Journal of Chromatography, 602 (1992) 21–25
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2520

Direct resolution of organic acid enantiomers on a novel polymer-based stationary phase

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ABSTRACT

A chiral packing for high-performance liquid chromatography was obtained by copolymerization of urea with formaldehyde in the presence of an optically active amino acid derivative. Spherical particles with a mean bead size of 6 μm were evaluated as stationary phases with respect to their chemical stability and chromatographic performance. Electrostatic interactions govern the overall kinetics of the retention in the chromatographic process, so that ion pairing seems to be responsible for the chiral recognition. Increasing the column temperature improves the chromatographic pattern considerably, and the resolution and peak shape are similar to those in the usual high-performance liquid chromatographic separations. The support is easy to pack and yields good column lifetimes. Its application to the resolution of some organic acid racemates is illustrated.

INTRODUCTION

Most high-performance liquid chromatographic (HPLC) methods for the resolution of enantiomers are done on silica-based packing materials because of their good chromatographic performance and the well established grafting reactions [1–4]. However, these materials have certain disadvantages which limit their use and shorten column lifetimes. These restrictions are related to the Si–O–Si–C bonds, which are unstable in acids and bases, and to the presence of residual surface silanol groups, which results in tailing peaks. As an alternative to silica gel packings, synthetic and natural organic polymers gels can be used as chiral stationary phases (CSPs), and a number of supports with characteristic enantioselectivity for several racemates have been reported [5–11]. These provide excellent chemical stability, but show low column efficiency, and are subject to shrinkage and swelling processes.

In a project aimed at the development of polymer gels that would match silica-bonded CSPs in the resolution of enantiomers and allow the use of eluents over wide ranges of pH and polarity, we undertook the preparation of a novel stationary phase which was obtained by copolymerization of urea with formaldehyde in the presence of L-leucinamide. The resolution of some organic acid racemates into their enantiomers is demonstrated.

EXPERIMENTAL

Apparatus

The liquid chromatographic system consisted of a Perkin-Elmer (Norwalk, CT, USA) Series 2B solvent-delivery pump equipped with a Rheodyne Model 7125 injection valve, connected to a Jasco (Tokyo, Japan) Uvidec-100-V variable-wavelength detector, combined with a Carlo Erba (Milan, Italy) Mega Series integrator.

Microscopic investigations of the gel particles were performed in the Centro di Studio per la Termodinamica Chimica alle Alte Temperature, CNR (Rome), with a Cambridge 100 scanning secondary electron microscope using the gold-sputtered procedure.

0021-9673/92/$05.00

Reagents and materials

Carboxylic acids and barbiturates were purchased from Sigma (St. Louis, MO, USA). L-Leucinamide was obtained from Bachem (Switzerland). All solvents and other reagents were of HPLC or analytical-reagent grade and obtained from Carlo Erba.

Preparation of chiral packing

Spherical gel particles were synthesized as follows. About 200 ml of distilled water were acidified with dilute hydrochloric acid, then urea (U) (17.5 g, 0.29 mol), 40% formaldehyde (F) (32 g, 1.06 mol) and L-leucinamide (3.6 g, 0.03 mol) were added. After the pH had been adjusted to 2 with hydrochloric acid, the solution was placed in a high-speed mixer and allowed to react with stirring at *ca.* 300 rpm for 20 h at 24°C. The resulting microspheres were filtered and washed three times with 200–300-ml portions of distilled water by sedimentation and decanting. The sorbent was filtered off under reduced pressure and washed with dilute hydrochloric acid, distilled water and methanol and dried for 2 h at 70°C under vacuum. About 6 g of material were rinsed with 300 ml of acetone and the suspension was placed in a 35.0 × 2.0 cm I.D. glass column. After 5 min a fraction of particles at low sedimentation speed was collected (4.5 g) and used for chromatography. Elemental analysis of the sorbent gave N 29.63, C 31.02, H 5.53%. The particle size distribution (4–7 μm) was calculated from optical micrographs of about 50 randomly selected beads. In addition, a microparticulate material was prepared by mixing urea and formaldehyde according to the above-described procedures. Elemental analysis gave: N 30.83, C 29.34, H 5.48%.

