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Journal of Chromatography A, 680 (1994) 175–179

JOURNAL OF CHROMATOGRAPHY A

Optimization of separation selectivity in capillary electrophoresis of flavonoids

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Abstract

The migration behaviour of selected flavonoids differing in their degree of hydroxylation was investigated. The influence of the solvent in the injected sample, the surfactant [sodium dodecyl sulphate (SDS)] and pH on the separation selectivity was studied. The organic solvent (methanol or 2-propanol) modifies the interaction between micelles and analytes, thus reducing migration times and resolution. SDS improves the separation at pH 8.3, whereas it has less or no effect at higher pH. At pH 10.5 the separation is mainly regulated by ionization of the hydroxyl groups and borate complexation of the carbohydrate residues.

1. Introduction

Flavonoids are ubiquitous secondary plant metabolites which occur in the free state (aglycone) or as glycosides. These compounds have the basic skeleton of 2-phenylbenzopyrone, and differ in their degree of saturation and the position of hydroxyl, methoxyl and sugar residues. Flavones and flavonols are widespread in different vegetables, fruits and medicinal plants [1], and their analysis has mainly been performed by reversed-phase high-performance liquid chromatography (HPLC) [2]. Recently, capillary electrophoresis (CE) has been proposed as a complementary technique, and the micellar mode introduced by Terabe *et al.* [3] is one of the most widely used CE modes. Micellar electrokinetic chromatography (MEKC) is a hybrid of electrophoresis and chromatography, as micelles originated by the surfactant added to a buffer provide both ionic and hydrophobic interactions. This technique has been applied to the separation of a number of flavonoid-containing drugs [4], and integration of the CE apparatus with UV diode-array detection (DAD) has permitted "on-line" structural information to be obtained as for HPLC [5]. So far, the migration behaviour of flavonoids has received little attention, mainly concerning the role of buffer concentration and sampling time [6,7]. In this work, the influence of pH, the surfactant [sodium dodecyl sulphate (SDS)] and the amount of organic solvent injected on the electrophoretic mobilities and resolution of selected flavonol glycosides was studied.

2. Experimental

2.1. Reagents

Methoxylflavonols (**8**, **9** and **10**) were isolated

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0021-9673/94/$07.00
SSDI 0021-9673(94)00285-H

from *Arnicae flos* according to ref. 8. Quercetin, kaempferol and isorhamnetin glucosides (**2**, **3**, **6** and **7**) and rutinosides (**1**, **4** and **5**) were purchased from Extrasynthese (Genay, France).

SDS and sodium tetraborate were purchased from Sigma (St. Louis, MO, USA). Methanol and 2-propanol were of HPLC grade.

The sample mixtures were prepared dissolving 0.5 mg of each standard in various percentages (10–100%) of organic solvent. Replicate injections ($n = 5$) were made.

2.2. Apparatus and conditions

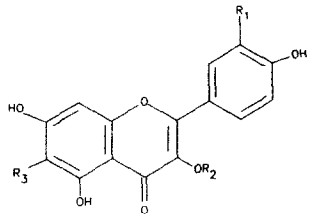
Capillary electrophoretic separations were carried out using a Model 270A apparatus from Applied Biosystems (San Jose, CA, USA) equipped with a 58 cm (to the detector) × 50 μm I.D. fused-silica capillary and a ^{3D}CE system from Hewlett-Packard (Waldbronn, Germany) equipped with a 50 cm (to the detector) × 50 μm I.D. fused-silica capillary. The running buffer was 20 m*M* tetraborate (pH range 8.3–10.5, SDS concentration range 0–100 m*M*). The voltage was 270–300 V/cm; the injection was by aspiration for the Model 270A and by positive pressure for the ^{3D}CE system. The temperature was 30°C and detection was performed at 260 nm.

