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Determination of Phenolic Constituents of Biological Interest in Red Wine by Capillary Electrophoresis with Electrochemical Detection

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A simultaneous determination of *trans*-resveratrol, (−)-epicatechin, and (+)-catechin in red wine by capillary electrophoresis with electrochemical detection (CE-ED) is reported. The effects of the potential of the working electrode, pH and concentration of running buffer, separation voltage, and injection time on CE-ED were investigated. Under the optimum conditions, the analytes could be separated in a 100 mmol/L borate buffer (pH 9.2) within 20 min. A 300 μm diameter carbon disk electrode has a good response at +0.85 V (vs SCE) for all analytes. The response was linear over 3 orders of magnitude with detection limit (S/N = 3) ranging from 2×10^{-7} to 5×10^{-7} g/mL for all analytes. This method has been used for the determination of these analytes in red wine without enrichment, and the assay result was satisfactory.

KEYWORDS: ***trans*-Resveratrol; (−)-epicatechin; (+)-catechin; capillary electrophoresis; electrochemical detection**

INTRODUCTION

Reduced mortality from coronary heart disease (CHD) among moderate consumers of alcohol is a well-established epidemiologic phenomenon (*1*−*4*). There is some evidence that those who regularly drink wine may have lower CHD mortality than those who prefer other alcoholic beverages (*5*, *6*). The latter possibility has provoked intense interest in constituents unique to wine that may be responsible for these putative effects. Among these, the antioxidant components of red wine rank high (*7*, *8*). Many of the latter, including (+)-catechin, (−)-epicatechin, and *trans*-resveratrol (**Figure 1**), have been shown to protect low-density lipoproteins (LDLs) against oxidation more effectively than α-tocopherol on a molar basis (*9*−*12*). It has been suggested that the moderate intake of wine provides protection against CHD because the antioxidant properties of the phenolic compounds of wine delay the onset of atherogenesis and regulate thrombotic tendencies. The "French paradox" (the apparent compatibility of a high-fat diet with a low incidence of CHD) has been attributed to the regular consumption of red wine (*13*, *14*). Recent interest in these phenolic constituents of red wine, *trans*-resveratrol, (−)-epicatechin, and (+)-catechin, has been stimulated by their potential beneficial effects on health.

Several analytical methods including high-performance liquid chromatography (HPLC) (*15*−*17*), thin-layer chromatography (TLC) (*18*), and high-speed countercurrent chromatography (*19*) have been employed for the determination of *trans*-resveratrol or the catechins in wines. HPLC, regarded as a prime separation method, has some shortcomings including long analysis time, low resolution, and short column lifetime owing to numerous coexistent interferences. Capillary electrophoresis (CE) is becoming increasingly recognized as an important analytical separation technique for its speed, efficiency, reproducibility, ultrasmall sample volume, and minimal consumption of solvent. In addition, with electrochemical detection (ED), CE-ED offers high sensitivity and good selectivity for electroactive analytes, and this method has been applied to the analysis of some phenols in wines (*20*). To our knowledge, so far the CE-ED method

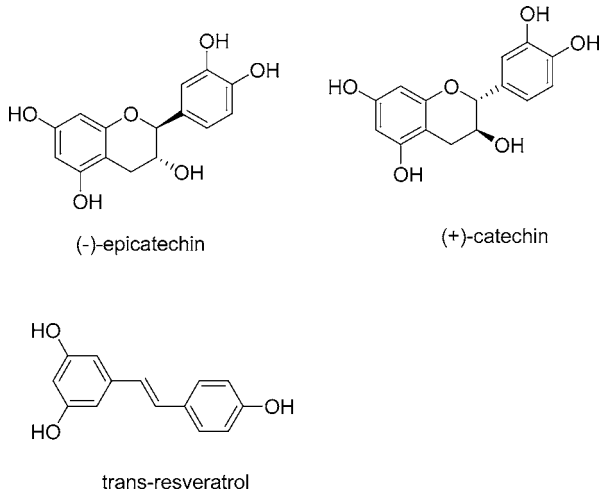

Figure 1. Molecular structures of *trans*-resveratrol, (–)-epicatechin, and (+)-catechin.

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has not been applied for the simutaneous determination *trans*-resveratrol, (+)-catechin, and (−)-epicatechin.

In this work, an alternative method for the determination of *trans*-resveratrol, (+)-catechin, and (−)-epicatechin in wines by using the CE-ED approach is described, which has been proven to be simple and convenient, as well as sensitive and selective.

MATERIALS AND METHODS

Apparatus. In this work, a CE-ED system has been constructed and is similar to that described (*21*) previously. A ±30 kV high-voltage dc power supply (Shanghai Institute of Nuclear Research, Shanghai, China) provided a voltage between the ends of the capillary. The inlet of the capillary was held at a positive potential, and the outlet end of the capillary was maintained at ground potential. The separations were undertaken in a 70 cm length, 25 μm i.d., and 360 μm o.d. fused silica capillary (Polymicro Technologies, Phoenix, AZ).

