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Journal of Chromatography A, 925 (2001) 279–289

JOURNAL OF CHROMATOGRAPHY A

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Chiral separation of six diastereomeric flavanone-7-*O*-glycosides by capillary electrophoresis and analysis of lemon juice

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Received 15 December 2000; received in revised form 11 June 2001; accepted 11 June 2001

Abstract

The diastereomers of six major flavanone-7-*O*-glycosides (naringin, prunin, narirutin, hesperidin, neohesperidin and eriocitrin) were completely separated for the first time by chiral capillary electrophoresis (CE) employing various buffers and chiral selectors on the basis of the results achieved in 1998 in our research group by Mellenthin. The following chiral additives to the background electrolyte (BGE) were examined: native cyclodextrins (CDs; α-, β- and γ-CD), neutral cyclodextrin derivatives (dimethyl-β-CD, hydroxypropyl-β-CD, hydroxypropyl-γ-CD) and charged cyclodextrin derivatives (carboxymethyl-β-CD, carboxyethyl-β-CD). The effect of CD type, CD concentration and pH value on chiral recognition will be discussed in the following article. In this work, lemon juice (*Citrus limon* L.) was also examined by chiral CE. Eriocitrin and hesperidin could be identified as characteristic flavanones and chiral separation of their diastereomers could be achieved according to the developed chiral method by capillary electrophoresis using a 0.2 *M* borate buffer at pH 10.0 and with 5 m*M* γ-CD as chiral selector.

Keywords: Capillary electrophoresis; Enantiomer separation; Fruit juices; Food analysis; Diastereomer separation; Flavanone glycosides; Glycosides

1. Introduction

(−)-2*S*-Naringenin (5,7,4′-trihydroxyflavanone) is a central intermediate in the biosynthesis of flavonoids [1]. In nature, only (−)-2*S*-naringenin, but not the 2*R*-enantiomer, seems to be formed in the enzymatical pathway, thereby the chalcon isomerase synthesizes stereospecifically the 2*S*-enantiomer of naringenin, starting from the corresponding 4,2′,4′,6′-tetrahydroxychalcon. The formation of many flavonoids proceeds from this vital compound.

Flavonoids constitute one of the largest groups of naturally occurring phenols commonly present in plants as flavonoid-*O*-glycosides. The large natural biodiversity of flavonoids and their very specific appearance in particular species of plants make them suitable indicators of quality in food analysis. They act sometimes also as gauges for adulteration as proof of origin for vegetable food products [2–4].

Flavanone-*O*-glycosides exist as a pair of diastereomers because of the presence of a chiral center in the aglycone (C2) and the optically active sugar residue (see Fig. 1). The chiral separation of this group of polyphenols opens up new analytical oppor-

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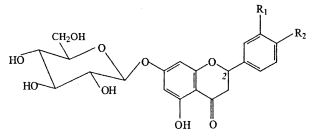
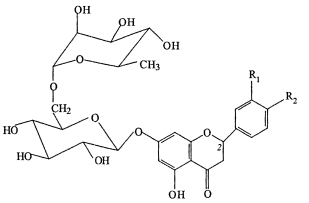
0021-9673/01/$ – see front matter
PII: S0021-9673(01)01038-X



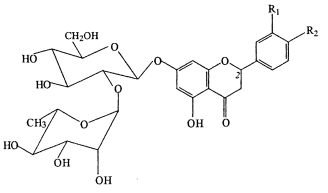
Glucoside: **1**: $R_1 = H$, $R_2 = OH$



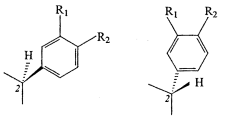
Rutinosides*): **2a**: $R_1 = H$, $R_2 = OH$

2b: $R_1 = OH$, $R_2 = OCH_3$

2c: $R_1 = OH$, $R_2 = OH$



whereby:



2*R*-form 2*S*-form

Neohesperidosides*): **3a**: $R_1 = H$; $R_2 = OH$

3b: $R_1 = OH$, $R_2 = OCH_3$

Fig. 1. Structures of selected flavanone-7-*O*-glycosides. **1** = Prunin (naringenin-7-*O*-glucoside); **2a** = narirutin (naringenin-7-*O*-rutinoside); **2b** = hesperidin (hesperetin-7-*O*-rutinoside); **2c** = eriocitrin (eriodictyol-7-*O*-rutinoside); **3a** = naringin (naringenin-7-*O*-neohesperidoside); **3b** = neohesperidin (hesperetin-7-*O*-neohesperidoside). *Rutinose (6-*O*-α-rhamnosyl-D-glucose) and neohesperidose (2-*O*-α-rhamnosyl-D-glucose).

tunities in food analysis. Furthermore, the enhancement of new insights on their chiral appearance is of great interest due to their key position in the biosynthesis of polyphenolic compounds. Their chiral separation with the help of HPLC was first achieved by Krause and Galensa in 1990 [5] and in recent years published in a few studies [6–8]. In various pieces of research was obtained the diastereomeric separation of flavanone-7-*O*-glycosides by HPLC only with the application of a chiral environment, a resolution of either diastereomeric forms was not achieved without a chiral stationary phase [6,9].

Also chiral CE is in some cases regarded as a good method for the separation of stereoisomers. Up to now, chiral separation of flavanones by CE has only been described by Mellenthin in 1998 [10]. In his studies he obtained the chiral resolution of naringin (naringenin-7-*O*-neohesperidoside) and prunin (naringenin-7-*O*-glucoside) with 0.05–0.1 m*M* borate buffers at pH 8.6 and 10 m*M* β-CD. Narirutin (naringenin-7-*O*-rutinoside) could not be separated with these buffers [10].

