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Determination of limiting ionic mobilities and dissociation constants of some local anaesthetics

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ABSTRACT

The limiting ionic mobilities and thermodynamic acid dissociation constants were calculated from isotachophoretic experiments for the local anaesthetics procaine, tetracaine, lidocaine, trimecaine, bupivacaine, cinchocaine, diperodone, diocaine, cocaine, psicaine-neu, tropacocaine, amylocaine, β-eucaine and leucinocaine. The pH values at which the local anaesthetics with very similar limiting ionic mobilities can be isotachophoretically separated were determined from simulated mobility curves. The measuring apparatus employed a high-frequency contactless conductivity detector.

INTRODUCTION

Capillary isotachophoresis has already been used to separate local anaesthetics (LAs), *e.g.*, in the control of the composition of various pharmaceuticals [1–3], the determination of trimecaine in plasma [4] and separations of model mixtures [5,6] (the LAs were characterized in terms of the relative zone heights at pH 4.75, 5.4 and 6.2). Hence the determination of the mobilities and dissociation constants of these compounds would be useful not only for isotachophoresis but also for other electromigration methods. The utility of isotachophoresis as a technique for the measurement of these physicochemical constants has already been reported [7–13]. It is based on the measurement of the observed R_E values ($R_E = R_S/R_L$, where R_S is the resistance of the sample zone and R_L is the resistance of the leading electrolyte) with different leading electrolyte pH_L values.

This work was aimed at the determination of the above characteristics for fourteen LAs as the limiting ionic mobilities are not available in the literature and only a few pK values, some of them approximate, are known. On the basis of these values, experimental conditions can be found for isotachophoretic separation of the LAs, including those with very similar ionic mobilities.

EXPERIMENTAL

The isotachophoretic measurements were carried out on an apparatus with a PTFE separating capillary tube and a high-frequency contactless conduc-

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tivity detector [14–16]. The sensing electrodes of the detector are not in galvanic contact with the electrolyte inside the capillary tube, hence electrode processes which may occur on the electrodes of direct-contact conductivity detectors, especially at high pH, are prevented. The measuring cell and the part of capillary tube inside the cell were thermostated at 25°C.

It follows from the structures of the LAs that they are mostly basic, forming stable crystalline salts with acids which are readily soluble in water. The LAs used were obtained from the Faculty of Medicine, Palacký University, Olomouc, Czechoslovakia, in the form of the hydrochlorides, except for leucinocaine, which was in the form of the methanesulphate. In isotachophoretic experiments, it is often difficult to find a suitably slow terminating ion, and therefore measured LAs were used in stead of samples as the terminating electrolytes, at a concentration of 0.005 *M*. The leading ion was Na^+ in all the measurement, its concentration in the leading electrolyte being 0.01 *M* and its limiting ionic mobility assumed to be $51.9 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}. Acetic acid, DL-α-alanine (both of analytical-reagent grade from Lachema, Brno, Czechoslovakia), 2-morpholinoethanesulphonic acid (Fluka, Buchs, Switzerland), 2-morpholinopropanesulphonic acid (Sigma, St. Louis, MO, USA), glycylglycine (Nutritional Biochemicals, Cleveland, OH, USA) and L-histidine (Merck, Darmstadt, Germany) were used one after another as the buffering anionic counter compounds in the leading electrolyte.

The same approach for the determination of the mobilities and p*K* values as described previously [7,8] was used, *i.e.*, measured R_E values were analysed by the computational program which iteratively fits the experimental data by a simulated curve using the least-squares method. Limiting ionic mobilities and pK_A values of all compounds used as input data in the calculations are listed in Table I.

The limiting anionic mobility of 2-morpholinopropanesulphonic acid has not been published previously, so it was determined isotachophoretically using system 0.01 *M* Cl^- plus ethanolamine as leading electrolyte. Ethanolamine (Sigma) was freshly distilled before measurement.

