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The degradation of Sephadex G-150 by 50% formic and acetic acids

We are currently engaged in the preparation of synthetic polypeptide collagen models for immunological studies. In the course of this work we have encountered the need to fractionate these polydisperse materials, which are insoluble in nearly all solvents except carboxylic acids and aqueous mixtures containing high proportions of carboxylic acids. Sephadex G media have often been used for preparative gel filtration of peptides using solvent mixtures containing large amounts of acetic or formic acid. To cite but a few examples, G-50 has been used with 45 %[1], 70 %[2] or 88 %[3] formic acid, G-25 with 50 % acetic acid[4], G-50 with 30 %[5] or 50 %[6–8] acetic acid, G-75 with 50 % acetic acid[9,10], and G-25 with phenol–acetic acid–water (1:1:1:1)[11,12]. Although the lability of dextran gels to aqueous mineral acids is well known,[13] it seemed implicit from the large number of examples in the literature that serious detriment was not caused by exposure to aqueous mixtures containing in the region of 50 % acetic or formic acid. We were therefore encouraged to use such a system for our purposes.

Although we are not acquainted with any published application of Sephadex

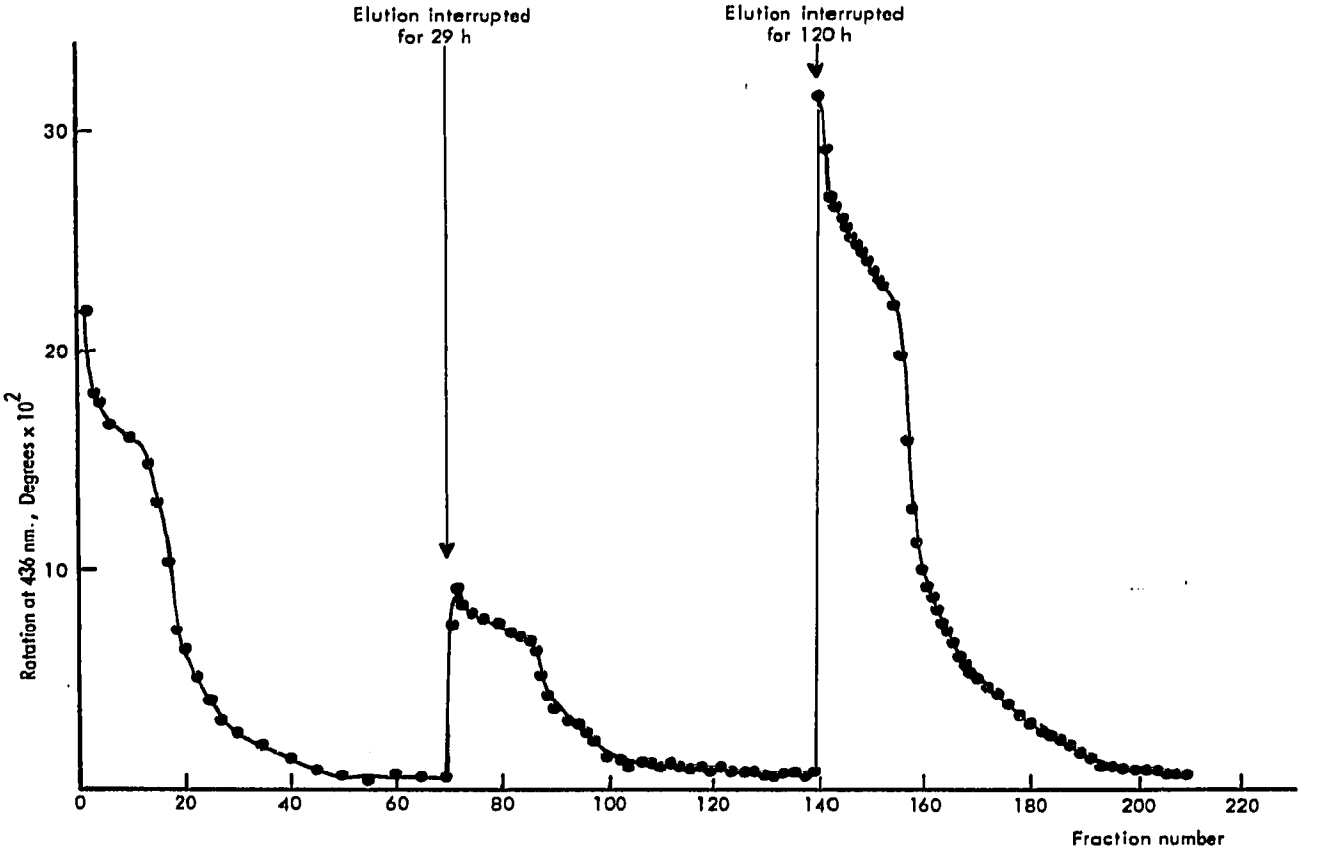

Fig. 1. Sephadex G-150 (lot No. 860) in 50 % (v/v) aqueous AnalaR formic acid. Bed dimensions, 1.2 × 76 cm; bed volume, 85 ml; void volume, 28 ml; fraction size, 1.4 ml; flow rate, 7 ml/h. The optical rotations of the fractions were determined in a Perkin-Elmer 141 polarimeter using a 1 dm cell at 25°.