A three-electrode electrochemical cell consisting of a 300 μm diameter carbon disk working electrode, a platinum auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode was used in combination with a BAS LC-3D amperometric detector (Biochemical System, West Lafayette, IN). Before use, the carbon disk electrode was polished with emery paper, sonicated in doubly distilled water, and finally carefully positioned opposite the outlet of the capillary with the aid of a micromanipulator (Correct, Tokyo, Japan) and arranged in a wall-jet configuration (*22*). The distance between the tip of the working electrode and the capillary outlet was as close as possible so that the CE effluent directly impinged upon the electrode surface. The electropherograms were recorded using a chart recorder (Shanghai Dahua Instrument Factory, China). CE was performed in a 100 mmol/L borate buffer (pH 9.2) used as the running buffer at a separation voltage of 12 kV. The potential applied to the working electrode was 0.85 V (vs SCE). Samples were injected electrokinetically at 12 kV for 6 s.

Reagents and Solutions. *trans*-Resveratrol, (−)-epicathin, and (+)-catechin were purchased from Sigma (St. Louis, MO). Stock solution of *trans*-resveratrol, (−)-epicathin, and (+)-catechin (1.0×10^{-3} g/mL each) was prepared in anhydrous ethanol (A.R. grade), stored in the dark and at 4 °C, and diluted to the desired concentrations with the running buffer (100 mmol/L borate buffer, pH 9.2), in which the carbon working electrode shows excellent response to phenolic compounds. Before use, all solutions were filtered through 0.22 μm nylon filters.

Sample Preparation. Three wine samples were Zhangyu dry red wine (1998), Dynasty dry red wine (2000), and Merlot dry red wine (1996). All of the wine samples were purchased from a supermarket in Shanghai. One milliliter of the wine samples was neutralized with NaOH and then diluted with the running buffer to 2.00 mL. After filtration through 0.22 μm nylon filters, the samples could be injected electrokinetically without preconcentration.

RESULTS AND DISCUSSION

Hydrodynamic Voltammograms (HDVs). In amperometric detection the potential applied to the working electrode directly affects the sensitivity, detection limit, and stability of this method. Therefore, the effect of working electrode potential on the peak current (calculated by measuring the peak height) of the analytes was investigated to obtain optimum detection. **Figure 2** illustrates the HDVs of *trans*-resveratrol, (−)-epicatechin, and (+)-catechin. When the applied potential reaches 0.60 V (vs SCE), the peak currents increase rapidly. However, when the potential exceeds 0.85−0.90 V (vs SCE), the current levels off. Although an applied potential of >+0.90 V (vs SCE) results in a larger peak current, the background current of the working electrode increases sharply. Hence, the applied potential of the working electrode was maintained at +0.85 V (vs SCE), at which the background current is not too high and the S/N ratio is highest.

Effects of the pH and Concentration of the Buffer. The acidity and concentration of the running buffer play an important role in CE for its effect on zeta potential (ζ), electroosmotic flow (EOF), and overall charge of all of the analytes, which affect the migration time and the separation of the analytes. Therefore, it is important to study their influences on CE in order to obtain optimum separations. On the basis of experiments, 100 mmol/L borate buffer (pH 9.2) was chosen as the running buffer after consideration of the peak current, resolution, and analytical time.

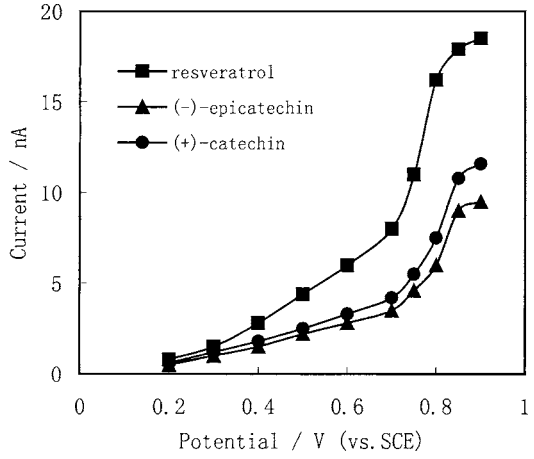

Figure 2. Hydrodynamic voltammograms (HDVs) for *trans*-resveratrol, (–)–epicatechin, and (+)-catechin in CE. Working conditions: fused-silica capillary, 25 μm i.d. × 70 cm; working electrode, 300 μm diameter carbon disk electrode; running buffer, 100 mmol/L borate buffer (pH 9.2); separation voltage, 12 kV; electrokinetic injection, 6 s (12 kV); concentrations, 2.5×10^{-5} g/mL for *trans*-resveratrol, 5.0×10^{-5} g/mL for (–)-epicatechin and (+)-catechin.