The objective of this study was to investigate the chiral recognition capacities of various CDs with six major flavanone-7-*O*-glycosides (naringin, prunin, narirutin, hesperidin, neohesperidin and eriocitrin, see Fig. 1) by CE, in order to achieve the separation of their 2*S*- and 2*R*-diastereomers. The chiral separation of these analytes should be optimized by the change of different parameters of CE. This dependence of the separation upon various CE-factors will be discussed in this paper.

Citrus contain different flavanone glycosides in the juice and in the peel. Lemon juice should also be analyzed by chiral CE.

In this work we used a high pH electrolyte, i.e. a borate buffer at the range 8–10. Under these conditions, the neutral CDs are swept along the capillary by a relatively rapid electroosmotic flow (EOF) towards the cathode, while the analytes migrate in the opposite direction of the EOF because their molecular charge is negative.

2. Experimental

2.1. Instrumentation

Chiral CE separations were carried out using a Beckman automated CE apparatus (P/ACE 5500 CE-System, Beckman Instruments, Fullerton, CA, USA) equipped with a diode array detection (DAD) system (Beckman Instruments). The scan range was 200–400 nm and electrophorograms were stored at 290 nm. Data were processed on an IBM personal computer with P/ACE Station software (Beckman Instruments) version 1.21.

Uncoated fused-silica capillaries of 375 μm O.D.×75 μm I.D. were obtained from Beckman and cut to two lengths [77 cm (effective length 70 cm) and 67 cm (effective length 60 cm)]. The untreated capillaries were washed first with 0.1 *M* HCl for 10 min, followed by 0.25 *M* NaOH for 20 min and finally with distilled water for 10 min. Between runs, the capillary was washed with 0.25 *M* NaOH for 3 min, then it was equilibrated with the running buffer for 4 min. For the overnight storage the liquid in the capillary was removed by using an air stream.

The capillary temperature was set to 25°C. Samples were injected hydrodynamically (20 p.s.i., 1.38 bar) for 2 s. The applied voltage ranged from 10 to 30 kV. Other electrophoretic conditions are described below the heading above each figure and table.

2.2. Chemicals and reagents

Standards of flavanone-7-*O*-glycosides including naringin (naringenin-7-*O*-neohesperidoside, M_r 580.5), prunin (naringenin-7-*O*-glucoside, M_r 414.3), narirutin (naringenin-7-*O*-rutinoside, M_r 580.6), hesperidin (hesperetin-7-*O*-rutinoside, M_r 610.5), neohesperidin (hesperetin-7-*O*-neohesperidoside, M_r 610.6) and eriocitrin (eriodictyol-7-*O*-rutinoside, M_r 596.5) were obtained from Roth (Karlsruhe, Germany). These commercial obtained compounds occur stereochemically as mixtures of both diastereomeric forms. Their 2*S*-/2*R*-ratio was varied in the different standards used. Solutions of these analytes were prepared in dimethylformamide–water (2:1; v/v) at a concentration of 0.2 mg ml^{-1}.

CDs including α-CD, β-CD, γ-CD, 2,6-di-*O*-methyl-β-CD (DM-β-CD), (2-hydroxypropyl)-β-CD (HP-β-CD), (2-hydroxypropyl)-γ-CD (HP-γ-CD), carboxymethyl-β-CD (CM-β-CD) and (2-carboxyethyl)-β-CD (CE-β-CD) were purchased from Fluka (Neu-Ulm, Germany).

Borate buffers that served as running background electrolyte (BGE) were prepared by dissolving appropriate amounts of boric acid in doubly distilled water to yield final concentrations of 0.10, 0.15 and 0.20 *M*, respectively. The pH was adjusted to 8.6, 9.3 or 10.0 by addition of 1 *M* or 0.25 *M* NaOH.

Extraction of phenolic compounds from lemon juice (*Citrus limon* L.) was obtained like described elsewhere [3] and purified afterwards by solid-phase extraction (SPE) with a polyamide cartridge. During the sample preparation the juice components are concentrated by a factor of five. A more detailed extraction procedure for a quantitative statement will be published in a subsequent work about the analysis of some fruit juices.

3. Results and discussion

After a series of CE experiments, we were able to separate the 2*R*- and 2*S*-diastereomers of the six flavanones (naringin, prunin, narirutin, hesperidin, neohesperidin and eriocitrin, see Fig. 1). By this means, flavanone-7-*O*-glycosides could be completely chiral resoluted for the first time by CE (see also Ref. [9]). To achieve this, various buffers and CDs were examined.

A diastereomeric separation of each flavonoid could be achieved with at least one of the tested CDs. There is no generally applicable CD to separate all these analytes, the one-time separation using only one CD type was not achieved yet. The six flavonoids showed a large discrepancy in their affinity to the different CDs in order to build complexes with them. It was observed that the separation also

depends on the amount of the CDs, the pH of the buffer and the concentration of the BGE. The dependence of the separation upon these electrophoretic parameters will be discussed in the following.

