Zirconium selenite as ion-exchanger

According to the literature[1] some zirconium salts have good ion-exchange properties. Since ZrO_2^{--} is precipitated by H_2SeO_3 to give sparingly soluble compounds, zirconium selenite was prepared from 2 *M* nitric acid solutions of H_2SeO_3 and $ZrOCl_2 \cdot 8H_2O$ (ratio H_2SeO_3:$ZrOCl_2$ 1:1.23). The precipitate was washed with water until the pH was 4 and then dried at room temperature.

The capacity was determined by running a 0.1 *M* solution of NaCl through a column and titrating the acid formed. The capacity was found to be 0.48 mequiv./g.

The behaviour of the IB-group was studied with zirconium selenite as exchanger, in the same way as previously with zirconium phosphate[2].

Paper impregnated with zirconium selenite was prepared as follows:

(1) Solution A: 12.3 g of $ZrOCl_2$ in 150 ml of 2 *M* HNO_3. Whatman No. 1 paper was impregnated with this solution, the excess drained off and the paper dried at room temperature.

(2) Solution B: 10 g of H_2SeO_3 in 150 ml of 2 *M* HNO_3. The paper impregnated with solution A was immersed in solution B. The paper was then washed with water until the pH was 4 and dried at room temperature.

With this impregnated paper the separation of Ag(I) and Cu(II), and of Cu(II) and Au(III) was studied.

(a) With 0.01 *M* HCl as eluent a good separation of Cu(II) (R_F = 0.25) and Au(III) (R_F = 0.66) was obtained. The salts employed were $CuCl_2$ and $AuCl_3$.

(b) With 0.1 *M* HCl as eluent, Ag(I) was precipitated (R_F = 0), while Cu(II) had an R_F of 0.78. The salts employed were Ag_2SO_4 and $CuSO_4$.

The results were similar to those obtained with zirconium phosphate[2].

Instituto de Alta Cultura, M. J. NUNES DA COSTA
Centro de Estudos de Química Nuclear, Porto (Portugal) M. A. S. JERÓNIMO

[1] P. PASCAL, *Nouveau Traité de Chimie Minérale*, Tome 5e, Masson et Cie., p. 726.
[2] M. J. NUNES DA COSTA AND M. A. S. JERÓNIMO, *J. Chromatog.*, 5 (1961) 456.

Received February 6th, 1961

Improved separation of amino acids with a new solvent system for two-dimensional paper chromatography

Some of the major advantages of the paper chromatographic method are its versatility of application to separate any complex group of substances, flexibility of the choice of the moving phase, and the ease and capacity with which information can be obtained. The vast amount of available literature on these subjects has been excellently

reviewed by BLOCK, DURRUM AND ZWEIG[1], LEDERER AND LEDERER[2] and others. Now it is possible to routinely separate most of the common naturally occurring amino acids either unidimensionally using multi-solvent and multi-run systems or two-dimensionally using a number of pairs of solvent systems. The former is comparatively laborious and time-consuming while in the latter the best recommended and the most widely used solvent systems include one or more of phenol, cresol, collidine, lutidine, pyridine, etc., which give offensive and toxic vapors and require special precautions and measures when in constant use. Moreover, in the two-dimensional systems known, which evidently have apparent advantages over the multi-solvent single dimensional technique, satisfactory separation of most of the amino acids is achieved at the cost of resolution of some amino acids, such as leucine and isoleucine, lysine and arginine, glycine and serine or glutamic acid and aspartic acid. The problem of a solvent system which can separate all the common naturally occurring amino acids on a single chromatogram has long remained unresolved.

In the present paper we will describe a new combination of solvent systems for two-dimensional paper chromatography which gives a reasonable resolution of 20 common amino acids as shown in Fig. 1.

The first solvent is *sec.*-butanol–*tert.*-butanol–2-butanone–water in the proportion 4:4:8:5 (v/v) which makes a miscible solvent and was routinely mixed with 0.5% diethylamine (v/v). The use of 0.5% ammonium hydroxide in the solvent instead of diethylamine was also found satisfactory. The second solvent consists of the popular

