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A quantitative protamine assay by diffusion in agarose gels containing DNA*

The determination of the concentration of protamine solutions for experimental, especially chromatographic, and for pharmaceutical purposes is complicated by several factors. Standard procedures, such as the Folin method or measurement of the extinction at 280 nm, cannot be employed (Fig. 1) because of the peculiar amino acid composition of these basic nuclear proteins. Kjeldahl determinations require special, time-consuming calibrations, as the N-content of the protamines is outside the normal protein range. Moreover they are non-immunogenic, so that immunological methods cannot be used.

A new assay for protamine, based on the diffusion of the sample in a layer of DNA-containing agarose and similar to the immunochemical technique used by

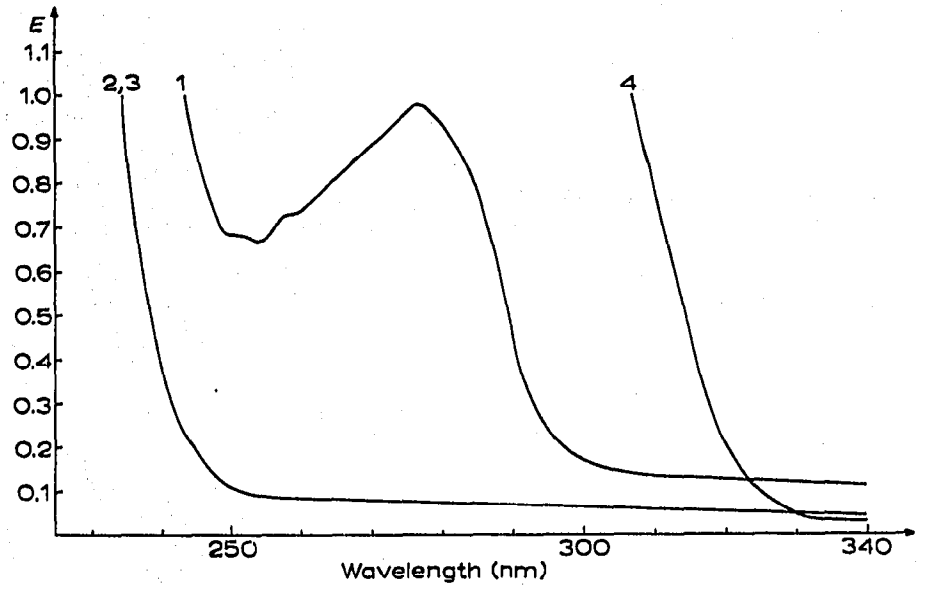

Fig. 1. UV-absorption curve of the protamine preparations investigated; the poly-lysine preparation and an albumin sample are included for comparison. 1 = albumin (Serva, human); 2 = salmine sulphate (Serva); 3 = poly-lysine HBr (Serva); 4 = "Protamine Roche" (pharmaceutical preparation of protamine HCl). Note the absence of the usual peak at 280 nm for 2 and 3 and the strong absorption for 4, possibly due to an additive for special purposes. Quantitative determination is not possible for 2, 3 and 4.

MANCINI[1] is described. Equal parts of a 2% solution of agarose (Behringwerke, Marburg, G.F.R.) and of a 0.2% solution of DNA (Serva, Heidelberg, G.F.R.; from salmon testes, high molecular), both in a pH 8.6 buffer, are thoroughly mixed at a temperature of 45°. 1.1 ml portions of this solution are evenly spread on carefully cleaned microscope slides (74 × 24 mm). After cooling, 2.6 mm diameter wells are punched out of the gel and the spare gel is removed. The slides are kept in a moist chamber at 4° until use.

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For the assay, 2 μl of the sample are placed in the wells by means of a microsyringe. After 3 h of diffusion in a moist chamber at 37° the diameters of the precipitation rings formed are determined by use of a calibrated loop. Diameters are measured twice at right angles and the mean value is employed for the assay. No dyeing procedure is needed as the precipitates formed are visible as thick, white haloes around the wells without further treatment. Comparison of the diameters with a standard curve (Fig. 2, No. 1) permits the concentration of the samples to be

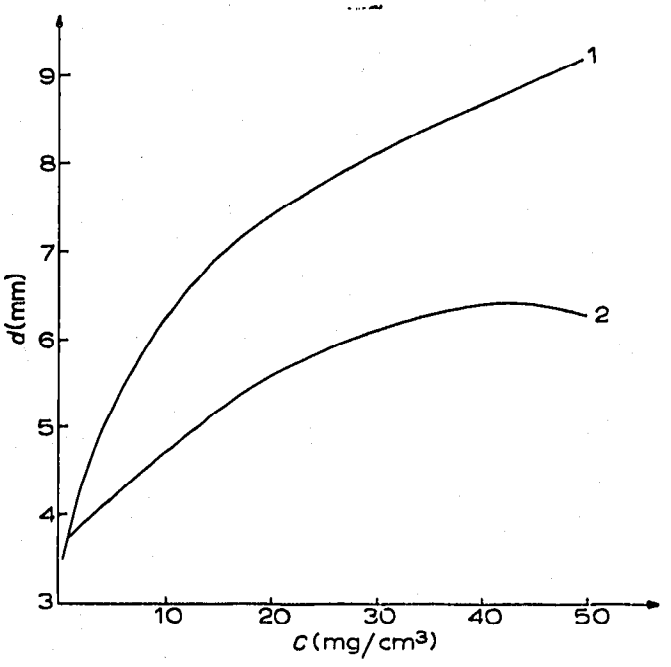

Fig. 2. Standard curves of the DNA-agarose diffusion method for "Protamine Roche" and salmine (No. 1) and for poly-lysine (No. 2).

read directly. The presence of blood plasma does not result in any visible precipitation rings; those formed by histone solutions are—under the experimental conditions described—not usable for concentration measurement. A standard curve for a poly-lysine preparation (Serva, mol. wt. = 100 000) is also included in the diagram (Fig. 2, No. 2). It is characterized by a peak at about 40 mg/cm^3 and a decline at higher concentrations. Therefore the method described, in the case of poly-lysine, is only useful at lower concentrations.

It should be mentioned that agar gels are not suitable for the diffusion assay because all basic proteins are precipitated by the acid groups, especially SO_4^{2-}, present. They are precipitated in a diffuse way not comparable to the precipitates formed by DNA.

The technique described here provides an easy method of determination of protamine concentrations in a relatively short period of time from samples of only 2 μl.

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mental Cancer Research, German Cancer Research Centre, Heidelberg, is gratefully acknowledged. The author thanks Miss U. REINLE for excellent technical assistance.

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