G-150 in conjunction with *ca.* 50% acetic or *ca.* 50% formic acids we chose these combinations for our work because preliminary small scale experiments on the fractionation of poly-(L-alanylglycyl-L-proline) showed them to be the most suitable for the molecular weight range of our material. Since this polymer has a high optical rotation and lacks convenient chromophores, eluants were monitored polarimetrically. It was observed that with both 50% acetic acid and 50% formic acid the eluant was significantly optically active before the void volume had emerged and remained so even after one bed volume had passed. Because it seemed likely that this was evidence of degradation of the gel we investigated this by running the solvent mixtures alone through fresh columns. A column of G-150, made up in 50% formic acid and stabilised by passage of several bed volumes of solvent, was allowed to stand at room temperature for 75 h before examination. The first fractions of eluant collected were highly optically active: the rotation fell as elution progressed, attaining a constant small positive value after one bed volume had passed (Fig. 1). The fractions corresponding to the first bed volume were pooled and lyophilised to give 105 mg of a pale yellow solid which was shown to be mainly carbohydrate by the anthrone reaction. When the flow of eluant was interrupted and resumed after 29 h the rotations of the first fractions then obtained were again highly optically active, falling off with continued elution. Repetition of the same operations caused recurrence of the same effects (Fig. 1). Use of 50% acetic acid gave qualitatively similar evidence for degradation

