CHROM. 4789

Chromatographic behavior of silicic acid on Sephadex columns

Gel chromatography has developed into a useful tool for the separation of organic macromolecules and for the estimation of their molecular weight. In recent years gel chromatography has been used for the investigation of inorganic polymers such as polyphosphates[1–4], polymeric ferric hydroxide[5,6] and polymeric molybdates[7].

When a sodium silicate solution, which is highly alkaline, is neutralized or acidified, monosilicic acid* polymerizes with time, but it is not clear whether polysilicic acid of low molecular weight can exist in a stable state. The present paper describes the application of gel chromatography to the study of polymerization of silicic acid.

Experimental

Sample solutions and reagents. All reagents used were reagent grade. A silicic acid solution was made by fusing 0.500 g of anhydrous silica with 4 g of sodium carbonate. The melt was dissolved in distilled water and diluted to 1000 ml. Blue Dextran 2000 solution was 0.2%. 0.1 *M* sodium chloride solution (pH 2) was adjusted with hydrochloric acid to make the eluent.

Sephadex column. Sephadex G-25 (50–150 μ) and G-100 (40–120 μ) were used after swelling for at least 48 h in distilled water. The column was a 1.5 × 60 cm glass tube with a porous polystyrene disc at one end. The column was wet-packed in the usual manner, using just enough gel to bring the packing to constant volume marked on the column at 57 cm. In addition, a small disc of filter paper served to reduce disturbance at the top of the bed.

Procedure. Experiments were carried out as follows. The eluent was passed through the column. An aliquot of alkaline silicic acid solution was neutralized by adding hydrochloric acid. After standing for a definite period, the pH of the sample solution was adjusted to 2 with hydrochloric acid. One ml of the sample solution made as described above was placed on the column bed just as the last layer of eluent soaked into the bed. Then the eluent was applied when the last portion of the sample solution soaked into the bed. Using a fraction collector, the effluent was collected in fractions of 1 ml, with flow rates of 20–26 ml/h (Sephadex G-25) and 5–6 ml/h (Sephadex G-100). The effluent fraction was transferred into a platinum crucible, and 0.2 ml of a 0.1 *M* sodium carbonate solution was added. The solution in the crucible was heated nearly to dryness on a sand bath. Polysilicic acid was thus converted to monosilicic acid. Distilled water was added to the crucible, and monosilicic acid in this solution was measured by colorimetry.

Results and discussion

Effect of pH on the polymerization of silicic acid. The rate of polymerization of

* In this paper, silicic acid which reacts with molybdate reagent in acid solution and gives a yellow color is defined as monosilicic acid for convenience. The reaction of silicic acid with molybdate reagent is used for a colorimetric determination of monosilicic acid. Silicic acid which does not react with molybdate reagent is defined as polysilicic acid.

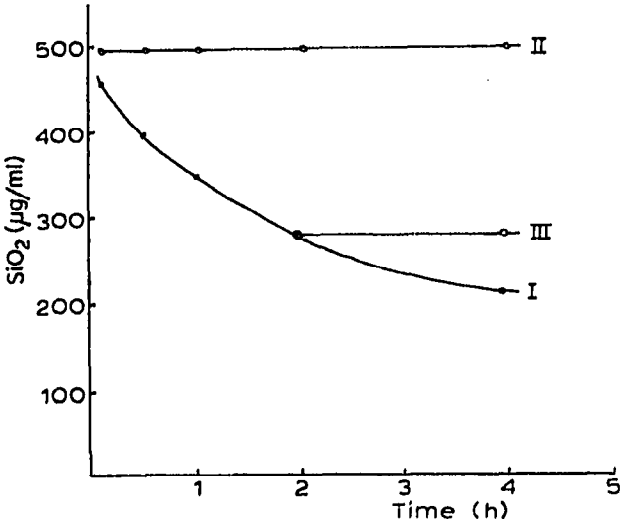

Fig. 1. Effect of pH on the polymerization of silicic acid. Sample I: neutral solution. Sample II: pH 2 solution. Sample III: pH 2 solution adjusted 2 h after neutralization.

