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NEW WATER-COMPATIBLE MODIFIED POLYSTYRENE AS A STATIONARY PHASE FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

CHARACTERIZATION AND APPLICATION

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SUMMARY

A new packing material for high-performance liquid chromatography (HPLC) was obtained by covalent bonding of neutral hydrophilic groups to the surface of porous polystyrene–divinylbenzene beads. Particles with a mean bead size of 7 μm and with mean pore diameters of 3 and 17 nm were evaluated as stationary phases as regards their rigidity, stability, chromatographic performance as well as the influence of pore size on column efficiency, etc. The modified polystyrene maintains the main advantages of polystyrene: it is ion-free and has high chemical stability. Noteworthy also are the increased rigidity, compatibility for solvent changes from aqueous to organic and the reverse and a broad application range in different chromatographic modes. The new hydrophilic polystyrene HPLC phase is easy to pack and yield good column lifetime. Its application to the separation of several complex mixtures is illustrated.

INTRODUCTION

Most high-performance liquid chromatography (HPLC) is done on silica-based packing materials due to their good chromatographic performance. However, it is well known that these materials have some drawbacks which limit their use, such as a short column lifetime and sometimes irreversible adsorption of a solute due to dissolution of the packing materials and to active sites on their surface. An alternative for silica gel as packing material would be cross-linked polystyrene, if the problems inherent to this material could be overcome[1].

Polystyrene packings for chromatography are organic porous spheres obtained by copolymerization of styrene and divinylbenzene. Such materials are chemically stable, and not soluble in solvents. Their surfaces are ion-free and have no active sites such as those which exist in silica-based packings. Ever since Moore[2] developed a process for making highly cross-linked polystyrene gels, they have been used for organic size-exclusion chromatography (SEC), and also as a starting material for strong and weak ion-exchange resins, *e.g.*, sulphonated polystyrene and even car-

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boxylated polystyrene[3]. More recently, improvements in the techniques used to synthesize polystyrene beads have led to the use of this resin as reversed-phase HPLC packing material[4–9]. A column packing method for PRP-1 (one kind of spherical polystyrene–divinylbenzene copolymer from the Hamilton company) was described by Lee and Kindsvater[4]. This method can also be used for a polystyrene–divinylbenzene copolymer from other sources[8]. However, some problems still limit the use of these polymers in the reversed-phase mode, especially for biochemical separations which are mostly performed in aqueous media. These limitations are caused by swelling and shrinking in the organic solvent and water respectively, by the high hydrophobicity of polystyrene as well as by difficulties in packing such columns. This is well recognized. Of the many efforts with more polar organic polymers, mention can be made of a recent contribution on polyacrylamide phases by Dawkins *et al.*[9].

Our intention is to make polystyrene bead packing materials more water-compatible by chemical derivatization but without introducing electric charges. This could overcome the difficulties mentioned above. In this paper we describe a new modified polystyrene and its chromatographic performance, as well as its application to several groups of compounds.

EXPERIMENTAL

Apparatus and chemicals

A Model 5000 liquid chromatograph (Varian, Palo Alto, CA, U.S.A.) equipped with a 7000-p.s.i. 10-μl loop injector (Valco Instruments, Houston, TX, U.S.A.) and a Vari-Chrom 50 variable-wavelength UV detector (Varian) and a Varian A-25 recorder was employed.

Mobile phases were prepared with deionized water and acetonitrile. They were degassed by agitation under vacuum in an ultrasonic bath before use.

Samples and reagents were obtained from commercial sources and were used as received, except for the alkaloids which are experimental products from GeChem (Louvain-la-Neuve, Belgium).

Packing material

Porous, spherical polystyrene beads were produced at this laboratory. Two batches were used with particle size of 7 μm and pore sizes of 3 and 17 nm. The specific surface area of these materials is a problem in the sense that its determination is strongly influenced by the techniques used. The BET approach (measuring in the dry state) gives figures which are too small. The SEC method (swelling in solvent) seems to be best for an HPLC material[10]. In this way a specific surface area of 320 m^2/g was found for the larger pore size base material. The pore sizes mentioned above were determined with the method of Halasz and Martin[11]. To this polystyrene matrix, a polar ligand was bound chemically, thus yielding a water-compatible, charge-free packing material. Details of the modification procedure will be published elsewhere.

This material was developed in collaboration with RSL and Alltech-Europe (Eke, Belgium). It will eventually be commercialized. A tentative name is Rogel-P.