Effect of Separation Voltage and Injection Time. The separation voltage affects the electric field strength, which in turn affects the EOF and the migration velocity of charged particles, which determine the migration time of analytes. Moreover, a higher separation voltage may result in a higher joule heating. The effect of separation voltage on the migration time of the analytes is shown in **Figure 3A**: increasing the voltage gives shorter migration times but also increases the background noise, resulting in a higher detection limit. Although the resolution of analytes can be improved to some extent, too low a separation voltage will increase the analytical time considerably, which in turn causes severe peak broadening. On the basis of experiments, 12 kV was chosen as the optimum voltage to accomplish a good compromise.

Injection time determines the amount of sample and affects both the peak current and peak shape. The effect of injection time on separation was investigated by changing the sampling time (2, 4, 6, 8, and 10 s at a voltage of 12 kV, as shown in **Figure 3B**). The peak current increases with the increase of the sampling time, and the peak width increases simultaneously. When the injection time is >6 s, the peak current levels off and peak broadening becomes more severe. Six seconds (12 kV) was, therefore, selected as the optimum injection time.

A typical electropherogram for the standard mixture solution under the optimum conditions is shown in **Figure 4A**. Baseline separation for all analytes can be achieved within 20 min.

Reproducibility, Linearity, and Detection Limit of the Analytes. A standard mixture solution of 2.5×10^{-5} g/mL *trans*-resveratrol, 5.0×10^{-5} g/mL (−)-epicatechin, and (+)-catechin was analyzed seven times to determine the reproducibility of the peak current and migration time for all analytes under the optimum conditions in this experiment. The relative standard deviations (RSDs) of peak current and migration time

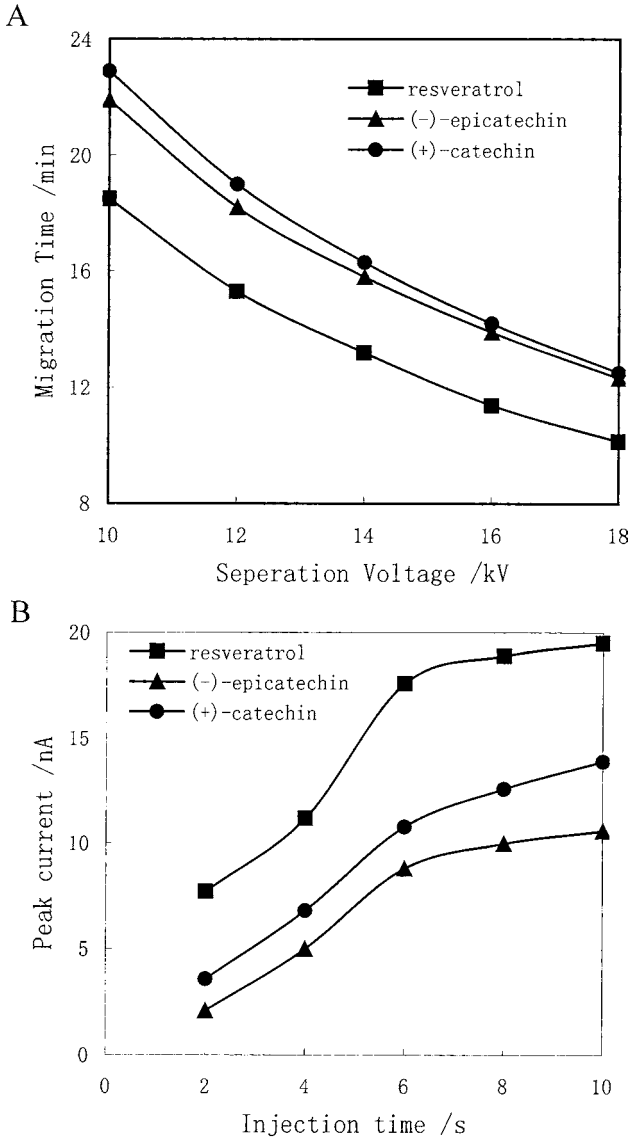

Figure 3. Effect of separation voltage on the migration time of *trans*-resveratrol, (–)-epicatechin, and (+)-catechin and effect of injection time on the analyte peak current. Working potential: +0.85 V (vs SCE); other conditions as in **Figure 2**.

were 2.5 and 0.9% for resveratrol, 3.8 and 1.2% for (−)-epicatechin, and 3.0 and 0.8% for (+)-catechin, respectively.

