CHROMATOGRAPHY IN THIN POLYAMIDE FILMS

P. D. Reshetov, G. G. Chestukhina,
S. Makhmudov, and A. S. Pyshkin

UDC 543.544

Polyamide salts fixed to glass or to a synthetic [Dacron – poly(ethylene terephthalate)] film, are being used more and more for the separation of many classes of organic compounds [1, 2] and – primarily – of a number of amino acid and peptide derivatives [3, 6].

TABLE 1. R_f Values of DNP-Amino Acids in Various Systems of Solvents

DNP-Amino acids	In systems*			
	1	2	3	4
Tryptophan	0,32	0,12	0,17	0,23
Lysine	0,35	0,14	0,14	0,15
Leucine	0,94	0,90	0,32	0,53
Isoleucine	0,94	0,90	0,31	0,56

*1) Benzene–acetic acid (4 : 1); 2) 35% formic acid–water (1 : 1); 3) carbon tetrachloride–acetic acid (4 : 1); and 4) butan-1-ol–acetic acid (9 : 1).

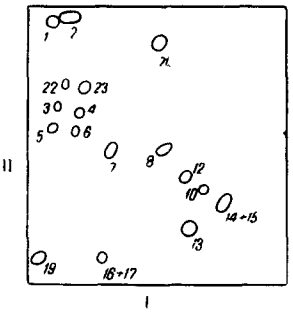

Fig. 1. Two-dimensional chromatogram of DNP-amino acids in a layer of polyamide (glass plates 10× 10 cm): I) benzene–acetic acid (4:1), 90 min (second direction); II) 85% formic acid–water (1 : 1), 60 min (first direction; charge, 1 μg in 1 μliter of methanol). For the numbering of the amino acids, see the caption to Fig. 2.

Similar films can be prepared from available materials of Russian manufacture. In the present paper we describe a method for the preparation of polyamide layers on glass and Dacron and also some examples of the effective separation of amino acid derivatives.

The results of preliminary investigations showed that satisfactory separation is achieved only for certain batches of polyamide. In a comparison of the materials that we had obtained with known samples (films of ε-caprolactam, Amilan CM 1011, Toyo Rayon Co.) it was found that the rates of absorption in them were practically the same. Thus, on a plate fixed to glass, system 1 (Fig. 1) travels 9 cm in 90 min, and system 2 in 60 min, these figures differing only slightly from those given in the literature [3, 7]. The R_f values of DNP-amino acids and, consequently, their mutual position on a two-dimensional chromatogram also correspond to the figures given in the literature. As can be seen from Fig. 1, we were unable to separate DNP-leucine and DNP-isoleucine, or DNP-tryptophan and di-DNP-lysine; however, from a comparison of the R_f values of these DNP-amino acids in the four systems of solvents it is obvious that system 4 can be recommended for the separation of these pairs (Table 1).

The separation of the DNS-amino acids (Fig. 2) was carried out as described by Woods and Wang [4]. By chromatography in only two systems we were unable to separate two pairs of compounds (see Fig. 2a) which, however, were well separated in system 3 (Fig. 2b). The combination of three systems (Fig. 2c) is fully adequate for the complete separation of a standard mixture of DNS-amino acids. ε-DNS-Lysine, separated in system 1, has a R_f value close to 1, while according to literature information its R_f should be 0 [4].

M. M. Shemyakin Institute of the Chemistry of Natural Compounds, Academy of Sciences of the USSR. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 66-68, January, 1971. Original article submitted August 10, 1970.

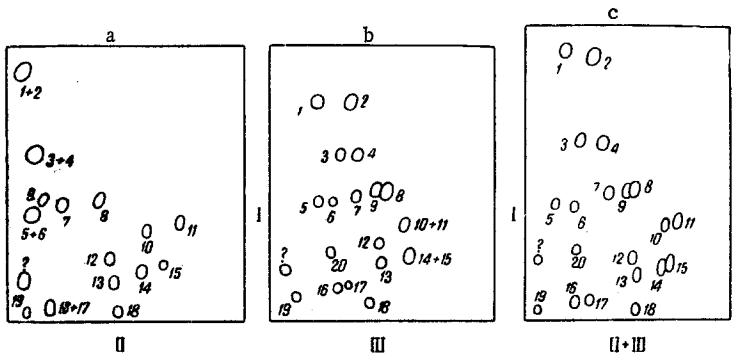

Fig. 2. Two-dimensional chromatograms of DNS-amino acids in a layer of polyamide fixed to Dacron: I) water–85% formic acid (100:1.6), 50 min; II) benzene–acetic acid (9:1), 90 min; III) n-heptane–butan-1-ol–acetic acid (3:3:1), 90 min. The DNP (see Fig. 1) and DNS derivatives of the amino acids were used for the separation: 1) arginine; 2) ε-lysine; 3) serine; 4) threonine; 5) aspartic acid; 6) glutamic acid; 7) glycine; 8) alanine; 9) o-tyrosine; 10) valine; 11) proline; 12) methionine; 13) phenylalanine; 14) leucine; 15) isoleucine; 16) tryptophan; 17) bis-lysine; 18) bis-tyrosine; 19) cystine; 20) N-tyrosine; 21) histidine; 22) asparagine; 23) glutamine.

The films that we obtained can be used repeatedly. Their washing in due time with acetone (with the addition of ammonia or formic acid) ensures that they can be used for more than six repetitions.

EXPERIMENTAL

The polyamide (22 g) was dissolved with shaking in 100 ml of formic acid (88.2 ml of 85% HCOOH and 12.5 ml of water), and the clear solution was deposited on glass plates (8 ml on a 10 × 10 cm plate) which were then placed in a tightly closed chamber (50 × 50 × 45 cm). A bath full of water was placed in the chamber together with the plates. After 24 h, the chamber was ventilated for 3-5 min, after which the plates were kept in a moist atmosphere for another day. Finally, the layer of polyamide was dried in the air for 2-3 h.

A Dacron film (40 × 10 cm, 75 μ thick) treated with No. 1 emery paper was slowly passed through a viscous solution of polyamide; then it was suspended above the cell for 3-5 min for the excess of solution to run off, and was placed in a chamber saturated with water vapor. After 24 h, the film was taken out and dried in the air for 2-3 h. Chromatography was carried out on 10 × 10 cm films, the solvent front migrating approximately 8 cm.

Mixtures of DNS- and DNP-amino acids were used as standards. Separation was performed in the recommended solvent systems [3, 4]. For re-use, the films were washed with a mixture of acetone and concentrated ammonia (9:1) for several hours.

SUMMARY

The applicability of Russian-made samples of polyamide for use as sorbents for the separation of some amino acid derivatives has been shown.

LITERATURE CITED

1. K. T. Wang, I. S. Y. Wang, and A. L. Lin, J. Chinese Chem. Soc., 13, Ser. II, 77 (1966).
2. K. T. Wang and I. S. Y. Wang, J. Chromatography, 27, 318 (1967).
3. K. T. Wang, J. M. K. Huang, and I. S. Y. Wang, J. Chromatography, 22, 362 (1966).
4. K. R. Woods and K. T. Wang, Biochem. and Biophys. Acta, 133, 369 (1967).
5. K. T. Wang, K. Y. Chen, and B. Weinstein, J. Chromatography, 32, 591 (1968).
6. Ch. Gerday and E. Robyns, J. Chromatography, 38, 408 (1968).
7. K. T. Wang and I. S. Y. Wang, J. Chromatography, 24, 460 (1966).