Column packing procedure

A 2.5-g amount of Rogel-P was suspended in 12 ml of water–acetonitrile (50:50). The suspension was sonicated for 10 min and packed into a 10 cm × 0.7 cm I.D. stainless-steel column connected with a 20 cm × 0.7 cm I.D. stainless-steel precolumn with a Valco coupling union, using an air-driven fluid pump DSHT-302A (Haskel, Burbank, CA, U.S.A.) at the packing pressure of 400 bar and flow-rate of 5 ml/min for half an hour. Pure distilled water was used as the packing eluent. The column was closed using 3/8 in. Valco fittings with a 2-μm stainless-steel frit.

RESULTS AND DISCUSSION

Efficiency

Fig. 1 shows curves of the height equivalent to a theoretical plate (HETP) *vs.* the velocity, u, for phenolic compounds on materials of different pore sizes, but the same particle size. With the column of large (17 nm) pore size (Fig. 1A), the efficiency for phenol was about 50000 plates per m, which corresponds to a reduced plate height of 3. Comparison of Fig. 1A and B reveals the influence of the pore size on efficiency. The efficiencies for phenol, resorcinol and epicatechol on the large pore (17 nm) column are respectively 2.8, 2.4 and 1.4 times larger than those on the small pore (3 nm) column at 0.2 ml/min, and 1.8, 1.9 and 2.0 times better respectively at 2.0 ml/min. It is apparent that the mass-transfer rate of the solutes is much better on the larger pore size packing material. The efficiency increases with decreasing number of hydroxyl groups of the solute, suggesting that the hydroxyl group contributes negatively to mass transfer. This behaviour can be improved and changed by increasing the column temperature, as shown in Fig. 2. The slope of the curves increases with the number of hydroxyl groups, showing that the influence of temperature on efficiency is larger for compounds having higher hydroxyl content.

Solvent compatibility and rigidity

This is an important feature, which often limits the application of polymeric columns in HPLC. Figs. 3 and 4 show the swelling test, devised in this laboratory[12],

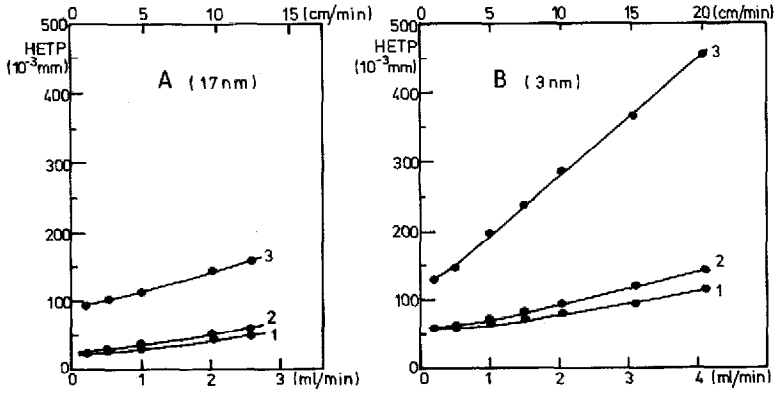
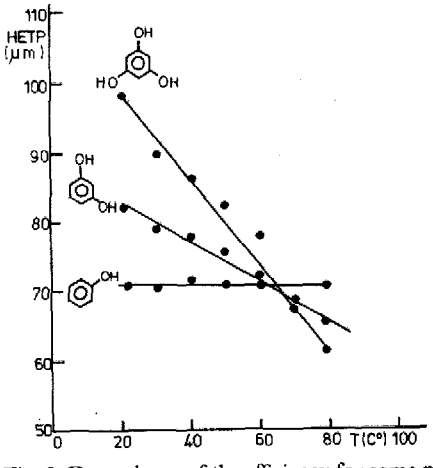


Fig. 1. HETP *vs.* u curves on the new modified polystyrene of particle size 7 μm, pore size 17 (A) and 3 nm (B). Column: 10 cm × 0.7 cm I.D. Eluent: acetonitrile–water (40:60). Detection: 280 nm at 0.2 a.u.f.s. Samples: 1 = phenol; 2 = resorcinol; 3 = epicatechol.

