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Highly sensitive analysis of flavonoids by zwitterionic microemulsion electrokinetic chromatography coupled with light-emitting diode-induced fluorescence detection

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ABSTRACT

A rapid zwitterionic microemulsion electrokinetic chromatography (ZI-MEEKC) approach coupled with light-emitting-diode-induced fluorescence (LED-IF, 480 nm) detection was proposed for the analysis of flavonoids. In the optimization process, we systematically investigated the separation conditions, including the surfactants, cosurfactants, pH, buffers and fluorescence parameters. It was found that the baseline separation of the seven flavonoids was obtained in less than 5 min with a running buffer consisting of 92.9% (v/v) 5 mM sodium borate, 0.6% (w/v) ZI surfactant, 0.5% (w/v) ethyl acetate and 6.0% (w/v) 1-butanol. High sensitivity was obtained by the application of LED-IF detection. The limits of detection for seven flavonoids were in the range of 3.30×10^{-8} to 2.15×10^{-6} mol L^{-1} without derivatization. Ultimately, the detection method was successfully applied to the analysis of flavonoids in hawthorn plant and food products with satisfactory results.

1. Introduction

Capillary electrophoresis (CE) is a powerful analytical approach with significant importance in the food, forensic, environmental and pharmaceutical sciences due to its unique advantages, such as its instrumentation simplicity, high separation efficiency, minimum operation cost and compatibility [1,2]. CE separation depends on the differing migrations of solutes in an electrical field, and electrophoresis is carried out in capillaries filled with a background electrolyte (BGE) [3]. Recently, the coupling of CE with light-emitting-diode-induced fluorescence (LED-IF), a spectroscopic approach used for the determination of molecular structures, flow visualization and the detection of selective species, has received attention. LED-IF possesses the advantages of being small in size, providing a stable output, being low-cost, having a long lifetime and consuming low amounts of energy [4].

Over the past several years, there has been a rapid increase in interest in the study of microemulsions due to their unique physicochemical properties, such as low interfacial tension, thermodynamic stability, optical transparency and strong solubilizing ability [5]. Microemulsion droplets, either water-in-oil or the more common oil-in-water, are generally comprised of nanoscale droplets of oil, water, surfactant and cosurfactant in a certain proportion and can be observed as the swell of micelles. Based on the difference in surfactants, microemulsions are divided into four forms: anionic, nonionic, cationic and zwitterionic (ZI) microemulsion. ZI microemulsions are induced by a three-phase system in which oil surfactant-poor phases coexist with excess water and a ZI surfactant. The ZI surfactant, whose polar hydrophilic heads carries both a positive and a negative charge, has wide applications in many fields [6]. The presence of both cationic and anionic active groups in the same molecule results in the head group hydrophilicity being an intermediate between the nonionic and ionic classes [7]. In addition, it is compatible with other types of surfactant and mild to skin and eyes.

Microemulsion electrokinetic chromatography (MEEKC) is a form of CE that utilizes microemulsion droplets as separation media to detect a wide range of analytes. Microemulsions are more

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flexible and can expand better than micelles, providing MEEKC with a higher resolution and a wider separation window than micellar electrokinetic chromatography [8]. In addition, a series of reports have shown the great potential of MEEKC for the separation of lipophilic and hydrophilic substances, such as polyaromatic hydrocarbons [9,10], steroids [11], fat-soluble vitamins [12,14], water-soluble vitamins [13,14], sugars [15] and proteins [16], as well as pharmaceuticals [12,17] and natural products [18,19]. However, no study has been reported on the use of ZI microemulsion as a pseudostationary phase (PSP) for CE.

To date, various detectors have been applied in MEEKC analysis, such as diode-array detection (DAD) [20], mass spectrometry (MS) [21], electrochemical detection (ED) [22] and laser-induced fluorescence (LIF) [23,24]. However, it is well known that the use of DAD in this method leads to low sensitivity. Meanwhile, MS requires a salt-free solution, and ED is easily influenced by the pH of the mobile phase and impurities. Although LIF provides high sensitivity, it is usually characterized by high cost, a limited lifetime and high power consumption. To the best of our knowledge, there have been no reports published on the application of LED-IF in MEEKC analysis to date. Therefore, the investigation of microemulsion-LED in CE is highly interesting, particularly for the analysis of complex samples.

