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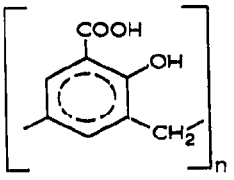
Fractionation of poly(methylene-2-hydroxybenzoic acid) by preparative layer chromatography

In our work on biologically active synthetic polymers, poly(methylene-2-hydroxybenzoic acid) [1] which showed a reasonable anti-inflammatory activity on localized edema caused by bradykinin[1] has been examined.



I

To investigate whether such a property should be attributed to the polymer as a whole or to some fraction of it, we developed a fractionation procedure using preparative layer chromatography. Satisfactory results were obtained using Silica Gel H as sorbent and a mixture of benzene–acetic acid–water (2:2:1) as eluent. Each fraction was removed from the chromatoplate together with the sorbent and eluted using a column. The molecular and the equivalent weights were determined for each fraction.

Experimental

Materials and methods. A Shandon equipment for preparative layer chromatography was used. Chromatoplates (100 × 20 cm) were prepared by the method described by STAHL[2], with Silica Gel H (Merck) as sorbent. A 750-μ layer was used on all plates which were then placed for 3 h in the pre-drying rack, heated at 110° for 1 h and stored in a desiccator. The chromatographic tank was lined with filter paper and equilibrated for 4–5 h before use.

Benzene–acetic acid–water (2:2:1) was employed as the solvent system. The solvents were mixed in a separatory funnel and allowed to equilibrate for 30–40 min. The benzene phase was placed at the bottom of the chromatographic tank, and the aqueous phase was placed in a beaker inside the tank.

TABLE I

CHARACTERIZATION OF POLY(METHYLENE-2-HYDROXYBENZOIC ACID) (PMSA) FRACTIONS

Fraction	*R_F value (× 100)*	*Equiv. wt.*	*Mol. wt.*
1	0– 4	158.8	1100
2	5–18	160.9	800
3	19–27	165.3	550
4	28–38	173.8	450
5	39–46	143.8	288[a]
PMSA	—	163.9	650
Salicylic acid	78	138.2	138

[a] This is the molecular weight of 3,3'-dicarboxy-4,4'-dioxydiphenylmethane.

About 0.3 g of polymer previously solubilized in 2 ml of an acetone–water (10:1) mixture was deposited along the length of each plate. The chromatoplates were developed in the stainless-steel preparative chromatotank which held one chromatoplate rack with five plates. In this way it was possible to fractionate 1.5 g of polymer at the same time. The plates were developed for 14–16 cm (*ca.* 45 min) by the ascending method.

After complete drying, five fractions, with R_F values as reported in Table I, were observed as bands on the plate by means of UV light at 366 mμ (Engelhard Hanovia Model 16).

Extraction and characterization of fractions. The fractions were removed from the plates and transferred into glass tubes (2 × 30 cm) drawn at one end. The fractions were dissolved in ethanol; by adding dilute HCl a precipitate was obtained. After centrifugation and repeated washing with water, the fractions were dried under vacuum at 40°; each fraction was collected as a white powder.

The equivalent weight was determined by a potentiometric titration[3] of the carboxylic group of each monomeric unit with potassium methylate as titrant and pyridine–benzene–methanol (2:2:1) as solvent*.

Table I shows the equivalent weights of the fractions and related molecular weights determined in methanol by an isopiestic method (Hitachi Perkin-Elmer, Model 115).

Discussion

The results summarized in Table I show that the polymer fractionation is a function of the molecular weight of each fraction. The procedure developed allows one to obtain reasonable quantities of each fraction in a relatively short time. By using a set of five plates, it is possible to fractionate 1.5 g of polymer in about 2 h. This simple technique can also be satisfactorily used for the molecular weight fractionation of other similar polymers.

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1 G. CIAMPA AND E. MIELE, *Rend. Accad. Naz. XL*, 16 (1966) 247.
2 E. STAHL (Editor), *Thin-layer chromatography*, Springer, Berlin and New York, 1962.
3 G. CIAMPA, C. GRIECO, F. MANNA, C. SILIPO AND A. VITTORIA, *Chim. Ind.*, 51 (1969) 595.
4 G. CIAMPA, C. GRIECO, C. SILIPO AND M. PENNAROLA, *Rend. Accad. Sci. Fis. Mat.*, IV, 36 (1969) 98.

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* The higher values of the equivalent weight as compared with the equivalent weights calculated from formula I can be explained by the presence of hydroxymethylenic groups, as confirmed by NMR[4].