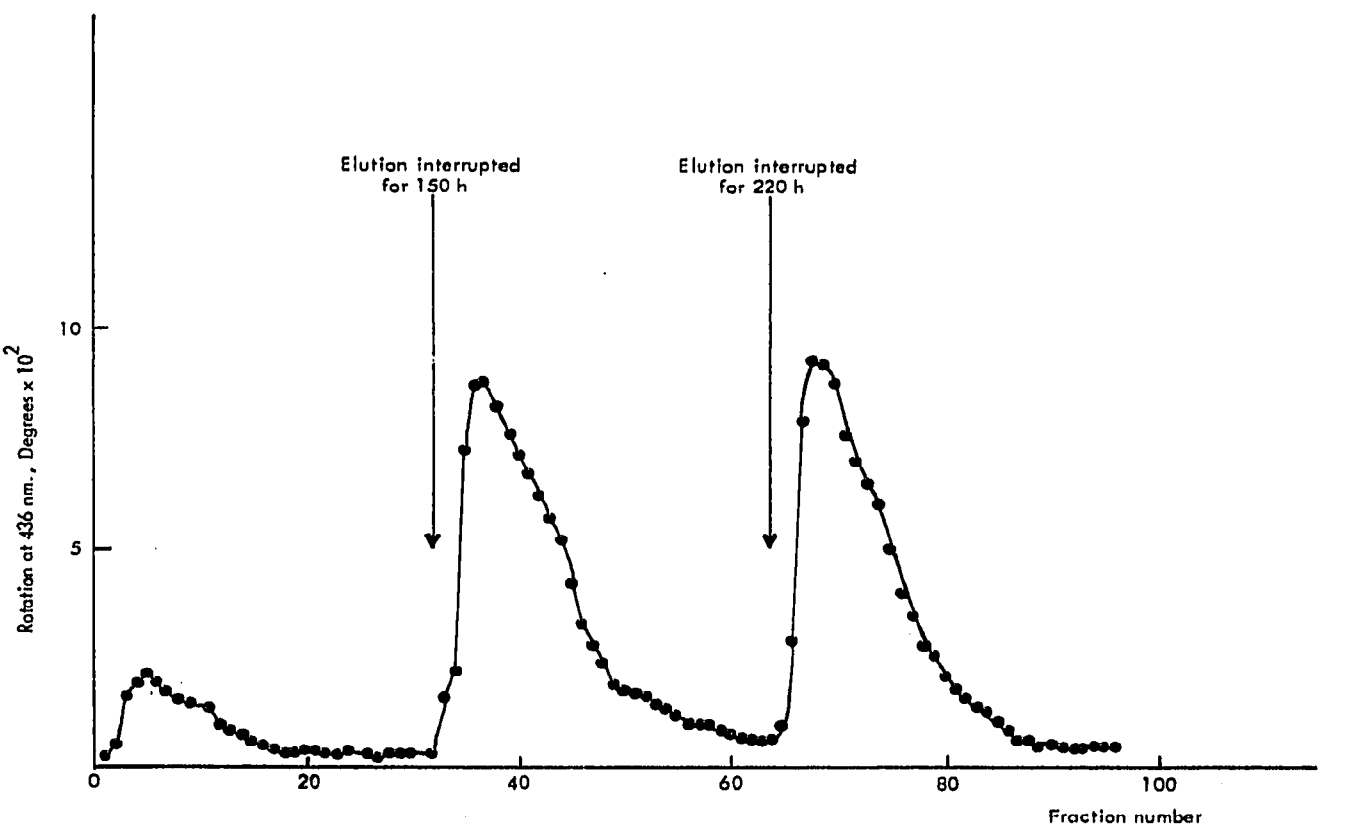

Fig. 2. Sephadex G-150 (lot No. 860) in 50% (v/v) aqueous AnalaR acetic acid. Bed dimensions, 1.9 × 11 cm; bed volume, 30 ml; void volume, 10.2 ml; fraction size, 1.25 ml; flow rate, 15 ml/h. The optical rotations of the fractions were determined in a Perkin-Elmer 141 polarimeter using a 1 dm cell at 25°.

of the gel (Fig. 2). Distilled water alone did not elute significant amounts of optically active material from a column of G-150.

We conclude that if contamination of the applied material is to be minimised when aqueous mixtures of acetic or formic acid are used as eluants with Sephadex G-150, then the column should be washed through with at least one bed volume of solvent immediately prior to use, since carbohydrate artefacts accumulate in the column on standing. Even if this procedure is followed, some contamination which would not be detected by standard monitoring techniques seems inevitable. For many purposes the introduction of carbohydrate traces is not disastrous, but our polypeptides are intended for immunological work in which the presence of small amounts of carbohydrate antigens is highly undesirable. We have therefore abandoned the use of dextran gels for fractionation of our polypeptides, and have not examined the stability to carboxylic acid mixtures of gels with lower exclusion limits than G-150. However, it seems probable that solubilisation of the more highly cross-linked gels by strong formic and acetic acids also occurs but has escaped notice because the most commonly applied procedures for examining peptide-containing eluants would not have detected the presence of carbohydrate.

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