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Sensitive analysis of curcuminoids via micellar electrokinetic chromatography with laser-induced native fluorescence detection and mixed micelles-induced fluorescence synergism

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ABSTRACT

Curcuminoids, the major bioactive constituents of traditional medicine known as turmeric, have exhibited extensive therapeutic benefits. Excited by violet-blue light, curcuminoids can emit native fluorescence, making them possible to be detected with high sensitivity and specificity by laser-induced native fluorescence (LINF). Here, a commercial 445 nm laser diode was used as an excitation source to construct a confocal laser-induced fluorescence (LIF) detector and then a complete capillary electrophoresis (CE) system coupled with LIF detection was established. With three major curcuminoids, curcumin, demethoxy curcumin (DMC) and bisdemethoxy curcumin (BDMC) as target analytes, a micellar electrokinetic chromatography (MEKC) method was proposed using mixed micelles consisting of Triton X-100 and SDS to sensitize the native fluorescence of curcuminoids and enhance their separation efficiency. Fluorescence spectra revealed that the mixed micelles induced fluorescence synergism could enhance the signals of three curcuminoids by 77-, 57-, and 47-fold for curcumin, DMC, and BDMC. After systematic investigation, the optimal separation buffer for curcuminoids was chosen as follows: 20 mM Triton X-100, 20 mM SDS, 30% (v/v) methanol in 10 mM borax solution at pH 10.0. Under these conditions, a baseline separation of three curcuminoids was achieved within 10 min and the detection limits were found to be 4.1, 2.6, and 0.4 ng/mL for curcumin, DMC, and BDMC, respectively. Furthermore, the developed MEKC-LINF method was validated in terms of precision, linearity, accuracy and successfully applied for the determination of three curcuminoids in turmeric, medicinal turmeric liniment, curry seasoning, and human urine samples.

1. Introduction

Capillary electrophoresis (CE), benefited from exceptional advantages such as high separation efficiency, short analysis time, versatile operation modes, low sample and reagent consumption, has developed into an effective separation technology that is widely applied in the fields of biomedicine, food, environment, and so on [1]. Limited to smaller capillary inner diameter (typically 25–100 μm), the injection volume is often in nanoliters, and the effective optical path-length for detection is usually in micrometers, thus leading to a low sensitivity for CE analysis by using traditional absorbance detection [2]. To overcome this shortcoming, many sample enrichment technologies based on capillary have been developed, but most of enrichment procedures are tedious, and then are difficult for application in complicated samples [3,4]. Furthermore, some sensitive detection methods such as mass spectrometry (MS) have also been utilized in CE to improve its sensitivity [5–7]. However, MS instruments are generally expensive and require specialized interfaces, which are not beneficial for their wide use in CE analysis.

Laser-induce fluorescence (LIF) detection is another sensitive detection technology for CE. The laser has the advantages of good monochromaticity and high intensity, and then is considered to be an excellent excitation light source for on-column detection in capillary with smaller inner diameter. Featured with fast, efficient and sensitive characteristics, CE-LIF is a promising technology for the analysis of trace substances in complex samples [8–10]. Nevertheless, the wavelength of available lasers is relatively single and meanwhile most analytes show no native fluorescence, and accordingly, different derivatization reactions are routinely employed to convert target analytes to their derivatives with suitable fluorescence properties [11,12]. Often, the derivatization reactions are

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time-consuming, and may produce unexpected interferences for the analysis, and as consequence, the sample derivatization procedures have become a bottleneck for limiting analysis time and sample throughput in CE-LIF. In addition, many analytes of interest cannot be derivatized due to lack of reactive groups or available fluorescent reagents. With the progress of laser technology, extensive lasers have been developed and become available on the market in recent years, in particular for those short-wavelength lasers such as ultraviolet laser and low cost semiconductor laser [13–15]. The advances in laser technology offer new possibilities for the application of LIF detection, in particular, label-free laser-induced native fluorescence (LINF) detection greatly simplifies the CE-LIF analysis process and expands the application of CE [16–18].

Turmeric, the rhizome of *Curcuma longa* L., is not only widely used in traditional Chinese medicine, but also plays an import role in Asian cuisine as a spice and condiment [19,20]. Recently, turmeric is more popular in the market of health products. The main active components of turmeric are curcuminoids, which possess the broad-spectrum abilities of anti-inflammatory, anti-oxidation, anti-virus, anti-cancer, anti-bacterial, anti-fibrosis, antidepressant, and other pharmacological activities, thus resulting in increasing interests in the medical researches [21–23]. However, a recent review queried the therapeutic benefits and claimed that curcuminoids were unstable, nonbioavailable and, therefore, a highly improbable lead [24]. Meanwhile, *Nature* published a news to appeal to prevent further wasted research on curcuminoids [25]. Soon afterwards, Heger and other scientists argued in a subsequent *Nature* correspondence [26] that, the clinical potential of curcuminoids should not be dismissed simply, their molecular targets and regulatory mechanisms warrant further investigation. These controversies have received widespread concern, and favored for potential directions on curcuminoids research.

