Capillary Electrophoresis for Simultaneous Determination of Emodin, Chrysophanol, and Their 8-β-D-Glucosides

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The simultaneous separation and determination of the major anthraquinones, emodin, chrysophanol, and their glucosides, of *Rumex japonicus* Houtt., and emodin and emodin glucoside, of *Cassia tora* L., *Rhamnus purshiana* DC., *Polygonum multiflorum* Thunb., and *P. cuspidatum* Sieb. et Zucc., were achieved by cyclodextrin modified capillary zone electrophoresis. The running electrolyte used in this method was 0.005 M α-cyclodextrin in 0.03 M borate buffer (pH 10.5) containing 10% acetonitrile, with an applied voltage of 20 kV.

In a continuation of our work on quinones that widely occur in the plant kingdom and that may have biological activities, we have developed a simple and rapid cyclodextrin modified capillary zone electrophoresis (CD-CZE) to separate and determine emodin, chrysophanol, emodin-8-β-D-glucoside, and chrysophanol-8-β-D-glucoside.

Emodin is perhaps the most ubiquitous natural anthraquinone, occurring in several higher plants, in fungi, and in lichens. In higher plants, it is chiefly present in glycoconjugates. Emodin and chrysophanol frequently occurred together in plants.

Some naturally occurring anthraquinones have already been examined by thin layer chromatography,[1] HPLC methods,[2—6] and capillary electrophoresis (CE).[7—11] However, to determine one component at one time is less efficient and the simultaneous determination of different groups of components is rarely reported. In this study, a simple and rapid CD-CZE method for the simultaneous separating and determining of the major anthraquinones, emodin, chrysophanol, and their glucosides of several natural drugs has been established using 0.005 M α-CD in 0.03 M borate buffer (pH 10.5) containing 10% acetonitrile. During the course of our screening for antitumor-active anthraquinones, it was found that emodin might be valuable as an anti-tumor-promoter and chemopreventive agent.[12,13]

Experimental

Reagents and Materials Sodium tetraborate, sodium dodecyl sulphate (SDS), sodium hydroxide, hydroxypropyl-β-CD (HP-β-CD), γ-CD, and HP-γ-CD were purchased from Wako (Osaka, Japan), α-CD and β-CD from Nacalai Tesque (Kyoto, Japan), and boric acid from Fluka (Buchs, Switzerland). Acetonitrile, methanol, and water were of HPLC grade. Emodin (**1**) and chrysophanol (**2**) were isolated from the root bark of *Cassia siamea*.[14] Emodin-8-β-D-glucoside (**3**) was isolated from the root of *Polygonum cuspidatum* Sieb. et Zucc.[15,16] and chrysophanol-8-β-D-glucoside (**4**) from Cascara Sagrada (the root of *Rhamnus purshiana* DC.).[17,18] Cassiae Semen (the seed of *C. tora* L.) and Polygoni Multiflori Radix (the root of *P. multiflorum* Thunb.) were purchased from The Iguchi Pharmacy (Kobe, Japan). Cascara Sagrada was purchased from The Nakaikoushindou Pharmacy (Kobe, Japan). The root of *Rumex japonicus* Houtt. and *P. cuspidatum* Sieb. et Zucc. were collected in Takatsuki, Osaka, Japan, in October 2001 and identified by Associated Professor Kiyoshi Tagahara.

Procedure for CE The CE analyses were carried out using a Beckman P/ACE System 5000 apparatus (Fullerton, CA, U.S.A.) equipped with a UV detector set at 254 nm, Diode Array detector, and a Beckman untreated fused-silica capillary (570 mm × 75 μm i.d.; 500 mm effective length).

The analytical conditions were as follows: sampling time, 5 s (hydrodynamic mode; 0.5 p.s.i.); applied constant voltage, 20 kV; column temperature, 20 °C. The CD-CZE electrolyte was a buffer solution prepared by mixing 0.03 M sodium tetraborate solution with the appropriate volumes of 10% sodium hydroxide (pH 10.5) followed by the addition of 0.005 M α-CD and 10% acetonitrile. Standard solutions for each anthraquinone were prepared by dissolving the compounds in the electrolyte at a concentration of *ca.* 10 ppm. Among the peaks, emodin, chrysophanol, emodin-8-β-D-glucoside, and chrysophanol-8-β-D-glucoside were identified by spiking with standards and comparing with a UV Similarity Index.

Sample Preparation of Extracts Each powdered material (10 g) was exhaustively extracted in a Soxhlet apparatus with methanol (100 ml) for 10 h. After concentration, the residue was diluted with methanol to 100 ml. This solution was passed through a 0.45 μm filter. After centrifugation, 1 ml of the methanol solution was diluted to 10 ml with borate buffer (pH 10.5), yielding the sample for CE analysis.

Results and Discussion

We have studied the application of CE to the isolation and the determination of anthraquinones, and found that due to the phenolic (and hence weak acidic) or neutral nature of the anthraquinones, the weak alkaline condition of the borate buffer as an eluent could provide a good resolution. It is well known that when the buffer concentration increases, the electro-osmotic flow decreases, and therefore the migration times increase.

