

## Chromatographic identification of dinitrophenylamino acids on polyester film supported polyamide layers\*

The separation of 31 dinitrophenyl (DNP)-amino acids together with 2,4-dinitrophenol and 2,4-dinitroaniline by polyamide layer chromatography has previously been described<sup>1-3</sup>. Excellent results were obtained, so that even DNP-leucine and DNP-isoleucine could clearly be separated on a 10 × 10 cm chromatogram. These polyamide layers were prepared by slow evaporation of a formic acid solution on glass plates<sup>4</sup>. Later, the polyamide layer was prepared by coating it on to a poly(ethylene-terephthalate) film<sup>5</sup>, which is also available as a commercial product\*\*. The application of this method to DNP-amino acids is described here.

### Methods and materials

*Preparation of the polyamide layer*<sup>5</sup>. Twenty grams of polyamide ( $\epsilon$ -polycaprolactam CM 1011 of Toyo Rayon Co., Tokyo, Japan) were dissolved in a mixture of

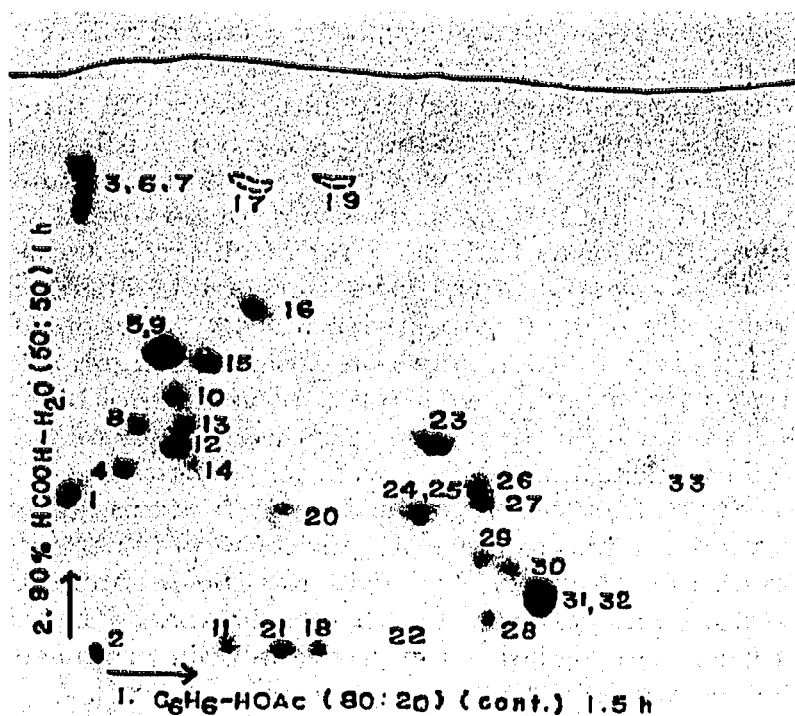


Fig. 1. Two-dimensional chromatogram. Solvent: 1st dimension: benzene-glacial acetic acid (80:20) (cont.), 1.5 h, 7.3 cm (2,4-dinitrophenol as internal standard); 2nd dimension: 90% formic acid-water (50:50), 1 h, 9.0 cm. Layer:  $\epsilon$ -polycaprolactam resin CM 1011 on polyester film. Loading: ca. 0.2  $\mu$ g of each DNP derivative. Numbers: 1 = DNP-CySO<sub>3</sub>Na; 2 = bis-DNP-Cys; 3 =  $\alpha$ -DNP-Arg; 4 = DNP-Asp; 5 = DNP-MetO<sub>2</sub>; 6 =  $\delta$ -DNP-Orn; 7 =  $\epsilon$ -DNP-Lys; 8 = DNP-Ser; 9 = DNP-AspNH<sub>2</sub>; 10 = DNP-Hypro; 11 = bis-DNP-Orn; 12 = DNP-allo-Thr; 13 = DNP-Thr; 14 = DNP-Glu; 15 = DNP-GluNH<sub>2</sub>; 16 = DNP-MetO; 17 = O-DNP-Tyr; 18 = bis-DNP-Lys; 19 = bis-DNP-His; 20 = DNP-Gly; 21 = DNP-Try; 22 = bis-DNP-Tyr; 23 = DNP-Sar; 24 = DNP-Ala; 25 = 2,4-dinitroaniline; 26 = DNP- $\beta$ -Ala; 27 = DNP-Pro; 28 = DNP-Phé; 29 = DNP-Met; 30 = DNP-Val; 31 = DNP-Leu; 32 = DNP-Ileu; 33 = 2,4-dinitrophenol.

