Journal of Chromatography, 520 (1990) 403–410
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 1808

Retention of some organic electrolytes in ion-pair reversed-phase high-performance liquid and reversed-phase high-performance thin-layer chromatographic systems

M. L. BIEGANOWSKA*, A. PETRUCZYNIK and M. GADZIKOWSKA
Department of Inorganic and Analytical Chemistry, Medical Academy, Staszica 6, 20-081 Lublin (Poland)

ABSTRACT

Cetyltrimethylammonium bromide (cetrimide) and tetrabutylammonium chloride were employed as ion-pairing reagents in reversed-phase ion-pair chromatography. The optimization of the retention and selectivity for some N-phenylamides of benzoylacetic acid was carried out by changing the content of the organic modifier (methanol) and the concentration of the ion-pairing reagent in the mobile phase.

INTRODUCTION

The chromatography of ionizable substances such as organic acids often presents problems in terms of retention, column efficiency and peak symmetry [1,2]. The theoretical dependence of the capacity factors on the pK_a values of the solutes, on the pH of the buffer and on the concentration of the ion-pair reagent has been discussed by several workers [3–7]. In this study, the optimization of the retention and selectivity for some N-phenylamides of benzoylacetic acid was carried out by changing the content of the organic modifier and of the ion-pairing reagent [cetyltrimethylammonium bromide (cetrimide) and tetrabutylammonium chloride (TBA-Cl)] in the mobile phase.

The compounds under investigation have a complicated structure (hydrophilic and hydrophobic substituents) and, owing to their biological activity [8], make an interesting set of substances to study in reversed-phase ion-pair chromatography and in structure–activity relationships.

The purpose of these investigations was to establish whether or not cetrimide and TBA-Cl are suitable ion-pairing reagents for chromatographic separation of N-phenylamides, and whether the retention data obtained can be used in studies of quantitative structure–activity relationships (QSAR) [9,10].

EXPERIMENTAL

Column high-performance liquid chromatography (HPLC) was performed using a liquid chromatograph (produced by the Institute of Physical Chemistry of the Polish Academy of Sciences, Warsaw, Poland) equipped with a 200-ml syringe pump,

0021-9673/90/$03.50

a 5-μl injector valve and a UV detector operated at 254 nm. The reference sample concentration for HPLC was about 0.1 mg/ml in the eluent. Stainless-steel columns (100 × 3.8 mm I.D. and 250 × 4 mm I.D.) were packed with 10-μm Lichrosorb RP-18 (E. Merck, Darmstadt, F.R.G.). The void volume was determined by injection of pure methanol. The mobile phase was passed through the chromatographic system until a constant retention of the test solutes was obtained; usually, 50 ml of eluent were sufficient. The flow-rate was 1.2 ml min^{-1}. All measurements were made at ambient temperature.

High-performance thin-layer chromatography (HPTLC) was carried out in sandwich chambers with a glass distributor, using 10 × 10 cm precoated HPTLC plates of RP-18 F_{254} (E. Merck). Samples of 1 μl of 0.1% (w/v) solutions of the solutes in methanol were spotted 1 cm from the edge and developed over a distance of 8.5 cm. The spots of the compounds were localized under UV light at 254 nm.

The compounds studied were synthesized in the Department of Pharmaceutical Chemistry of the Medical Academy of Cracow, Poland and their biological activities were determined in the Department of Pharmacology of the Medical Academy of Cracow.

RESULTS AND DISCUSSION

Fig. 1 shows plots of the capacity factors (k') of N-phenylamides of benzoylacetic acid as a function of the volume percentage of methanol in the mobile phase.

