CAPILLARY ISOTACHOPHORESIS OF PIPERIDINOETHYLESTERS OF THE ALKOXYPHENYLCARBAMIC ACIDS

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Conditions for the separation and determination of N-[2-(alkoxyphenylcarbamoyloxy)-ethyl]-piperidinium chlorides (analogues of heptacaine) by the capillary isotachophoresis have been studied. For the successful separation of lower alkoxy derivatives the acetate buffer solution, $pH_L = 4{\cdot}75$, was found to be a suitable leading electrolyte (K^+ as the leading ion) and β-alanine a suitable terminator. The separation can be achieved even in the acid medium with H^+ as the leading ion and with a voluminous terminating cation. For instance, pseudoaconitine or N-[2-(2-heptyloxyphenylcarbamoyloxy)-ethyl]-benzylpiperidinium chloride were found to be acceptable. The steric hindrance of the solvation, characteristic for 2-alkoxy isomers, makes their separation from 3- or 4-alkoxy isomers possible, with the exception of methoxy derivatives.

Čižmárik and Borovanský[1] prepared the series of piperidinoethylesters of the 2-, 3-, and 4-alkoxyphenylcarbamic acids. These compounds exhibit local anaesthetic and antimicrobial effects. The basic physico-chemical parameters of these compounds (the solubility of the base, pH of turbidity, surface tension, and the distribution constants) were determined by Pešák and coworkers[2] and the TLC data were published by Čižmárik and coworkers[3]. The results of the pharmacological and physico-chemical research revealed that the biological activity and certain physico-chemical parameters of these compounds depend on the nature and position of the alkoxy group on the benzene ring. The highest activity in course of the topical and infiltration anaesthesia is exhibited by heptacaine (substance *XIX*) that has also an anti-arhythmic effect. The basic analytical properties of heptacaine were published by Šubert and coworkers[4], who proposed their determination using the extraction photometry and acidimetry.

However, difficulties will arise if heptacaine has to be separated and determined in mixtures with its homologues and isomers or with other local anaesthetics. The R_F values of some of its homologues or isomers for the separation chromatography and particularly for the adsorption thin-layer chromatography[3] differ only negligibly. In the case of gas chromatographic separation the labile carbamates are thermally decomposed so that the use of suitable derivatives (*e.g.*, of N-methyl derivatives) of the substances that are to be separated is unavoidable[5,6].

Capillary isotachophoresis has been used for the separation of only some local anaesthetics[7]. For similar purposes also the isotachophoresis on a carrier has been used[8]. Due to a sufficiently high basicity of the piperidine nitrogen atom the series of substances under study can be separated using the electromigration methods. The aim of this study was to find the conditions for the separation and determination of the mixtures under study using the capillary isotachophoresis.

EXPERIMENTAL

The apparatus for capillary isotachophoresis (Research Laboratories and Workshops of the Palacký University, Olomouc), with coupled columns was used in these experiments. The currents in the pre-separation PTFE capillary column (230 × 0·8 mm I.D.) and in the analytical capillary column (230 × 0·3 mm I.D.) were adjusted to 200 μA and 50 μA, respectively. Both capillary columns were equipped with the conductivity detectors. Microsyringes Hamilton were used for sampling and the sampling volumes varied from 1 to 10 μl.

Solutions of the substances under study and their model mixtures were prepared using the deionized water. All reagents used in the working systems were of the analytical grade.

RESULTS AND DISCUSSION

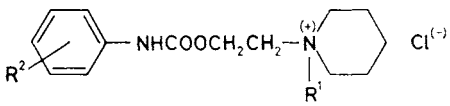
List of substances under study and some of their constants are given in Table I. The practically identical dissociation constants of homologues and isomers (*cf.* Table I) mean that only the difference in their molecular weight (homologue increment) can be exploited for their separation by isotachophoresis. The rather high molecular weight of the derivatives requires to carry out the separation at pH < 6 that ensures their total protonization. The suitable working systems are listed in Table II.

In the system A that is suitable for both the identification and determination of the individual members of the series of homologues all the derivatives from 2-methoxy up to 2-heptyloxy derivative can be separated (Fig. 1, curve *a*), the derivatives from 3-methoxy up to 3-pentyloxy derivative (Fig. 1, curve *b*), and the derivatives from 4-methoxy up to 4-butoxy (Fig. 1, curve *c*). The derivatives mentioned above give linear calibration graphs for sample amounts from 1 to 100 nmol. The higher homologues in individual isomer series form mixed zones (M), the total length of which does not correspond (not even approximately) to the sum of the injected amounts. The formation of the "mixed" zone can be ascribed to the fact that the critical micellar concentration of the higher members in the series of homologues is surpassed due to the concentration isotachophoretic effect. The number of separable homologues in the given series of isomers is in agreement with the published data for the surface tension and for the lipo-hydrophilic parameter π. The surface tension is substantially higher for 2-alkoxy isomers so that the ability to form micelles is lower.

