Separation of Derivatives of 1-(2-Hydroxypropyl)-2-methyl-5-nitroimidazole by Capillary Zone Electrophoresis

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Chiral derivatives of 1-(2-hydroxypropyl)-2-methyl-5-nitroimidazole substituted on the 2-hydroxypropyl chain were separated by capillary zone electrophoresis; the effect of background electrolyte concentration and addition of cyclodextrins and achiral pairing reagent were studied. The best resolution was observed with compound having thiophenyl substituent attached to the aliphatic moiety.

Compounds with a 2-methyl-5-nitroimidazole moiety in their structure are useful chemotherapeutics, antibacterial or antiprotozoal agents [1—6]. Most of these compounds have a substituted 2-hydroxypropyl chain attached to N-1 of the heterocyclic nucleus. Due to the presence of an asymmetrically substituted 2-hydroxypropyl chain in these structures, they all possess one chiral carbon and, therefore, they exist in two enantiomeric forms. Capillary electrophoresis is an excellent method for separation of racemates. It was used for determination of chiral purity of drugs, especially in the drug development process [7—10].

The aim of this paper was the separation of 1-(2-hydroxypropyl)-2-methyl-5-nitroimidazoles *I—VII* (Formula 1) by capillary zone electrophoresis in the presence of cyclodextrins.

Derivatives of 1-(2-hydroxypropyl)-2-methyl-5-nitroimidazole *I—VII* may be divided into two groups according to the heteroatom in position 3 of the 2-hydroxypropyl chain: *i*) compounds *I, III, IV, V* with basic nitrogen, *ii*) compounds *II, VI, VII* with sulfur or chlorine. All these compounds were well separated and their peaks were well resolved by capillary zone electrophoresis (Table 1). Migration times of compounds of the first group which have common basic moiety and differ only in the quality of the side chain should be a function of some physicochemical parameters, *e.g.* log$\{P\}$, solubility parameter, polarity, percent of hydrophilic surface or dipole moment. In fact, we found a good correlation between these calculated data and the observed migration times but only when we used the data calculated for structure *III*, not for aldehyde *VIII*, the proposed product of hydrolysis of the parent compound in the background acidic electrolyte (Scheme 1). Therefore, we believe
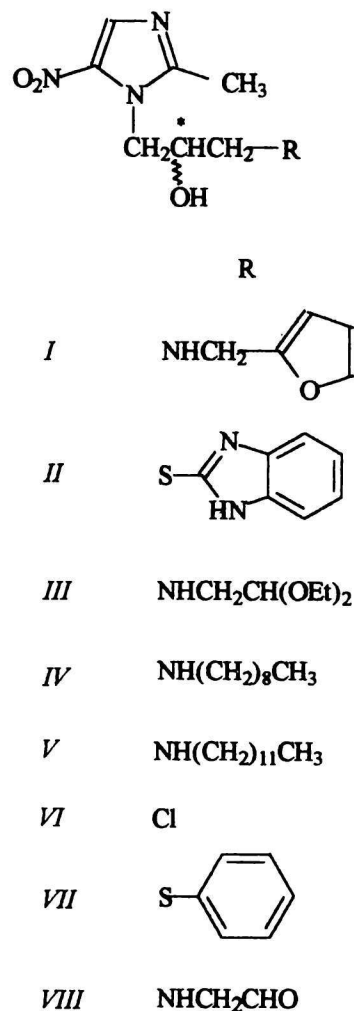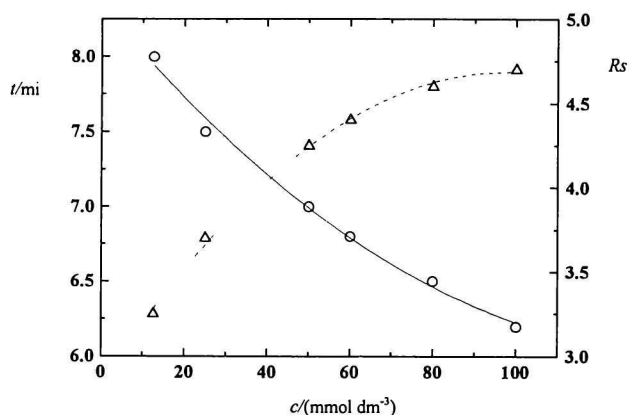

Formula 1

Table 1. Separation of Compounds $I—VII$ by CZE. Capillary 40 cm, c(TRIS/Phosphate Buffer) = 100 mmol dm^{-3}, pH 2.25, 30 kV

Compound	t/min	Rs
I	3.37	
II	3.59	3.98
III	3.76	2.60
IV	3.96	2.23
V	4.30	2.91
VI	6.20	15.71
VII	6.95	6.05

Fig. 1. Dependence of migration time of compound VI (——) and resolution of peaks of compounds VI and VII (·······) on the concentration of background electrolyte.

that the migration time 3.76 min may be ascribed to the acetal structure III and not to aldehyde $VIII$.

