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Note

High-speed gel filtration chromatography of polymers formed by β-lactam antibiotics

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β-Lactam antibiotics, such as penicillins and cephalosporins, are susceptible to polymer formation by cleavage of the β-lactam ring, and this has been thought to be one of the causes of their acquisition of antigenicity and allergenicity[1–4]. The formation of polymers is accelerated in the presence of water, *e.g.*, in a concentrated aqueous solution or in a water suspension[1–6]. In β-lactam antibiotic preparations for clinical use, therefore, the presence of a small amount of polymers is often detected and this is probably responsible for the major adverse reaction, anaphylaxis.

For the determination of polymers in the preparation, gel chromatography on cross-linked dextran, Sephadex or polyacrylamide gel, Bio-Gel, has been widely used. Recently, however, gels for high-pressure gel filtration chromatography have been developed and used successfully to separate synthetic polymers or oligomers and viruses and various proteins[7–12]. In this study, we applied TSK-GEL G2000SW, a hydrophilic porous silica-based gel, to the analysis of β-lactam antibiotic polymers and found that it offers high resolution and rapid separations.

EXPERIMENTAL

Materials

β-Lactam antibiotics were obtained from commercial sources: penicillin G (PcG) (Penicillin G; Meiji, Tokyo, Japan); ampicillin (ABPC) (Solcillin; Takeda, Osaka, Japan); cephalothin (CET) (Keflin; Shionogi, Osaka, Japan).

Procedure

The antibiotics were dissolved in water to give 25% (w/v) solutions, which were stored at room temperature for 2 weeks. Samples of constant volume were taken from the solutions immediately after dissolution and subsequently at 3- or 4-day intervals and subjected to gel filtration on columns of TSK-GEL G2000SW and Sephadex G-25. The TSK column (600 × 7.5 mm I.D.; Toyo Soda, Tokyo, Japan) was equipped with a Model 635 LC detector (Hitachi, Tokyo, Japan) operating at

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250 nm. Samples were injected using a U6K injector (Waters Assoc., Milford, MA, U.S.A.) and eluted with 0.1% sodium chloride solution at a flow-rate of 1 ml/min using a 6000A solvent delivery system (Waters Assoc.). The Sephadex column was prepared by packing a column (1000 × 15 mm I.D.) with Sephadex G-25, fine (Pharmacia, Uppsala, Sweden) and 0.5% sodium chloride solution was used as the eluent at a flow-rate of 1 ml/min[1]. The peaks were monitored with a Model 124 spectrophotometer (Hitachi) operating at 230 or 254 nm.

RESULTS AND DISCUSSION

An aged 25% solution of PcG, 14 days after dissolution, was applied to a G2000SW column. Elution patterns were drawn by varying the concentration of sodium chloride in the eluent. Several typical patterns are shown in Fig. 1. When the eluent contained no sodium chloride, the antibiotic was eluted within 15 min without separation from its polymers. With increase in sodium chloride concentration up to at least 0.3%, the elution time was increased accompanied by an improved separation. At higher concentrations, however, the peaks broadened and the chromatogram became blurred. Optimal separation was achieved with 0.05–0.3% sodium chloride solution.

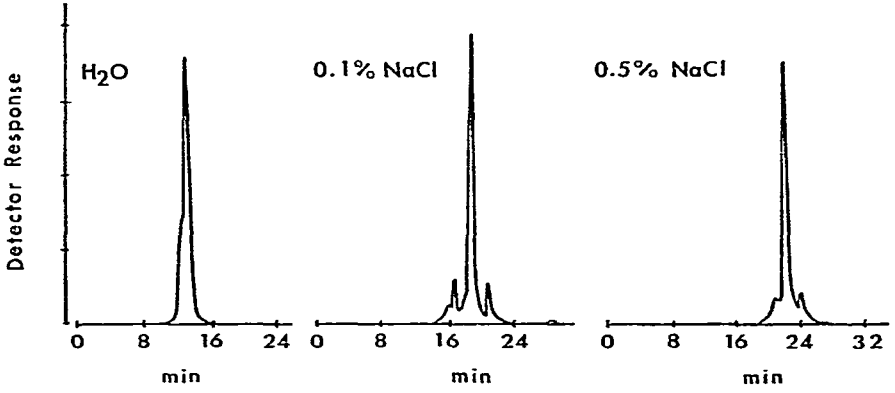

Fig. 1. Effect of sodium chloride concentration on the elution profile of an aged solution of PcG using TSK-GEL G2000SW.

When phosphate buffer (pH 7.0) or ammonium formate solution was used as the eluent, a similar change in the elution pattern was observed on varying the solute concentration. With 0.02 *M* solutions of these salts, patterns comparable to that obtained with 0.1% sodium chloride solution were obtained and a further increase in concentration gave poorer results. This kind of change in pattern with variation in the concentration of electrolytes in the eluent has been reported for the elution of proteins[10,11], probably being caused by the ionizing character of silanol, as G2000SW is silica-based. At a suitable ionic strength, *e.g.*, 0.1% sodium chloride solution, the ion-exclusion effect of silanol may work synergistically with gel filtration to result in good separations.