by measuring the change in column back pressure on the original unmodified polystyrene and on the modified polystyrene when changing the mobile phase from pure water to pure tetrahydrofuran (THF) at 3.0 ml/min. In Fig. 3 the column back pressure rises abruptly to 350 atm, the maximum working pressure of the chromatograph, when the gradient has reached only 30% THF. In this case, it is impossible to perform a whole gradient from 0 to 100% THF. This column gave a pressure as high as 228 atm when the flow-rate was reduced to 0.5 ml/min with 100% THF. In contrast, on the new modified polystyrene packing material the curve is reproducible and can be reversed, *i.e.*, by changing the mobile phase from pure THF to pure water again. The pressure is 62 atm at 100% water, and only 48 atm at 100% THF; the maximum pressure is 160 atm. This change corresponds to the viscosity change of the binary solvent system. The swelling propensity (SP) of the modified polystyrene material is about 0.4. This is comparable with silica-based material and better than those of most commercially available polymeric packing materials[12]. It means that the new material swells very little when changing from aqueous to organic solvents. In addition, Fig. 5 demonstrates a good linear relationship between the column back pressure and the mobile phase velocity, up to nearly 350 atm. This suggests that the new material is quite rigid, as required for use in HPLC.

Selectivity

Figs. 6 and 7 show plots of k' (capacity factor) against the percentage of organic modifier (acetonitrile) on the unmodified and modified polystyrene respectively. The selectivity is greatly changed by the modification. An interesting phenom-



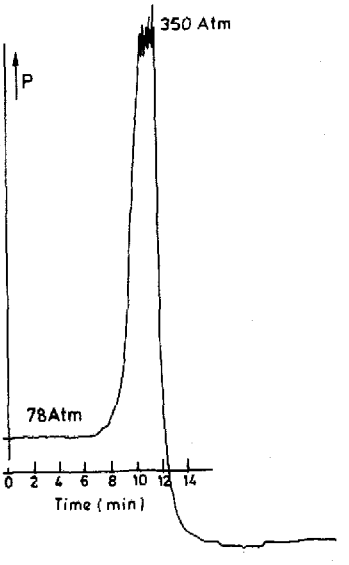
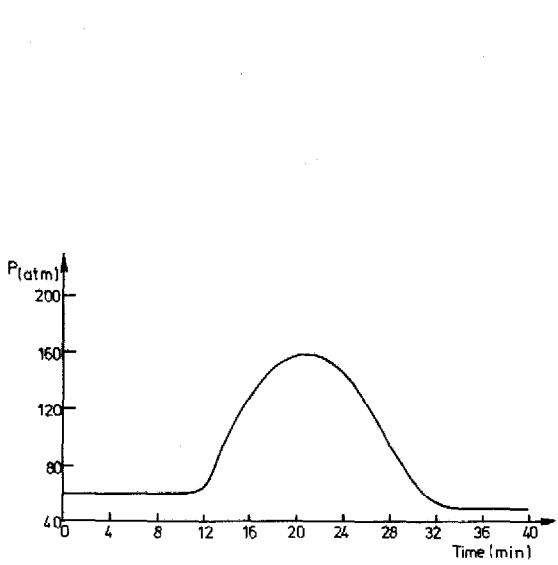


Fig. 2. Dependence of the efficiency for some phenolic compounds on temperature. Column: 10 cm × 0.7 cm I.D. (7-μm particles, 3-nm pores). Flow-rate: 1.0 ml/min. Other details as in Fig. 1.

Fig. 3. Influence of solvent composition on the column back pressure for the unmodified polystyrene. Column: 10 cm × 0.7 cm I.D. (7-μm particles, 3-nm pores). Flow-rate: 3 ml/min. Eluent: mixture of water and THF, isocratic at 100% water for 5 min and then linear gradient to 100% THF in 20 min at room temperature.



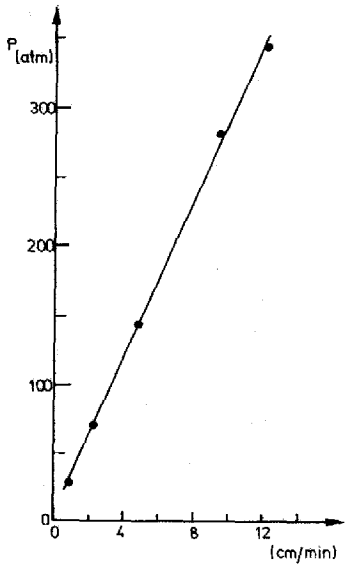
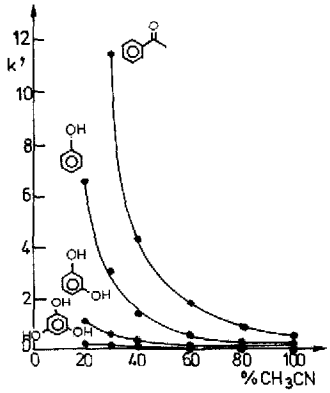


Fig. 4. Influence of solvent composition on the column back pressure for the new modified polystyrene column. Conditions as in Fig. 3.