silicic acid differs with the pH of solution. In a strongly alkaline solution, monosilicic acid does not polymerize, but polysilicic acid rapidly forms in neutral solution. The polymerization of silicic acid is least rapid in an aqueous solution at pH 2. Below about pH 2, the rate of polymerization increases with greater acidity[8,9]. An example is shown in Fig. 1. The ordinate is the concentration of monosilicic acid, determined by a colorimetric method. The abscissa is the time after the pH is adjusted. Monosilicic acid has decreased rapidly in the pH 7 solution (Sample I). In the pH 2 solution, monosilicic acid has not decreased at least within 10 h (Sample II), but polysilicic acid has formed very gradually over a long period. In the case of Sample III, 2 h after neutralization the pH of the sample solution was adjusted to 2. Polysilicic acid rapidly formed until 2 h after neutralization, but monosilicic acid did not decrease within a few hours after adjustment to pH 2. From the results mentioned above, it can be assumed that the state of silicic acid in the solution is just "fixed" when the pH of solution is adjusted to 2.

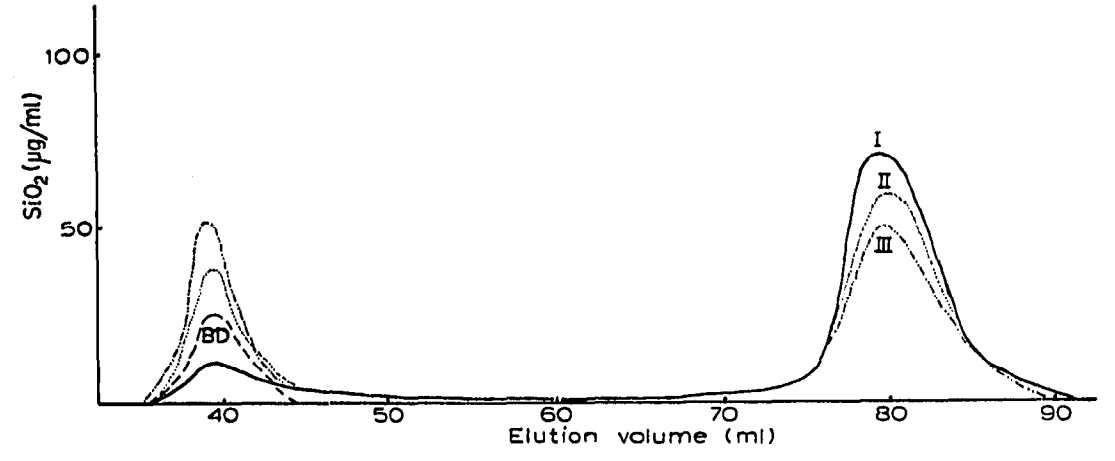

Fig. 2. Elution curves of silicic acid chromatographed on a column of Sephadex G-25. Sample I: 1 h after neutralization. Sample II: 2 h after neutralization. Sample III: 4 h after neutralization. BD: Blue Dextran.

Sephadex G-25. Fig. 2 shows typical chromatograms for silicic acid samples obtained at different periods after neutralization on Sephadex G-25. Two peaks were obtained. Peaks on the right in Fig. 2 were due to monosilicic acid, and peaks on the left were due to polysilicic acid. The volume for polysilicic acid appeared to be identical to that for Blue Dextran. It can be assumed that the elution volume of Blue Dextran is equal to the void volume of the bed.

An approximate separation range (shown by molecular weight) for Sephadex G-25 measured by peptides and globular proteins is given as 1000–5000 (ref. 10). If it can be assumed that polysilicic acid has spherical particles and that an approximate separation range for silicic acid is the same as that for globular proteins, the molecular weight of the peak given by polysilicic acid is over 5000. An absence of any peak between two peaks shows that the particle size of polysilicic acid grew rapidly and that polysilicic acid of a low molecular weight (under 5000) could not exist in a stable state. The results described above suggest that monosilicic acid can be separated from polysilicic acid using a Sephadex column.