Selected flavanone-7-*O*-glycosides have the same basic structure (see Fig. 1), but they differ in degree and position of hydroxyl and methoxyl groups in the aglycone and in their 7-*O*-sugar residues. They are, like flavonoids and other phenolics in general, weak acids with pK_a values in the range from 9 to 10. According to Morin et al., their ionization constants lie in the pH range of 10–12 and their apparent charge depends on their pK_a values and on the pH of the running buffer [11]. Knowing this, the analytes can be expected to be uncharged at the selected pH range from 8.6 to 10.0. However, a borate buffer was chosen as BGE, because of its ability to build negatively charged complexes with polyphenols. Thereby all hydroxyl groups on adjacent carbon atoms can build complexes with borate. Nevertheless, only vicinal hydroxyl groups with a *cis* configuration can be stably complexed [12]. The most favorable configuration for complex building in flavanone-7-*O*-glycoside molecules will be the vicinal hydroxyl pair at C3′ and C4′ in the aglycone, as in the case of eriocitrin, and the *cis*-oriented hydroxyl pair at C2 and C3 in the rhamnose molecule, as in the case of all rutinosides and neohesperidosides (see Fig. 1). Polyphenols cannot only build 1:1 complexes, they can also form 1:2 complexes [11]. Thus, the analytes are negatively charged at the selected pH range and migrate in the reverse direction of the EOF, towards the anode. The EOF in this system is large and its mobility is higher than that of the analytes, thus these reach eventually the detector. The migration behavior of these compounds can therefore be explained by the ionization of some hydroxyl groups and mainly by borate complexation.

The result of a chiral CE separation can be characterized by the peak resolution (R_s), which depends on the peak efficiency (N) and on the apparent separation factor (α_{app}). These parameters are dependent on a number of electrophoretic factors such as the analyte, CD, nature, concentration, pH value of the buffer, among others. The CE factors should be optimized in order to obtain the highest possible peak resolution [13]. The apparent mobility (μ_{app}) of one diastereomer is the algebraic sum of the electrophoretic mobility (μ_e) and that of the EOF (μ_{EOF}). The apparent mobility μ_{app}, the resolution factor R_s and the apparent separation factor α_{app} were calculated using the following equations respectively:

$$\mu_{app} = l/tE = lL/tV \quad (1)$$

$$R_s = 2(t_2 - t_1)/(W_1 + W_2) \quad (2)$$

$$\alpha_{app} = \mu_{app\,1}/\mu_{app\,2} = t_2/t_1 \quad (3)$$

where $t_1$and t_2 are the migration times of the first and second diastereomer (s), W_1 and W_2 are the corresponding widths at the peak base, L is the total length of capillary (cm), l is the effective length to the detector (cm), V is the applied voltage (V) and E is the electric field.

3.1. Chiral separation in the standard solutions with various CDs

The effect of the type of CD on the chiral recognition of selected diastereomeric compounds was first studied by using a 0.1 *M* boric acid buffer at three different pH values in the range from 8.6 to 10.0, to which each CD was added. Naturally occurring CDs (α-CD, β-CD, γ-CD), neutral CD derivatives (DM-β-CD, HP-β-CD, HP-γ-CD) and charged CD derivatives (CM-β-CD, CE-β-CD) were successively tested as chiral selectors. The choice of suitable chiral selectors is one of the most decisive factors in achieving a successful resolution of both diastereomers. The search of these additives requires a great number of experiments, for there is no way to predetermine whether a certain CD type can be used as a useful chiral selector for an analyte.

Chiral recognition of the selected flavanone-7-*O*-glycosides was observed with six CDs, except for α- and HP-γ-CD (see Table 1). These CDs seemed to have an unfavorable spatial shape for interacting with flavanones. The size of cavity and the type of linkage of the hydroxyl groups with different residues might be relevant for the chiral recognition.

The 2*S*- and 2*R*-diastereomers of neohesperidin could be resolved by using six different CDs and their resolution was always excellent (see Table 1). Fig. 2 shows this chiral separation with three differ-

Table 1
Separation parameters for the diastereomeric resolution of selected flavanone-7-*O*-glycosides in standard solutions

Flavanone	pH range	CD type/conc. (m*M*)	R_s	α_{app}
Naringin	8.6–10.0	β-CD/5–15	≤0.88	1.01
	10.0	DM-β-CD/10	≤0.54	1.01
	9.3–10.0	HP-β-CD/1.5–25	≤1.02	1.01
Prunin	9.3	β-CD/10 –15	≤1.04	1.01
	9.3–10.0	DM-β-CD/1.5–15	≤0.65	1.00
Narirutin	10.0	γ-CD/10–25	≤0.77	1.01
	9.3	DM-β-CD/1.5–25	≤0.65	1.00
Hesperidin	9.3–10.0	β-CD/1.5–15	≤1.57	1.01
	9.3–10.0	HP-β-CD/1.5–25	≤1.63	1.01
Neohesperidin	8.6–10.0	β-CD/1.5–15	≤3.10	1.03
	9.3–10.0	γ-CD/5–25	≤1.35	1.01
	9.3–10.0	DM-β-CD/1.5–25	≤2.48	1.02
	9.3–10.0	HP-β-CD/1.5–25	≤3.34	1.04
	8.6–9.3	CM-β-CD/5–25	≤1.88	1.03
	8.6–10.0	CE-β-CD/1.5–25	≤1.07	1.01
Eriocitrin	9.3–10.0	β-CD/5–15	≤0.75	1.01
	10.0	γ-CD/3–15	≤0.92	1.01
	9.3–10.0	DM-β-CD/3–10	≤0.60	1.01
	9.3–10.0	HP-β-CD/1.5–15	≤0.63	1.01

Conditions: uncoated fused-silica capillary [77 cm (effective length 70 cm)×75 μm I.D.]; electrolyte: 0.1 *M* borate buffer; voltage: 20 kV; detection: 290 nm; temperature: 25°C; injection: pressure, 2 s.

ent CDs as chiral selectors. Again, the maximally achieved chiral resolution of the diastereomers of narirutin by adding γ- and DM-β-CD was not sufficient yet. Until now the formation of CD complexes with flavanone glycosides is not well clarified. Flavanones differ in their chemical structure. Thus, it is evident that they also differ in their spatial shape, and consequently in their ability to build inclusion complexes with different CDs.

