oxidation during sample pretreatment. This oxidation was minimized by acidic pH or addition of ascorbic acid or addition of ethylenediaminetetraacetic acid (EDTA) [11]. Rapid sample preparation steps could reduce the exposure of sample to oxygen and consequently its oxidation. In our previous work [10], CE coupled with a modified high sensitivity cell was used for the simultaneous determination of C and EC in human plasma. The popularity of the high sensitivity cell is still limited and its installation or maintenance is a hard work. Accordingly, the development of a simple, fast, sensitive and selective CE method to analyze tea catechins in biological samples would be interested.

In electrokinetic chromatography (EKC), as a mode of CE, the capillary is usually pre-filled with a separation solution of pseudostationary phase (PS) and buffer [12,13]. Then, the analytes prepared in a solution without PS were hydrodynamically or electrokinetically injected into the separation solution [12]. The PS could be micelles to form the micellar electrokinetic chromatography mode (MEKC) [14]. MEKC was successfully applied to the determination of organic compounds in complex matrices with satisfied selectivity. In the last decades, sweeping and stacking were used to enhance the sensitivity of MEKC [15]. Sweeping-MEKC involves the picking up and accumulation of sample ions in a small zone through the chromatographic interactions and electrophoresis to induce interactions [16,17]. The extent of focusing is dictated by the strength of the interactions (k, retention factor) during sweeping and the large sample plug length [18]. However, in the case of reduced electric conductivity in the sample region, not only the retention factor of the analyte but also the conductivity ratio (γ, through stacking) can have an impact on the sensitivity enhancement factor (SEF) [19,20]. The conductivity ratio factor (γ) is the ratio of electric conductivity in the sample zone and in the BGE (BGE/sample) [15]. In this case, a stacking boundary (SB) in the interface between sample and BGE is formed. When the sample ions passed through the SB, the ions are slowed down and further concentrated into a short zone [21]. Therefore, the search about micelles that are adequate to enhance the sensitivity of MEKC by sweeping and stacking without compromise the separation efficiency is the main target of the current work.

Ionic liquids (ILs) are attracted the interesting of scientists that they have special physical and chemical properties [22,23], such as high conductivity, high thermal stability, and form micelles with low critical micelle concentration (CMC). Furthermore, ILs possessed hydrophobic alkyl tails with cationic hydrophilic head groups that enable ILs to emerge as new cationic micelles in CE [24] superior to the traditional cationic surfactants such as alkyl trimethyl ammonium bromides. Furthermore, ILs have ability to form versatility of interactions with analytes including electrostatic, π–π, ion-dipole or hydrogen bonding by the imidazolium cation head group besides the inclusion complexation [25]. These properties make ILs as fruitful micelle forming agents for analyte preconcentration by sweeping-MEKC [26–28]. The long alkyl chain ILs such as 1-tetradecyl-3-methylimidazolium (C14MIm) were successfully employed as sweeping carriers for on-line analyte preconcentration by sweeping-MEKC [23,29–32].

Therefore, in the current work, we examined, for the first time, the use of three long alkyl chains of C14MIm bromide, C14MIm acetate and C14MIm hydrogensulfate micelles for the high sensitive determination of seven catechins by sweeping and stacking online preconcentration in biological matrix. The separation medium was acidic to stabilize catechins without compromise the separation efficiency. Accuracy and precision of the proposed sweeping-MEKC method in the complex medium were evaluated. Moreover, the current sweeping-MEKC was successfully applied to the detection of trace amounts of catechins in human plasma.

2. Experimental

2.1. Chemicals and reagents

(+)-catechin (C), (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (−)-epigallocatechin gallate (EGCG), (−)-gallocatechin (GC), (−)-gallocatechin gallate (GCG) and tetradecyltrimethylammonium bromide (TTAB) were purchased from Sigma–Aldrich (Milwaukee, WI, USA). 1-tetradecyl-3-methylimidazolium bromide (C14MImBr), 1-tetradecyl-3-methylimidazolium acetate (C14MImAc) and 1-tetradecyl-3-methylimidazolium hydrogensulfate (C14MImHSO4) was synthesized as described elsewhere [25]. Purity of ILs was checked with NMR and FTIR analysis [33]. FTIR spectra were recorded using BIORAD FTS-40A spectrometer (Kleve, Germany) with KBr as reference. Nuclear magnetic resonance (NMR) spectra were measured using JEOL Lambda 400 NMR spectrometer (California, USA). All other chemicals were of analytical grade. Ultrapure water purified by Millipore, MA, USA was used for the preparation of all sample and buffer solutions.

2.2. Apparatus

All separations were carried out using a 1600 CE system (Agilent Technologies, Germany) equipped with a photodiode-array detector. CE ChemStation software was used for instrument control, data acquisition and data analysis. An uncoated fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 28 cm total length, 19.5-cm effective length and 50-μm internal diameter was used. The applied voltage was maintained at 10 kV (positive polarity) with controlled temperature at 25 °C. The CE sampling was performed by hydrodynamic injection at 50 mbar for 150 s. The detection wavelength was 200 nm.

Prior to first use, the capillary was conditioned by flushing with 1.0 mol L^{-1} sodium hydroxide for 30 min at 40 °C and finally water with10 min. During analysis, flushing procedure was optimized to give precise analysis by the following: at the beginning of day, procedure was 10 min of 0.1 mol L^{-1} HCl, 10 min H_2O and 10 min running buffer; between runs it was 3 min of 0.1 mol L^{-1} HCl, 4 min H_2O and 4 min running buffer; at the end of day it was 5 min of 0.1 mol L^{-1} HCl and 20 min H_2O.

2.3. Preparation of standards and plasma samples

Standard stock solutions of the different catechins (100 μg mL^{-1}) were prepared in 15 mmol L^{-1} at pH 4.5 and stored carefully at −20 °C refrigeration. The working standard sample solutions required to form the desired sweeping sample matrices were made by dilution in the same phosphate buffer. The separation background electrolyte (BGE) was prepared from sodium dihydrogen phosphate (15 mmol L^{-1}) at pH 4.5 and 5 mmol L^{-1} of 1-tetradecyl-3-methylimidazolium bromide (C14MImBr). Phosphoric acid (1 mol L^{-1}) was used as an acidifying agent. All solutions were filtered through a 0.45 μm filter and then directly injected into the electrophoretic system.

Commercial samples of green tea were collected from the local market (Jeddah, Saudi Arabia). Solutions were prepared by immersing a packet of tea (2 g dry weight) in boiled distilled water (40 mL) at 95 °C for 3 min. Plasma samples were obtained from 5 healthy males adult volunteers (35–40 years old, 60–80 kg body weight and nonsmokers) who were fully informed about all procedures before their written informed consent was obtained. The subjects did not ingest tea-related beverages, food, or supplements for at least two days prior to the experiment [34]. On an empty stomach, the volunteers ingested green tea solutions. Afterwards, no other beverages were ingested except water (the volume was not