Journal of Chromatography, 114 (1975) 297–300

CHROM. 8412

Note

A paper electrophoretic study of ion-pair formation

IX. The migration of small organic ions in strong acids

BRUNO RINALDUZZI
Laboratorio di Cromatografia del C.N.R., Via Romagnosi 18A, Rome (Italy)
(Received May 13th, 1975)

In previous papers in this series, we have shown that the separation of cobalt(III) complexes by paper electrophoresis is strongly influenced by the anion of the electrolyte used[1–3]. We also examined the interactions between a series of inorganic anions with mono-, di- and tri-charged cations (used as electrolytes), the separation of alkaloids and the separation of quaternary ammonium salts of the synthetic curare type. Interactions that change the quality or sequence of the separation were found under different circumstances[4–6].

In this work, we wanted to obtain some data on the possible effects of ion-pair formation when small organic ions are separated in paper electrophoresis.

The experimental conditions that are necessary in order to obtain unambiguous results are rather stringent. We decided that working with buffers to which other ions are added should be avoided so that one can be sure that the ionization equilibria involved are not upset. Strong bases are unsuitable for use in paper electrophoresis, leaving only strong acids as the electrolytes that could be examined. As examples of small organic ions we decided to study a range of amino acids and amines. We could thus examine the effect of a large range of functional groups.

Some results have already been presented in previous papers in this series. There is no measurable difference in the ratio of the movements of tetramethylammonium and tetraethylammonium ions in a wide range of electrolytes[5]. Acetate, formate and oxalate were studied together with a series of inorganic anions and some interactions were recorded, which could also have been due to complex formation, however[4].

EXPERIMENTAL AND RESULTS

Paper electrophoresis was carried out in a Camag high-voltage electrophoresis apparatus as described previously[1–3]. The operating conditions are given in the tables and figures.

The migration of a number of amino acids in various mineral acids relative to the movement of lysine is shown in Fig. 1. Lysine was run on all sheets and thus differences between various runs could be corrected.

There seems to be little difference between the different acids studied except for

