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Effect of temperature on the porosity of dextran gels

Gel chromatography is generally considered to be unaffected by changes in temperature[1]. This has been well demonstrated with macroporous or heteroporous gels such as Styragel® (ref. 2). Since in other gels, for instance Sephadex® (cross-linked dextran) and Bio-Gel (cross-linked acrylamide), the porosity is produced by swelling, it appeared plausible that these gels might be affected by changes in temperature.

Sephadex LH-20 in organic solvents and Bio-Gel P-2 in water show a greater swelling at higher temperatures (Figs. 1a and b). In contrast, all Sephadex gel types exposed to water shrink when the temperature is raised. The shrinkage is especially observed with Sephadex LH-20 in water. (It should be pointed out that ÖBRINK *et al.*[3] also observed such behaviour with the Sephadex type G-200 (ref. 2).) Addition of an electrolyte increases the shrinkage (Fig. 1b, solvent II). Shrinking and swelling are reversible. No product of hydrolysis was found in the solvent with the anthrone test during the heat treatment.

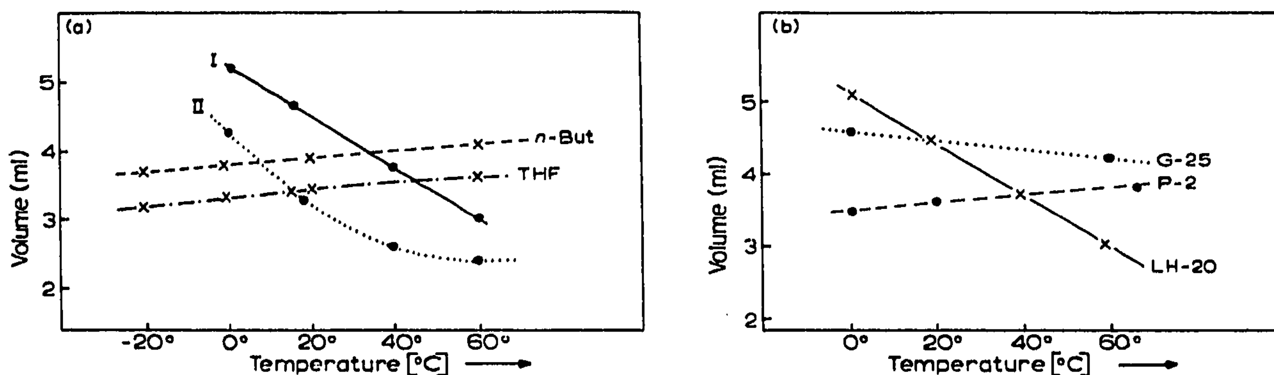

Fig. 1. Temperature dependence of the swelling of gels in different media. (a) 1 g of Sephadex LH-20 swollen in tetrahydrofuran (THF), *n*-butanol (*n*-But), water (I), and water + 2.5 *M* KCl (II). (b) 1 g of Sephadex LH-20 (LH-20), 1 g of Sephadex G-25 (G-25), and 1 g of Bio-Gel P-2 (P-2) swollen in water.

Fig. 2 (top) gives the separation characteristics of the two gels that show opposing behaviour, Bio-Gel P-2 (swells slightly at higher temperatures) and Sephadex LH-20 (shrinks strongly when heated in water). A straight line, independent of temperature, is obtained for Bio-Gel P-2, while for Sephadex LH-20 the middle part of the S-shaped curve at 60° is flatter than the one at 20°, which means that at higher temperatures the separation volumes are smaller and consequently the separation is poorer.

The lower half of the diagram in Fig. 2 represents a schematic elution pattern (Fig. 2, bottom). The hatched section is the gel volume which is not available to gel chromatography. Only the distance between V_0 and H_2O (representing the molecule of smallest size) indicates the volume where separation by size occurs according to the rules of gel chromatography. (Elution with a buffer, water, was started on top of the column and could easily be detected by a refractometer.) Sephadex LH-20 has a smaller volume available for size separation at 60° than at room temperature.