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\*\* Chen Chin Trading Co., Ltd., No. 75, Section 1, Hankow St. Taipei, Taiwan, Republic of China.

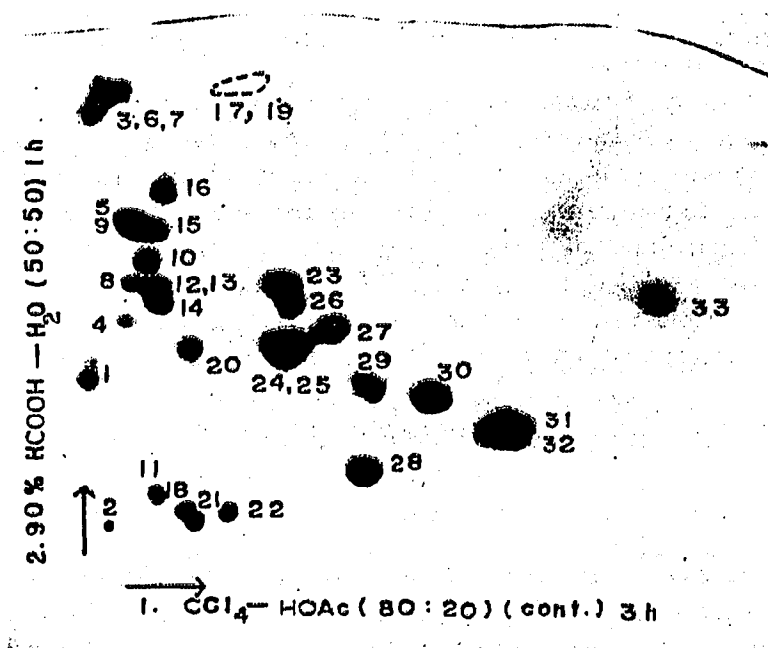


Fig. 2. Two-dimensional chromatogram. Solvent: 1st dimension: carbon tetrachloride-glacial acetic acid (80:20) (cont.), 3 h, 7.4 cm (2,4-dinitrophenol as internal standard); 2nd dimension: 90% formic acid-water (50:50), 1 h, 8.5 cm. Layer:  $\epsilon$ -polycaprolactam resin CM 1011 on polyester film. Loading: *ca.* 0.2  $\mu$ g of each DNP derivative. Numbers: *cf.* Fig. 1.

