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Short communication

Study on capillary electrophoresis–amperometric detection profiles of different parts of *Morus alba* L.

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Abstract

A high-performance capillary electrophoresis with amperometric detection (CE–AD) method has been developed for the determination of the pharmacologically active ingredients in different parts of *Morus alba* L. after a relatively simple extraction procedure. This method was also used in the comparison of bioactive constituent difference in the five parts, based on their electropherograms or characteristic "CE–AD profiles". The effects of several factors such as the acidity and concentration of running buffer, separation voltage, applied potential and injection time were investigated to find the optimum conditions. Method detection limits (S/N = 3) ranged from 1.5×10^{-7} to 1.4×10^{-8} g/mL for all 10 analytes, and the assay results were satisfactory.

Keywords: Capillary electrophoresis; Amperometric detection; CE–AD profiles; *Morus alba* L.; Mulberry

1. Introduction

Traditional Chinese medicines have been extensively used to prevent and cure human disease for over a millennium in oriental countries. Because of its low toxicity and good therapeutical performance, traditional Chinese medicines have attracted considerable attention in many fields [1]. *Morus alba* L. (mulberry), as a non-toxic natural therapeutic agent, belongs to the family of Moraceae. The whole plant of *M. alba* L. possess multiple medicinal values including root (radix mori), bark (cortex mori radicis), branch (ramuli mori), leaf (folia mori) and fruit (fructus mori or mulberry) [2]. For instance, the leaves and mulberry of *M. alba* L. possess hypoglycemic, hypotensive, diuretic, bacteriostatic and antivirotic properties, and they have been applied widely in clinic, which has important values to gerontal diseases and delayed consenescence [3–5].

Pharmacological studies reveal that flavonoids, cumarins, phenols and terperols are the main bioactive constituents in *M. alba* L. [2]. Modern research has revealed that flavonoids [6–8] and phenolic acids [9,10] show speeding cruor, antioxidant, radioprotective, antimutagenic and anticarcinogenic effects, respectively, and that cumarins possess antibacterial, hypotensive, calmative and spasmolytic functions. Yet, their toxicity to animal cells is low.

HPLC, as a prime analytical method, has been applied to analyze the flavonoids [11–15], cumarins [16] and chlorogenic acid [17] in fruit, bark and leaves of *M. alba* L. However, HPLC used in the analysis of traditional Chinese medicines often has some shortcomings, including long analysis time, low resolution and short column lifetime owing to easy contamination [18]. Chromatometry [19–21], spectrophotometry [22] and capillary electrophoresis (CE) with UV approaches [23–25] have also been used for this purpose. These methods rely on photoabsorption detection, and the sensitivity is relatively low. Capillary electrophoresis is increasingly recognized as an important analytical separation technique because of its speed, efficiency, reproducibility, ultra-small sample volume, little consumption of solvent and simple cleaning-up. In addition, with amperometric detection (AD), CE–AD affords high sensitivity and good selectivity for electroactive species [26–28].

In this work we have successively developed a sensitive, simple, and dependable method for the determination of 10 bioactive ingredients in different parts of *M. alba* L. by employing CE–AD, and for the identification of these compounds based on their electropherograms or characteristic "CE–AD profiles".

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2. Experimental

2.1. Apparatus

The laboratory-built CE–AD system used in this work has been constructed and described previously [28,29].

2.2. Reagents and solutions

Umbelliferone, kaempferol, apigenin, luteolin, morin, chlorogenic and caffeic acids were purchased from Sigma (St. Louis, MO, USA). Rutin was purchased from Aldrich (Milwaukee, WI, USA), quercetin and gallic acid were obtained from Shanghai Reagent Factory (Shanghai, China), and were all used as received. *M. alba* L. samples were purchased from a drug store in Shanghai (China). Stock solutions of 10 analytes (1.00×10^{-3} g/mL, each) were prepared in anhydrous ethanol (A.R. grade), and were diluted to the desired concentration with the running buffer. Before use, all solutions were filtered through 0.22 μm syringe filter.

2.3. Sample preparation

Each sample was ground into powder in a mortar and accurately weighed, then extracted with 10 mL anhydrous ethanol (A.R. grade) and water (4:1) for 30 min in an ultrasonic bath. Extracted samples was filtered through filter paper first, and then through a 0.22 μm syringe filter. After filtration, the solutions were injected directly to the CE–AD system for analysis. Before use, all solutions were stored in the dark and at 4 °C.

3. Results and discussion

In amperometric detection the potential applied to the working electrode directly affects the sensitivity, detection limit and stability of this method. Therefore, hydrodynamic voltammetry was investigated to obtain optimum detection results. When the applied potential exceeds +500 mV (versus SCE), all analytes can generate oxidation current at the working electrode, and the oxidation currents of analytes increase rapidly except chlorogenic, caffeic and gallic acids. However, when the applied potential is greater than +1000 mV (versus SCE), both the baseline noise and the background current increase very strongly, which is a disadvantage for sensitive and stable detection. Therefore, the applied potential to the working electrode was maintained at +950 mV (versus SCE).

