Notes

Centrifugal chromatography

IX. Centrifugal paper chromatography of soluble collagens*

The existence of two soluble degradation products of collagen has been repeatedly reported[2,3]. Recent work by OREKHOVICH[4] indicated that these fractions could be separated by means of precipitation. PIEZ *et al.*[5] described the chromatographic behaviour of these substances: for chromatographic separation they applied ion-exchange chromatography on a column of carboxymethyl-cellulose, the results being verified by ultracentrifugal analysis. The separation technique, however, was unsuitable for routine analysis because of its complexity.

We recently described simple ion-exchange chromatography of soluble collagens[6]. The time-wasting character of this separation led us to develop a quick type of paper

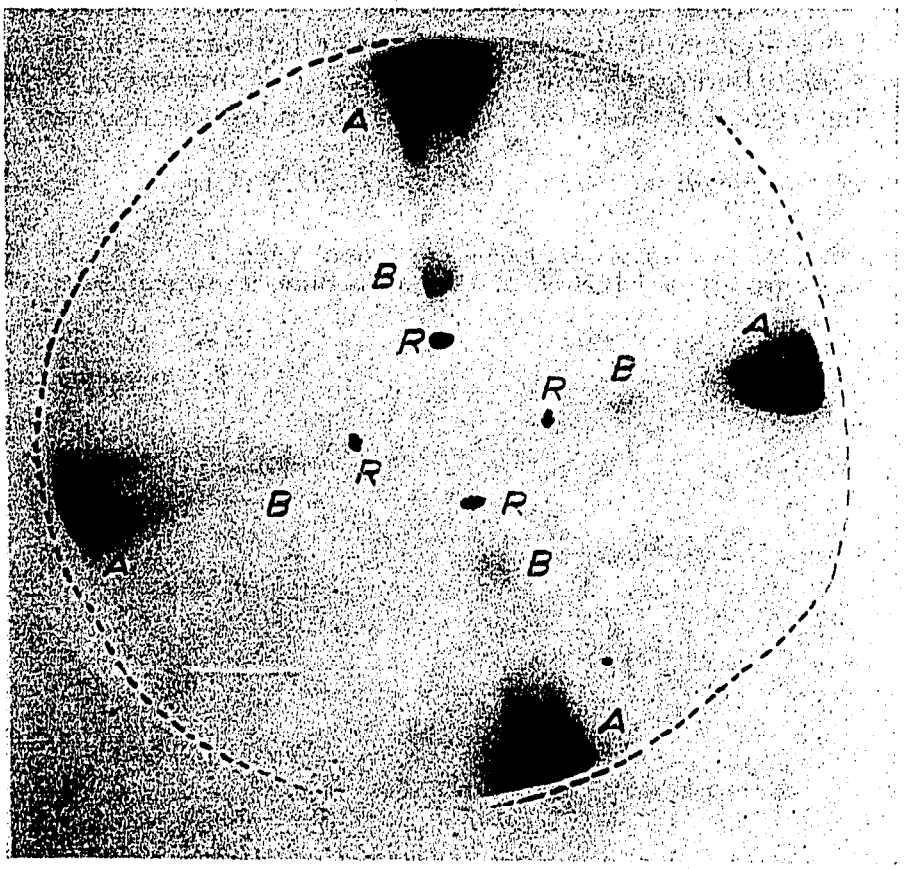

Fig. 1. Centrifugal chromatographic separation of soluble collagens (A = α fraction; B = β fraction). Paper W-3; gradient elution. Run 17 min. Detection: bromphenol blue[8].

* For Part VIII see ref.[1].

chromatography. Using the pressureless apparatus with radial development for centrifugal chromatography, which has been described elsewhere[7], it is possible to obtain perfect results within 20 min.

The bovine collagen (2.5 g) destroyed partially by alkali was extracted with 20 ml of acetate buffer pH 4.8 ($\mu = 0.1$) at 38° for 24 h. The extract was centrifuged and the supernatant liquid applied directly on the chromatogram. Whatman paper No. 3 was found to be the most suitable. Samples of the collagenous mixture were applied in quantities of 10 μl to the start, which was 3 cm from the center of the chromatographic disc. Up to six samples could be developed at a time.

The mobile-phase gradient was made by running glacial acetic acid into 5 ml of amyl alcohol–acetic acid mixture (2:1). The necessary mobile-phase inlet was 1.2 ml per min.

Fig. 1 is a chromatogram on which the separation of two collagenous fractions is shown. In order to identify the separated fractions with those isolated by PIEZ[5] on carboxymethyl-cellulose we made parallel separations on the carboxymethyl-cellulose column. The results obtained by means of ion-exchange chromatography agreed with our results and therefore we designated the first fraction ($R_F = 0.9$) as the α fraction of collagen and the second ($R_F = 0.45$) as the β fraction. Further investigation being made in our laboratory is necessary for the identification of soluble collagens from various sources.

Thanks are due to Dr. MILOŠ CHVAPIL of the Institute of Industrial Hygiene and Occupational Diseases, Prague, for his help with the classification of soluble collagens.

*Central Research Institute of Food Industry,** ZDENĚK DEYL
Prague, Smichov (Czechoslovakia) JAN ROSMUS

[1] Z. DEYL, M. PAVLÍČEK AND J. ROSMUS, *Chem. Listy*, in the press.
[2] V. N. OREKHOVICH AND V. O. SPHIKITER, *Dokl. Akad. Nauk SSSR*, 101 (1955) 529.
[3] E. H. L. CHUN AND P. DOTY, *Abstracts, Miami Meeting of the Am. Chem. Soc.*, April 1957.
[4] V. N. OREKHOVICH AND V. O. SPHIKITER, *Biokhimiya*, 23 (1958) 286.
[5] K. A. PIEZ, E. WEISS AND M. S. LEWIS, *J. Biol. Chem.*, 235 (1960) 1987.
[6] Z. DEYL AND J. ROSMUS, *Biokhimiya*, in the press.
[7] M. PAVLÍČEK, J. ROSMUS AND Z. DEYL, *J. Chromatog.*, 7 (1962) 19.
[8] I. M. HAIS AND K. MACEK, *Papírová Chromatografie* ČSAV, 1959.

Received July 20th, 1962

* Director, Ing. F. VONEŠ.

A method for diazotising (2,4-dinitro-5-aminophenyl)-amino acids on thin layer chromatograms

Recently, BERGMANN AND BENTOV[1] introduced 2,4-dinitro-5-fluoroaniline as a reagent for the formation of amino acid derivatives. Its behavior is similar to that of 2,4-dinitrofluorobenzene and it can be used in similar circumstances. The dinitroaminophenyl (DNAP) derivatives have a free amino group which can be diazotised and coupled with phenols to form intensely colored azo compounds.