Sephadex G-100. Fig. 3 shows the elution profiles of silicic acid chromatographed

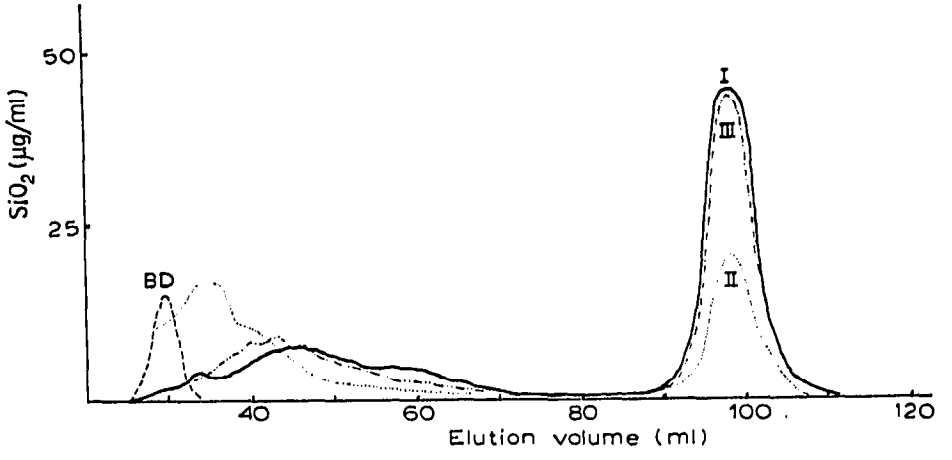

Fig. 3. Elution curves of silicic acid chromatographed on a column of Sephadex G-100. Sample I: 2 h after neutralization. Sample II: 48 h after neutralization. Sample III: adjusted to pH 2 2 h after neutralization and allowed to stand 12 days. BD: Blue Dextran.

on Sephadex G-100. Peaks on the right in Fig. 3 were due to monosilicic acid, and broad peaks on the left were due to polysilicic acid. An approximate separation range for Sephadex G-100 is given as 4000–150 000 (ref. 10). Peaks due to polysilicic acid indicate a broad distribution of molecular weight (size). It is seen that the molecular weight of polysilicic acid in Sample I ranges from 5000 to 150 000 because the lower limit for the molecular weight of polysilicic acid was given by the results obtained for Sephadex G-25.

The molecular weight distribution of polysilicic acid in Sample II shifted to 150 000 or larger compared with that of Sample I. It seems reasonable to assume that the broad distribution of molecular size, indicated by peaks due to polysilicic acid, arises from a random condensation of all the polymeric species.

The shape of the curve for Sample III is similar to that for Sample I. This

means that aggregation of polysilicic acid as well as polymerization of monosilicic acid in the pH 2 solution is strongly retarded.

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Resolution of some closely related prednisolone derivatives by thin-layer chromatography

One of the most important tests of steroidal drugs is the related foreign steroids test[1–3]. In this test use is made of thin-layer chromatography (TLC) to detect the presence of structurally related compounds that may exist in the therapeutically active steroid. As a result of the interest in this laboratory in the determination of steroid purity and absence of closely related compounds by TLC, solvent systems were reported previously for the separation of some estrogens[4] and some closely related hydroxycorticosteroids[5]. The semiquantitation of the closely related steroids as well as the estimation of flurandrenolone acetonide purity by TLC, were also achieved[6]. Several TLC systems for cortical steroids have been reported[7–10]. None of these were found to be completely adequate for the complete resolution of a mixture of closely related prednisolone derivatives in which we were interested. This paper describes a new developing system—and its application—for the complete resolution of this particular mixture by TLC on silica gel.

Materials

Reagents. All solvents and chemicals were reagent grade.

Developing system. Ethyl propionate.

Spray reagent. Methanolic sulfuric acid, prepared as mentioned previously[4].

Equipment. Pre-coated 250 μ thin-layer plates (Silica Gel F_{254}) supplied by Brinkmann Instruments Inc. Micro-pipets, Microcaps (Drummond Scientific Compa-