Effectivity of the separation process in capillary zone electrophoresis is dependent *inter alia* on concentration of the background electrolyte. We observed that with increasing concentration of the electrolyte the migration times decreased and resolution of peaks increased (Fig. 1). However, concurrently increased also the electrical current according to equation: $\{i\} = 9.32 + 1.37\{c\} - 0.005\{c\}^2$ (where i = current in μA, c = concentration of the background electrolyte in range 10—100 mmol dm^{-3}) which causes nonuniform tem-

perature gradient across the capillary, local changes in viscosity, and subsequent zone broadening.

The presence of cyclodextrins in background electrolyte may substantially affect the resolution of peaks of the studied compounds due to different ability of individual compounds to form complexes with these selectors. Because cyclodextrins, cyclic saccha-
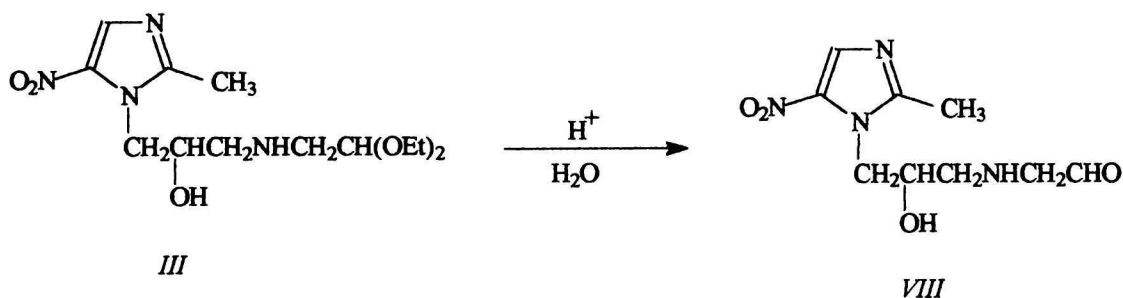
Scheme 1. Hydrolysis of acetal III.

Table 2. Migration Times of the Studied Compounds in the Presence of Cyclodextrins. c(TRIS/Phosphate Buffer) = 60 mmol dm^{-3}, pH 2.45, 30 kV, c(Cyclodextrin) = 20 mmol dm^{-3}

Compound	t/min

Compound	–	A	B	C	D	E
I	3.70	3.96	3.91	4.22	4.04	4.04
II	4.02	4.55	4.37	5.35	4.78	4.59
II^*	–			5.40	4.81	–
III	4.12	4.41	4.32	4.69	4.48	4.49
IV	4.35	8.60	6.06	10.39	9.60	6.32
V	4.74	9.04	8.17	10.83	10.67	9.40
VI	8.85	10.03	9.87	11.81	10.30	10.45
VI^*	–			11.94		–
VII	9.77	20.18	16.56	27.58	17.46	16.77
VII^*	–		16.63		–	17.34

A: B-CD, B: G-CD, C: D-CD, D: HP-B-CD, E: HP-G-CD.
* Data for enantiomers.

Table 3. Migration Times and Resolution of Peaks of Compounds *IV* and *V* in the Presence of Cyclodextrins. c(TRIS/Phosphate Buffer) = 100 mmol dm^{-3}, pH 2.60, 30 kV, c(Cyclodextrin) = 20 mmol dm^{-3}

Cyclodextrin	t/min		Rs
	IV	*V*	
	4.70	5.03	4.66
G-CD	6.54	8.29	6.75
HP-G-CD	6.70	9.00	9.46
HP-G-CD/S	7.30	11.80	16.02
B-CD	8.38	8.65	1.35
HP-B-CD	8.93	9.45	2.21
D-CD	10.25	10.54	1.03

HP-G-CD/S = (2-hydroxypropyl)-γ-cyclodextrin + sodium dodecyl sulfate (20 mmol dm^{-3}).

Fig. 2. Electrophoreogram of derivatives of 1-(2-hydroxypropyl)-2-methyl-5-nitroimidazole. Capillary 48.5 cm × 0.05 mm, c(TRIS/phosphate buffer) = 100 mmol dm^{-3}, pH 2.5, c(HP-G-CD) = 15 mmol dm^{-3} (*1 = I, 2 = III, 3 = IV, 4 = V, 5a, 5b* = enantiomers of compound *VII*.)

Fig. 3. Dependence of resolution of peaks of compounds *I* and *III* (– – –), *IV* and *V* (········), (+)- and (–)-*VII* (——) on the concentration of HP-G-CD.

ride oligomers, are chiral, the formed complexes with particular enantiomers may have different stability which is advantageous for analysis of chiral compounds.

