Interaction of substances during the process of paper chromatography

The fundamental principle of paper chromatography is that the compounds move independently of each other on the paper strip, and their quality is thus determined by their position. Consequently, the substances appear as discrete spots. In contrast, there frequently appear more stretched bands—homogeneous or structured—or the spots are of the so-called "comet" shape, instead of being discrete spots[1-6]. These phenomena may be explained partly by dissociation of the compounds, partly by interaction of the substances (eventually that of the substances and the solvent). It may be noted that the same phenomenon is encountered in the paper chromatography of both inorganic[7-9] and organic substances.

HACKMAN AND LAZARUS in referring to one of our papers[4]—where the phenomena occurring during the simultaneous chromatographing of different amino acids were explained as being due to their interaction—wrote as follows[10]: "If this explanation is correct then the interpretation of paper chromatography would become extremely complicated, results of earlier workers might have to be reassessed and the usefulness of paper chromatography for the quantitative and qualitative analysis of amino acids would be considerably reduced".

First of all, it may be noted that the basis of this conclusion is not the explanation but the phenomenon itself. *The impressive results and vast usefulness of paper chromatography as a microanalytical method—especially in analysis of amino acids—is beyond doubt.* However, the results of the paper chromatography may be considered adequate only if the fundamental condition mentioned above, *i.e.* the independent movement of the substances, is fulfilled.

HACKMAN AND LAZARUS suggest interaction between the glutamic acid and the phenol and effect of change of pH as a possible explanation for the phenomenon. We can also confirm the multiple spots phenomenon with glutamic acid alone, using phenol as solvent. In our opinion, however, the interaction of the amino acids is proved by the following experiments. On simultaneous chromatographing of the glycine and glutamic acid (solvent: butanol-acetic acid) three spots appeared, at the mol ratios: 3:1, 4:1, 5:1, but when the glycine and glutamic acid were separately chromatographed only a single spot appeared in every case.

The arginine and glutamic acid being simultaneously chromatographed with phenol, the change of R_F values is most pronounced at 1:4 mol ratio, according to FRANKEL's statement, *i.e.* these amino acids form a so-called molecule compound at this mol ratio[11]. The change of R_F values and stretching of spots may be observed if the amino acids are soluble either in buffer solution or water, and is most pronounced at pH 3.2 and 7. (The pH of the saturated solution of the molecule compound is 3.2.) Naturally, the phenomenon cannot be observed at extreme low and high pH values owing to the decomposition of the molecule compound. However, the multiple spots phenomenon appears in the presence of larger amount of amino acids only. In these and the above-mentioned experiments the amount of amino acids were about 10–20 γ and in no case more than 50 γ.

Our explanation of the role of amino and carboxyl groups in the interaction is consistent with the following experiment also: if these groups are blocked by metal complex formation the interaction (change of R_F values) cannot be observed[12]. In our opinion the chromatography of amino acid-metal complexes instead of free amino acids is one of the most promising methods for the more exact paper chromatography of the amino acids.

Institute for Inorganic and Analytical Chemistry, M. T. BECK
Pediatric Department, University of Szeged, Szeged (Hungary) P. ÉBREY

1 J. W. H. LUGG AND B. T. OVERELL, *Nature*, 160 (1947) 87.
2 S. ARONOFF, *Science*, 110 (1949) 590.
3 G. ZIMMERMANN, *Z. anal. Chem.*, 138 (1953) 321.
4 M. T. BECK AND P. ÉBREY, *Magyar Kém. Folyóirat*, 59 (1953) 350; *Acta Chim. Acad. Sci. Hung.*, 4 (1954) 231.
5 P. ÉBREY AND M. T. BECK, *Kisérletes Orvostudomány*, 7 (1955) 145.
6 G. B. WEST AND J. F. RILEY, *Nature*, 174 (1954) 882.
7 E. C. MARTIN, *Anal. Chim. Acta*, 13 (1955) 353.
8 M. T. BECK, *Acta Chim. Acad. Sci. Hung.*, 3 (1953) 187.
9 B. ERDEM AND H. ERLENMEYER, *Helv. Chim. Acta*, 37 (1954) 2220.
10 R. H. HACKMAN AND M. LAZARUS, *Biochim. Biophys. Acta*, 17 (1955) 147.
11 M. FRANKEL, *Biochem. Z.*, 242 (1931) 67.
12 M. T. BECK AND J. CSÁSZÁR, *Acta Chim. Acad. Sci. Hung.*, 7 (1955) 465.

Received January 12th, 1956