In addition, the effect of H_3BO_3–$Na_2B_4O_7$ running buffer pH on the migration time of the analytes was investigated in the pH range of 8.7–9.5. At pH 9.2, all 10 analytes can be well separated within a relatively short time. Besides the pH value, the running buffer concentration is also an important parameter. The effect of the running buffer concentration on migration time was studied ranging from 20 to 100 mmol/L (at pH 9.2), and the optimum running buffer concentration is 50 mmol/L (pH 9.2).

In addition to the running buffer and applied potential, the effect of separation voltage and injection time on CE–AD was also investigated. Under the optimum conditions, 10 analytes

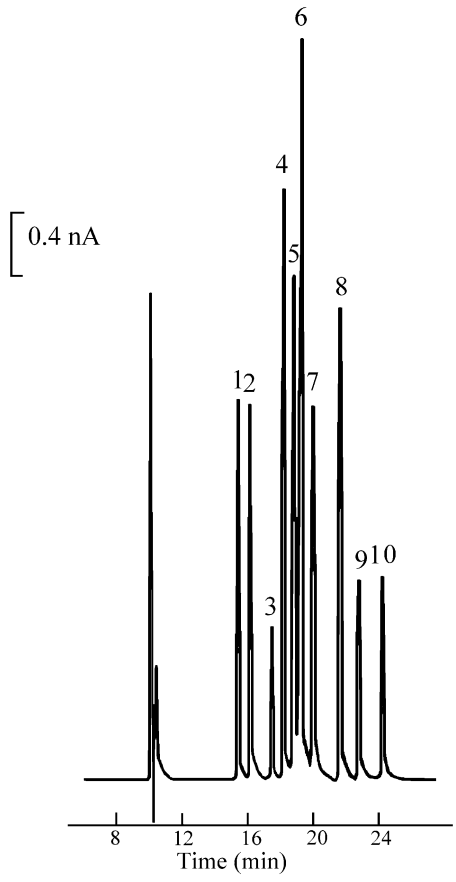

Fig. 1. Electropherograms of a standard mixture solution (1.0×10^{-5} g/mL each). Fused-silica capillary: 25 μm i.d. × 75 cm; working electrode: 300 μm diameter carbon disk electrode; working electrode potential: +950 mV (vs. SCE); running buffer: 50 mmol/L (pH 9.2); separation voltage: 16 kV; injection time: 8 s/16 kV; concentration of analytes: 1.0×10^{-5} g/mL each. Peak identification: (1) rutin, (2) umbelliferone, (3) chlorogenic acid, (4) kaempferol, (5) apigenin, (6) luteolin, (7) quercetin, (8) morin, (9) caffeic acid, and (10) gallic acid.

can be well separated within 25 min at the separation voltage of 16 kV in a 75 cm length capillary with injection time of 8 s (at 16 kV), and the typical electropherogram for a standard mixture solution was shown in Fig. 1.

To determine the linear range of CE–AD response to the 10 analytes, a series of standard solutions from 5.0×10^{-7} to 2.0×10^{-4} g/mL were tested. The calibration curves exhibit very good linear behavior over the concentration range 5.0×10^{-7} to 5.0×10^{-5} g/mL for rutin and umbelliferone, 2.0×10^{-6} to 1.0×10^{-4} g/mL for chlorogenic acid, 5.0×10^{-7} to 1.0×10^{-4} g/mL for kaempferol, apigenin, luteolin, quercetin, and morin, and 1.0×10^{-6} to 1.0×10^{-4} g/mL for caffeic acid and gallic acid, respectively. The linear equations are $Y = 2.20 \times 10^5 X - 0.09$ ($R = 0.9995$, rutin), $Y = 2.03 \times 10^5 X + 0.16$ ($R = 0.9992$, umbelliferone), $Y = 1.05 \times 10^5 X - 0.17$ ($R = 0.9990$, chlorogenic acid), $Y = 2.74 \times 10^5 X + 0.12$ ($R = 0.9995$, kaempferol), $Y = 3.05 \times 10^5 X - 0.20$ ($R = 0.9990$, apigenin), $Y = 3.47 \times 10^5 X + 0.32$ ($R = 0.9993$, luteolin), $Y = 2.36 \times 10^5 X - 0.17$ ($R = 0.9993$, quercetin), $Y = 2.24 \times 10^5 X + 0.30$ ($R = 0.9991$, morin), $Y = 1.39 \times 10^5 X + 0.02$ ($R = 0.9998$, caffeic acid), $Y = 1.03 \times 10^5 X + 0.13$ ($R = 0.9993$, gallic acid), where Y is