We tried to separate the extracts containing emodin, chrysophanol, and their glucosides by CZE, micellar electrokinetic chromatography (MEKC), CD-MEKC, and CD-CZE, changing the buffer solutions to different pHs, composition, buffer concentration, organic solvent, and other modifiers. It was found that a standard mixture of four anthraquinones could be resolved by CZE, MEKC, CD-MEKC, and CD-CZE. However, only CD-CZE was established for the qualitative and quantitative determination of anthraquinones of the herbal extract. After examining a series of buffer solutions differing in pH (pH 8—11), concentration (0.005—0.1 M), and the nature (α, β, γ, HP-β-CD, HP-γ-CD) and concentration of CD (0.005—0.03 M), it was found that a 0.03 M borate buffer (pH 10.5) containing 0.005 M α-CD and 10% acetonitrile could resolve emodin (**1**), chrysophanol (**2**), emodin-8-β-D-glucoside (**3**), and chrysophanol-8-β-D-glucoside (**4**) in the extracts. The migration times of four anthraquinones were almost similar under a 0.03 M borate buffer (pH 10.5) containing 10% acetonitrile (CZE) and a 0.03 M borate buffer (pH 10.5) containing 0.005 M α-CD and
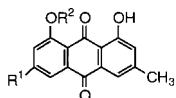
1 $R^1 = OH$, $R^2 = H$ emodin
2 $R^1 = R^2 = H$ chrysophanol
3 $R^1 = OH$, $R^2 = $ glc   emodin-8-β-D-glucoside
4 $R^1 = H$, $R^2 = $ glc   chrysophanol-8-β-D-glucoside

Chart 1.   Structures of Compounds **1**—**4**
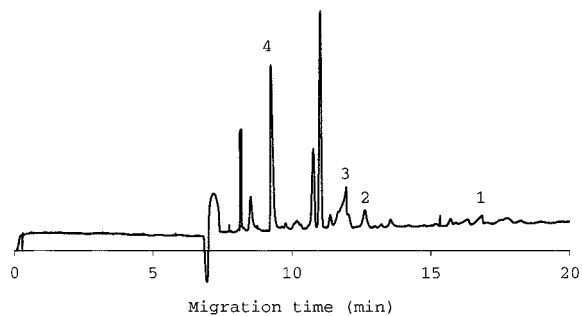
Fig. 1.   Electropherogram Showing the Separation of Anthraquinones from the Extract of *R. japonicus* Houtt.

Using 0.03 M borate buffer (pH 10.5) containing 0.005 M α-CD and 10% acetonitrile. Peaks are identified by substance number as indicated in Chart 1.

Table 1.   Concentration of Emodin, Chrysophanol, Emodin-8-glucoside and Chrysophanol-8-glucoside

Sample	Emodin (μg/g)	Chrysophanol (μg/g)	Emodin-8-glucoside (μg/g)	Chrysophanol-8-glucoside (μg/g)
Rumex japonicus Houtt.	141.32	154.56	1059.20	4260.10
Cassia tora L.	81.94	68.90	97.23	ND
Rhamnus purshiana	1433.16	ND[a]	606.45	ND
Polygonum multiflorum	165.83	ND	2022.01	ND
Polygonum cuspidatum S.	163.20	ND	3766.96	ND

a) Not detected.

10% acetonitrile (CD-CZE). In CZE, the main factor affecting the velocity of a molecule is determined by the molecular size/charge ratio. CDs have been successfully applied for the separation of several hydrophobic compounds. The CD's shape is similar to that of a truncated cone with a relatively hydrophobic cavity which is able to host analytes, and a hydrophilic outside region due to the presence of hydroxyl groups. In the inclusion-complexation mechanism, the entire molecule fits into the CD cavity or with its hydrophobic part. In this work, the α-CD may have an important role in the separation process of the herbal extracts with a large number of co-existent interferences. The migration time of **3** is longer as the concentration of the buffer and pH increased. This could be explained by forming an anionic borate complex with the *ortho*-dihydroxy group of the glucopyranosyl unit[19] and alkaline conditions are required to induce hydroxyl group ionization. An electropherogram of the extract of *R. japonicus* Houtt. is shown in Fig. 1. (Similarity Index (with UV); **1**: 0.9912, **2**: 0.9959, **3**: 0.9984, **4**: 0.9983).

For the quantitative analysis, correlations between the peak area and the sample concentrations (**1**, **2**, **3**, **4**) were studied.

The curves (peak-area ratios, *Y*, *vs.* concentration, *x*, μg/ml) were constructed in the range of 5—50 μg/ml for **1**, 5—75 μg/ml for **2**, 25—150 μg/ml for **3**, and 50—500 μg/ml for **4**. The regression equations of these curves and their correlation coefficients were calculated as follows: **1**, $Y = 2000x + 2307$ ($r = 0.994$); **2**, $Y = 334x + 5941$ ($r = 0.991$); **3**, $Y = 618x + 848$ ($r = 0.996$); **4**, $Y = 220x - 7043$ ($r = 0.991$).

The extraction recovery was tested by adding known amounts of **1** and **2**. The ranges for the recovery of **1** were 96.1—105.3% for *R. japonicus* Houtt., Cassiae Semen, Cascara Sagrada, Polygoni Multiflori Radix, and *P. cuspidatum* Sieb. *et* Zucc., and that of **2** 99.2—103.3% (*n* = 3) for *R. japonicus* Houtt. and Cassiae Semen. The amount of

Table 2.   Reproducibility of Migration Times and Areas of Compounds **1**—**4**

Compound	Migration time (min)	RSD of migration time (%) (*n* = 3)	RSD of areas (%) (*n* = 3)
1	16.18	0.47	2.95
2	12.86	0.46	0.89
3	12.01	0.43	2.61
4	9.27	0.41	2.21

emodin, chrysophanol, and their glucosides found in these sample extracts are shown in Table 1.

The reproducibilities, expressed as the relative standard deviation (RSD) of this method calculated on the basis of peak area over three replicate injections are shown in Table 2. For a series of three consecutive injections, the migration time reproducibility for an individual compound was between 0.41 and 0.47% RSD.

In conclusion, the simultaneous CD-CZE method described here has proved to be a useful technique for investigating mixtures of emodin, chrysophanol, and their glucosides as it is rapid, simple and reproducible.

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