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Note

Use of silicone-coated porous glass for exclusion chromatography in an aqueous medium

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Manufactured porous glass would be a valuable matrix for exclusion chromatography[1]. However, proteins are well adsorbed on the surfaces by two major forces, ionic amine–silanol bonding and a cooperative aggregative force, and the amounts of proteins adsorbed on 1 g of porous glass in distilled water are 100–200 mg[2,3]. Porous glass has therefore been used for the adsorption chromatography of proteins[4,5]. It has been shown that this surface activity can be minimized by coating the glass with polyethylene glycol[6]. However, the stability of the coating with polyethylene glycol is not permanent and some proteins are still adsorbed on the coated glass.

In the blood clotting reaction, the surfaces of the glassware are usually coated with silicone in order to overcome the surface activity of the glass. The coating with silicone is almost permanent but porous glass beads coated with silicone float on the surface of water and are not useful for exclusion chromatography in an aqueous medium. This paper reports a method for using silicone-coated porous glass in an aqueous medium and shows the lack of adsorption of proteins on silicone-coated porous glass.

MATERIALS AND METHODS

The controlled-pore glass used was CPG-10 (pore diameter 240 Å; 100-μm particles) obtained from Electro-Nucleonics (Fairfield, NJ, U.S.A.) with a surface area of 97 m^2/g. After being washed with 0.1% sodium dodecylsulphate (SDS), water and chromic acid, and then washed thoroughly with distilled water to neutrality in a column, the glass was dried at 180°C in an evaporating dish. The silicone oil used was KF 96 (dimethylpolysiloxane), obtained from Shinetsu Chemicals (Tokyo, Japan).

A 1-g amount of CPG was added to 3 ml of carbon tetrachloride containing 0.01, 0.05 or 0.2 ml of silicone oil and mixed. After evaporation of the carbon tetrachloride, the glass was tightly coated with silicone by heating at 300°C for 5 min. Elution of samples on a silicone-coated glass column was carried out at a flow-rate of 1.5 $ml/cm^2 \cdot min$ at room temperature.

RESULTS AND DISCUSSION

The best proportions of silicone and CPG of the three tried was 50 mg of

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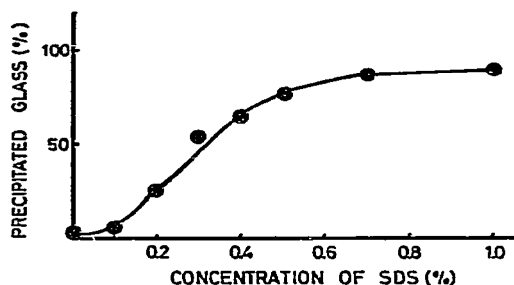
silicone per 1 g of CPG, and was used in subsequent experiments. These proportions correspond to about 30 molecules of silicone per 10000 Å^2 of glass surface area. With proportions of 10 mg of silicone per 1 g of CPG, the coated glass still adsorbed proteins in aqueous medium on elution of a protein solution through the silicone-coated glass column. With the proportions of 200 mg of silicone per 1 g of CPG, the pores of the CPG were partially clogged by silicone and the molecules of low molecular weight were eluted at the void volume of the silicone-coated porous glass column during gel chromatography.



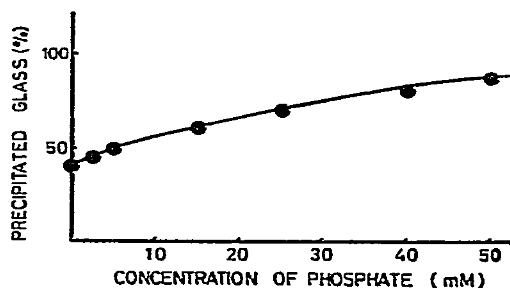


Fig. 1. Relationship between percentage of glass deposited and concentration of sodium dodecylsulphate in 0.05 *M* phosphate buffer (pH 7.3).

Fig. 2. Relationship between percentage of glass deposited and concentration of phosphate in 0.7% sodium dodecylsulphate.

The conditions for the preparation of silicone-coated porous glass in aqueous medium were as follows. The glass (200 mg) was added to 1 ml of 0.05 *M* phosphate buffer (pH 7.3) containing SDS at various concentrations and the mixtures were warmed to dissolve the SDS. The glass was deposited at the bottom of a tube by mixing. Fig. 1 shows the relationship between the percentage of glass deposited and the concentration of SDS. The glass was almost completely deposited at concentrations of SDS higher than 0.7%. This deposition was caused by hydrophobic bonding between dodecyl residues of SDS and silicone on the glass surfaces and the effect of the water repulsion was lost. At a concentration of 0.7% SDS in distilled water, the percentage of the glass deposited was low (approximately 40%). The addition of a salt such as phosphate was essential for deposition of the silicone-coated porous glass as well as SDS. Fig. 2 shows the relationship between the concentration of phosphate (pH 7.3) and the percentage of glass deposited in 0.7% SDS solution. The glass was almost completely deposited at concentrations of phosphate higher than 0.05 *M* (ionic strength 0.13 μ). From these results, the silicone-coated porous glass was deposited using 0.05 *M* phosphate buffer (pH 7.3) containing 0.7% SDS.