Fig. 5. Plot of column back pressure against mobile phase velocity for the new modified polystyrene. Conditions as in in Fig. 1A.



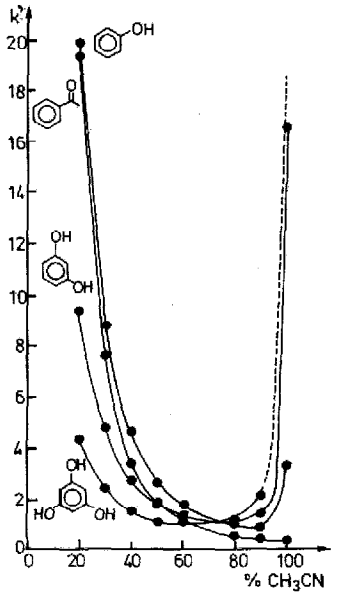


Fig. 6. Capacity factor, k', *vs.* mobile phase composition on the unmodified polystyrene. Column: 10 cm × 0.7 cm I.D. (7-μm particles, 17-nm pores). Eluent: acetonitrile–water. Flow-rate: 1.0 ml/min. Detection: 280 nm. Samples: phloroglucinol; resorcinol; phenol; acetophenone.

Fig. 7. Capacity factor, k', *vs.* mobile phase composition on the modified polystyrene with pore diameter 3 nm. Other conditions as in Fig. 6. The 17-nm pore material gives similar results.

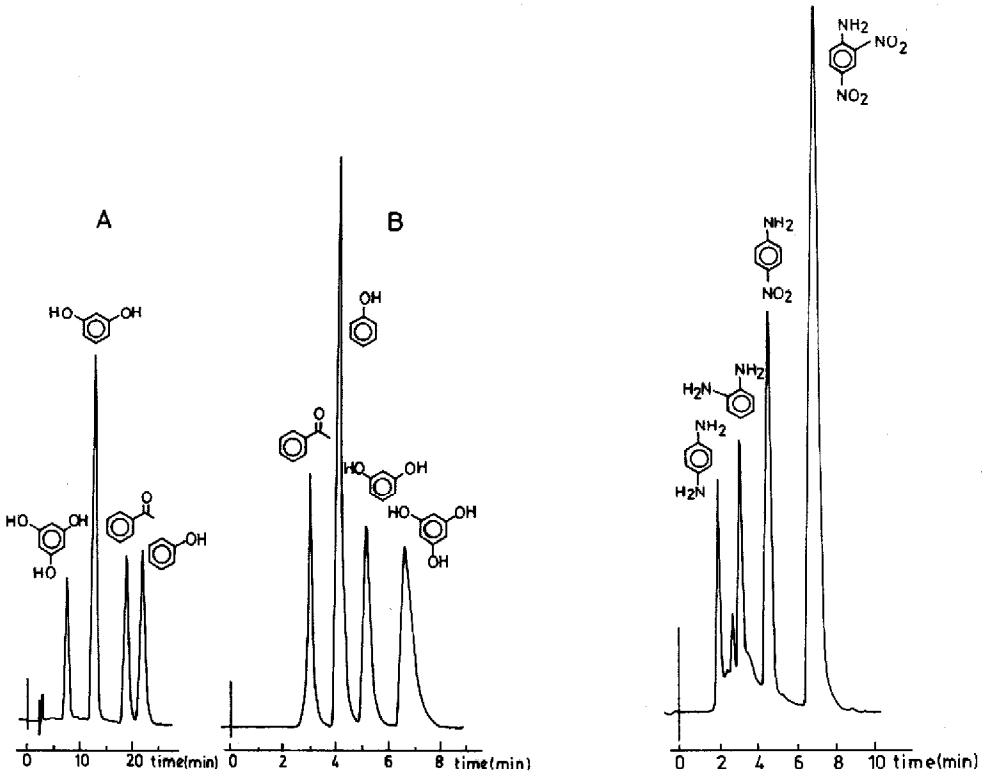


Fig. 8. Chromatograms in reversed-phase (A) and normal-phase modes (B). Conditions as in Fig. 7 at acetonitrile–water (30:70) (A) and acetonitrile–water (90:10)(B).