The calculated peak resolution R_s and the apparent separation factor α_{app} are summarized in Table 1 according to the above specified formulae for all the achieved chiral resolutions in this work.

3.2. Dependence of the separation parameters on the CD concentration

The chiral results were strongly dependent on the added CD amount to the buffer. Thus, the separation of each flavanone was optimized by the change in this factor in a range of 1.5–25 m*M* CD. The derived CDs are generally more soluble than the native CDs, β-CD in particular is insufficiently soluble in aqueous buffers, its maximal solubility amounts to 1.85 g/100 ml. For this reason, the separation systems with β-CD could be produced with, at the most, 15 m*M* of this CD.

The separation was not always improved with an increase in the CD concentration, in some cases the

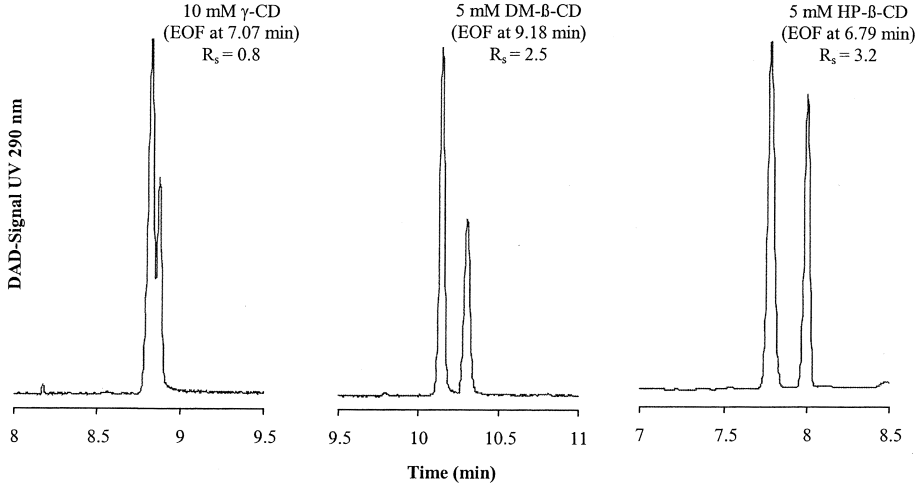


Fig. 2. Chiral separation of the 2*R*- and 2*S*-diastereomers of neohesperidin with a 0.1 *M* borate buffer, at pH 9.3 and with three different CDs. Other conditions as shown in Table 1.

resolution was improved, in other cases it deteriorated (see Fig. 3). The diastereomeric separation of neohesperidin by various concentrations of γ-CD, given on each trace in Fig. 3A, was dependent on the CD concentration added to the BGE. Under selected conditions, peak resolution increased constantly with an increase in the concentration of γ-CD. The separation of the diastereomers of neohesperidin was at its best with the highest CD concentration (25 m*M*; $R_s = 1.52$). Contrary to this, the peak resolution of 2*S*- and 2*R*-eriocitrin increased first with a growth in the amount of γ-CD, but reached a maximum at CD concentration of about 5 m*M* ($R_s = 0.92$) and then decreased with a further growth in the CD amount (see Fig. 3B).

Fig. 4 shows the dependence of the separation resolution (R_s) on the CD concentration in the range from 0 to 25 m*M* for the six selected flavanone-7-*O*-glycosides with two different running buffers. Each analyte shows different behavior due to the different complex binding constants of its two diastereomers with the tested CD or to the different mobilities of its CD complexes built. Naringin, hesperidin, neohesperidin and eriocitrin could be separated in their 2*S*- and 2*R*-diastereomers with 1.5–5.0 m*M* HP-β-CD (see Fig. 4A). In contrast, the best range to resolve neohesperidin, eriocitrin and narirutin with γ-CD was higher, to be exact from 10 to 15 m*M* (see Fig. 4B).

The effect of the CD concentration on chiral separation in CE is considered to be crucial and has therefore been the subject of many theoretical and experimental studies [13]. In 1992, Wren and Rowe introduced the mobility difference model, a simple mathematical model to describe the chiral separation in CE with neutral CDs [14,15]. This model assumes the same mobility of uncomplexed *R*- and *S*-analyte (μ_f) and of complexed diastereomers (μ_c), but different complex binding constants ($K_R \neq K_S$).

The difference in the apparent electrophoretic mobilities between two diastereomers, which determine their chiral separation by CE, is described by this theory as:

$$\Delta\mu_{app} = \mu_{appR} - \mu_{appS} = \frac{[C](\mu_f - \mu_c)(K_S - K_R)}{1 + [C](K_S + K_R) + K_R K_S [C]^2} \quad (4)$$

This equation shows that a chiral separation will be impossible when $K_R = K_S$, $\mu_f = \mu_c$, $[C] = 0$ or $[C]$ is very high. Therefore, maximum separation can be achieved at a certain CD concentration between these two extremes. The optimum concentration is given mathematically as [14]:

$$[C]_{opt} = (K_R K_S)^{-1/2} \quad (5)$$

Experimental data from several studies have supported this model well [16–18]. It should be noted, however, that the preconditions do not always hold and only a concentration dependence of the interactions between the analyte and the CD is rationalized by this model. Therefore, it cannot explain completely all experimental results.

Naringin, hesperidin, neohesperidin and eriocitrin showed with HP-β-CD a characteristic CD concentration dependence of the value R_s (see Fig. 4A). It increased initially with increasing CD amount before reaching a maximum, then decreasing at higher CD concentrations. A sharp maximum was observed for hesperidin and neohesperidin and a broad maximum for naringin and eriocitrin. With γ-CD eriocitrin also showed a characteristic CD concentration dependence with a maximum (see Fig. 4B). The CD concentration which maximizes R_s is different for each flavanone glycoside, as is the maximum degree of R_s obtained.