It is well known that the ability of cation tensides towards the formation of micelles decreases with the decreasing pH. Indeed, in the acid medium a higher number of

homologues can be separated. Thus, in the system B the series from 2-methoxy up to 2-nonyloxy derivatives was successfully separated (Fig. 2). This system represents an analogue of the working systems used for the separation of the relatively mobile inorganic cations with Li^+ or $Tris^+$ as terminating ions (*cf.*[9]). However, with pseudo-

TABLE I
Compounds under study



Compound	R^1	R^2	M_r	pK_a[a]	log K_d[a]
IR-1	H	H	284·79	—	2·69
I	H	2-OCH_3	314·79	8·86	2·71
II	H	3-OCH_3	314·79	8·86	2·91
III	H	4-OCH_3	314·79	8·87	2·55
IV	H	2-OC_2H_5	328·81	8·88	3·17
V	H	3-OC_2H_5	328·81	8·87	3·35
VI	H	4-OC_2H_5	328·81	8·90	3·00
VII	H	2-OC_3H_7	342·84	—	3·81
VIII	H	3-OC_3H_7	342·84	—	4·06
IX	H	4-OC_3H_7	342·84	—	3·78
X	H	2-OC_4H_9	356·88	—	4·37
XI	H	3-OC_4H_9	356·88	—	4·62
XII	H	4-OC_4H_9	356·88	—	4·27
XIII	H	2-OC_5H_{11}	370·89	—	4·97
XIV	H	3-OC_5H_{11}	370·89	—	5·08
XV	H	4-OC_5H_{11}	370·89	—	4·90
XVI	H	2-OC_6H_{13}	384·95	—	5·31
XVII	H	3-OC_6H_{13}	384·95	—	5·53
XVIII	H	4-OC_6H_{13}	384·95	—	5·43
XIX	H	2-OC_7H_{15}	398·98[b]	—	—
XX	H	3-OC_7H_{15}	398·98	—	—
XXI	H	4-OC_7H_{15}	398·98	—	—
XXII	H	2-OC_8H_{17}	413·50	—	—
XXIII	H	3-OC_8H_{17}	413·50	—	—
XXIV	H	4-OC_8H_{17}	413·50	—	—
XXV	H	2-OC_9H_{19}	427·03	—	—
XXVI, N-25	C_5H_{11}	2-OC_5H_{11}	485·81	—	—
XXVII, H + B	$H_2CC_6H_5$	2-OC_7H_{15}	489·10	—	—

[a] Ref.[2]; [b] heptacaine.

TABLE II

Optimum working systems

System[a]	Leading ion (c, mol l^{-1})	Counterion (c, mol l^{-1})	pH_L[b]	Terminator (c, mol l^{-1})
A	K^+ (0·01)	acetate	4·75	β-alanine (0·03)
B	H^+ (0·005)	Cl^- (0·005)	≈2·5	pseudoaconitine (0·001)
C	H^+ (0·005)	Cl^- (0·005)	≈2·5	N-25[c] (0·005)
D	H^+ (0·01)	Cl^- (0·01)	≈2·0	H + B[c] (0·003)

[a] The leading electrolyte contained always 0·05% of polyvinylalcohol Gohsenol GM-14 L (Nippon Gohsei, Osaka, Japan) as an additive; [b] pH_L means the value of pH for the leading electrolyte; [c] *cf.* Table I.