The formation of polymers in a 25% aqueous solution of PcG on storage at

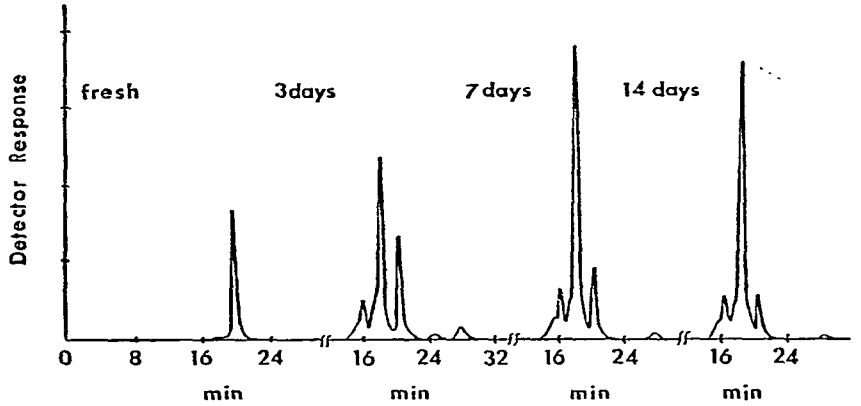

Fig. 2. High-speed gel filtration chromatography of aged solutions of PcG on TSK-GEL G2000 SW.

room temperature was followed by using the above procedure and the results are shown in Fig. 2. The peaks of PcG decreased in height monotonously with increasing time whereas the peaks due to its polymers increased in height up to 7 days. The apparent decrease in peak height from 7 to 14 days can be ascribed to a shift in the absorption maximum due to polymers caused by concomitant degradation.

For comparison, the same sample was subjected to conventional gel filtration on Sephadex G-25 (Fig. 3). It can be seen that the Sephadex method required more than six times longer than the G2000SW method and still gave a considerably inferior peak.

To establish whether the order of elution of the polymers and/or degradation products of PcG is the same or not on G2000SW and G-25 columns, 12 ml of an aged PcG solution was applied to a large G-25 column (1000 × 50 mm I.D.) and the eluate was fractionated into six parts, as shown in Fig. 4. After re-chromatography

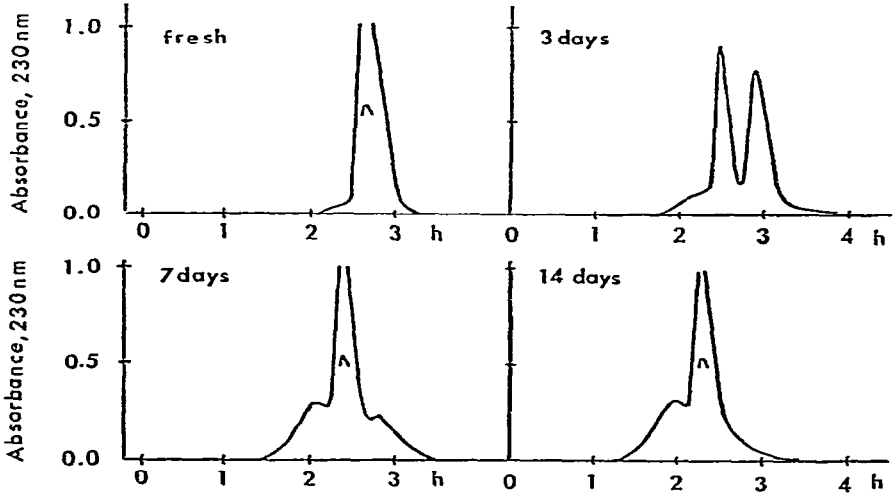

Fig. 3. Gel filtration chromatography of aged solutions of PcG on Sephadex G-25.