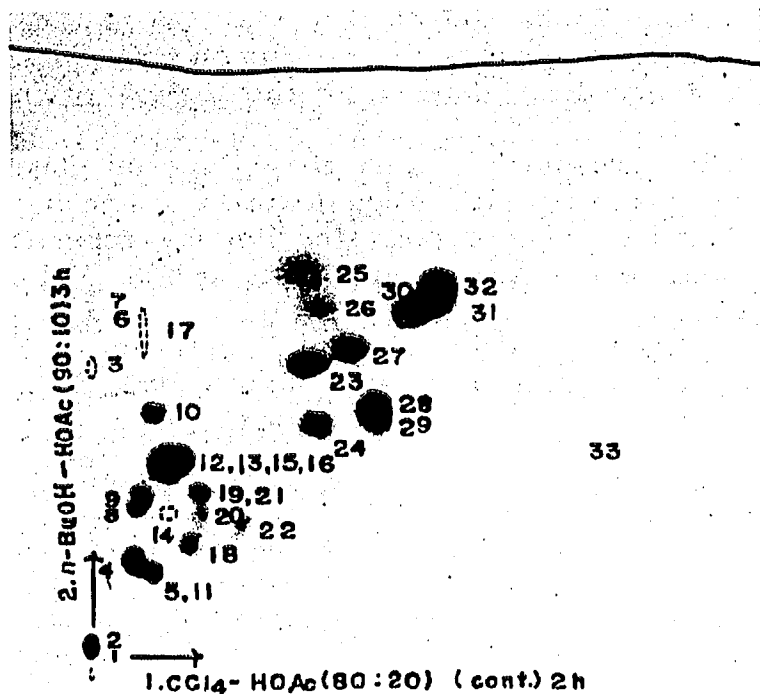


Fig. 3. Two-dimensional chromatogram. Solvent: 1st dimension: carbon tetrachloride-glacial acetic acid (80:20) (cont.), 2 h, 6.0 cm (2,4-dinitrophenol as internal standard); 2nd dimension: *n*-butanol-glacial acetic acid (90:10), 3 h, 9.1 cm. Layer:  $\epsilon$ -polycaprolactam resin CM 1011 on polyester film. Loading: *ca.* 0.2  $\mu$ g of each DNP derivative. Numbers: *cf.* Fig. 1.

100 g 90 % formic acid and 13 ml distilled water with stirring. After several hours, a viscous and homogeneous solution was obtained.

Five hundred millilitres of the above solution were poured into a dish (30 × 20 × 4 cm) and an etched poly(ethyleneterephthalate) film (23 × 18 × 0.018 cm, Lumilar, Toyo Rayon Co., Tokyo, Japan) was dipped into it. Both sides of the film were covered homogeneously by the polyamide solution. The film was hung for 2 min over the dish to let the excess solution drain back. It was then suspended in a water-saturated cabinet (50 × 45 × 45 cm) and kept at  $26 \pm 2^\circ$  for one day. After air-drying for another day, the layer was cut 1 cm from the bottom, 7 cm from the top and 1.5 cm from both sides to give a square of 15 × 15 cm. These polyamide layers can be stored for a long period.

*Chromatography of DNP-derivatives.* The chromatographic techniques were the same as used in the earlier work<sup>2,3</sup>.

### *Results and discussion*

Figs. 1, 2 and 3 are the U.V. contact photographs<sup>2</sup> of two-dimensional chromatograms of DNP-amino acids on a poly(ethyleneterephthalate) film/polyamide layer. The amount of sample applied was about 0.2  $\mu$ g for each DNP-derivative. Because the spot diffusion is much smaller than on a glass plate supported layer, clearer resolution is obtained, and an 8-cm run is sufficient for ordinary purposes instead of the 10-cm development used previously. In quantitative work there is no difficulty in eluting the separated substances, because the spots can be cut up for extraction. We have always found higher recovery percentages for DNP-amino acids on polyester film supported layers than on glass supported ones<sup>5</sup>. Both sides of the film are independent of each other. For one-dimensional work a 15 × 15 cm film is equivalent to a layer of 30 × 15 cm in size and thirty samples can be chromatographed simultaneously on both sides. For two-dimensional work, one side can be used as reference for the analysis of an unknown mixture.

Because the layers on polyester film are much thinner than those on glass plates, the drying time between the two runs in two-dimensional work is faster and more efficient. Usually 5 min are sufficient for layers on polyester film as compared with 15 min in former work. For light- and heat-sensitive compounds such as DNP-amino acids this also means less destruction.

From our experience, a polyamide layer on polyester film is better than a polyamide layer supported on a glass plate in qualitative and quantitative work. In preparative work<sup>7</sup>, however, a thick layer of polyamide is necessary.

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