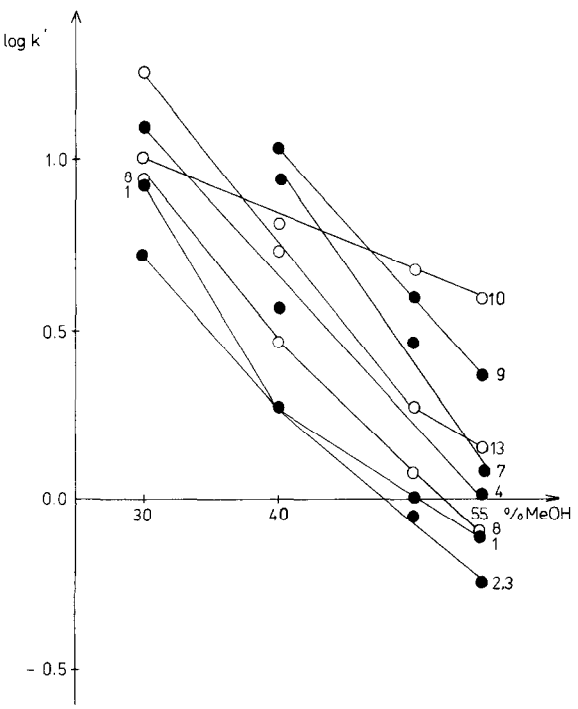


Fig. 1. Plots of log k' *vs.* % (v/v) methanol (MeOH) in the aqueous mobile phase. Column: 100 × 3.8 mm I.D. For identification of solutes, see Table I.

The effect of solvent composition in these systems (water or buffer solution + organic modifier) is frequently expressed by the semi-empirical equation [11,12]

$$\log k' = \text{constant} + n(\%\ \text{water}) \quad (1)$$

which has been reported in previous studies on liquid–liquid systems [13]. An increase in the concentration of methanol causes a decrease in the retention times. The plots are linear and sometimes cross, indicating changes in the sequence of the solutes and in their selectivity of separation. The best results were obtained with 40% of methanol.

Fig. 2 shows the plot of k' as a function of the pH of the mobile phase. The buffer pH stated is that measured in the undiluted buffer and not in the final eluent. A strong retention is observed at low pH, when the ionization of the compounds is suppressed.

Fig. 3 shows plots of logarithm of capacity factor as a function of the volume percentage of methanol in the mobile phase containing 0.2% cetrimide and 0.005 *M* phosphate buffer. For ion-pairing systems, in spite of strong ionization at neutral pH (7.38), the retention is suitable owing to the formation of ion pairs with the ammonium cations. In almost all instances linear relationships were obtained according to eqn. 1.

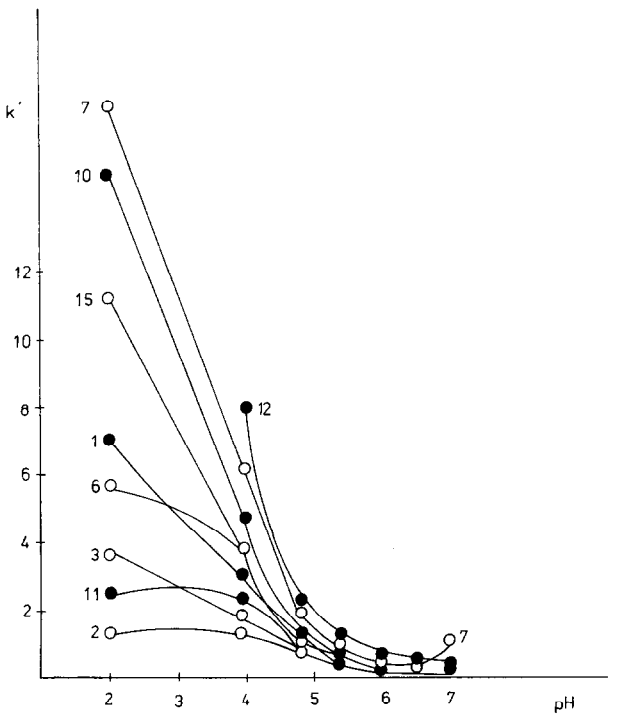


Fig. 2. Plots of k' *vs.* pH of the mobile phase containing 65% methanol and 0.01 *M* phosphate buffer. Column: 250 × 4 mm I.D.

