Separation and Determination of Rutin and Quercetin in the Flowers of *Sophora japonica* L. by Capillary Electrophoresis with Electrochemical Detection

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Key Words

Capillary electrophoresis
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Summary

Capillary electrophoresis with amperometric detection has been evaluated for the simultaneous determination of rutin and quercetin. The cyclic voltammogram, hydrodynamic voltammogram, and the effects of pH, concentration of buffer and sodium dodecyl sulfate (SDS), and amount of organic modifier on the separation and the detection were studied. The optimized conditions were: detection potential 1.2 V, separation at 12 kV, 5 s at 15 kV for sample injection, running electrolyte 20 mmol L^{-1} borate buffer, pH 8.8, containing 40 mmol L^{-1} SDS and 10% acetonitrile. The detection limit of the method was low, 0.001 and 0.0005 mg mL^{-1}, for rutin and quercetin, respectively; the linear ranges were wide – 0.005 – 0.5 and 0.005 – 0.4 mg mL^{-1}, respectively. The variations in peak current and migration time for eight consecutive injections of a standard solution containing 0.1 mg mL^{-1} of each compound were 4.78 and 3.63%, and 6.50 and 2.59% for rutin and quercetin, respectively. The levels of the two compounds in traditional Chinese herbal drugs were easily determined.

Introduction

Flavonols occur widely in edible plants, foods, and beverages and are vital constituents of the non-energy part of the human diet. Many flavonol-containing species have been used as traditional medicines for a long time. Several studies have revealed that flavonols, which act as antioxidants in biological systems, are very efficient free-radical scavengers in vitro. Analysis of flavonols in medicinal plants plays an essential part of any research into the efficacy, safety, and therapeutic reproducibility of preparations made from these plants [1, 2]. During recent years much attention has been devoted to the analysis of flavonol-containing plants for identification and quantification purposes [3–6]. This analysis has been performed by use of different techniques, from paper chromatography to HPLC. Because of the inherent structural similarity of most flavonols, and the complex characteristics of the sample matrices, gradient elution is often required if efficiency in HPLC is to be adequate.

Capillary electrophoresis (CE) with electrochemical detection (ED), based on the electrochemical reaction of analytes at the electrode surface, is one of the most sensitive methods of detection in CE. CE-ED has recently been applied to the determination of variety of compounds with different electrode-capillary behavior [7–13], and the application of CE-ED has been reviewed many times [14, 15]. Carbon-based electrodes have often been used in amperometric detection, because of their low background current, high stability, and resistance to passivation. Many papers have reported applications of CE separation with amperometric detection at a carbon-fiber electrode.

Rutin and quercetin (Figure 1) are flavonol compounds which occur in the flowers of *Sophora japonica* L.; it is be-

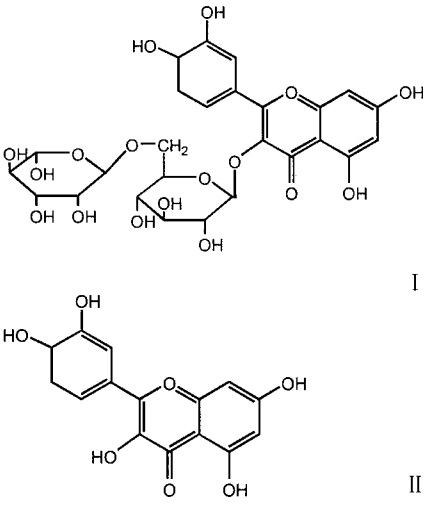

Figure 1. The structures of the analytes: **I.** rutin; **II.** quercetin.

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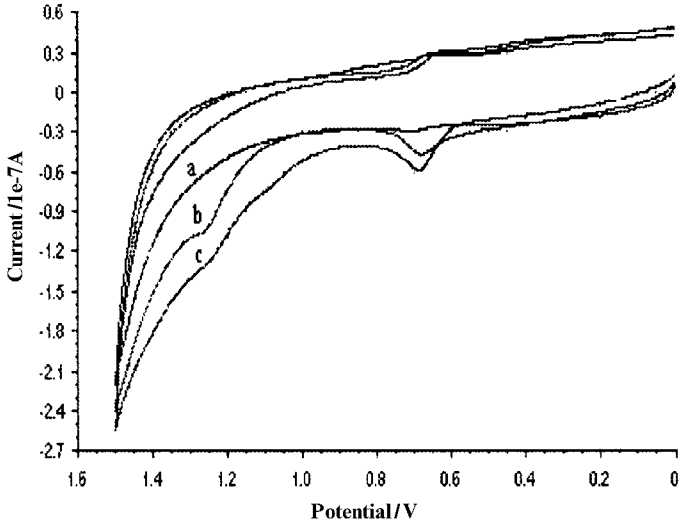

Figure 2. Cyclic voltammograms obtained from quercetin and rutin in 20 mmol L^{-1} borate buffer, pH 8.8, at the 30 μm carbon-fiber electrode. Scan rate: 100 mV s^{-1}: **a**. blank solution; **b**. rutin; **c**. quercetin.