Crataegus pinnatifida Bge. var. *major* N.E.Br., also called hawthorn, has been widely used in medicines and food in China and Europe and is thought to promote blood circulation, improve digestion and resolve blood stasis in both traditional and modern medicine [25]. The main chemical components of hawthorn are flavonoids and phenolic acids [26], and pharmacological studies of flavonoids indicate that they possess a number of useful effects, such as hypolipidemic [27], cardiotonic [28] and antioxidative activities [29]. In this study, a ZI-MEEKC method coupled with LED-IF was developed for the rapid simultaneous separation of seven flavonoids (kaempferide, apigenin, quercetin, isovitexin, apigenin 8-C-glucoside, isoquercitrin and hyperoside) without derivatization. The separation of the analytes was obtained using the optimized surfactant, cosurfactant, buffer, pH and fluorescence parameters, and the analytical performance of this method was investigated in terms of selectivity, recovery, linearity and precision. Ultimately, the developed detection method was successfully applied to the analysis of flavonoids in hawthorn plant and food products with satisfactory results.

2. Experimental

2.1. Chemicals and reagents

N-dodecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate (DAPS), 3-(*N*,*N*-dimethylmyristylammonio)propanesulfonate (MAPS), 3-(*N*,*N*-dimethylpalmitylammonio)propanesulfonate (PAPS), sodium dodecyl sulfate (SDS) and sodium borate were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Brij 35 was obtained from ANPEL Scientific Instrument Co., Ltd. (Shanghai, China). All other chemicals used (chromatography reagents), including 1-butanol, methanol, ethyl acetate, 2-propanol, 1-pentanol and cyclohexanol, were provided by Tianjin Siyou Fine Chemical Co. Ltd. (Tianjin, China). Standards of kaempferide, apigenin, quercetin, isovitexin, apigenin 8-C-glucoside, isoquercitrin and hyperoside were purchased from Shanghai Winherb Medical Science and Technology Development Co., Ltd. (Shanghai, China). The purity of each standard was determined to be higher than 98% by normalization of the peak areas detected by MEEKC-LED-IF. The structures of these seven analytes are shown in Fig. 1. Hawthorn plant powder was obtained from local pharmaceutical stores (Hangzhou, China), and hawthorn food products (appetizing hawthorn, iron hawthorn, hawthorn maltose, mini hawthorn and

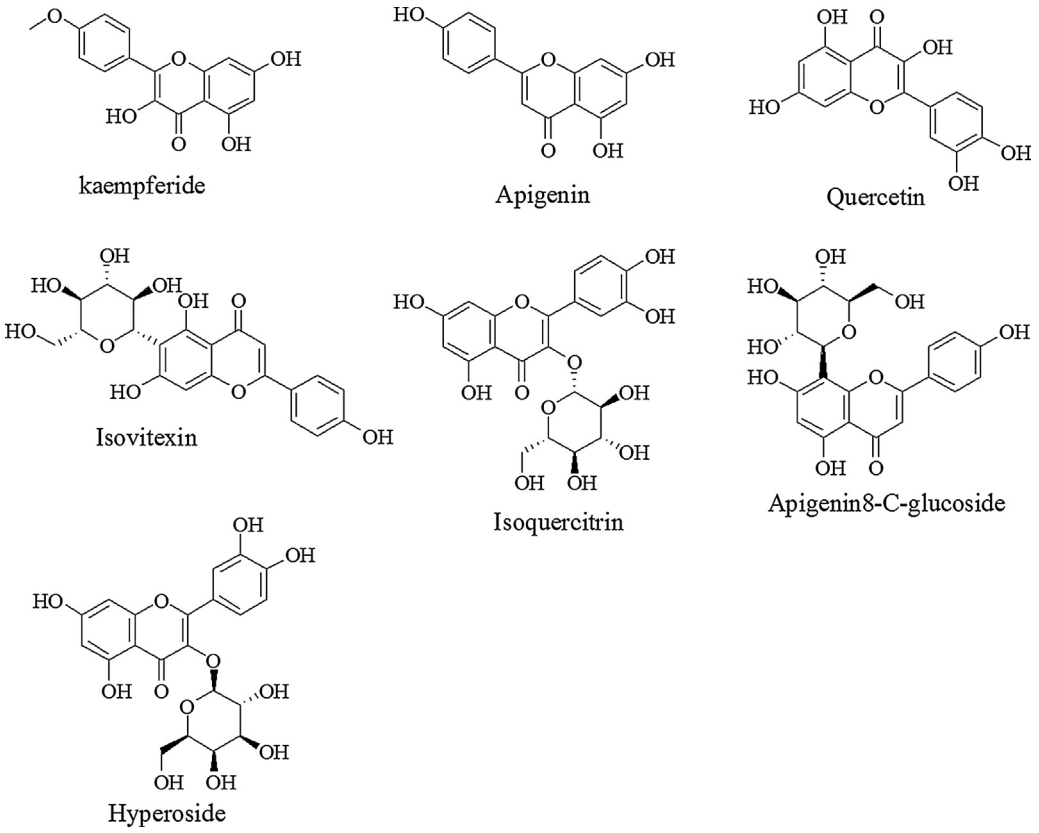

Fig. 1. Chemical structures of the seven compounds investigated.