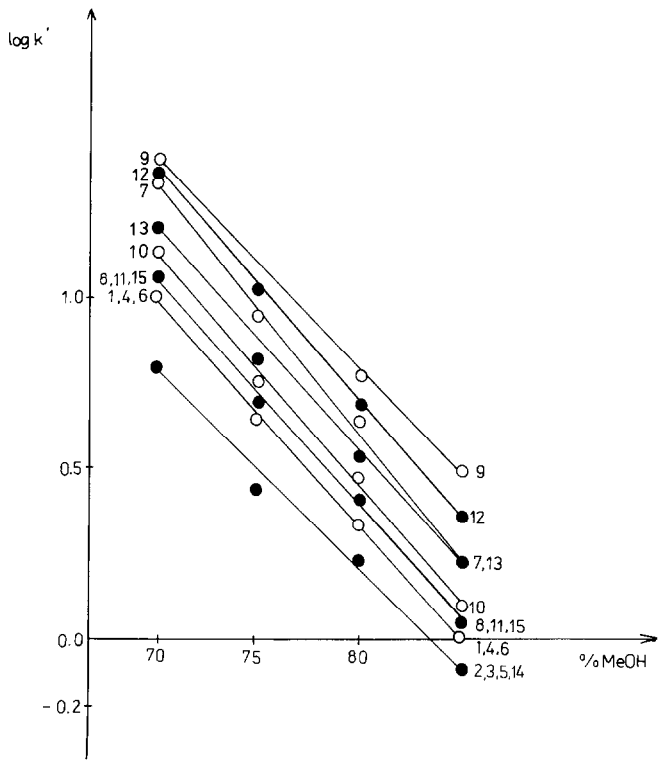


Fig. 3. Plots of log k' *vs.* % (v/v) methanol in the mobile phase containing 0.2% cetrimide and 0.005 *M* phosphate buffer (pH = 7.38). Column: 100 × 3.88 mm I.D.

Fig. 4 illustrates that an increase in cetrimide concentration results in stronger retention. The highest increases in retention were obtained for 0.02–0.05% cetrimide and the best selectivity with *ca.* 0.1% cetrimide in the eluent.

For HPTLC (Fig. 5), similar results were obtained to those in HPLC (Fig. 4). Changes in the lower concentration ranges of the ion-pair reagent cause the largest increase in retention. Comparison of the R_M and log k' data is of practical interest owing to the potential use of reversed-phase ion-pair TLC as a pilot technique for the optimization of reversed-phase ion-pair column chromatography.

Good results were also obtained for TBA-Cl as ion-pairing reagent (Figs. 6 and 7). The chromatogram of a mixture of solutes shows symmetrical peaks and relatively good separation, which indicates the high efficiency of this system. Both TBA-Cl and cetrimide ion-pairing reagents are suitable; the selectivities differ and the choice depends on the set of compounds to be analysed. The following equations for correlations between partition coefficient (P), k' and biological activity were obtained:

$$\log P = 2.03 \log k' + 1.79; \quad n = 12, r = 0.870, \text{S.D.} = 0.47 \tag{2}$$

$$-\log C_i = 4.54 \log k' - 4.84; \quad n = 12, r = 0.841, \text{S.D.} = 1.10 \tag{3}$$

$$-\log C_b = 3.53 \log k' - 4.84; \quad n = 12, r = 0.928, \text{S.D.} = 0.78 \tag{4}$$

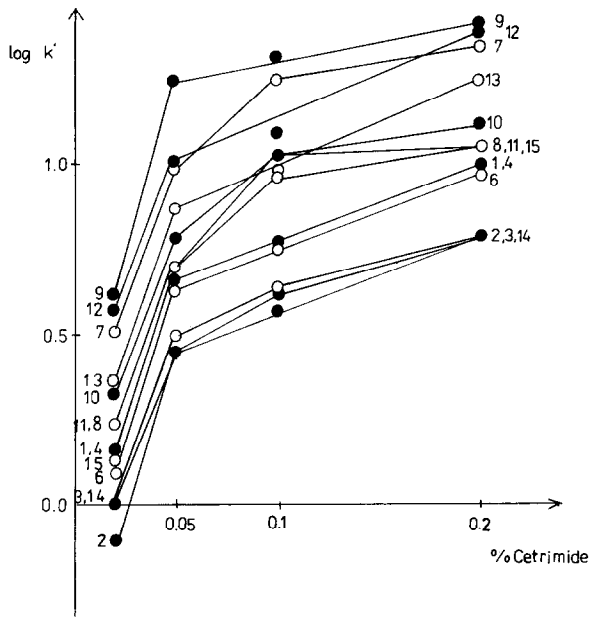


Fig. 4. Plots of log k' *vs.* % (v/v) cetrimide in the mobile phase containing 70% methanol and 0.005 M phosphate buffer (pH = 7.38). Column: 100 × 3.8 mm I.D.