3. Results and discussion

Quercetin, kaempferol and isorhamnetin are very common flavonols and they occur mainly as glycosides, where the sugar moiety is either a monosaccharide residue (3-O-glucosyl, 3-O-galactosyl) or a disaccharide residue such as 3-O-rutinosyl (Fig. 1). For this reason, compounds **1–10** were considered for investigating the effect of the injected solvent, SDS and pH on electrophoretic mobilities and separation.

3.1. Influence of the organic solvent

Sample preparation is a crucial step in the analysis of flavonoid-containing drugs, and often after a solid-phase purification step the samples are dissolved in solvents, such as methanol or



	R_1	R_2	R_3	Compound
1	OH	Rutinose	H	Quercetin-3-O-Rutinoside
2	H	Glucose	H	Quercetin-3-O-Glucoside
3	OH	Galactose	H	Quercetin-3-O-Galactoside
4	H	Rutinose	H	Kaempferol-3-O-Rutinoside
5	OCH_3	Rutinose	H	Isorhamnetin-3-O-Rutinoside
6	H	Glucose	H	Kaempferol-3-O-Glucoside
7	OCH_3	Glucose	H	Isorhamnetin-3-O-Glucoside
8	OH	Glucose	OCH_3	Quercetin-6-methoxy-3-O-Glucoside
9	H	Glucose	OCH_3	Kaempferol-6-methoxy-3-O-Glucoside
10	OCH_3	Glucose	OCH_3	Isorhamnetin-6-methoxy-3-O-Glucoside

Fig. 1. Structures of the investigated flavonol-3-O-glycosides.

2-propanol. The presence of this organic modifier may cause problems in the separation, and its effect on migration time and resolution needs to be known.

Rutin (**1**) was chosen as a reference standard, and several injections were made keeping constant either the sampling time or the percentage of the solvent present in the sample. As shown in Fig. 2, the migration times decrease linearly with increasing amount (0.2–2 nl) of injected solvent. The organic solvent influences the resolution, as exemplified in Fig. 3, which shows how the separation of quercetin-3-O-glucoside (**2**) and quercetin-3-O-galactoside (**3**) is related to the percentage of methanol used to dissolve the sample. For an injection time of 1 s (about 4 nl of sample), optimum resolution of this critical pair is achieved using less than 30% methanol. Analogous results can be obtained by injecting decreasing volumes (less than 4 nl) of a 100% methanolic solution, as the amount of methanol injected is an essential parameter for the resolution.

For routine analysis of flavonoid drugs, it may be suggested that the sample is dissolved in 30%

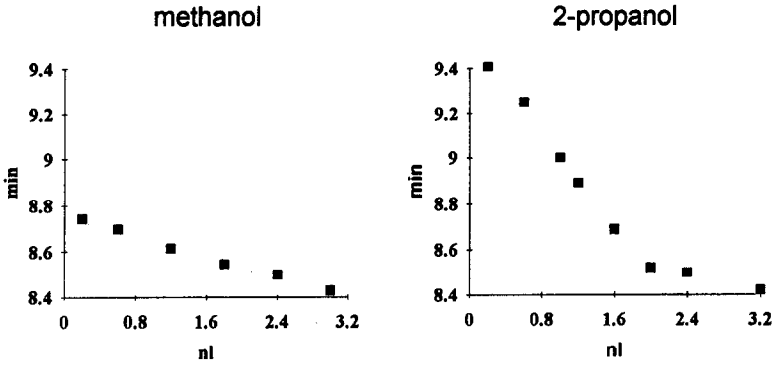


Fig. 2. Influence of the solvent injected on migration times. CE conditions: apparatus, Model 270A equipped with a 58 cm (to the detector) × 50 μm I.D. fused-silica capillary; running buffer, 20 m*M* borate–70 m*M* SDS (pH 8.3); voltage, 270 V/cm; standard, rutin (**1**).

methanol with injection times of 0.5–1.5 s (volumes ca. 2–6 nl).