TABLE I

PHYSICO-CHEMICAL CONSTANTS USED AS INPUT DATA IN CALCULATION (25°C)

U^0 = Limiting ionic mobility (10^{-9} m^2 V^{-1} s^{-1}); pK_A = thermodynamic acid dissociation constant; HAc = acetic acid; MES = 2-morpholinoethanesulphonic acid; MOPS = 2-morpholinopropanesulphonic acid; Gly–Gly = glycylglycine; His = L-histidine; Ala = DL-α-alanine; EA = ethanolamine.

Compound	U^0	pK_A
Cl^-	−79.1[a]	−2[a]
HAc	−42.4[a]	4.756[a]
MES	−28.0[a]	6.095[a]
MOPS	−26.9[b]	7.2[c]
Gly–Gly	−31.5[d]	8.2[c]
His	−28.3[e]	9.33[e]
Ala	−32.2[e]	9.857[e]
EA	44.3[a]	9.498[a]

[a] Values taken from ref. 11.
[b] Value obtained isotachophoretically.
[c] Values taken from ref. 17.
[d] Value taken from ref. 13.
[e] Values taken from ref. 12.

RESULTS AND DISCUSSION

The results of the measurements, *i.e.*, R_E^{expt} values, together with the experimental conditions are listed in Table II. The R_E^{theor} values, effective mobilities (U_{eff}) and pH_S values in the sample zones calculated on the basis of a least-squares fit are also given. The determination of the limiting ionic mobilities (U^0) and pK_A values was performed according to the isotachophoretic steady-state model and the values obtained are given in Table III. The dissociation constants found in the literature [18,19] are also given for comparison.

When the pH_L value of the leading electrolyte is low and thus the pH_S values in the zones are also low, then the weak bases are virtually completely dissociated; the LAs are in the cationic form and the ionic mobilities equal the effective mobility (U_{eff}). The mobility curves (U_{eff} *vas* pH_S; Fig. 1a–d) were calculated for four groups of LAs according to the limiting mobilities.

It is apparent from the mobility curves that diperodone differs from the other LAs and can be separated from them in systems with a pH_L of the leading electrolyte from 6 to 7. The effective mobility of the other LAs is efficiently influenced in systems with pH *ca.* 7–8. However, LA separations at higher pH values show certain limitations. The concen-

TABLE II

OBSERVED AND CALCULATED PHYSICO-CHEMICAL VALUES FOR LOCAL ANAESTHETICS (25°C)

pH_L = Experimentally measured pH of the leading electrolyte; buffer = compound used as buffer in the leading electrolyte (abbreviations as in Table I); R_E^{exptl} = experimentally measured R_E value; R_E^{theor} = theoretically calculated R_E value; U_{eff} theoretically calculated effective mobility corrected for ionic strength (10^{-9} m^2 V^{-1} s^{-1}); pH_S = theoreticaly calculated pH in the sample zone.