According to the model by Wren and Rowe, these results could be partly explained by the different values of K_R and K_S. The magnitude of R_s increases as the percentage difference between K_S and K_R increases. The CD concentration required to maximize R_s varies greatly, with smaller values of K_R and K_S requiring higher CD concentrations.

For narirutin, no optimum value of R_s was observed over the γ-CD concentration range studied, for neohesperidin a continuously increasing R_s value was observed (see Fig. 4B). Probably, neohesperidin and narirutin have a maximum at a γ-CD concentration which exceeds the upper limit of the concentration used in this work. According to this model, this effect could arise because of the low complex binding constants of these compounds with γ-CD.

3.3. Dependence of diastereomeric separation on pH of the running buffer

The role of the pH of the running buffer where the

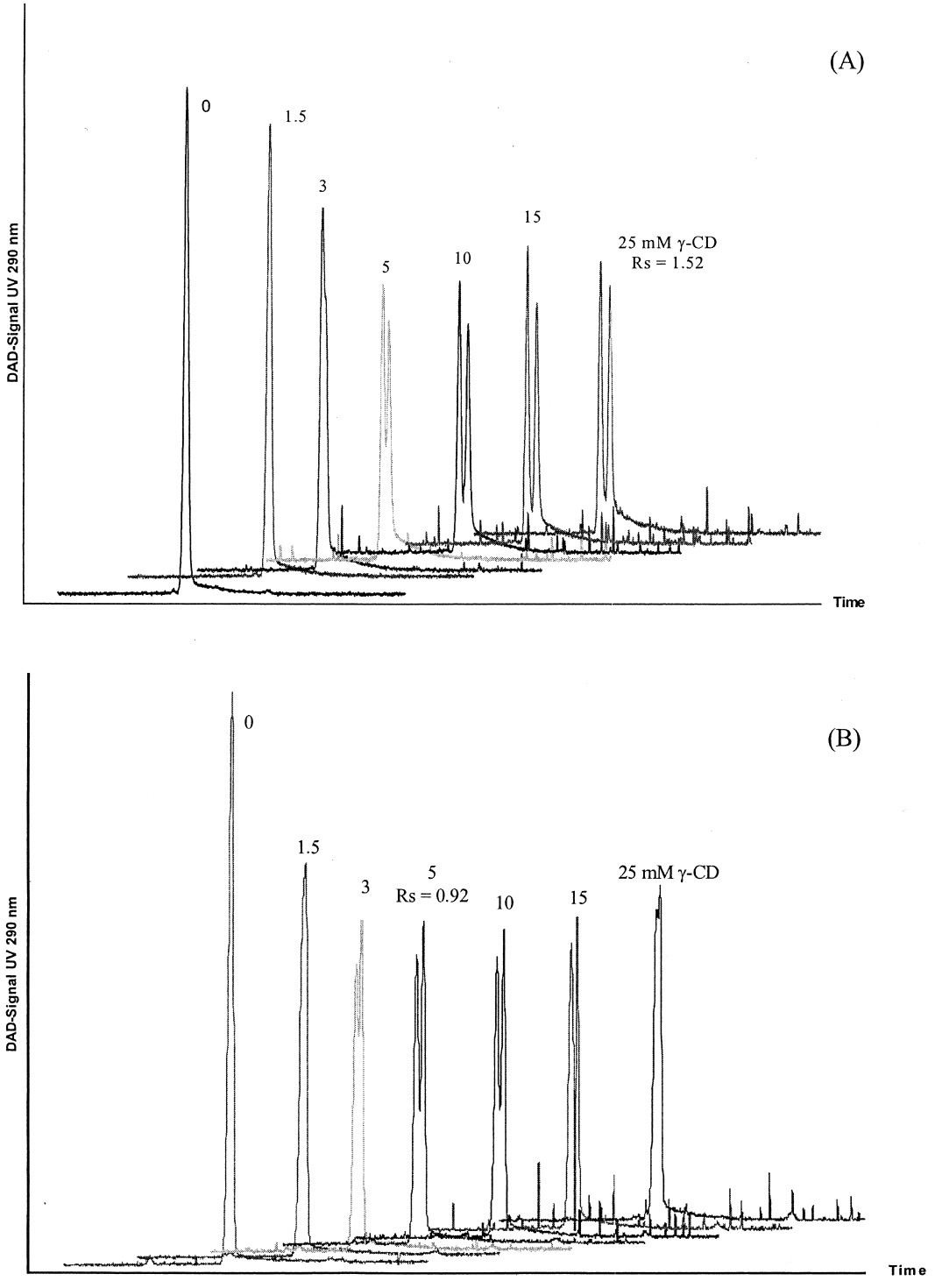


Fig. 3. Electrophorograms of diastereomer separation of neohesperidin (A) and eriocitrin (B) in the presence of increasing concentrations of γ-CD added to the running buffer. Other conditions: Electrolyte: 0.1 *M* borate buffer, pH 10.0; capillary: uncoated fused-silica [67 cm (effective length 60 cm)×75 μm I.D.]; voltage: 20 kV; detection: 290 nm; temperature: 25°C; injection: pressure, 2 s; EOF: 7.30–8.50 min.