3.2. *Influence of the surfactant*

A standard mixture of six flavonol-3-O-glucosides was analysed using running buffers with or without SDS. The presence of SDS is crucial for the separation of all components (Fig. 4), as its effect is related to the degree of hydroxylation. SDS decreases the mobilities of kaempferol and isorhamnetin glycosides as compared with quercetin glycosides (Fig. 5), the migration order being Q-Rut (**1**) > Q-Glu (**2**) > K-Rut (**4**) > I-Rut (**5**) > K-Glu (**6**) > I-Glu (**7**). In contrast, in the absence of SDS, kaempferol and isorhamnetin derivatives migrate faster than quercetin derivatives, and with the same sugar moiety [*i.e.*,

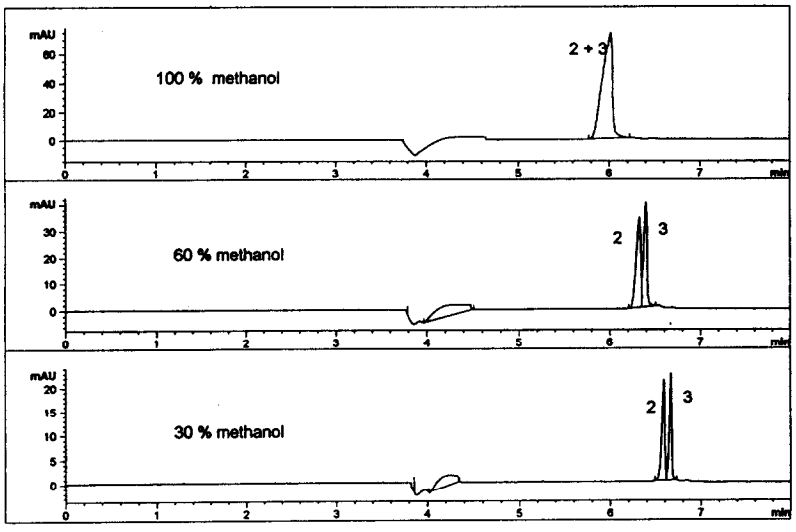


Fig. 3. Influence of percentage of solvent in the sample on resolution. CE conditions: apparatus, ^{3D}CE equipped with a 50 cm (to the detector) × 50 μm I.D. fused-silica capillary; running buffer, 20 m*M* borate–50 m*M* SDS (pH 8.3); voltage, 300 V/cm; injection, 50 mbar, 1 s; for peak identification, see Fig. 1.

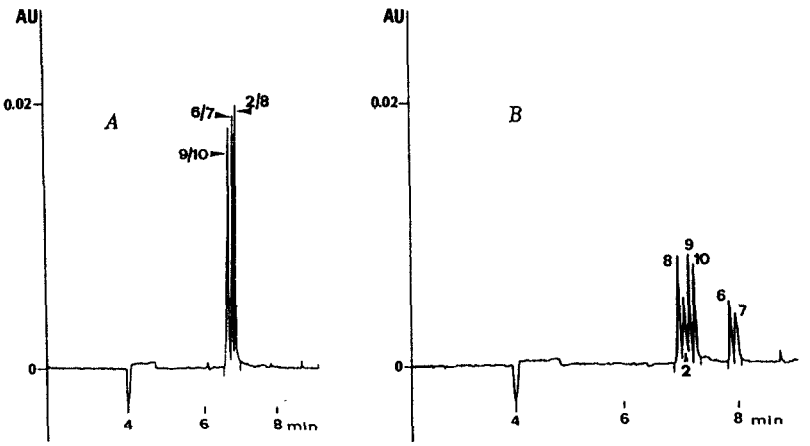


Fig. 4. Influence of the surfactant (SDS) on resolution and mobilities of glucosylflavonols: (A) without SDS and (B) with 50 m*M* SDS. For CE conditions, see Fig. 2; for peak identification, see Fig. 1.