A series of the standard mixture solutions of *trans*-resveratrol, (−)-epicatechin, and (+)-catechin with a concentration range of 5.0×10^{-7}−1.0×10^{-3} g/mL were tested to determine the linearity for all analytes at the carbon disk electrode in this method. The results of regression analysis on calibration curves and detection limits are presented in **Table 1**. The determination limits are evaluated on the basis of a signal-to-noise ratio of 3. The calibration curves exhibit excellent linear behavior over the concentration range of ∼3 orders of magnitude, with the detection limits ranging from 2×10^{-7} to 5×10^{-7} g/mL for all of the analytes.

Sample Analysis. Under optimum conditions, the determination of *trans*-reveratrol, (−)-epicatechin, and (+)-catechin in red wine samples was carried out according to the procedures described earlier. A typical electropherogram of wine sample is shown in **Figure 4B**. By comparison with the electropherogram of the standard solution (**Figure 4A**), the active ingredients, namely, *trans*-resveratrol, (−)-epicatechin, and (+)-catechin, can be determined. The assay results are listed in **Table 2**.

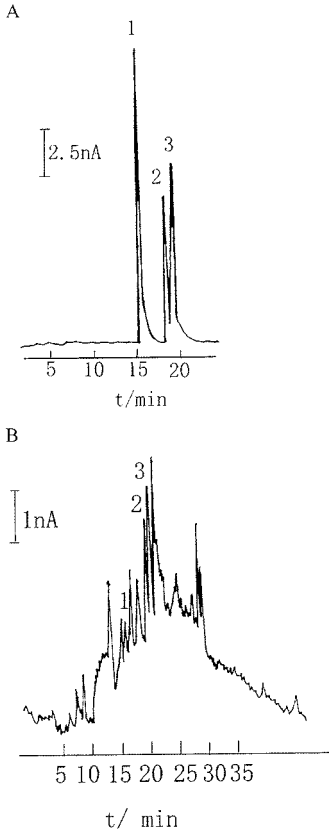

Figure 4. (A) Electropherogram of a standard mixture solution [2.5×10^{-5} g/mL for *trans*-resveratrol, 5.0×10^{-5} g/mL for (–)-epicatechin and (+)-catechin] and (B) typical electropherogram of the real sample (Zhangyu red wine, dilution = 1:2). Experimental conditions are the same as in **Figure 3**. Peaks: 1, *trans*-resveratrol; 2, (–)-epicatechin; 3, (+)-catechin.

Table 1. Results of Regression Analysis on Calibration and Detection Limits[a]

compound	regression eq, $y = a + bx^b$	correlation coefficient	linear range (μg/mL)	detection limit[c] (g/mL)
trans-resveratrol	$y = 672320x + 0.656$	0.9994	0.5–1000	2×10^{-7}
(–)-epicatechin	$y = 171310x + 0.205$	0.9996	1–1000	5×10^{-7}
(+)-catechin	$y = 209250x + 0.297$	0.9996	1–1000	5×10^{-7}

[a] Working potential is 0.85 V (vs SCE). Other conditions as in **Figure 2**. [b] y and x are the peak current (nA) and concentration of the analytes (g/mL), respectively. [c] Corresponding to concentrations giving signal-to-noise ratio of 3.

Table 2. Assay Results of the Analytes in Red Wines ($n = 3$, 10^{-6} g/mL)[a]

sample	*trans*-resveratrol	(+)-catechin	(–)-epicatechin
Zhangyu dry red wine	2.12	35.8	35.0
Dynasty dry red wine	2.50	32.5	45.8
Merlot dry red wine	2.18	28.0	36.5

[a] Working potential is 0.85 V (vs SCE). Other conditions as in **Figure 2**.

Recovery and reproducibility experiments under optimum conditions were also conducted to evaluate the precision and accuracy of the method. Recovery was determined by using the standard addition method. Accurate amounts of *trans*-resveratrol, (−)-epicatechin, and (+)-catechin were added to the sample solution of red wine, and the recovery values were obtained using their peak currents from the calibration curve under the same conditions. The average recoveries and RSDs for *trans*-

Table 3. Results of the Recovery of This Method ($n = 3$, 10^{-6} g/mL)[a]

compound	original amount	added amount	found amount	recovery (%)	RSD (%)
trans-resveratrol	2.12	2.00	4.08	98	2.5
(+)-catechin	35.8	30.0	64.3	95	3.8
(−)-epicatechin	35.0	30.0	63.8	96	2.0

[a] Working potential is 0.85 V (vs SCE). Other conditions as in **Figure 2**.

resveratrol, (−)-epicatechin, and (+)-catechin are listed in **Table 3** ($n = 3$). The average recoveries for the analytes in the other two samples are from 98 to 105%. The above assay results indicate that this method is accurate, sensitivr, and reproducible, providing a useful quantitative method for the analyses of active ingredients in red wines.

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