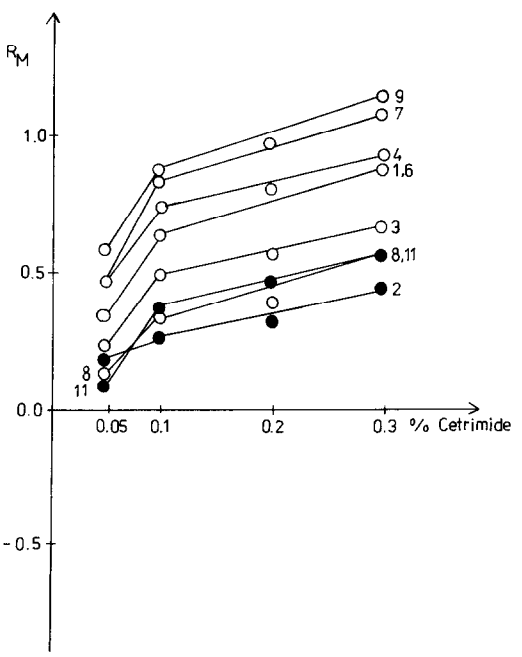


Fig. 5. Plots of R_M (HPTLC–RP-18) values *vs.* % cetrimide in the mobile phase containing 75% methanol and 0.01 M phosphate buffer (pH = 7.38).

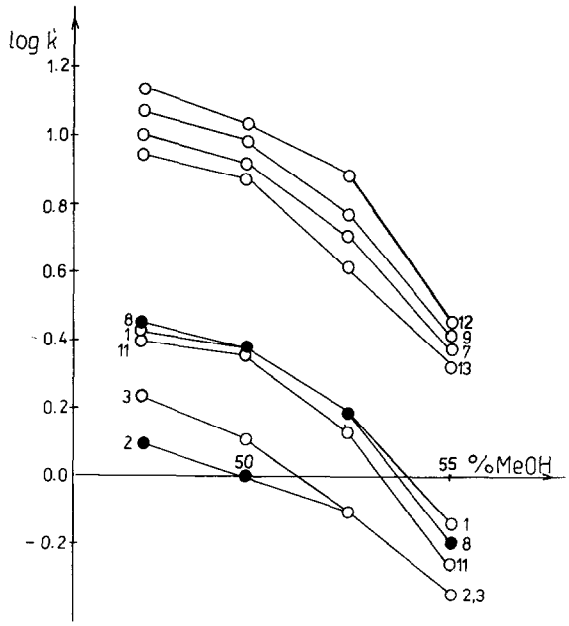


Fig. 6. Plots of log k' *vs.* % (v/v) methanol in the mobile phase containing 0.005 *M* TBA-Cl and 0.005 *M* phosphate buffer (pH = 7.38). Column: 250 × 4 mm I.D.

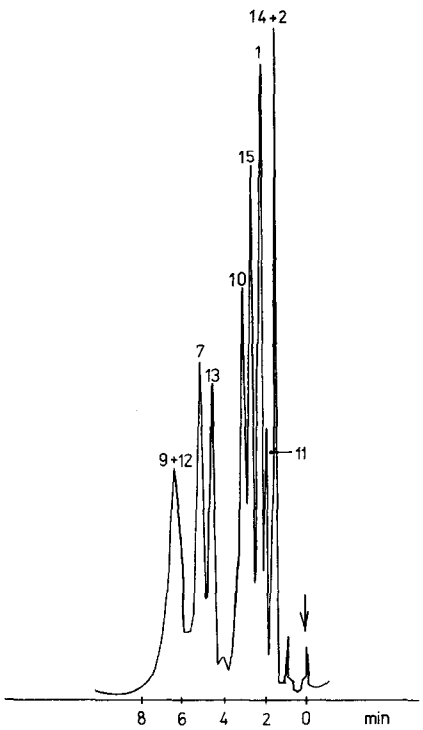
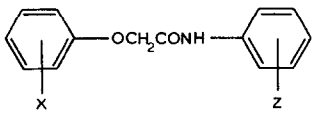


Fig. 7. Chromatogram for the separation of a mixture of solutes. Mobile phase: 52.5% (v/v) methanol, 0.01 *M* TBA-Cl and 0.005 *M* phosphate buffer (pH = 7.38). Column: 250 × 4 mm I.D.