In order to remove SDS from the glass, the deposited glass was packed in a column and washed with about 100 column volumes of degassed hot water. If washing is carried out by decantation, part of the deposited glass floats, and the glass lacking SDS or not-binding SDS also floats on contact with air. Therefore, the glass in the deposited state should be washed in a column used for exclusion chromatography.

After washing with distilled water, it was confirmed that SDS did not remain on the glass by elution of 0.2% barium chloride solution[7]. SDS is bound and precipitated by Ba^{2+} ions, but the Ba^{2+} ions passed through the column, indicating that it was free of SDS. This lack of binding of SDS on the washed glass was also confirmed by floating of the glass on contact with air.

Conservation of the pores in the glass was confirmed. Fig. 3 shows the results of gel chromatography by elution of 0.1 ml of solution containing 1 mg of inosinic acid (a marker of the inner volume), 1 mg of bovine serum albumin and 2 mg of Blue Dextran 2000 (a marker of the void volume) in 0.01 *M* phosphate on a silicone-coated porous glass column (10 × 0.8 cm). All of the inosinic acid was eluted at the inner volume and not at the void volume, indicating that the glass fully retained the pores. Albumin was eluted at the void volume with Blue Dextran and this result was coincident with the results obtained using non-coated CPG using a buffer preventing adsorption[8]. However, the pores should be reduced in size on coating with silicone, and the extent of this reduction must be about 10–20Å, corresponding to the thickness of silicone on the glass surface.

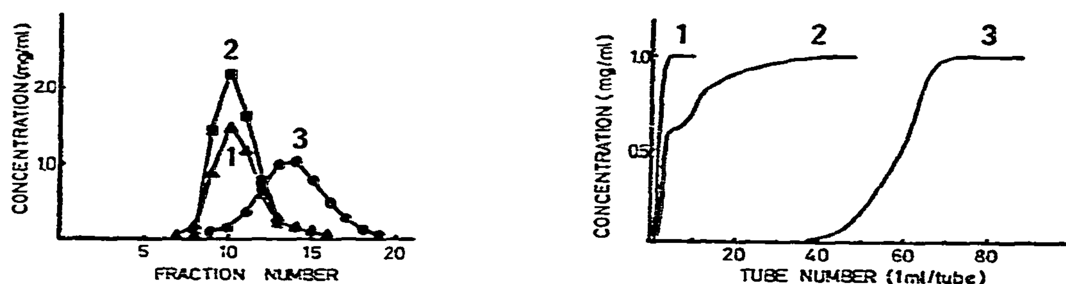


Fig. 3. Elution profiles of a mixture composed of dextran marker, bovine serum albumin and inosinic acid on a silicone-coated CPG column (10 × 0.8 cm) in 0.01 *M* phosphate buffer (pH 7.3). The fraction volume was 0.2 ml. 1 = Bovine serum albumin; 2 = Blue Dextran 2000; 3 = inosinic acid. The concentration of albumin was determined by the Lowry method, and those of dextran marker and iosinic acid were measured at 620 and 250 nm, respectively.

Fig. 4. Adsorption profiles of serum albumin (1 mg/ml) on a porous glass column (4 × 0.6 cm) in distilled water. 1 = Silicone-coated glass; 2 = glyceryl-CPG; 3 = non-coated CPG. In each instance 0.5 g of porous glass was used.

The elution pattern of a protein solution obtained using the silicone-coated porous glass is shown in Fig. 4. The amount of proteins adsorbed on 1 g of the glass in distilled water was less than 1.0 mg, and this was less than the amount (136 mg) of albumin adsorbed on non-coated glass and also less than that (9 mg) on glyceryl-CPG. This low adsorption of proteins is reproducible with an ionic strength of the solutes lower than 0.02 μ. Proteins are bound on the glass at concentrations of salt higher than 0.05 μ by hydrophobic bonding. A low concentration of the solute is essential for preventing adsorption of proteins. If proteins are adsorbed, the glass column can be regenerated by washing with hot 0.05 *M* phosphate solution containing 0.7% SDS, water and chromic acid, and then washing well with water.

CONCLUSION

Porous glass (1 g) was coated with 50 mg of silicone. Most of the silicone-coated glass was precipitated by mixing with 0.05 *M* phosphate (pH 7.3) containing 0.7% SDS. The coated glass with bound SDS was packed in columns and the SDS was completely removed from the glass by washing with distilled water. The silicone-coated CPG in an aqueous medium retained the pores for exclusion chromatography of proteins and did not bind proteins in aqueous medium at concentrations of salt lower than an ionic strength of 0.02 μ.

NOTE ADDED IN PROOF

The addition of glutamic or aspartic acids (1–10 m*M*) is effective for prevention of adsorption of proteins on the coated glass.

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