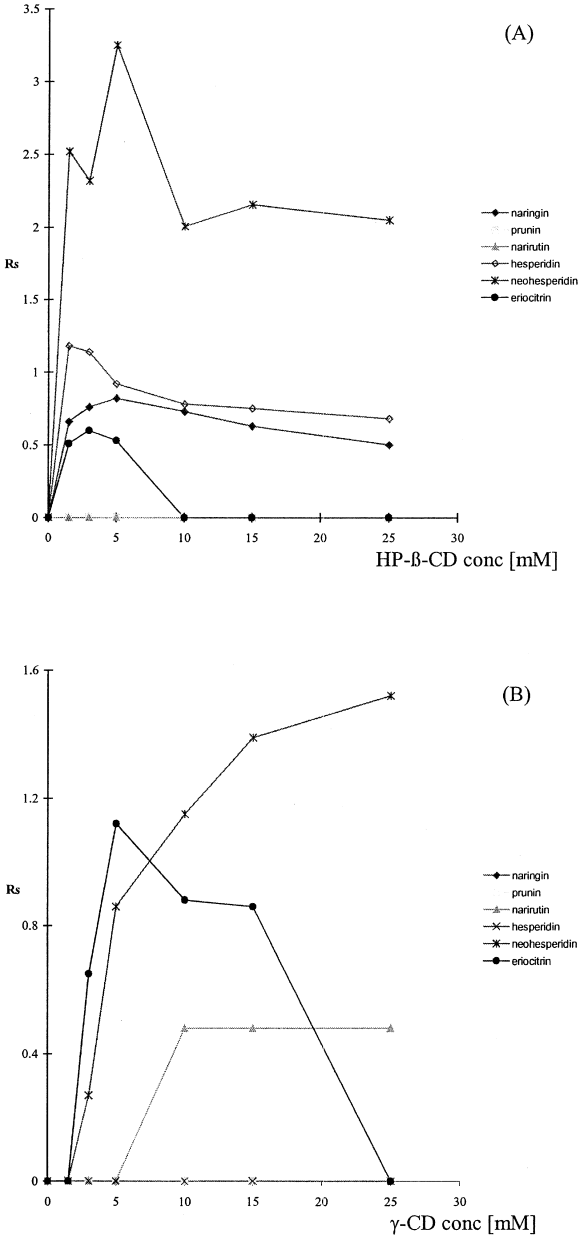


Fig. 4. Peak resolution (R_s) of diastereomeric analytes as a function of CD concentration in the buffer. Conditions: electrolyte: 0.1 *M* borate buffer, pH 9.3 with different HP-β-CD concentrations (A) and pH 10.0 with different γ-CD concentrations (B); capillary: uncoated fused-silica [67 cm (effective length 60 cm)×75 μm I.D.]; voltage: 20 kV; detection: 290 nm; temperature: 25°C; injection: pressure, 2 s.

chiral separation takes place is also one of the most important factors in order to achieve a considerable resolution. The effective charge and, consequently, the mobility of the analytes depend directly on the pH of the medium. Furthermore, the EOF and the self-mobility of chargeable CDs (like CM-β-CD and CE-β-CD) are also pH-dependent.

Flavanone-7-*O*-glycosides are — as aforementioned — weak acids with pK_a values in the range of 9–10. In addition, they may react with boric acid to form borate complexes with negative charge. This borate complexation is a strongly pH-dependent equilibrium. With increasing pH value the formation of complexes is favored, resulting in a greater electrophoretic mobility towards the anode and thus a delay in the flavanone migration [19]. For these reasons the apparent charge of analytes depends on several different factors: the pK_a values of flavanones, their ability to form complexes with borate and the pH of the running buffer.

An alkaline borate buffer system was chosen (0.1 *M* borate, pH 8.6–10.0) to ensure an adequate degree of dissociation of hydroxyl groups and borate complexation. Under these conditions, anionic analytes move towards the anode with the velocity of self electrophoretic migration minus that of EOF. In all the experiments performed we observed an increase of the migration time of flavanones with increasing pH. This proves that the apparent charge of selected analytes depends on the pH-value.

We also compared the achieved peak resolution as a function of pH value. A general increase of resolution was observed with increasing pH for most of the experiments (see Fig. 5) except for those which were carried out with charged CDs like CM-β-CD and CE-β-CD. In these cases the peak resolution became worse at higher pH values.

The general effect of an enhancement in the resolution can be explained by an increase in the velocity of the EOF (which supports the uncharged CDs) towards the cathode and a simultaneous increase in the apparent mobility of flavanone–borate complexes towards the anode with increasing pH in this alkaline range. The analytes stay longer within the capillary and consequently have more chance to interact with the chiral selectors in the BGE. This causes the increase of peak resolution using uncharged CDs.