TABLE I

LOG *P* VALUES AND PHARMACOLOGICAL DATA FOR THE INVESTIGATED COMPOUNDS



No.	Compound		Inhibition of synthetase of prostaglandins, ID_{50} (log μM)	Strength of binding to albumin, ID_{50} (log μM)	Log *P*
	X	Z			
1	4-CH_3	2-COOH	1.58	2.30	2.863
2	4-CH_3	3-COOH	2.70	3.67	2.863
3	–	2-COOH	2.04	2.78	2.336
4	2-CH_3	2-COOH	2.70	2.65	2.863
5	2-CH_3	3-COOH	2.70	3.56	2.863
6	3-CH_3	2-COOH	2.70	2.40	2.863
7	4-CH_3	2-COOH, 4-Cl	0.28	1.40	3.610
8	4-CH_3	2-COOH, 6-Cl	2.12	2.75	3.610
9	4-CH_3	2-COOH, 4-Br	0.00	1.34	3.819
10	4-CH_3	2-COOH, 4-CH_3	0.94	2.00	3.390
11	4-CH_3	2-COOH, 6-CH_3	2.70	2.80	3.390
12	4-Cl	2-COOH, 4-Cl	0.04	1.40	3.838
13	4-CH_3	2-COOH, 5-NO_2	–	–	–
14	2-CH_3	4-COOH	–	–	–
15	4-Cl	2-COOH	–	–	–

Eqn. 2 illustrates the correlation of log *P* (partition coefficient calculated from hydrophobic fragmental constants [14]) (Table I) with log k' values (obtained for 75% of methanol in the eluent, Fig. 3) (expressed by Collander's equation [15]: $\log P = b\log k' + a$), which confirms, as expected, that as in reversed-phase systems the hydrophobicity of these compounds is a dominant factor; reversed-phase ion-pair chromatographic systems can thus be used in studies of structure–activity relationships.

Eqns. 3 and 4 show correlations between chromatographic data and pharmacological activity, expressed by two effects: the inhibition of synthetase of prostaglandins (i), and the strength of binding to albumin (ii) (Table I); *C* denotes the micromolar concentration of the investigated solutes corresponding to the ID_{50}; *n* is the number of compounds and the regression coefficients, *r*, indicate that reversed-phase ion-pair chromatographic systems can be used in this approach to predict the activities of a congeneric series of compounds.

REFERENCES

1 B. A. Bidlingmeyer, *J. Chromatogr. Sci.*, 18 (1980) 525.
2 R. Gloor and E. L. Johnson, *J. Chromatogr. Sci.*, 15 (1977) 413.
3 Cs. Horvath, W. Melander and I. Molnar, *Anal. Chem.*, 49 (1977) 142, 2295.
4 J. H. Knox and R. A. Hartwick, *J. Chromatogr.*, 204 (1981) 3.

5 A. Bartha, Gy. Vigh, H. A. H. Billiet and L. de Galan, *Chromatographia*, 20 (1985) 587.
6 H. A. H. Billiet, J. Vuik, J. K. Strasters and L. de Galan, *J. Chromatogr.*, 184 (1987) 153.
7 S. H. Hansen and P. Helboe, *J. Chromatogr.*, 285 (1984) 53.
8 M. Bieganowska, *J. Liq. Chromatogr.*, 5(1) (1982) 39.
9 C. Hansch and A. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley-Interscience, New York, 1979.
10 T. Braumann, *J. Chromatogr.*, 373 (1986) 191.
11 B. L. Karger, J. N. Le Page and N. Tanaka, in Cs. Horváth (Editor), *High-Performance Liquid Chromatography*, Vol. 1, Academic Press, New York, 1980, pp. 113–205.
12 C. S. Horvath and W. R. Melander, *J. Chromatogr. Sci.*, 15 (1977) 393.
13 E. Soczewiński and C. A. Wachtmeister, *J. Chromatogr.*, 7 (1962) 311.
14 R. F. Rekker, *The Hydrophobic Fragmental Constant: Its Derivation and Application–A Means of Characterizing Membrane Systems*, Elsevier, Amsterdam, 1977.
15 R. Collander, *Acta Chem. Scand.*, 5 (1951) 774.