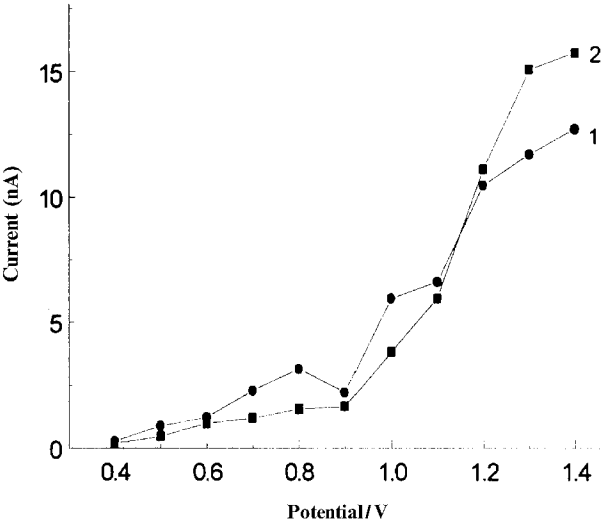

Figure 3. Hydrodynamic voltammograms obtained from quercetin and rutin in 20 mmol L^{-1} borate buffer at pH 8.8, containing 40 mmol L^{-1} SDS and 10% acetonitrile. The separation was performed at 12 kV, sample injection was for 5 s at 15 kV, and the separation capillary was 50 cm × 50 μm i. d. **1**. rutin, **2**. quercetin.

lieved that these compounds can reduce inflammation and have antiviral activity [16]. Simultaneous determination of these compounds by CE with electrochemical detection has not been reported. This paper reports the development of a simple and rapid method for the identification, separation, and quantification of these bioactive compounds, and the systematic investigation of the effects of buffer pH, and the concentration of sodium dodecyl sulfate (SDS) and borate buffer, on the results obtained. The compounds have been determined in an extract of the flowers of *Sophora japonica* L.

Experimental

Reagents

Rutin and quercetin were purchased from the National Institute for Control of Pharmaceutical and products, Beijing, China. The flowers of *Sophora japonica* L. were purchased from Bangding Medicine Shop, China. All other chemicals were of analytical reagent grade. Double-distilled water was used throughout.

Apparatus

The CE-ED system was assembled in this laboratory [17]. Electrophoresis was driven by a 35-high-voltage power supply (The Applied Chemistry of Graduate School of Chinese Academy of Science, Beijing, China). The uncoated fused-silica capillary (50 cm × 50 μm i. d.) was obtained from Yongnian Optical Fiber Factory (Hebei, China). The construction of the chemical detection cell and the working electrode has been described in detail elsewhere [17]. The outlet end of the capillary was always maintained at ground potential. The electrochemical cell was shielded in an iron box to reduce external disturbance. A three-electrode system was used. An Ag/AgCl electrode and a platinum wire were used as reference electrode and counter electrode, respectively. The electrode were connected to an amperometric detector (The Institute of Applied Chemistry, Chinese Academy of Science, Beijing, China) which provided the constant potential applied and measured the resulting current.

Procedure

In CE, a capillary with an improved high-voltage electric decoupler was used. The carbon-fiber array electrodes were inserted into the outlet of the separation capillary, which had been etched to a distance of approximately 0.5 mm. Before first use a new capillary was washed successively with 0.1 mol L^{-1} NaOH, deionized water, and the running electrolyte, each for 5 min, and then equilibrated with the running electrolyte at the separation voltage for approximately 2 h, i. e. until the migration times of analytes did not change significantly. When the running electrolyte was altered, these steps were repeated. After every analysis the capillary was washed with the running electrolyte for 5 min. The buffer in the anodic and cathodic reservoirs was renewed every five runs at the same time. Before use carbon-fiber array electrodes were pretreated electrochemically to activate the surface. Reactivation was achieved by cyclic potential scanning for 2 min between −0.5 V and 1.5 V (relative to the Ag/AgCl electrode) at a scan rate of 100 mV s^{-1} and then maintaining the detection potential for 3 min.