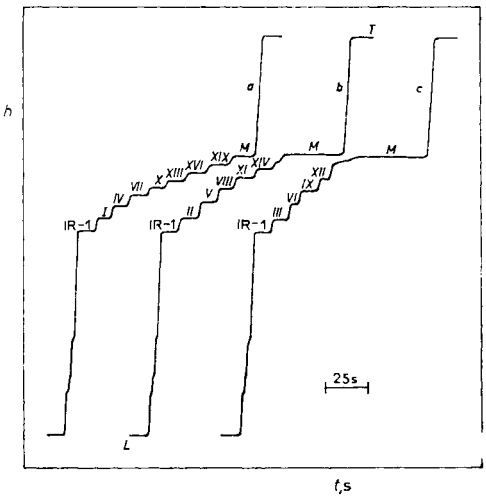


FIG. 1

Isotachophoresis of piperidinoethylesters of alkoxyphenylcarbamic acid. *a* — 2--alkoxy; *b* — 3-alkoxy; *c* — 4-alkoxy derivatives. Working system A, denotation *cf.* Table I. 2 nmol of each substance were sampled. The minor zones correspond to impurity traces in the leading electrolyte

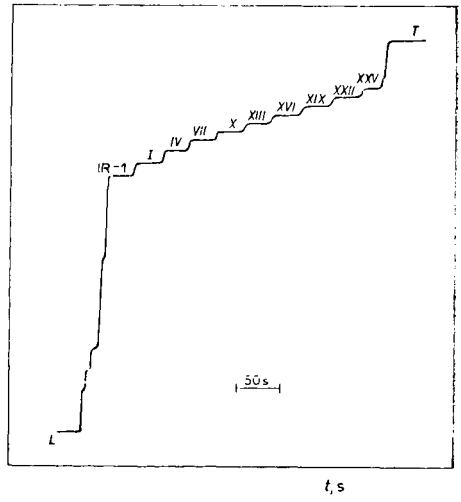


FIG. 2

Separation of 2-alkoxy derivatives. Working system B, denotation *cf.* Table I. 2 nmol of each substance were sampled. Minor zones — *cf.* Fig. 1

aconitinium chloride as terminator (M_r of cation = 630·3) even voluminous organic cations can be separated. The conditions of the isotachophoretic analysis with H^+ as the leading ion are not correctly adjusted and the reproducibility of the quantitative analysis depends on the buffer capacity of the terminating electrolyte that must be frequently exchanged (replaced). Pseudoaconitinium chloride, that is not easily available, can be replaced by other cations of high molecular weight. The use of some quatenary ammonium salts of piperidine ethylesters of the alkoxyphenylcarbamic acids for this purpose can serve as an example. The *XXVI* (N-25 from ref.[10]) and *XXVII* (H + B from ref.[10,11]) derivatives (Table I) used as terminators (systems C and D) can be used for the separation of the homologues from 2-methoxy up to 2-heptyloxy derivatives (Figs 3 and 4).

Isomers with identical molecular weights and with practically identical pK_a values (Table I) should not be separable by isotachophoresis. Indeed, the compounds *I*, *II*, and *III* had not been separated. Starting with ethoxy derivatives, the 2-alkoxy isomers have a higher effective mobility than the respective 3- or 4-alkoxy isomers. We obtain two zones, the first one corresponds to the content of 2-alkoxy derivative and the second one to the sum of 3-alkoxy and 4-alkoxy derivatives (Fig. 5). The difference between the relative step heights of the zones increases from the ethoxy

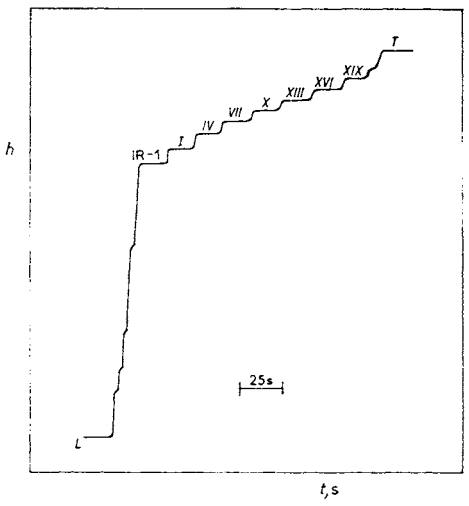


FIG. 3

Separation of 2-alkoxy derivatives. Working system C, denotation *cf.* Table I. 2 nmol of each substance were sampled. Minor zones — *cf.* Fig. 1

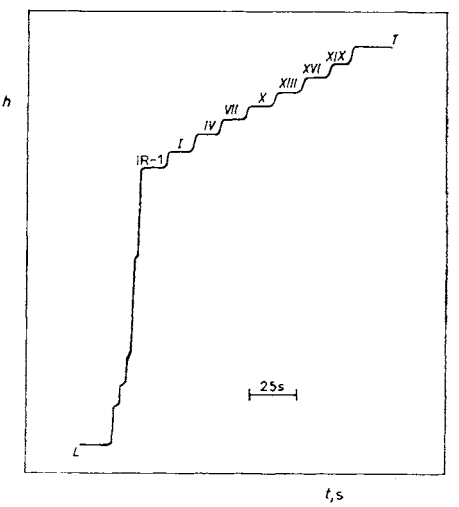


FIG. 4.