Fig. 9. Separation of amino- and nitrobenzenes. Column: 10 cm × 0.7 cm I.D. (7-μm particles, 3-nm pores). Mobile phase: acetonitrile–water (70:30). Flow-rate: 1.0 ml/min. Detection: 280 nm.

enon is the U-shape of the plot in Fig. 7, *i.e.*, the elution order of phloroglucinol, resorcinol and phenol reverses at about 75.5% of acetonitrile in the mobile phase. They are eluted in a reversed-phase mode before this point, and in a normal-phase mode after it. Corresponding chromatograms are shown in Fig. 8. This change is due to the contribution of hydrophilic groups introduced on the surface of the packing material. It means that the hydrophobic interactions are dominant at low acetonitrile contents and the hydrophilic interactions dominate at high contents. Melander and Horváth[13] explained this phenomenon in 1980. It was noted later by Hearn and Grego[14] and by Jandera *et al.*[15–17] on different organic polymers. Recently, Engelhardt and Muller[18] reported examples for proteins and peptides on reversed-phase silica gel. Our case is very clear-cut. At high water concentration the hydroxyl groups of the phase and the solutes are completely solvated and there is no adsorption force between them. The hydrophobic polystyrene matrix, not wetted by water, is the adsorbing part of the phase. The interaction with the solutes is hydrophobic in character. At higher acetonitrile content the opposite reasoning can be employed; in this situation the adsorption occurs on the polar hydroxyl sites of the phase.

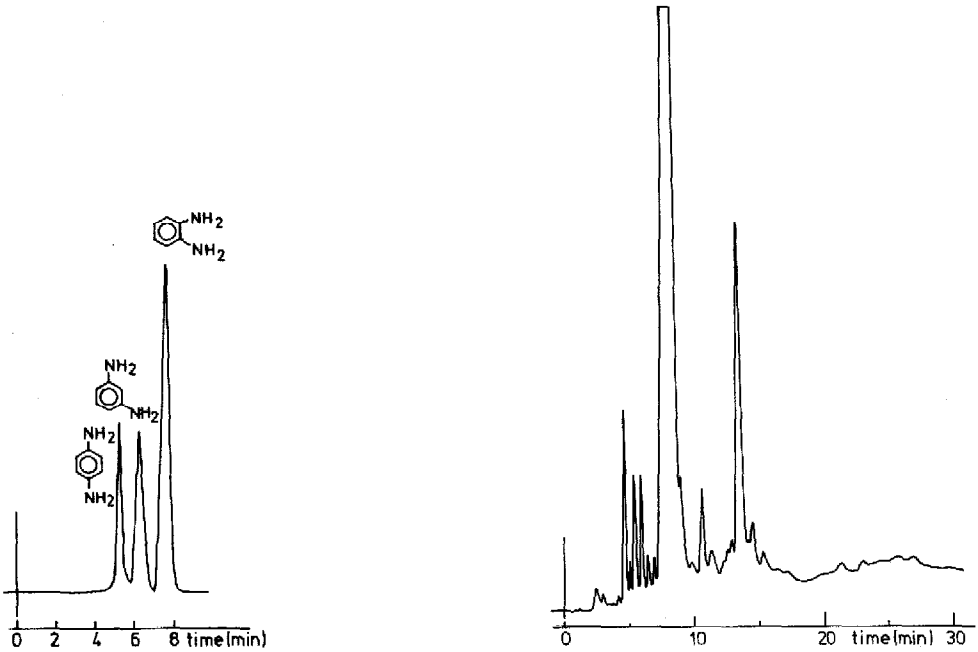


Fig. 10. Separation of three diaminobenzene isomers. Column: 10 cm × 0.7 cm I.D. (7-μm particles, 17-nm pores). Mobile phase: acetonitrile (containing 5% 2-hydroxyethylamine)–water (containing 5% 2-hydroxyethylamine) (30:70), pH 12. Flow-rate: 0.5 ml/min. Detection: 280 nm.