Sample Preparation

Dried flowers of *Sophora japonica* L. were ground to powder in a mortar. The powder (1.3905 g, accurately weighed) was then extracted with methanol (2 × ca. 20 mL) at room temperature for 5 h with continuous stirring. The extracts were combined, diluted to 50 mL, and the solution (the 'stock solution') was stored at 4 °C.

Results and Discussion

Voltammetric Characteristics of Rutin and Quercetin at the Carbon-Fiber Electrode

The cyclic voltammograms obtained from rutin and quercetin are shown in Figure 2, from which it is apparent that both com-

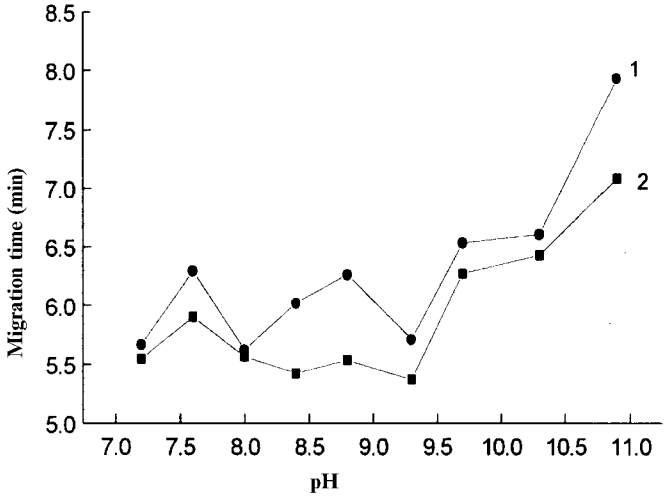

Figure 4. Effect of buffer pH on migration time. The detection potential was 1.2 V and the other conditions were as for Figure 3. **1**. quercetin, **2**. rutin.

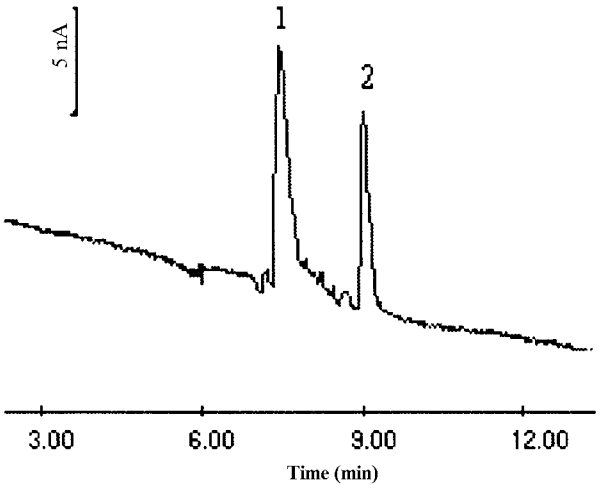

Figure 5. Capillary electropherogram obtained from a standard mixture. Peaks: **1**. rutin (0.1 mg mL^{-1}), **2**. quercetin (0.1 mg mL^{-1}). The analytical conditions were as for Figure 3.

pounds have oxidation peaks at 0.8 V and 1.2 V. To optimize the detection potential, the hydrodynamic voltammograms of the compounds were also studied by changing the detection potential from 0.4 V to 1.2 V in intervals of 0.1 V (Figure 3). It is apparent that when the applied potential was increased the peak current also increased. For applied potentials from 0.4 V to 0.9 V, the peak current was very low; it increased rapidly when the potential was > 0.9 V and reached a plateau at 1.2 V. To obtain a high current response and low background noise for both compounds 1.2 V was selected as the detection potential.

Effect of the Concentration and pH of the Running Buffer Solution

Buffer solution pH is one of the most important conditions affecting separations. Electroosmotic flow (EOF), the charge on the solute, and the analysis time are all affected by changing the buffer pH. To discover the effect of buffer pH on migration behavior, experiments were performed with 20 mmol L^{-1} borate containing 40 mmol L^{-1} SDS as background electrolyte. It is apparent from Figure 4 that good separation of the analytes was obtained at pH 8.8; this pH was therefore selected, because the separation time was short. Study of the effect of borate concentration on migration time revealed that when the borate concentration was increased from 5 to 25 mmol L^{-1} the migration time of the analytes increased rapidly; 20 mmol L^{-1} was therefore selected.