Separation of 2-alkoxy derivatives. Working system D, denotation *cf.* Table I. 2 nmol of each substance were sampled. Minor zones — *cf.* Fig. 1

derivatives up to pentyloxy derivatives, for which it reaches the maximum value. However, for higher members of the series the difference cannot be correctly determined as the zones for 3- and 4-alkoxy derivatives are in fact "micellar".

This interesting behaviour can be apparently explained only considering the steric hindrance of the piperidine nitrogen solvation. The measured value of the distribution constant[2] for the first member (IR-1) of the series is evidently lower (log K_D =

TABLE III

Differences between the relative step heights of zones of 2-alkoxy and 3-alkoxy isomers in the working system A

Alkoxy group	rsh, %[a] 2-alkoxy	rsh, % 3-alkoxy	Difference %
$-OCH_3$	54·5	54·5	0·0
$-OC_2H_5$	57·6	58·5	0·9
$-OC_3H_7$	60·0	61·8	1·8
$-OC_4H_9$	62·0	64·7	2·7
$-OC_5H_{11}$	63·8	66·7	2·9
$-OC_6H_{13}$	65·7	68·5[b]	2·8
$-OC_7H_{15}$	67·8	70·5[b]	2·7

[a] Relative step height (rsh) is expressed with regard to the rsh of the terminating zone in % (rsh of β-alanine = 100%); [b] the micellar nature of the zone is involved.

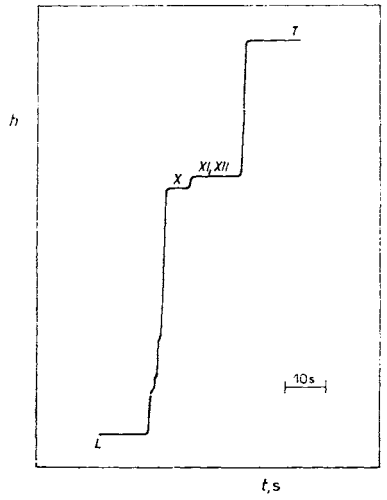
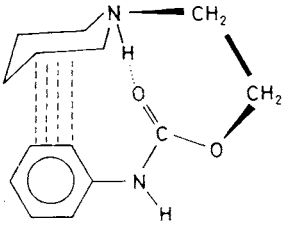


FIG. 5

Separation of 2-, 3-, and 4-butoxy isomers. Working system A, denotation *cf.* Table I. 1 nmol of each substance was sampled. Minor zones — *cf.* Fig. 1

= 2·69) than the value calculated under the assumption of the additivity of the distribution constant logarithms[12]. The sum obtained for the ethylester of phenylcarbamic acid ($\log K_D = 2{\cdot}30$) and piperidine ($\log K_D = 0{\cdot}85$) is substantially higher(for the distribution between n-octanol and water). The decrease of the actual value of $\log K_D$ for such structures with a side chain is explained by the interaction of dipoles of the side chain with π electrons of the aromatic nucleus and is denoted as "folding"[12].

This effect is also in agreement with *ab initio* SCF computations[13] of the electron distributions for 1-[2-(2-methoxyphenylcarbamoyloxy)-ethyl]-piperidine, (piperidinoethylester of 2-methoxyphenylcarbamic acid). According to these computations the aromatic nucleus carries a rather high negative charge[13,14] and the hydrogens of the piperidine ring have a rather positive charge. Assuming that the —NH—CO— group is in its more stable *trans* configuration[15,16], the folding in the case of the derivatives under study can be illustrated by the following structure:



Still more substantial difference has been found between the calculated and observed value of $\log K_D$ of dimethylaminoethylester of phenylcarbamic acid[17], where the folding value for the $-N(CH_3)_2$ group ($\log K_D = -0{\cdot}95$) gives much better results than the common value for this group on an aliphatic chain ($\log K_D = -0{\cdot}30$). The end of the oriented ethylcarbamate chain comes evidently very close to the 2-alkoxy group, the alkyl part of which is moreover bound by the hydrophobic interaction with the piperidine ring. The cation charge becomes screened, which is the cause of its higher mobility. The differences of the relative step heights of zones of the 2-alkoxy and 3-alkoxy isomers are given in Table III.

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