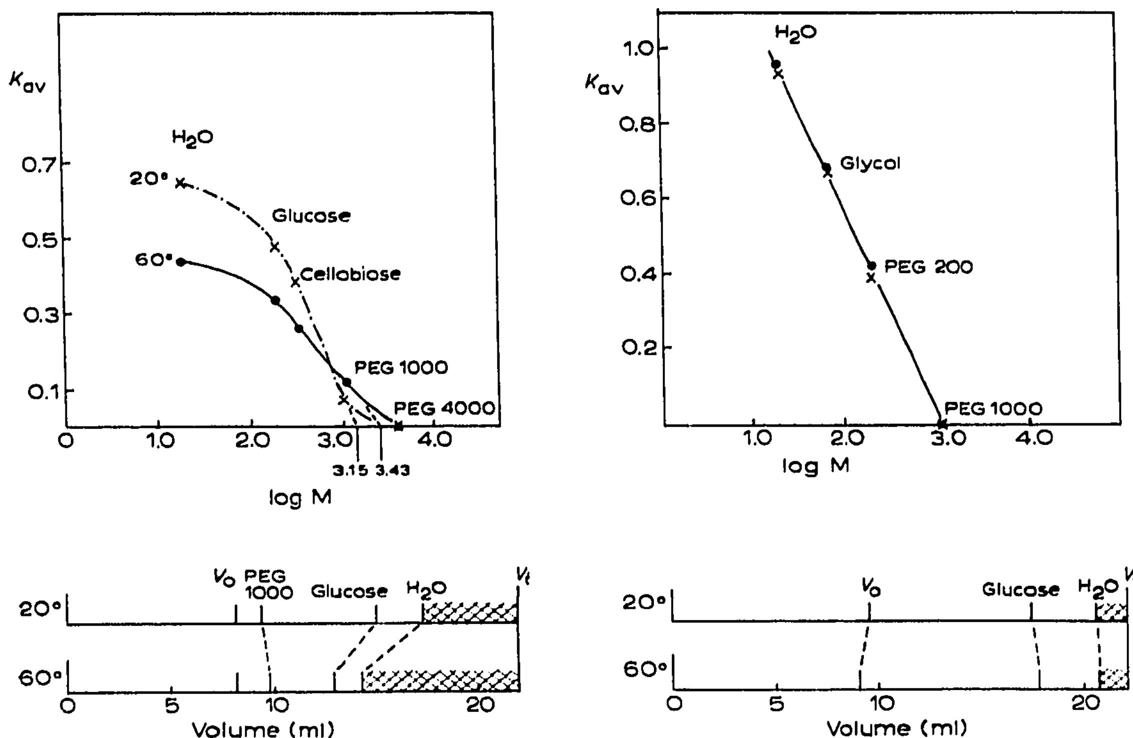

Fig. 2. Top: Separation characteristics of Sephadex LH-20 and Bio-Gel P-2 at 20° and at 60°. K_{av} represents the elution parameter, log M is the logarithm of the molecular weight. Column, 0.86 × 38 cm; V_t = 22.0 ml; elution by 0.002 N HCl, pH 2.5; sample volume, 0.15 ml; concentration, 1 mg/ml of each substance; flow rate, 7.5 ml/h; detector, Waters R-4 refractometer. Bottom: Schematic graph of the elution diagrams. The vertical lines symbolise the maximum of the elution peaks of the given substances. The hatched area marks the volume not available for gel chromatography. V_0, the void volume, was measured by PEG 4000 (polyethylene glycol, average molecular weight 4000). V_t, the total volume of the column filling, is 22.0 ml.

Nevertheless, the separation of substances of higher molecular weight improves. In Fig. 2 (bottom) the maxima of V_0 and PEG 1000 are 1.1 ml apart at 20° and 1.55 ml at 60°. From Fig. 2 (top), it can be seen that the exclusion limit for Sephadex LH-20 in water can be graphically calculated to a molecular weight of 1500 at 20° and to molecular weight of 2700 at 60°.

These findings indicate that, while the sum of the pore volume decreases, larger pores result in the case of LH-20. Fig. 3 represents the proposed structural change of the gel at the two temperatures. The lines represent the polymer threads. An increase in temperature causes a force to act upon the system which brings the polymer

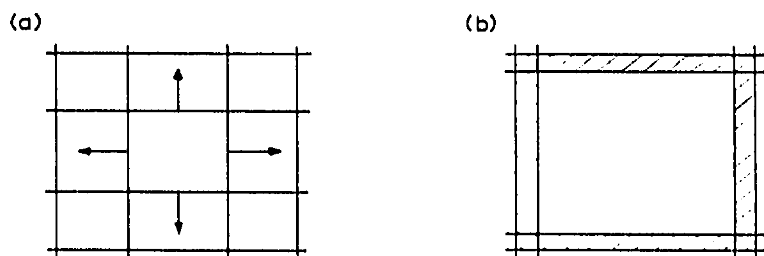

Fig. 3. Schematic diagram of the structural change in Sephadex LH-20 caused by increase in temperature. (a) 20°; (b) 60°.

threads closer together. As a result, small pores might be eliminated in favour of larger ones.

Fig. 3b represents the scheme of the inner structure of heteroporous gels as suggested by DETERMANN[1] and JOUSTRA[4]. The separation characteristics of macroporous gels are flatter than those of swellable gels[5]. The lower separation characteristics of Sephadex LH-20 at 60° in Fig. 2 (top) indicate that this gel has undergone a structural change towards heteroporosity. It is suggested that hydrophobic bonding is the force which causes the structural change. The three-dimensional network of Sephadex consists of hydrophilic dextran chains cross-linked by lipophilic ether bridges. In Sephadex LH-20, the remaining hydroxyl groups are converted to hydroxyalkyl ether groups. These lipophilic regions are responsible for the unusual behaviour of the gel in water. The ether oxygen is able to form hydrogen bonds with water at low temperatures in the form of an oxonium structure, thus explaining the swelling ability of the gel. At higher temperatures these organised zones will be destroyed and hydrophobic bonding brings the lipophilic parts of the gel closer together.

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