LA	pH_L	Buffer	R_E^{exptl}	R_E^{theor}	U_{eff}	pH_S
Cinchocaine	4.42	HAc	2.81	2.77	17.33	4.11
	4.72	HAc	2.80	2.78	17.30	4.43
	5.62	MES	2.79	2.81	17.13	5.41
	6.25	MES	2.80	2.81	17.09	6.04
	6.60	MES	2.80	2.82	17.14	6.38
	6.87	MES	2.81	2.83	17.04	6.63
	7.11	MOPS	2.87	2.87	16.75	6.89
	7.46	MOPS	2.96	2.95	16.27	7.24
Diperodone	4.42	HAc	2.79	2.74	17.54	4.12
	4.72	HAc	2.80	2.75	17.46	4.43
	5.62	MES	2.82	2.86	16.80	5.40
	5.95	MES	2.87	2.95	16.34	5.70
	6.30	MES	3.04	3.13	15.41	5.98
	6.60	MES	3.32	3.32	14.49	6.17
	6.87	MES	3.60	3.50	13.79	6.28
Diocaine	4.42	HAc	2.70	2.70	17.83	4.13
	4.72	HAc	2.72	2.70	17.79	4.44
	6.25	MES	2.74	2.73	17.62	6.05
	6.60	MES	2.70	2.72	17.70	6.40
	6.87	MES	2.71	2.73	17.67	6.66
	7.11	MOPS	2.76	2.75	17.47	6.91
Bupivacaine	4.42	HAc	2.57	2.56	18.77	4.15
	4.72	HAc	2.57	2.57	18.73	4.46
	5.62	MES	2.58	2.59	18.56	5.43
	6.25	MES	2.58	2.60	18.47	6.06
	6.60	MES	2.59	2.61	18.46	6.39
	6.87	MES	2.65	2.64	18.28	6.63
	7.11	MOPS	2.72	2.70	17.80	6.90
	7.46	MOPS	2.85	2.83	16.96	7.23
	7.72	Gly–Gly	2.99	2.98	16.21	7.45
	7.90	Gly–Gly	3.11	3.13	15.39	7.59
Leucinocaine	5.03	HAc	2.53	2.51	19.13	4.78
	6.25	MES	2.53	2.53	18.99	6.07
	7.00	MOPS	2.54	2.54	18.93	6.82
	7.53	MOPS	2.55	2.57	18.73	7.36
	7.90	Gly–Gly	2.58	2.58	18.67	7.69
	8.12	Gly–Gly	2.63	2.62	18.38	7.90
Psicaine-neu	4.42	HAc	2.46	2.47	19.43	4.14
	5.03	HAc	2.48	2.48	19.36	4.79
	5.62	MES	2.48	2.50	19.23	5.44
	6.25	MES	2.53	2.51	19.19	6.07
	7.00	MOPS	2.56	2.54	18.92	6.82
	7.53	MOPS	2.63	2.64	18.23	7.33
Trimecaine	4.42	HAc	2.44	2.42	19.86	4.17
	5.62	MES	2.45	2.45	19.65	5.45
	6.25	MES	2.46	2.47	19.47	6.07
	6.87	MES	2.52	2.53	19.05	6.61
	7.11	MOPS	2.63	2.63	18.29	6.89
	7.46	MOPS	2.85	2.83	17.01	7.20
	7.72	Gly–Gly	3.02	3.06	15.77	7.42
	7.90	Gly–Gly	3.21	3.28	14.69	7.55
	8.24	Gly–Gly	3.81	3.81	12.65	7.76
	8.39	Gly–Gly	4.14	4.07	11.85	7.83

(Continued on p. 268)

TABLE II (*continued*)