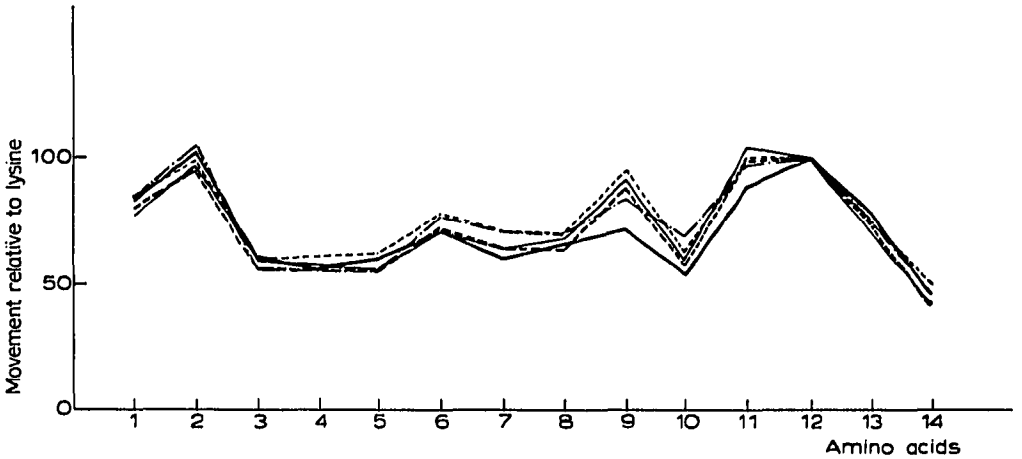

Fig. 1. Electrophoretic movement of amino acids (relative to lysine = 100) in high-voltage paper electrophoresis. A voltage of 500 V was applied for 3 h at 6° on Whatman No. 1 filter-paper. The spots were detected with ninhydrin. 1 = DL-α-Alanine; 2 = glycine; 3 = DL-methionine; 4 = L-glutamic acid; 6 = L-leucine; 6 = DL-serine; 7 = DL-threonine; 8 = DL-valine; 9 = L(+)-arginine·HCl; 10 = L-aspartic acid; 11 = L-histidine·HCl; 12 = L(+)-lysine·HCl; 13 = L-proline; 14 = L(−)-tyrosine. ———, 0.5 *N* HCl; — — —, 0.5 *N* $HClO_4$; ———, 0.5 *N* H_2SO_4; - - - -, 0.5 *N* HNO_3; -·-·-·, 0.5 *N* CCl_3COOH.

arginine and histidine, which are retarded in 0.5 *N* sulphuric acid compared with their migration in the other acids. Clearly, the difference in aliphatic chain length and the presence of a benzene ring, hydroxyl groups, etc., do not affect the interaction with the anions of inorganic acids in a measurable way. Only when two basic groups are present are differences noted and even these differences are not great.

Table I shows the electrophoretic movement of four dibasic compounds of varying molecular size. We can assume that in 0.5 *N* strong acids all of the compounds are completely ionized and hence it is interesting to see that the sequence of movement in all acids corresponds to the variation in molecular size. Only in sulphuric acid are the two smaller molecules, ethylenediamine and propylenediamine, retarded with respect to the other ions. Table I also gives results for two amino acids methylated

TABLE I

ELECTROPHORETIC MOVEMENT OF DIPYRIDYL, *o*-PHENANTHROLINE, ETHYLENEDIAMINE AND 1,2-PROPYLENEDIAMINE IN 0.5 *N* MINERAL ACIDS

Electrophoresis for 1 h at 500 V and 6° on Whatman No. 1 filter-paper. Results are distances travelled in millimetres.

Compound	*HCl*	*HNO_3*	*H_2SO_4*	*$HClO_4$*
Dipyridyl	30	28	29	31
o-Phenanthroline	24	21	25	22
Ethylenediamine	96	92	55	90
1,2-Propylenediamine	77	77	45	71
Trimethylaminovaleric acid	28	29	28	27
Dimethylaminocaproic acid	24	25	26	26

to different extents on the nitrogen atom. This variation in methylation seems to have little effect on the mobility. The concentration of sulphuric acid required in order to achieve this effect is not very critical; essentially the same results are obtained in 0.1, 0.5 and 1 *N* sulphuric acid, as shown in Table II.

TABLE II

ELECTROPHORETIC MOVEMENT OF SOME DIBASIC COMPOUNDS IN SULPHURIC ACID OF VARIOUS CONCENTRATIONS AS ELECTROLYTE

Electrophoresis for 1 h at 500 V and 6° on Whatman No. 1 filter-paper. Results are distances travelled in millimetres.

Compound	*Concentration of H_2SO_4 (N)*		
	0.1	*0.5*	*1*
Dipyridyl	31	31	23
o-Phenanthroline	24	27	20
Ethylenediamine	51	54	50
1,2-Propylenediamine	42	45	39

Table III shows the movement of a number of polyamines. It is interesting that again the molecular size has a much greater effect than the number of ionized basic groups. If the movement of the polyamines is expressed relative to the fastest, *i.e.* ethylenediamine, one can see that the ratios are the same (within the limits of the accuracy of the method) except in sulphuric acid, in which all polyamines are retarded although cadaverine and putrescine are less retarded than the others.

TABLE III

ELECTROPHORETIC MOVEMENT OF SOME POLYAMINES IN VARIOUS MINERAL ACIDS AS ELECTROLYTE

Electrophoresis for 1 h at 500 V and 6° on Whatman No. 1 filter-paper. Acid concentration 0.5 *N*. The distances moved are given in millimetres and the movements relative to ethylenediamine are given in parentheses.

