lyzed against dilute $(NH_4)HCO_3$-buffer pH 7.0 and lyophilized. The yield of several chromatographic separations is combined, taken up in cacodylate buffer pH 6.00 and rechromatographed. Both B.RNase B and B.RNase A move as a single component (Fig. 1), indicating that each enzyme really corresponds to a different protein entity. Both peaks, however, emerge slightly ahead of their respective positions in the original chromatography; this effect is probably due to the high viscosity of the initial enzyme solution.

The RNase-activity in the eluate between the two B.RNase-peaks does not decrease to zero, even when the column length is increased. Tailing of B.RNase B is unlikely to be the cause, as the other enzymes assayed as well as the RNases upon rechromatography emerge in nearly symmetrical peaks. As yet not enough material from this intermediate region could be collected to allow rechromatography.

The separation of two active B.RNases is similar to several reported heterogeneities of enzymic activity, mostly demonstrated by means of elution analysis. Chemical and enzymological studies indicate that B.RNase B and A are closely related. Moreover, the chromatographic behaviour is not changed upon addition of 0.01 *M* thioglycol to the eluent. The proteolytic activity in the extract is negligibly small. Hence the most likely explanation for the observed heterogeneity is that B.RNase A contains one or a few more amide functions than B.RNase B, although the formation of a strongly bound complex is not excluded.

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Laboratory of Enzymology, State Agricultural High School, Ghent (Belgium) W. FIERS

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"Window" origin spots in paper electrophoresis and chromatography

In this communication a method is described for preparing nearly identical origin spots for use in paper electrophoresis or chromatography. The difficulty of obtaining nearly identical spots by the conventional method adversely affects separation and quantitative results, where the latter are required.

In the following method the origin positions are marked by a pencil dot, and a

paper circle of 3–5 mm diameter is cut out around the dot with a cork borer. The paper circles are put in a known volume of sample and dried in an oven at a suitable temperature. After drying the paper circles are saturated with the sample, and can be kept, in a desiccator if necessary, for many months.

When the sample is to be separated by electrophoresis, the sheet of paper with holes is saturated with buffer solution and the wet sheet is put into the apparatus. The dry paper circles are then fitted in the holes. When the circles have become wet by capillary action, electrophoresis is carried out in the usual way. Fig. 1 shows a separation of amino acids carried out in this way.

In the case of separation by paper chromatography, the circles are inserted in the dry paper sheet and pressed in tight with a spatula. The chromatogram is then run as usual. Fig. 2 shows a separation of sugars carried out in this way.

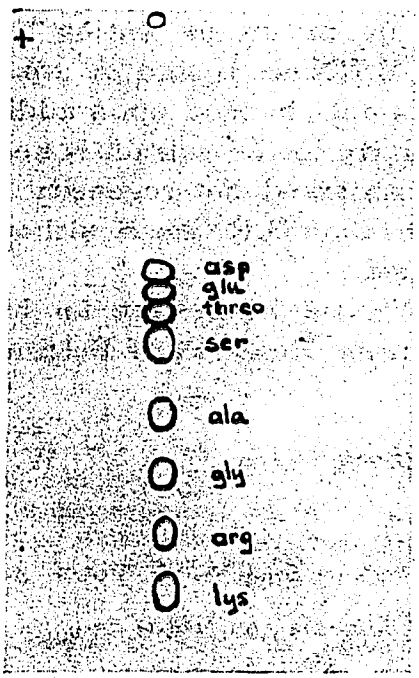

Fig. 1. "Window" origin spots in the high-voltage electrophoresis of a mixture of amino acids, using the Wieland-Pfleiderer apparatus. 2000 V, 8 mA, 60 min, —5°, formic acid–acetic acid buffer. Detection: ninhydrin.

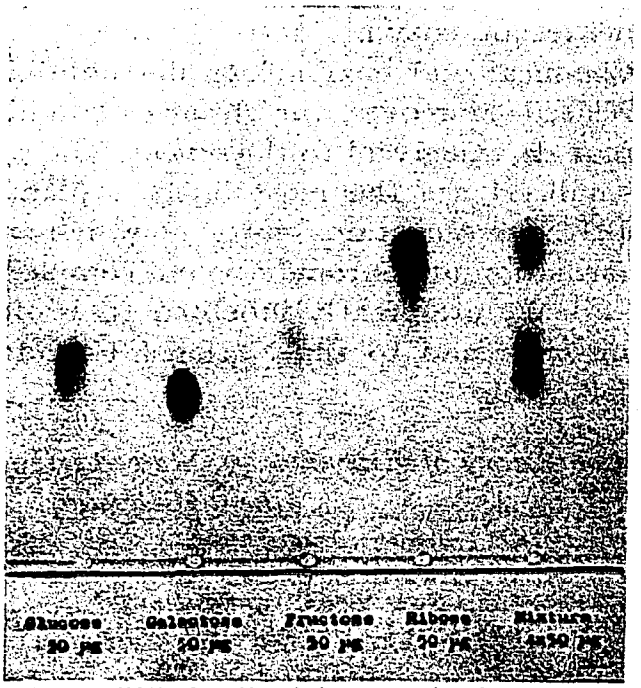

Fig. 2. "Window" origin spots in the chromatography of sugars solvent: butanol–pyridine–water; ascending method. Detection: aniline phthalate.

The spots obtained by the use of "window" origin spotting are sharper, smaller and more distinct than those obtained by the usual method.

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Department of General Chemistry, Medical Academy, Białystok (Poland) JULIUSZ POPOWICZ

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