LA	pH_L	Buffer	R_E^{expti}	R_E^{theor}	U_{eff}	pH_S
β-Eucaine	5.03	HAc	2.36	2.34	20.54	4.80
	6.25	MES	2.37	2.35	20.42	6.09
	7.53	MOPS	2.39	2.37	20.24	7.38
	8.12	Gly–Gly	2.39	2.40	20.10	7.93
	8.67	Gly–Gly	2.48	2.51	19.11	8.41
	9.06	His	2.70	2.76	17.44	8.80
	9.33	His	3.03	2.98	16.18	8.99
	9.55	His	3.21	3.20	15.02	9.12
	9.83	His	3.48	3.48	13.81	9.24
	10.12	Ala	4.28	4.28	11.23	9.48
Tetracaine	4.42	HAc	2.35	2.31	20.77	4.19
	5.62	MES	2.36	2.33	20.59	5.46
	6.60	MES	2.38	2.35	20.50	6.42
	7.00	MOPS	2.41	2.40	20.01	6.82
	7.46	MOPS	2.50	2.52	19.04	7.26
	7.72	Gly–Gly	2.59	2.63	18.28	7.48
	7.90	Gly–Gly	2.72	2.77	17.44	7.63
	8.14	Gly–Gly	2.96	2.99	16.14	7.80
	8.39	Gly–Gly	3.27	3.26	14.78	7.95
	8.64	Gly–Gly	3.60	3.55	13.61	8.07
Lidocaine	5.03	HAc	2.34	2.29	20.97	4.81
	6.25	MES	2.33	2.34	20.57	6.08
	6.71	MOPS	2.38	2.40	20.02	6.53
	7.11	MOPS	2.50	2.52	19.05	6.89
	7.53	MOPS	2.75	2.80	17.18	7.24
	7.86	Gly–Gly	3.17	3.21	15.03	7.51
	8.05	Gly–Gly	3.50	3.51	13.71	7.63
	8.28	Gly–Gly	3.96	3.90	12.36	7.75
Cocaine	5.03	HAc	2.28	2.25	21.37	4.82
	6.25	MES	2.28	2.27	21.21	6.09
	7.11	MOPS	2.29	2.30	20.92	6.95
	7.53	MOPS	2.33	2.36	20.39	7.36
	7.82	Gly–Gly	2.41	2.43	19.83	7.65
	8.28	Gly–Gly	2.62	2.65	18.22	7.99
	8.69	Gly–Gly	2.96	2.94	16.34	8.23
	8.81	His	3.43	3.38	14.23	8.45
	9.06	His	3.83	3.91	10.79	8.61
	9.33	His	4.49	4.45	10.81	8.74
Amylocaine	5.03	HAc	2.13	2.10	22.78	4.84
	6.25	MES	2.15	2.14	22.42	6.10
	7.00	MOPS	2.23	2.25	21.33	6.83
	7.53	MOPS	2.46	2.50	19.22	7.28
	7.86	Gly–Gly	2.77	2.83	17.06	7.55
	8.05	Gly–Gly	3.03	3.07	15.66	7.68
	8.28	Gly–Gly	3.43	3.40	14.21	7.80
	8.45	Gly–Gly	3.69	3.64	13.20	7.89
Procaine	4.72	HAc	2.13	2.09	22.98	4.53
	6.60	MES	2.10	2.10	22.94	6.46
	7.53	MOPS	2.17	2.15	22.36	7.39
	8.14	Gly–Gly	2.23	2.25	21.43	7.94
	8.39	Gly–Gly	2.34	2.34	20.58	8.15
	8.72	His	2.62	2.62	18.41	8.47
	9.01	His	2.88	2.94	16.38	8.68
	9.19	His	3.19	3.19	15.11	8.79
	9.33	His	3.42	3.40	14.19	8.86

TABLE II (*continued*)

LA	pH_L	Buffer	R_E^{exptl}	R_E^{theor}	U_{eff}	pH_S
Tropacocaine	5.03	HAc	2.12	2.07	23.22	4.85
	6.25	MES	2.10	2.08	23.10	6.12
	7.53	MOPS	2.13	2.10	22.85	7.40
	8.12	Gly–Gly	2.14	2.14	22.50	7.95
	8.69	Gly–Gly	2.27	2.29	21.03	8.42
	9.06	His	2.55	2.57	18.69	8.80
	9.33	His	2.83	2.82	17.09	8.97
	9.55	His	3.02	3.05	15.75	9.09
	9.83	His	3.34	3.33	14.42	9.20
	10.12	Ala	4.23	4.22	11.38	9.43

tration of the non-ionized free base in the zone increases with increasing pH_L. The non-ionized form of some bases is poorly soluble in water and may precipitate in the zone at a certain pH. With a common concentration of the leading electrolyte of 0.01 M, diperodone precipitates at about pH_L 7.0, diocaine at pH_L 7.4, cinchocaine at pH_L 7.7, psicaine-neu at pH_L 7.8, bupivacaine at pH_L 8.1 and leucinocaine at pH_L 8.3. The pH_L values at which the sample precipitates can be increased by decreasing the leading electrolyte concentration; therefore, at higher pH_L of the leading electrolyte, analysis should be performed with a lower concentration of the leading electrolyte.