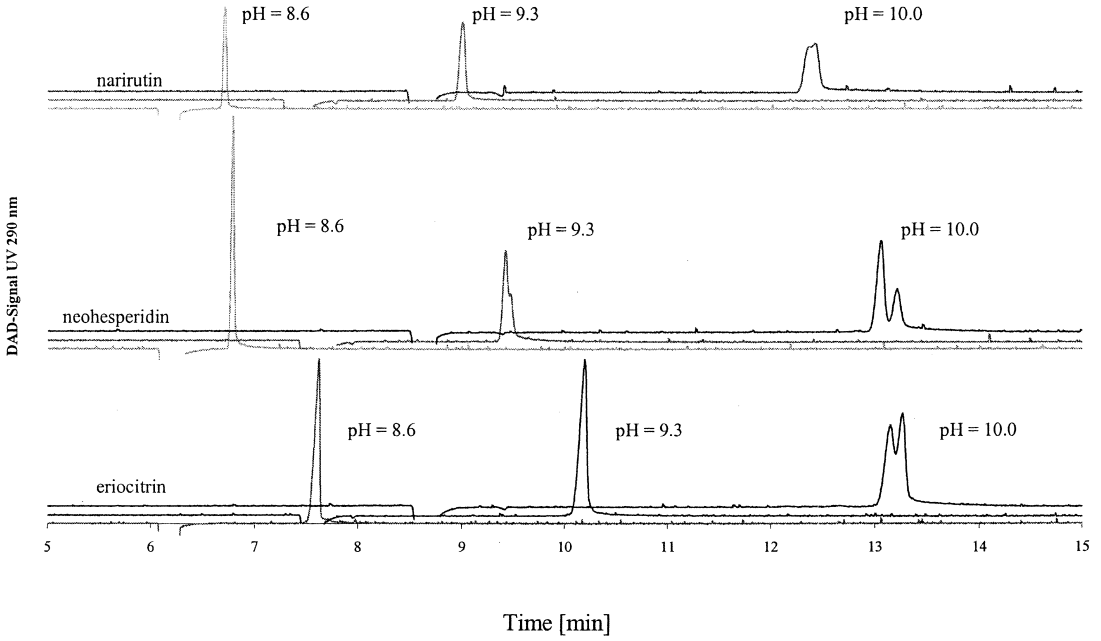


Fig. 5. Diastereomeric separation of narirutin, neohesperidin and eriocitrin at pH 8.6, 9.3 and 10.0 in the presence of 10 m*M* γ-CD as chiral selector. Other conditions as shown in Table 1. EOF: 6.14 min at pH 8.6; 7.46 min at pH 9.3 and 8.60 min at pH 10.0.

Used chargeable CDs are negative at the whole selected pH range. The analytes charge more negatively with increasing pH and thus migrate faster in the same direction as the CDs. On that account, flavanones and chiral selectors could not interact equally well under these conditions. Better peak resolutions were achieved with charged CM-β-CD and CE-β-CD at pH value 8.6, at which point the analytes are less charged.

Neohesperidin shows — as sole exception — with 5 m*M* CE-β-CD as chiral selector in a 0.1 *M* borate buffer a reverse of the diastereomer migration order (see Fig. 6). The diastereomers could not be identified in their *R*- or *S*-form, because a chiral detector for the CE is not for sale yet. Thus, we designate both chiral forms as A and B diastereomer.

It was observed that the form A of neohesperidin at pH 8.6 migrates slower to the detector than the form B. However, at pH 10.0 the form A is faster than the form B. One possible explanation for this reverse could be the increase in self electrophoretic mobility of the uncomplexed, negatively charged analyte with increasing pH value. At pH value 8.6, neohesperidin is charged slightly negative and therefore migrates with a low mobility towards the anode, so it is carried fast (within 12 min) to the detector by the EOF. The diastereomer A seems to build complexes preferentially with the chiral selector, which is the cause for its lower mobility. At pH 10.0 neohesperidin is noticeably charged and gets a higher self electrophoretic mobility towards the anode. The diastereomer A, complexing mainly with the negatively charged CE-β-CD, is carried along fast from the EOF and is detected under these conditions as the first peak, while the diastereomer B, which does not interact as much with the CD, migrates more slowly than the diastereomer A because of its stronger self mobility towards the anode. At an average pH value of 9.3 the effective mobilities of both diastereomers are too close for them to be resolved, they arrive at the detector simultaneously and cannot be resolved under these conditions.

3.4. Chiral analysis of the major flavanone-7-O-glycosides in lemon juice

The described results of chiral CE-separation can be applied to the examination of flavanone-7-*O*-

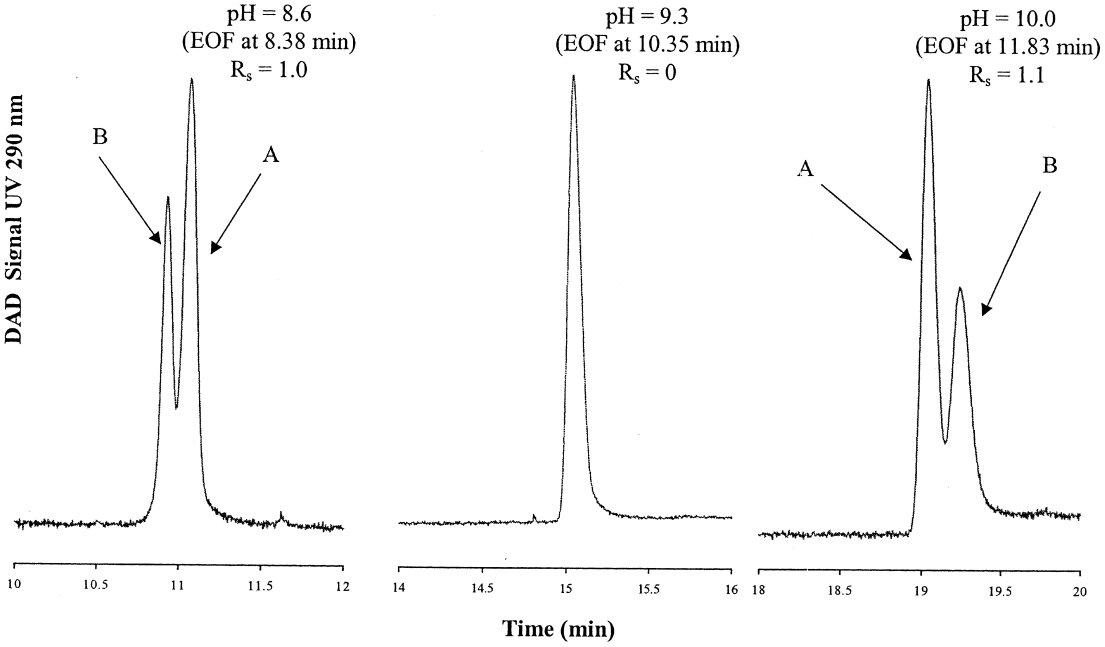


Fig. 6. Chiral separation and reversal of the migration order of 2*R*- and 2*S*-neohesperidin diastereomers at three different pH values. Conditions: electrolyte: 0.1 *M* borate buffer, 5 m*M* CE-β-CD; capillary: uncoated, fused-silica [77 cm (effective length 70 cm)×75 μm I.D.]; voltage: 20 kV; detection: 290 nm; temperature: 25°C; injection: pressure, 2 s.

