Short Communications

HPLC of Biopolymers on Columns of Agarose*

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In 1961 agarose was introduced as a supporting medium for electrophoresis¹ and immuno-electrophoresis² and one year later for chromatography.³ Very soon – and particularly when agarose became commercially available – it became routinely employed in most biochemical laboratories for these and similar purposes. The broad applicability of agarose can be ascribed to its low adsorption of most biopolymers, high rigidity and high porosity. For instance, a 12% (w/v) agarose gel has a more open structure than dextran and polyacrylamide gels of this concentration – and yet it is more rigid. This explains why agarose gels – and particularly the cross-linked ones^{4,5} – can be used for HPLC with its requirement of hard and small gel beads for high resolution also at relatively high flow rates.^{6,7} An example is shown in Fig. 1. The beads were prepared as described in Ref. 8. Even at the high flow rate used (0.6 ml/min) the resolution was satisfactory although it was better at lower flow rates, as expected.

There is an extensive literature on methods for the derivatization of agarose gels for their use in conventional affinity chromatography, ion exchange chromatography, hydrophobic interaction chromatography, *etc.* All these derivatization methods can be applied to the high-concentration, crosslinked agarose beads. The way is therefore open to transform the above chromatographic methods [and others, including molecular-sieve chromatography (Figs. 1 and 2)] to the HPLC mode with agarose as a matrix.^{6,7} Since agarose is comparatively inexpensive one can expect that not

Fig. 1. High performance molecular-sieve chromatography of model proteins on 12% cross-linked agarose. Sample: a mixture of thyroglobulin (mol. wt. 669,000), catalase (232,000), aldolase (158,000), albumin (67,000), ovalbumin (43,000), chymotrypsinogen A (25,000), and ribonuclease A (14,000). Bed dimensions: 6 (i.d.) $\times$ 300 mm. Bead diameter: 1–10 μ m. Sample volume: 6 μ l. The amount of each protein: 3–25 μ g. Buffer: 0.1 M sodium phosphate, pH 7.4. Pressure: 28 atm. Flow rate: 0.6 ml/min.

Fig. 2. Separation of monomer, dimer and trimer of human albumin by high performance molecular-sieve chromatography on 12% cross-linked agarose. Bed dimensions: 6 (i.d.) $\times$ 340 mm. Bead diameter: 2–10 μ m. Sample volume: 10 μ l. The amount of protein: 40 μ g. Buffer: 0.2 M sodium phosphate, pH 6.8. Flow rate: about 0.1 ml/min. This diagram illustrates that a simple peristaltic pump (which was used in the experiment) can be employed for elution when there is no definite requirement of very short running times. A similar result was obtained on a column only 130 mm long (i.d. = 6 mm) when the flow rate was decreased to 0.02 ml/min (the run took about 3 h) and somewhat smaller non-cross-linked beads were used.

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only analytical but also preparative HPLC of biopolymers on agarose beds will be widely used in the future. An attractive feature of agarose columns is that they have a relatively low flow resistance, which means that they need not be operated by high pressure pumps but by cheaper peristaltic pumps, provided that moderate flow rates can be accepted (see Fig. 2). Another advantage is that the column tubes can be made from transparent glass or Plexiglass instead of stainless steel.

One can expect a higher resolution if the experiments shown in Figs. 1 and 2 are performed with gel beads of a more uniform size.

This communication is not a final report, but a presentation of work in progress.

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