Effect of Sodium Dodecyl Sulfate and β-Cyclodextrin Concentrations

To optimize the effect of SDS concentration on migration time and separation efficiency, 20 mmol L^{-1} borate buffer at pH 8.8 containing concentrations of SDS ranging from 0 to 40 mmol L^{-1} was used to separate the analytes. Complete separation of the analytes was obtained when the concentration of SDS was > 40 mmol L^{-1}. Because the migration time of all the components increased with increasing SDS concentration, 40 mmol L^{-1} was selected for shorter migration times.

The effect of the concentration of β-cyclodextrin (β-CD) on migration time was also studied. It was found that although the migration times of both compounds increased with increasing β-CD concentration the difference between their migration times barely changed, and so β-CD was not used.

Effect of Organic Modifier

It has been reported that addition of an organic modifier to the buffer can improve separation selectivity, efficiency, and resolution [18, 19], because it affects the partition coefficient, mobile phase polarity, and electroosmotic flow. Investigation of the effects of several organic modifiers revealed that full separation could not be obtained without use of a modifier. Full separation was obtained with acetonitrile as modifier at concentrations of 5–20%. Because the migration times of the analytes increased rapidly with the increasing modifier concentration, 10% acetonitrile was selected.

Analytical Characterization

Under the optimum conditions – 1.2 V detection potential, separation at 12 kV, 20 mmol L^{-1} borate buffer at pH 8.8, 40 mmol L^{-1} SDS, and 10% acetonitrile – the two compounds were successfully separated (Figure 5). Detection limit, linearity, and reproducibility were determined. The calibration plots were linear over the ranges 0.005–0.5 mg mL^{-1} and 0.005–0.4 mg mL^{-1}, with correlation coefficients of 0.9986 and 0.9996, for rutin and quercetin, respectively; the respective detection limits were 0.001 and 0.0005 mg mL^{-1}. The reproducibility was determined from eight consecutive injections of a standard solution containing 0.1 mg mL^{-1} rutin and quercetin. The variations in peak current and migration time were 4.78 and 3.63%, and 6.50 and 2.59%, for rutin and quercetin, respectively.

Sample Analysis

The method was used to determine rutin and quercetin in an extract of the flowers of *Sophora japonica* L (Figure 6). Peaks were identified by two means:

(i) by comparison of the migration times of the unknown peaks with those of the standard substances under the same conditions; and

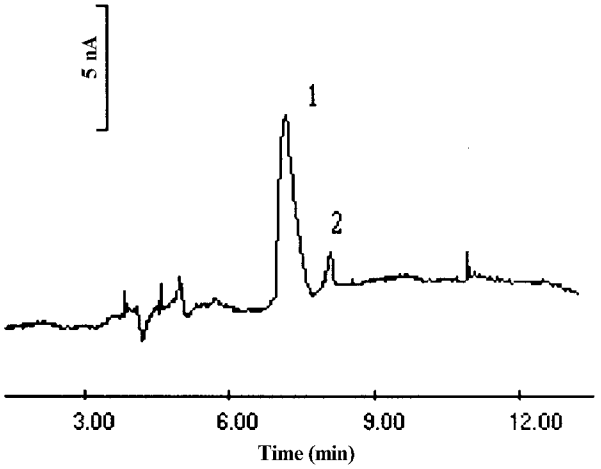

Figure 6. Capillary electropherogram obtained from an extract of the flowers of *Sophora japonica* L. **1** = rutin, **2** = quercetin. The analytical conditions were as for Figure 3.

(ii) by spiking the sample with stock standard solutions of rutin and quercetin.

The amounts of the compounds detected, as determined by addition of standard analytes, were 0.205 and 0.008 mg mL^{-1} for rutin and quercetin after 1:4 dilution of the extract of flowers of *Sophora japonica* L. The actual amount of the components in the powder, on a dry mass basis, were therefore 14.74 and 0.57 mg g^{-1} for rutin and quercetin, respectively.

Conclusion

The results demonstrated that the CE-ED method developed is a useful, simple, and rapid technique for identification, separation, and quantification of rutin and quercetin in Chinese herbal drugs. The operating conditions, i. e. buffer pH and concentration, SDS concentration, and amount of organic modifier, were optimized. The proposed CE-ED method is a good alternative to other chromatographic methods for simultaneous analysis of bioactive components in traditional Chinese herbal drugs. The method also seems suitable for the quality control of these materials.

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