glycosides in lemon juice. Eriocitrin and hesperidin can be identified as characteristic flavanones and be separated in their 2*S*- and 2*R*-diastereomers according to the achieved chiral method (see Fig. 7)

Both compounds, eriocitrin and hesperidin in their standard solutions in DMF–water, could be separated with different CDs in a 0.1 *M* borate buffer (see Table 1). However, these achievements are not fully transferable to the analysis of lemon juice because of its completely different accompanying matrix. The method has to be optimized in order to achieve the diastereomer resolution in this sample. We chose the borate concentration as a parameter for this optimizing treatment. The amount of boric acid was increased from 0.1 to 0.2 *M*. The applied voltage should correspondingly be adjusted a little lower (from 20 kV to 15 kV) to prevent a high Joule heating which would produce an undesirable dispersion, band broadening, and consequently a lower performance. The electrolyte concentration showed, as expected, a remarkable effect on the migration times and the resolution of the selected analytes. The viscosity of the running buffer at a higher borate concentration increased markedly and accordingly the EOF decelerated. A separation was not achieved with low ionic strength, but was performed with a borate concentration of 0.2 *M*. Under these conditions, a run can take up to 40 min, nevertheless a comparatively good resolution of diastereomers was achieved (see Fig. 7).

A quantitative statement about this topic will be published in a subsequent work.

4. Conclusions

The complete separation of the 2*R*- and 2*S*-diastereomer of six selected flavanone-7-*O*-glycosides (naringin, prunin, narirutin, hesperidin, neohesperidin and eriocitrin) was demonstrated by chiral CE for the first time. The chiral resolution of these analytes in standard solutions was achieved by employing various buffers and CDs. It was observed that the separation decisively depends on the type and the amount of the CDs, the pH of the buffer, and the concentration of the BGE. The dependence of the

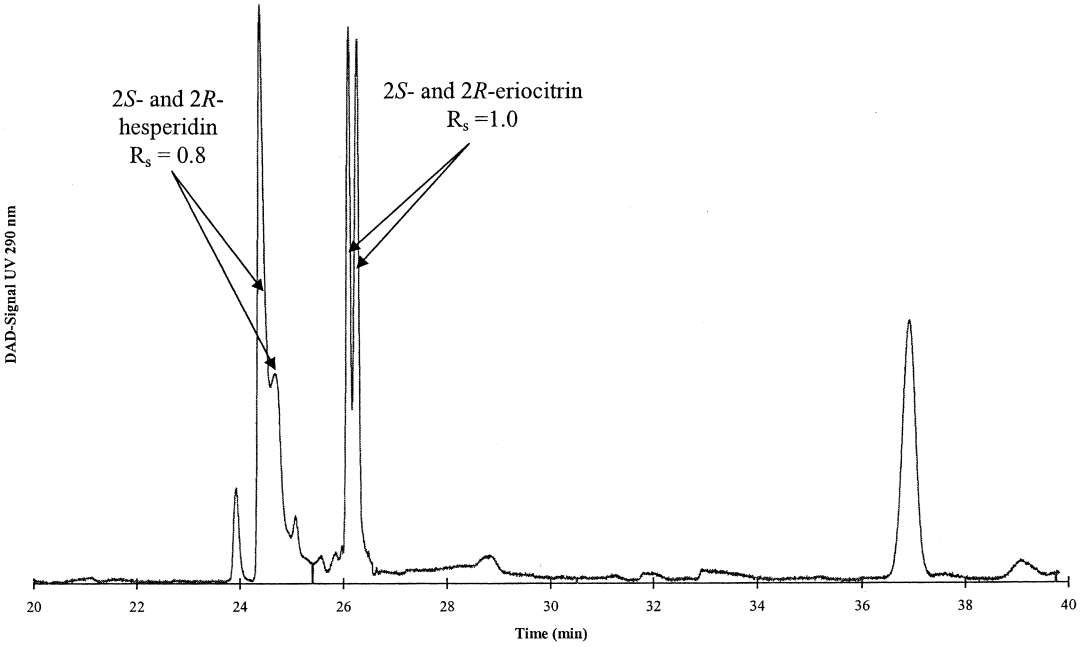


Fig. 7. Chiral separation of 2*S*- and 2*R*-diastereomers of eriocitrin and hesperidin in lemon juice (concentration factor, $F=5$, see Section 2.2). Conditions: electrolyte: 0.2 *M* borate buffer, 5 m*M* γ-CD, pH=10.0; capillary: uncoated, fused-silica [67 cm (effective length 60 cm)×75 μm I.D.]; voltage: 15 kV; detection: 290 nm; temperature: 25°C; injection: pressure, 2 s; EOF at 14.27 min.

separation upon these electrophoretic parameters was discussed in this article. A practical example of this chiral method was performed with lemon juice, thereby eriocitrin and hesperidin could be separated in their diastereomers. A quantitative statement about this topic will be published in a subsequent work together with the analysis of some other fruit juices.

Acknowledgements

We would like to thank the ''Deutsche Forschungsgemeinschaft'' (DFG) for the financial support of this work.

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