K-Glu (**6**), I-Glu (**7**) and K-Rut (**4**), I-Rut (**5**)] they are not resolved.

3.3. *Influence of pH*

The pH range 8–11 was chosen to exploit better the influence of the electroosmotic flow on the electrophoretic mobility. At pH 8.3 both the aglycone structure and the sugar type have an impact on migration, whereas at pH 9.3 the electrophoretic behaviour is mainly influenced by the linked carbohydrate. At this pH the presence of SDS is less important, and the order of migration of K-Glu (**6**) and I-Glu (**7**) is inverted. On increasing the pH to 10.5, the migration order depends on linked sugars and changes substantially (Fig. 6); the surfactant has no influence on mobilities and resolution.

From these data, it may be concluded that the amount of the organic solvent injected influences the separation as a consequence of modified partitioning of the analytes between micelles and buffer. Further, the surfactant plays an important role at pH 8.3, as it interacts preferentially with highly hydrophobic kaempferol and isorhamnetin derivatives. Higher pH values improve the complexation of flavonols by tetra-

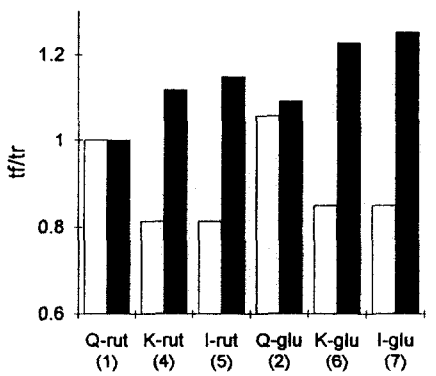


Fig. 5. Effect of SDS on mobilities and resolution of flavonol glucosides and rutinosides at pH 8.3 with 20 m*M* borate: (□) without SDS and (■) with 50 m*M* SDS. t_f = Migration time of the investigated flavonol; t_r = migration time of rutin (**1**).

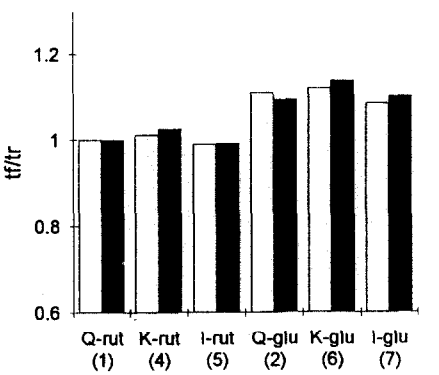


Fig. 6. Electrophoretic behaviour of flavonol glucosides and rutinosides at pH 10.5 with 20 m*M* borate: (□) without SDS and (■) with 50 m*M* SDS.

borate, and the effect of the surfactant is reduced (pH 9.3) or cancelled (pH 10.5).

Acknowledgement

The authors are grateful to CNR–P.F. "Chimica Fine" for providing funds (U.O.P.L. Mauri).

References

[1] J.B. Harbone (Editor), *The Flavonoids, Advances in Research Since 1986*, Chapman & Hall, London, 1994.
[2] P.G. Pietta, P.L. Mauri, E. Manera and P.L. Ceva, *J. Chromatogr.*, 513 (1990) 397.
[3] S. Terabe, K. Otsuda and T. Ando, *Anal. Chem.*, 57 (1985) 834.
[4] P.G. Pietta, P.L. Mauri, A. Rava and G. Sabbatini, *J. Chromatogr.*, 549 (1991) 367.
[5] P.G. Pietta, P.L. Mauri, A. Bruno and L. Zini, *J. Chromatogr.*, 638 (1993) 357.
[6] U. Seitz, P. Oefner, S. Nathakarnkitkool, M. Popp and G. Bonn, *Electrophoresis*, 13 (1992) 35.
[7] P. Morin, F. Villard and M. Dreux, *J. Chromatogr.*, 628 (1993) 153.
[8] I. Merfort, *Phytochemistry*, 31 (1992) 2111.