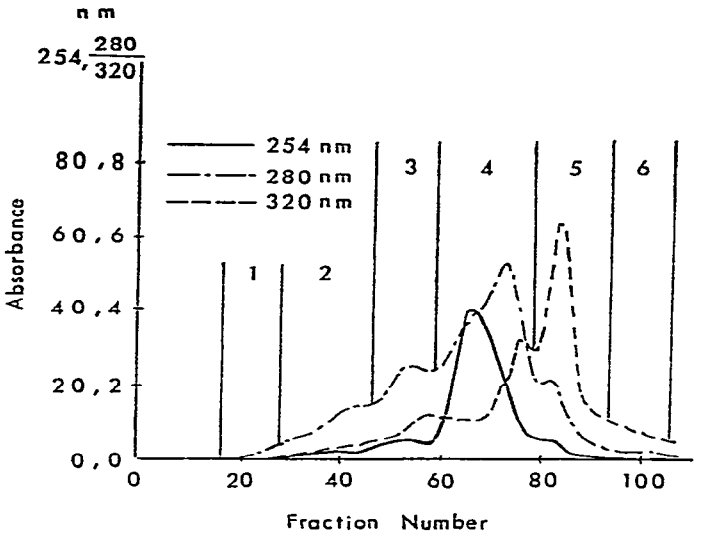

Fig. 4. Preparative gel filtration chromatography of a 14-day-old solution of PcG on a large Sephadex G-25 column using 0.5% sodium chloride solution as the eluent. A 15-ml volume of the eluate were collected. Chromatograms were obtained by measuring the UV absorption at 254, 280 and 320 nm.

through G-25, each fraction was analysed on a G2000SW column. The results (not shown here) indicated that the order of elution is the same on both columns and the polymer peaks at elution times of 1.4–2.2 h on G-25[5] corresponded to those at 14–17 min on G2000SW. These results suggest the usefulness of the G2000SW column

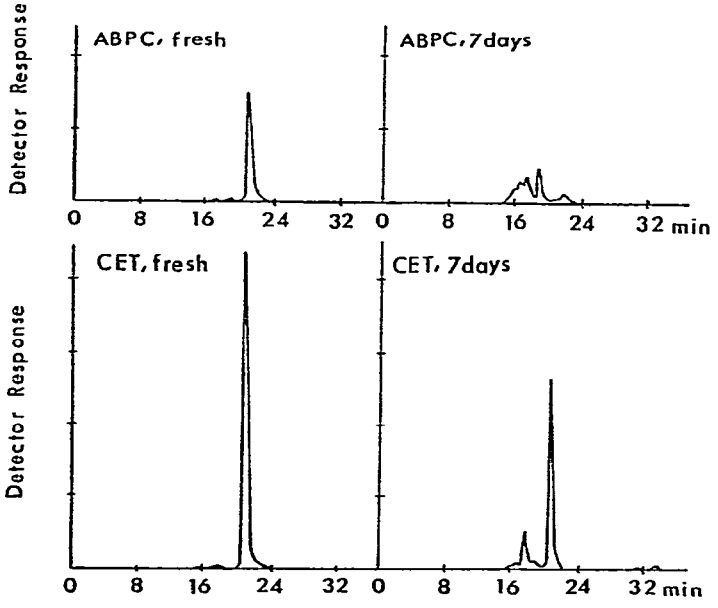

Fig. 5. High-speed gel filtration chromatography of polymers formed by ABPC and CET on TSK GEL G2000SW.

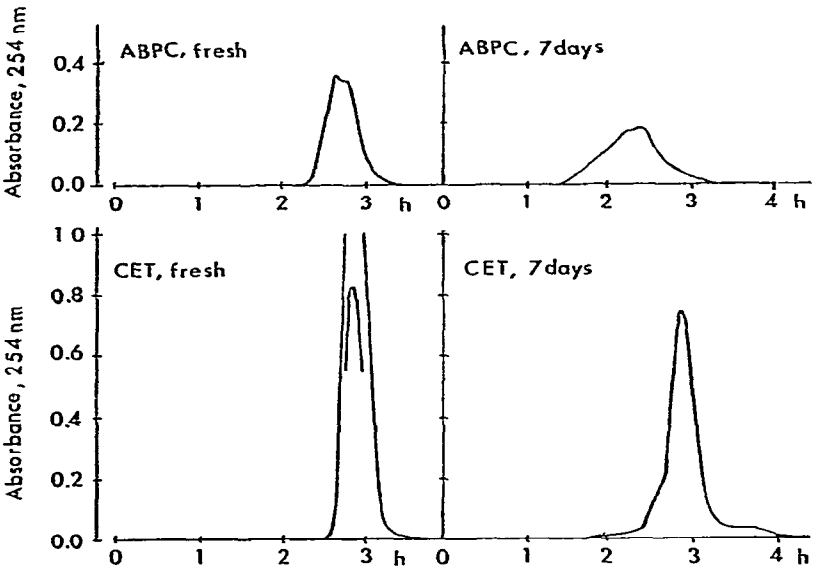

Fig. 6. Gel filtration chromatography of polymers formed by ABPC and CET on Sephadex G-25.

for following the formation of polymers in PcG solution and prompted us to apply it to some other β-lactam antibiotics. Figs. 5 and 6 show the chromatograms of fresh and 7-day-old solutions of ABPC and CET obtained on columns of G2000SW and G-25. The chromatograms show clearly the superiority of the G2000SW method over the G-25 method. Thus, the G2000SW column should be a powerful tool for the analysis of β-lactam antibiotic polymers in view of its higher separation efficiency and rapidity.

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