In light of these considerations, developing an analysis method with high sensitivity and specificity will promote the pharmacological studies of curcuminoids. Given the native fluorescence of curcuminoids, a commercial 445 nm laser diode is used here as an excitation source to construct a confocal LIF detector and then a complete CE-LIF system was established. In order to obtain higher sensitivity, the fluorescence synergism effect of different micro-heterogeneous systems (micelles and cyclodextrins) on curcuminoids was investigated using fluorimetric method. Based on this, a mixed micellar system consisting of Triton X-100 and SDS was proposed to sensitize the native fluorescence of curcuminoids and enhance their separation efficiency. Subsequently, the developed mixed micellar electrokinetic chromatography (MEKC) was successfully applied in some complex samples for the analysis of curcumin, demethoxy curcumin (DMC), and bisdemethoxy curcumin (BDMC), the major active components of turmeric. LINF detection ensures high sensitivity, and also can effectively avoid the interference of other components in the samples, which makes the developed MEKC-LINF method have great potential in the pharmacological research and quality control of turmeric.

2. Experimental

2.1. Materials

Curcumin, demethoxy curcumin (DMC), and bisdemethoxy curcumin (BDMC) were obtained from Shanghai Shfeng Biological Technology (Shanghai, China), and their chemical structures were shown in Fig.1. Triton X-100 was purchased from Aladdin (Shanghai, China), and sodium dodecyl sulfate (SDS) was supplied by Kermel Chemical Reagent (Tianjin, China). Borax was the product of Xi'an Chemical Reagent (Xi'an, China). Hydroxypropyl-β-cyclodextrin (HP-β-CD) was obtained from Zhiyuan Biotechnology

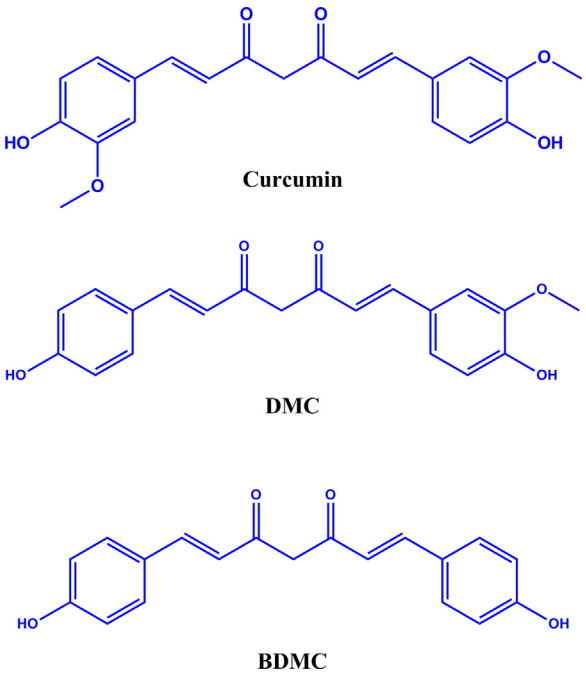

Fig. 1. Chemical structures of curcumin, demethoxy curcumin (DMC), and bisdemethoxy curcumin (BDMC).

(Binzhou, Shandong, China). All other chemicals were of analytical reagent grade and used without further purification. Unless otherwise stated, the ultrapure water with a resistivity of 18.25 MΩ•cm (Aquapro ALH-6000-U) was used throughout all experiments.

2.2. Solutions

Stock standard solutions of curcumin, DMC, BDMC with the concentration of 200 μg/mL were prepared by dissolving the appropriate amount of these analytes in methanol, stored at 4 °C in dark, and diluted to the desired concentration with water before use. The running buffers employed for the separation were prepared from the stock solutions of 100 mM Triton X-100, 100 mM SDS, 100 mM borax, and methanol. All buffers were adjusted accurately to the desired pH value with 1.0 and 0.1 M HCl or 1.0 and 0.1 M NaOH, and filtered through a 0.45 μm pore size cellulose acetate membrane prior to use.

2.3. Sample preparation

The traditional Chinese medicine, turmeric and its preparation, turmeric liniment (Guiyang Shumeida Pharmaceutical) were purchased from a local pharmaceutical store. The curry seasoning (Inner Mongolia Red Sun Food) was purchased from a local market.

The crude turmeric was finely powdered, and then 0.50 g powder was extracted with 5 mL methanol in an ultrasonic bath for 30 min. After centrifugation at 8000 rpm for 10 min, the extract was collected and the residues were extracted for one more time with 5 mL methanol. The extracts were combined together and filtered through a 0.45 μm pore size membrane filter. For curry seasoning, 1.00 g sample was accurately weighted and then was prepared following the same procedure as described above. When injected into a capillary, the turmeric and curry sample solution were diluted by 200 and 5 times, respectively. Turmeric liniment was diluted 5 times with water and then injected directly into the capillary. Human urine sample was collected in the morning from a healthy male volunteer and then frozen for 24 h at -20 °C. After thawing, the

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