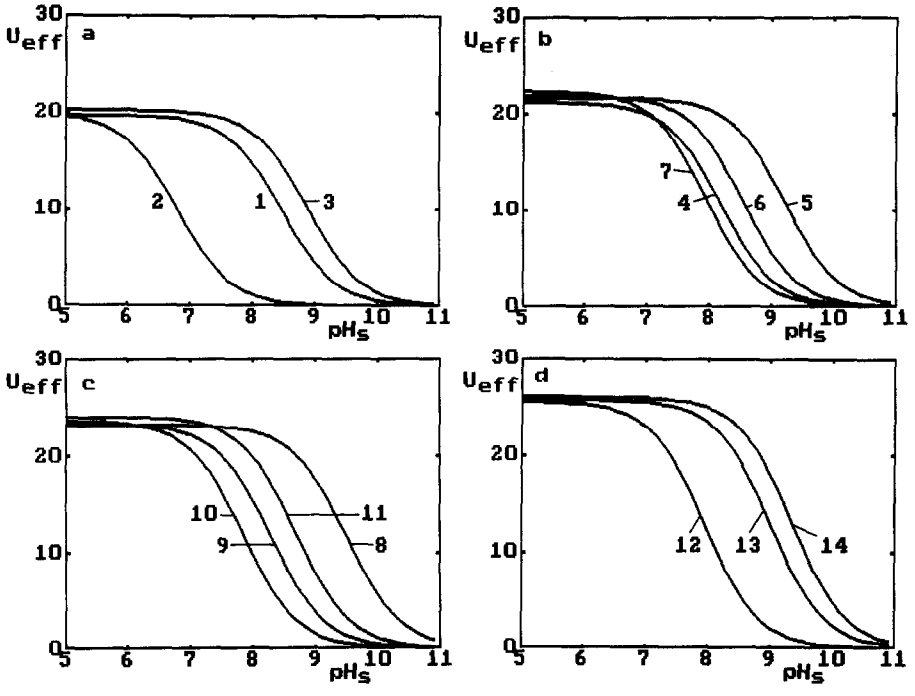

Fig. 1. Mobility curves for the local anaesthetics. U_{eff} = effective mobility (10^{-9} m^2 V^{-1} s^{-1}), pH_S = pH of the sample zone. (a) 1 = Cinchocaine; 2 = diperodone; 3 = diocaine; (b) 4 = bupivacaine; 5 = leucinocaine; 6 = psicaine-neu; 7 = trimecaine; (c) 8 = β-eucaine; 9 = tetracaine; 10 = lidocaine; 11 = cocaine; (d) 12 = amylocaine; 13 = procaine; 14 = tropacocaine. The U_{eff} and pK values are not corrected for the ionic strength.

TABLE III

CALCULATED LIMITING IONIC MOBILITIES, U^0, AND THERMODYNAMIC ACID DISSOCIATION CONSTANTS, pK_A (25°C)

U^0 in 10^{-9} m^2 V^{-1} s^{-1}; pK_{lit} = dissociation constant from literature; σ = standard deviation of a single measurement.

Compound	U^0	σ	pK_A	σ	pK_{lit}
Cinchocaine	19.7	0.1	8.47	0.08	8.31[a]
Diperodone	19.9	0.3	6.79	0.07	8.44[a]
Diocaine	20.2	0.1	8.88	0.34	–
Bupivacaine	21.2	0.1	8.19	0.02	–
Leucinocaine	21.7	0.1	9.21	0.05	9.4[b]
Psicaine-neu	21.9	0.1	8.54	0.09	–
Trimecaine	22.4	0.2	7.95	0.02	7.96[a]
β-Eucaine	23.2	0.1	9.50	0.02	9.35[a]
Tetracaine	23.3	0.2	8.29	0.02	8.5[b]
Lidocaine	23.6	0.2	7.85	0.02	7.84[a]
Cocaine	24.0	0.2	8.69	0.02	8.4[b]
Amylocaine	25.5	0.2	7.96	0.02	8.09[a]
Procaine	25.7	0.2	9.01	0.02	8.98[a]
Tropacocaine	26.0	0.2	9.36	0.01	9.9[b]

[a] From ref. 19.
[b] From ref. 18.

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