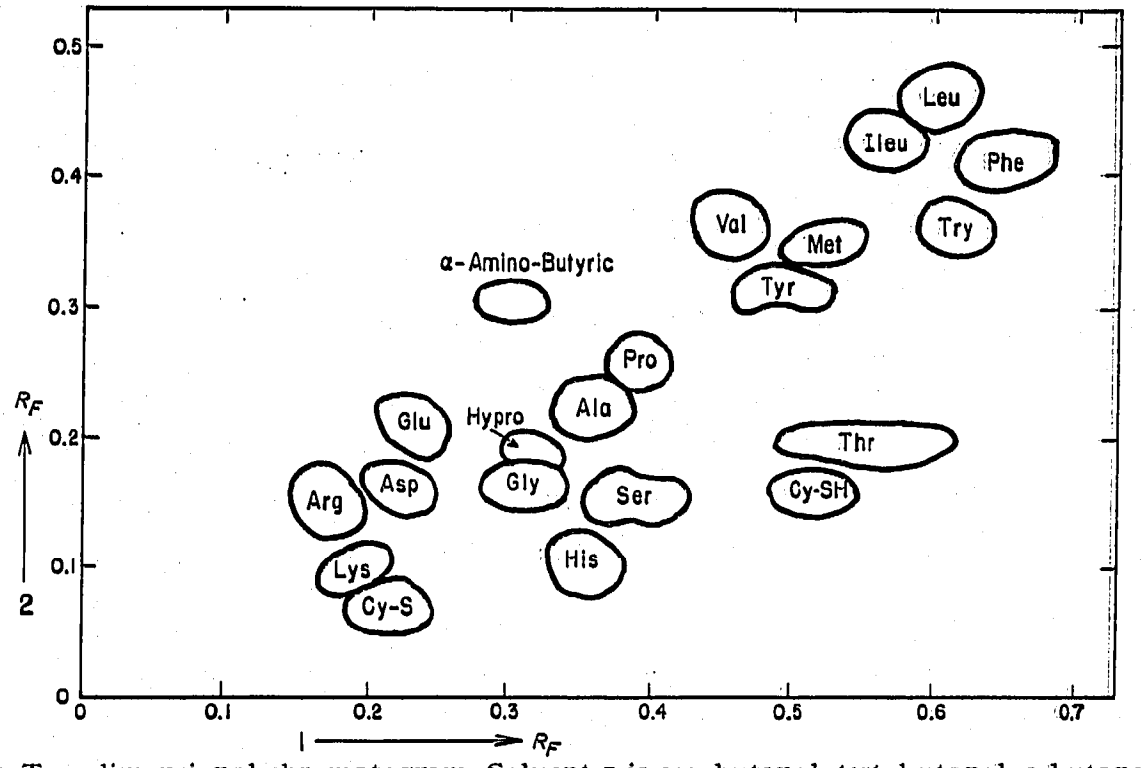

Fig. 1. Two-dimensional chromatogram. Solvent 1 is *sec.*-butanol–*tert.*-butanol–2-butanone–water in the proportion 4:4:8:5 (v/v) + 0.5% diethylamine. Solvent 2 is the upper layer of *n*-butanol–acetic acid–water in the proportion 4:1:5.

solvent mixture containing *n*-butanol–acetic acid–water in the proportion 4:1:5, which separates into two layers. The upper solvent layer was used. Reco chromatographic chambers were found very convenient for descending two-dimensional chromatography, although any general equipment of ascending or descending techniques should be satisfactory. A constant temperature oven of 55–75° was used for heating the chromatograms.

Although hydration in a steam hood gave better definition and more compact spots, this was not found essential. Also, previous saturation of the chamber was not a strict requirement if the chamber is leak proof. About a 15 hour run each way was found quite sufficient to give the necessary separation, and this could be routinely accomplished overnight.

For the best results, a few general precautions recommended in any paper chromatographic procedure were found necessary. The acidity of the hydrolysate, if not neutralized with excess of ammonia, results in streaking and gross distortion. This is best achieved by spotting an equal volume of 6 *N* ammonium hydroxide on the paper followed by aeration by a blower to remove the excess. Diethylamine or ammonia remaining after the first run interferes with the second acid solvent and results in a blue background when sprayed with ninhydrin. This can be avoided by heating the papers for about 2 hours between 55–65°, and this was routinely carried out. After the usual developments, the chromatograms were sprayed with 0.25 % ninhydrin in acetone and the color developed at 55–65° for 30 minutes. For quantitative purposes, 0.5 % ninhydrin in acetone is recommended.

All the three grade papers gave satisfactory resolution of amino acids with varying definition of the spots. Filter paper, Whatman No. 3, was found to be the most suitable, giving more compact and well-defined spots, its thickness permitting a large concentration of the sample to respond. On No. 1 paper, the spots were somewhat elongated; more so on No. 4. The rate of travel of both the solvents was similar on paper Nos. 1 and 3, but much faster on paper No. 4.

The assistance of Mrs. R. M. Cox is appreciated. This research was supported by the Quartermaster Food and Container Institute for the Armed Forces.

Department of Food Science and Technology, University of California, Davis, Calif. (U.S.A.)

K. S. AMBE
A. L. TAPPEL

1 R. J. BLOCK, E. L. DURRUM AND G. ZWEIG, *A Manual of Paper Chromatography and Paper Electrophoresis*, 2nd Ed., Academic Press, New York, 1958.
2 E. LEDERER AND M. LEDERER, *Chromotagraphy*, 2nd Ed., Elsevier Publ. Co., Amsterdam, 1957.

Received February 8th, 1961

Editor's note: This paper was received before the February issue containing a similar paper (M. MUZELL AND S. B. SIMPSON, JR., *J. Chromatog.*, 5 (1961) 157) had appeared.