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Determination of glycosides and sugars in Moutan Cortex by capillary electrophoresis with electrochemical detection

Gang Chen [a,*], Luyan Zhang [b], Yizhun Zhu [b]

[a] *Department of Chemistry, Fudan University, 220 Handan Road, Shanghai 200433, China*
[b] *School of Pharmacy, Fudan University, 138 Yixueyuan Road, Shanghai 200032, China*

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Abstract

A method based on capillary electrophoresis with electrochemical detection has been developed for the separation and determination of paeoniflorin, sucrose, paeonoside, glucose, and fructose in Moutan Cortex for the first time. Effects of several important factors such as the concentration of NaOH, separation voltage, injection time, and detection potential were investigated to acquire the optimum conditions. The detection electrode was a 300 μm diameter copper disc electrode at a working potential of +0.60 V (versus saturated calomel electrode (SCE)). The five analytes can be well-separated within 12 min in a 40 cm length fused silica capillary at a separation voltage of 12 kV in a 75 mM NaOH aqueous solution. The relation between peak current and analyte concentration was linear over about 3 orders of magnitude with detection limits (S/N = 3) ranging from 0.9 to 1.3 μM for all analytes. The proposed method has been successfully applied to monitor glycoside and sugar contents in the real plant samples with satisfactory assay results.

Keywords: Moutan Cortex; Glycoside; Sugar; Capillary electrophoresis; Electrochemical detection

1. Introduction

As a commonly used traditional Chinese medicine (TCM), Moutan Cortex is the dried root cortex of *Paeonia suffruticosa* Andrews, which belongs to the paeoniaceae family. It can cool blood, promote blood circulation, and remove blood stasis without inducing bleeding [1]. Moutan Cortex has been frequently used as an important ingredient in many traditional prescriptions. Recent investigation demonstrated its effects such as antiaggregatory [2], radical scavenging [3], and inhibition of phenylhydroquinone-induced oxidative DNA cleavage [4], etc. A variety of physiologically active compounds (such as paeonoside, paeonolide, apiopaeonoside, paeoniflorin, oxypaeoniflorin, benzoyloxypaeoniflorin, benzoylpaeoniflorin, paeonol, etc.) have been found presented in Moutan Cortex. They have close relation with the quality of the herbal drug because different functions of the herbal drug come from different active constituents [5]. As the primary metabolites, sucrose, glucose, and fructose are found widely presented in plants. It has been demonstrated that higher contents of sugars can indicate the better quality of some herbal drugs [6]. Hence, it is interesting to establish some rapid, simple, and accurate approaches for the determination of the bioactive substances and some co-existent constituents in Moutan Cortex.

Liquid chromatography (LC) was the most commonly used method for the determination of bioactive constituents in Moutan Cortex [7], composite herbal preparations with Moutan Cortex as their ingredient [8], and biological samples [9,10]. In addition, micellar capillary electrophoresis [11] and gas chromatography [12] have also been employed for the quantitative determination of paeonoside and some co-existent bioactive constituents in Moutan Cortex and multi-component traditional Chinese herbal medicines. Separation and determination of various constituents in plant drugs is always a complicated and challenging task. Nowadays, the application of CE for the separation of various active constituents in medicinal plants has become increasingly widespread because of its minimal sample volume requirement, short analysis time and high separation efficiency [13,14]. Electrochemical detection (ED) typically operated in the amperometric mode can be coupled with CE to provide high

* Corresponding author. Tel.: +86 21 6466 1130; fax: +86 21 6564 1740.
E-mail address: gangchen@fudan.edu.cn (G. Chen).

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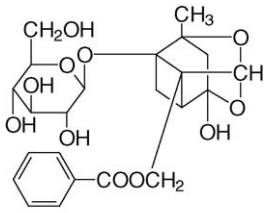
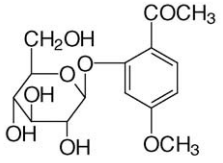


Paeoniflorin



Paeonoside

Fig. 1. Molecular structure of paeoniflorin and paeonoside.

sensitivity and selectivity for the determination of electro-active substances [15,16]. Nowadays, it is of high importance to control the quality of herbal drugs based on their active constituents and some co-existent substances. In 2000, the U.S. Food and Drug Administration (FDA) published a draft of Guidance for Industry Botanical Drug Products. Before a plant drug can become legally marketed, its spectroscopic or chromatographic fingerprints and chemical assay of the characteristic markers are required. CE should find more applications in this area.

