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Note

High-performance liquid chromatography on continuous polymer beds

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Current research in this laboratory is directed toward the investigation of various possible means to increase performance in electrophoresis and chromatography. These methods are studied in parallel because the separation mechanisms are analogous[1–3], which means that solutions of methodological problems in electrophoresis are often applicable to analogous problems in chromatography and *vice versa*. In the chromatographic field we give an high priority to practical solutions (with the aid of theoretical considerations) of the following fundamental questions:

(1) Is it possible to design chromatographic beds such that the resolution is independent of or even increases with an increase in the flow-rate and bead size? Classical chromatographic theory says no. However, the experiments described in refs. 4–6 and forthcoming papers show that compressed beds of non-porous agarose beads have the desired unique relationship between the resolution, flow-rate and bead-size.

(2) Is it possible to design a chromatographic bed by bulk polymerization directly in the chromatographic tube? It has been taken for granted that a chromatographic bed must be built up of granulated particles, preferably in spherical form. Even when the spheres are monodisperse the packing is never perfect. The theoretical maximum resolution can therefore never be attained. Further disadvantages of packed beds are the time-consuming and expensive steps required for preparation of the beads, the sieving of the beads to select the desired size (if not monodisperse in the preparation) and the packing of the column with the beads.

A continuous gel plug with channels sufficiently large to permit an hydrodynamic flow might be the ideal chromatographic column. One could then expect the zones to be almost as sharp as those obtained in agarose or polyacrylamide gel electrophoresis. Unfortunately, the latter continuous gels cannot be used for chromatography, since they collapse when pressure is applied, *i.e.*, water cannot be pressed through them. However, more than 20 years ago we prepared a polyacrylamide gel (cross-linked in a special way) directly in a glass tube and on this gel plug separated monomers and dimers of albumin by molecular-sieve chromatography. The flow-rate was relatively low, which limited its usefulness. We have now resumed these experiments and at the same time have tried to improve the mechanical properties of the gel plug so that it will withstand higher pressures and thereby permit higher flow-rates. The polymerization technique is still under development and will be published else-

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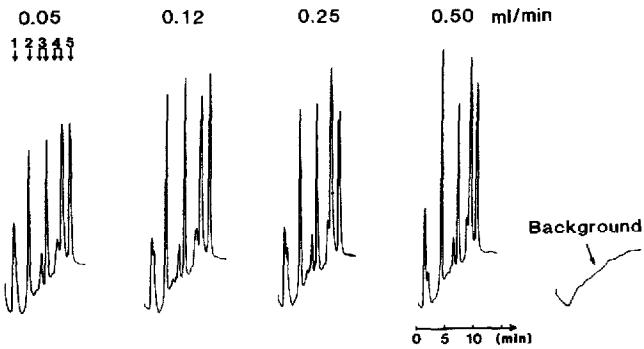

Fig. 1. High-performance cation-exchange chromatography of model proteins on a compressed continuous gel at the flow-rates indicated.

where. However, since the experiments are promising we present some preliminary results in order to focus interest on columns of continuous polymers.

EXPERIMENTAL AND RESULTS

The amphiphilic, macroporous gel plug consisted of a copolymer of acrylic acid and N,N'-methylenebisacrylamide. The gel plug was strongly compressed to a bed height of 3 cm (the importance of compressing a bed to increase its resolution is discussed in ref. 7). The diameter of the gel plug was 0.6 cm. This gel column was utilized for a cation-exchange chromatography experiment performed in the following way.

After equilibration with 0.01 *M* sodium phosphate, pH 6.4, a 40-μl sample [about 10–15 μg of each of the proteins alcohol dehydrogenase (1), horse skeletal muscle myoblobin (2), whale myoglobin (3), ribonuclease A (4) and cytochrome *c* (5)] was applied. Elution was performed with a linear gradient formed from the equilibration buffer and 0.01 *M* sodium phosphate, pH 6.4, containing 0.25 *M* sodium chloride. The flow-rates were 0.50, 0.25, 0.12 and 0.05 ml/min. The gradient volume was constant at 5.0 ml. The chromatograms are shown in Fig. 1. The relationship between the pressure and flow-rate is presented in Fig. 2.

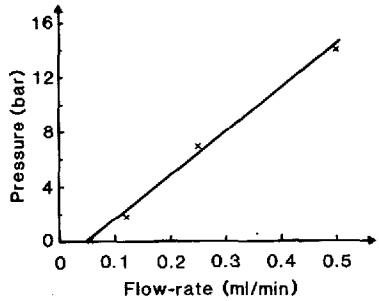

Fig. 2. The relationship between the flow-rate and pressure for the gel column used in the experiment shown in Fig. 1.

DISCUSSION

From Figs. 1 and 2 one can draw several conclusions:

(1) It is possible to prepare by bulk polymerization a continuous gel with channels large enough to permit passage of buffer when a pressure is applied to the bed.

(2) The bed is sufficiently rigid to give high flow-rates at moderate pressures.

(3) It is possible to prepare directly in the chromatographic tube a gel bed useful for ion-exchange chromatography. No subsequent step is required for attachment of ligands.

(4) The resolution on the continuous gel is roughly independent of the flow-rate, which is in sharp contrast to what is observed on columns of macroporous beads[8]. The reasons are probably that a gel plug has a more homogeneous structure than a packed bed of beads and that the gel plug was compressed, which, analogously to a compressed bed of agarose beads[7], has a favourable effect on the resolution. It is also likely that the gel plug is non-porous, *i.e.*, the "walls" of the channels in the gel are impermeable to proteins, which in combination with compression of the bed gives a resolution with the attractive flow-rate dependence mentioned above[4–6].

We are working on the preparation of continuous polymer beds for both hydrophobic-interaction and anion-exchange chromatography as well as for other kinds of chromatography.

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