Fig. 11. Chromatogram of vinblastine derivatives. Column as in Fig. 10. Mobile phase: 0.6 ml triethylamine and about 0.3 ml phosphoric acid (85%, w/w solution in water) in 100 ml water pH 3.1 (solvent A) and acetonitrile (solvent B). Gradient: from 15 to 30% B in 15 min, to 35% B in 10 min and to 50% B in another 15 min. Flow-rate: 1.0 ml/min. Detection: 290 nm at 0.5 a.u.f.s.

Applications

The new material was applied successfully to several groups of compounds including aromatic, phenolic, amino- and nitro-aromatic compounds, as well as alkaloids and proteins.

Fig. 9 shows the separation of a mixture of amino- and nitrobenzenes. In Fig. 10 a separation of three diaminobenzene isomers is obtained with a basic mobile phase, pH 12. This is not possible with silica-based materials. It should also be noted that the separation was not achieved with other mobile phases tested, *e.g.*, mixtures of water or water containing 0.1% TFA (trifluoroacetic acid) and acetonitrile in different proportions.

Figs. 11 and 12 demonstrate the separation of vinblastine derivatives and vinca rosea alkaloids with an acidic mobile phase of pH 3.1.

Another important application of the material is for protein separation. The chromatogram of Fig. 13 illustrates this. The proteins are separated in the SEC mode. More details of the separation of proteins on this new material will be published elsewhere.

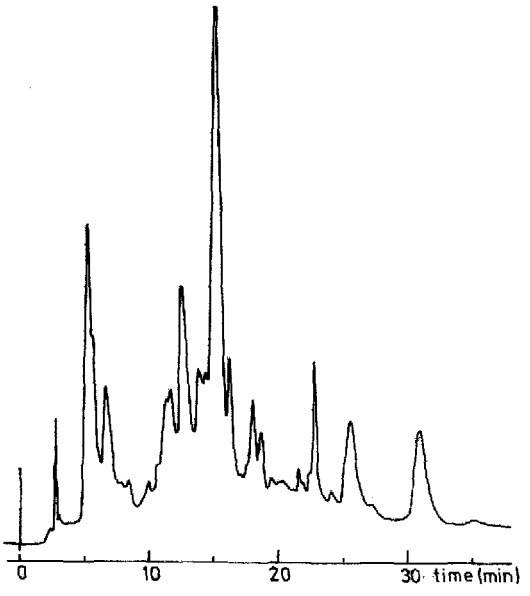


Fig. 12. Separation of vinca rosea alkaloids. Conditions as in Fig. 11 except gradient from 15 to 50% B in 35 min.

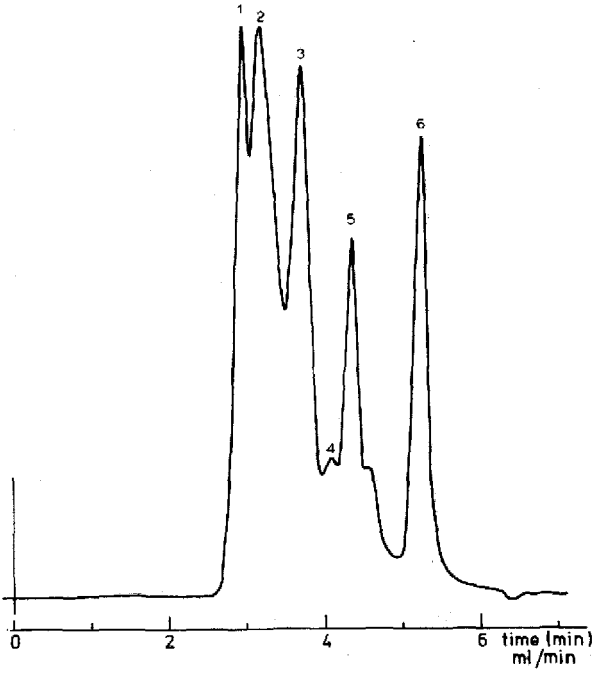


Fig. 13. Separation of proteins on the new phase in the SEC mode. Column: 25 cm × 0.7 cm I.D. (7-μm particles, 17-nm pores), packed with a Varian 5000 liquid chromatograph at 60°C. Mobile phase: mixture of 30% (0.1% TFA in 95% acetonitrile and 5% isopropanol) and 70% (0.1% TFA in 95% water and 5% isopropanol). Flow-rate: 1.0 ml/min. Detection: 220 nm. Samples: 1 = thyroglobulin; 2 = γ-globulin; 3 = chymotrypsinogen A; 4 = insulin; 5 = bacitracin; 6 = tryptophan.

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