In this study, CE-ED was employed for the determination of paeoniflorin, sucrose, paeonoside, glucose, and fructose (the molecular structures of paeoniflorin and paeonoside are shown in Fig. 1) in Moutan Cortex without derivatization for the first time. Because all the five constituents in the crude drugs contain nearby hydroxyl groups that are electro-active at modest oxidation potential on copper electrode in alkaline medium, ED was employed for their sensitive and selective detection in this work. This method is simple, sensitive, selective, and efficient, providing not only a way for evaluating the quality of Moutan Cortex and plant medicines made from Moutan Cortex in marketplaces, but also an alternative approach for quality control in medicinal factories and constituent investigations of other related plants. In a previous report, CE-ED has been employed for the determination of paeoniflorin in Radix Paeoniae Alba, the dried root of *Paeonia lactiflora* Pall [17]. To our best knowledge, there are no earlier reports published on the determination active constituents in Moutan Cortex by CE-ED. The optimization, detailed characterization, and advantages of the CE-ED approach are reported in the following sections in connection to the measurement of the five active constituents in the crude drugs.

2. Experimental

2.1. Reagent and solutions

Paeoniflorin and paeonoside were supplied by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) while sucrose, glucose, and fructose were all purchased from Sigma (St. Louis, MO, USA). All aqueous solutions were made up in doubly distilled water. Other chemicals were of analytical grade.

Stock solutions of paeoniflorin, sucrose, paeonoside, glucose, and fructose (20 mM) were all prepared in doubly distilled water and were kept in a 4 °C refrigerator. They were stable for at least 1 month. The *background electrolyte* was 75 mM NaOH aqueous solution unless mentioned otherwise. The stock solutions were diluted to the desired concentration with the background electrolyte just prior to use.

2.2. Apparatus

The CE-ED system used has been described previously [17,18]. A ±30 kV high-voltage dc power supply (Shanghai Institute of Nuclear Research, China) provided a separation voltage between the ends of the capillary. The inlet of the capillary was held at a positive potential and the outlet of capillary was maintained at ground. The separations were carried out in a 40 cm length of 25 μm i.d. and 360 μm o.d. fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA).

A three-electrode electrochemical cell consisting of a laboratory-made 300 μm diameter copper disc working electrode, a platinum auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode, was used in combination with a BAS LC-4C amperometric detector (Bioanalytical Systems Inc., West Lafayette, IN, USA). The filter of the detector was set at 0.1 Hz. The working electrode was positioned carefully opposite the outlet of the capillary with the aid of a micromanipulator (CORRECT, Tokyo, Japan) and arranged in a wall-jet configuration. The distance between the tip of the working electrode and the capillary outlet was adjusted to ~25 μm by comparison with the bore (25 μm) in the capillary while being viewed under a microscope. The electropherograms were recorded using a LKB·REC 1 chart record (Pharmacia, Sweden). A YS 38–1000, 220 V alternate constant-voltage power supply (Shanghai Instrumental Transformer Factory, Shanghai, China) was employed to suppress the voltage fluctuation of the power line. The whole system was assembled in a *laboratory* that was air-conditioned at 25 °C to minimize the *temperature fluctuation*.

2.3. Sample preparation

Two samples of Moutan Cortex were obtained from Sun-Tian-Tang Traditional Chinese Medicine Store (Shanghai, China). They were all dried at 60 °C for 2 h and then were pulverized. About 2.0 g of the powder was weighed accurately and dispersed in 100 ml doubly distilled water. The mixture was kept in a 90 °C water bath for 2 h. After cooling, it was sonicated for 30 min and filtered through a filter paper. The extract was diluted using 75 mM NaOH aqueous solution at a ratio of 5 (1–5) just prior to CE analysis.

2.4. Procedures

Before use, the copper disc electrode was successively polished with emery paper and alumina powder and sonicated in