Compound	*HCl*	*HNO_3*	*H_2SO_4*	*$HClO_4$*	*CCl_3COOH*
Spermine	71 (69)	56 (72)	30 (61)	67 (64)	54 (63)
Spermidine	73 (72)	59 (76)	33 (67)	71 (68)	58 (66)
Cadaverine	76 (75)	62 (79)	46 (94)	77 (73)	60 (68)
Putrescine	84 (82)	68 (87)	48 (98)	84 (80)	68 (77)
Ethylenediamine	102 (100)	78 (100)	49 (100)	105 (100)	88 (100)
1,2-Propylenediamine	84 (82)	64 (82)	40 (82)	87 (83)	70 (80)

The practical significance of the difference between sulphuric acid and the other strong acids is best illustrated in the electropherograms shown in Fig. 2. The mixtures of amino acids run next to the polyamines are comprised such that they encompass the range of amino acids in general. While in hydrochloric acid there is a good separation of all diamines from the amino acids and also a good separation of ethylenediamine from the other diamines, in sulphuric acid the diamines are hardly separated from each other and are much closer to the amino acids.

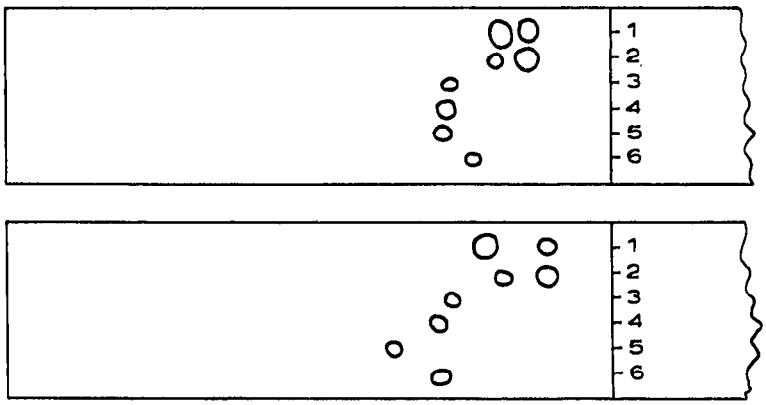

Fig. 2. Electropherograms of some polyamines and amino acids. Top: in 0.5 *N* H_2SO_4 for 1 h at 500 V and 6° on Whatman No. 1 filter-paper. Bottom: in 0.5 *N* HCl. 1 = Mixture of methionine and glycine; 2 = mixture of L(+)-leucine and L-histidine·HCl; 3 = cadaverine; 4 = putrescine; 5 = ethylenediamine; 6 = 1,2-propylenediamine.

In conclusion, ion pairing between small organic molecules and inorganic acids was not found to occur to such an extent that it would improve electrophoretic separations, with the possible exception of diamines, which seem to interact with sulphuric acid and are retarded to a measurable degree in this acid. The "hydrophobic interaction" noted between alkaloids or curare-like quaternary ammonium compounds on the one hand and perchloric or trichloroacetic acid on the other seems to be negligible in the electrophoresis of smaller organic molecules such as amino acids and polyamines.

ACKNOWLEDGEMENT

We thank Dr. J. L. Frahn for useful discussions which prompted us to publish these results.

REFERENCES

1 M. Mazzei and M. Lederer, *J. Chromatogr.*, 31 (1967) 196.
2 M. Lederer and M. Mazzei, *J. Chromatogr.*, 35 (1968) 201.
3 T. Prosperi, M. Sinibaldi and M. Lederer, *Gazz. Chim. Ital.*, 103 (1973) 995.
4 M. Lederer, L. Morpurgo and L. Ossicini, *J. Chromatogr.*, 50 (1970) 475.
5 M. Mazzei and M. Lederer, *J. Chromatogr.*, 37 (1968) 292.
6 M. Sinibaldi and B. Rinalduzzi, *Anal. Lett.*, 4 (1971) 125.