Migration time of the studied compounds *I—VII* varied according to cyclodextrin added into the electrolyte (Table 2). The cyclodextrins had remarkable effect on separation of compounds *IV* and *V* which differ only in number of methylene groups in the side chain. The best resolution was achieved with selectors composed of eight glucose units (γ-cyclodextrins: G-CD and HP-G-CD) (Table 3). Surprisingly, complexes of these compounds with β-cyclodextrins (B-CD, HP-B-CD, D-CD) are more stable than those of the previous group but the selectivity is substantially lower. The highest resolution of peaks of compounds *IV* and *V* was observed in the background electrolyte with added HP-G-CD and achiral ion-pairing hexane-1-sulfonic acid which in some cases enhanced the resolution of enantiomers [11]. 1-(2-Hydroxypropyl)-2-methyl-5-nitroimidazole derivatives, classified into group *i*, were not resolved into enantiomers in our experiments, opposite to compounds belonging into group *ii*. The best resolution of enantiomers was obtained for racemate *VII* (Fig. 2), however, resolution was dependent on concentration of the used selector. Concentration of HP-G-CD had specific effect on resolution of peaks of the studied compounds; resolution of compounds *IV* and *V* was enhanced while that of pair *I* and *III* decreased with increasing concentration of the selector. Higher concentration of HP-G-CD improved also the resolution of enantiomers of (±)-*VII* (Fig. 3); resolution of these enantiomers was pH-dependent; it was highest at pH 2.4 and decreased with increasing pH (Rs = 0 at pH 4.9).

Probably the proximity of an ionized nitrogen to the chiral carbinol carbon in compounds of the group *i* was the reason of unsuccessful separation of their enantiomers by capillary zone electrophoresis.
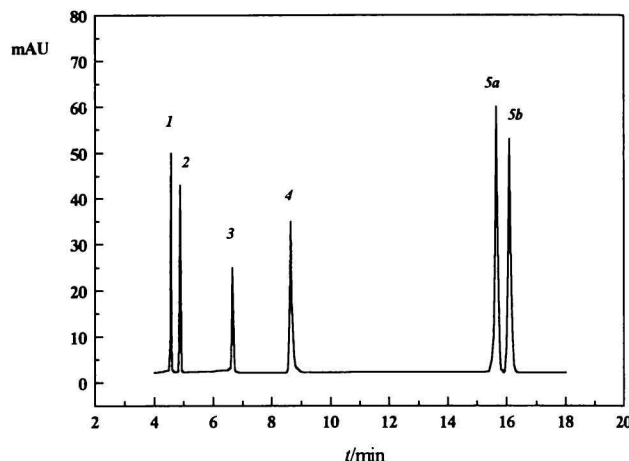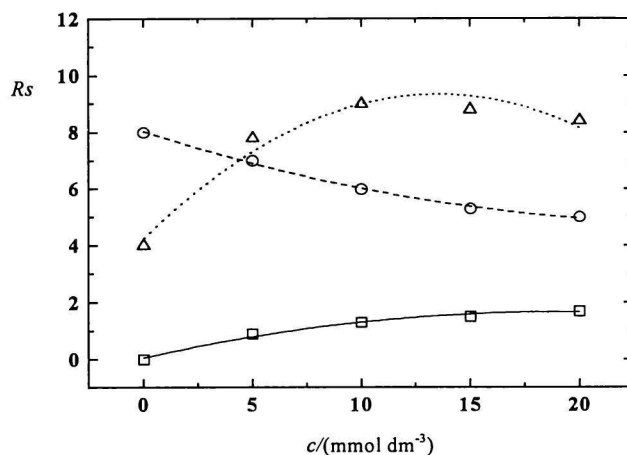
EXPERIMENTAL

An HP ^{3D} Capillary Electrophoresis System (HP ^{3D} CE, Waldbronn, Germany) equipped with diode array detector (190—600 nm) was used for analysis. The electrophoreograms were collected at fixed UV wavelengths of 215 nm and 320 nm with data processed on an HP ChemStation. A 48.5 cm (effective length 40 cm) untreated fused silica capillary tube (Hewlett—Packard) with 0.05 mm ID was used for analyses. The background electrolyte consisted of TRIS/phosphate buffer. The sample solutions were prepared by dissolving 10 mg of the selected compound in 2 cm^3 of methanol and filling up to 10 cm^3 with the appropriate background electrolyte. Before pressure injection (7500 Pa s), the solution was filtered through a 0.20 μm nylon membrane filter.

Computations of physicochemical parameters were carried out with a Molecular Modeling Pro™ from ChromMaSoft (Erpolzheim, Germany). Resolution of peaks was calculated according to equation $Rs = 2(t_2 - t_1)/(w_1 + w_2)$, where t = migration time, w = baseline peak width (in time), migration time reproducibility varied in range ± 6 %.

β-Cyclodextrin (B-CD), (2-hydroxy-propyl)-β-cyclodextrin (HP-B-CD), (2-hydroxypropyl)-γ-cyclodextrin (HP-G-CD), γ-cyclodextrin (G-CD), sodium dodecyl sulfate were from Fluka, Switzerland, heptakis(2,6-di-O-methyl)-β-cyclodextrin (D-CD) and heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (T-CD) were purchased from Sigma, USA.

1-(3-Alkylamino-2-hydroxypropyl)-2-methyl-5-nitroimidazole derivatives *I—VII* were prepared in our laboratory [12, 13].

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