The amount of chiral ligand immobilized on the copolymer was determined by comparing the elemental analysis of the materials prepared in the presence and absence of L-leucinamide. The results indicated that the chiral packing contains at least 2.7% (w/w) of the functional component, corresponding to 0.2 mmol per gram of resin. Because urea reacts with formaldehyde to form a polymeric network according to the following equation [12]:

$$n\ H_2NCONH_2 + n\ HCHO \rightarrow \rightarrow (\text{–}HNCONHCH_2NHCONH\text{–})_n$$

one leucinamide moiety links to the copolymer approximately every 20 urea–methylene units.

The U–F–L-leucinamide sorbent, suspended in 2-propanol, was packed in a stainless-steel tube (250 × 4.6 mm I.D.) at 8000 p.s.i., using *n*-hexane to pressurize the slurry into the column. In order to test the chemical stability of the gel, 0.1 *M* sodium hydroxide solution (pH 13) was passed through the column for 8 h. No reduction in chromatographic or enantioselective performance was observed.

Chromatographic procedure

Sample solutions were prepared in methanol at concentrations of *ca.* 1 mg/ml, and 1–3 μl of these solutions were injected. Chromatographic runs were performed with a UV detector set at 230 or 254 nm. The column was thermostated at different temperatures using an HPLC temperature control system (Fiatron, Oconomovoc, WI, USA).

RESULTS AND DISCUSSION

Stout *et al.* [13] reported the preparation of microspheres obtained by copolymerization of urea and formaldehyde in the presence of silica sol, which was successively removed in order to produce porous organic particles. They concluded that the UF matrix surrounding the pores imprinted by the sol particles is like a foam with micropores of probably 3–12 Å. Other investigators reported on a similar microporous structure present in polystyrene divinylbenzene copolymers [14].

In this work, urea and formaldehyde combined with L-leucinamide were allowed to copolymerize in the absence of silica, so that presumably a wholly microporous foam structure has to be ascribed to the gel particles, and stronger enantiodiscriminative activity to the superficial chiral sites. The shape and the size distribution of the gel beads are shown in the Fig. 1 and 2, respectively.

A number of organic acid racemates were employed to characterize the resolving properties of the stationary phase. As can be seen in Fig. 3, enantioselectivity (α) is strongly influenced by the pH of the buffer in the mobile phase and the size of the substituents bound to the asymmetric carbon atom. It seems that L-leucinamide participates in the formation of zwitterion pairs with the optically active counter ions, operating as a chiral ion-pairing

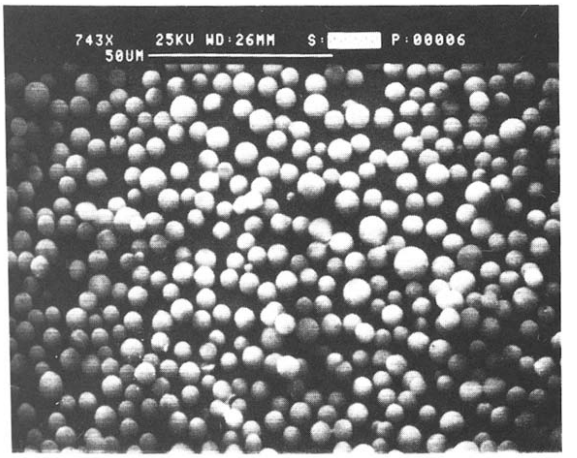

Fig. 1. Scanning electron micrograph of the spherical gel particles.

agent. This is evidenced by the improved resolution, as with barbiturates, when the amount of methanol in the eluent is increased. The chromatograms of a series of racemates are shown in Figs. 4 and 5. Fig. 4C shows the partial resolution of thiopental. In accord with literature findings, this is the first example reporting the enantiodiscrimination of a barbiturate which does not have the chiral centre in the

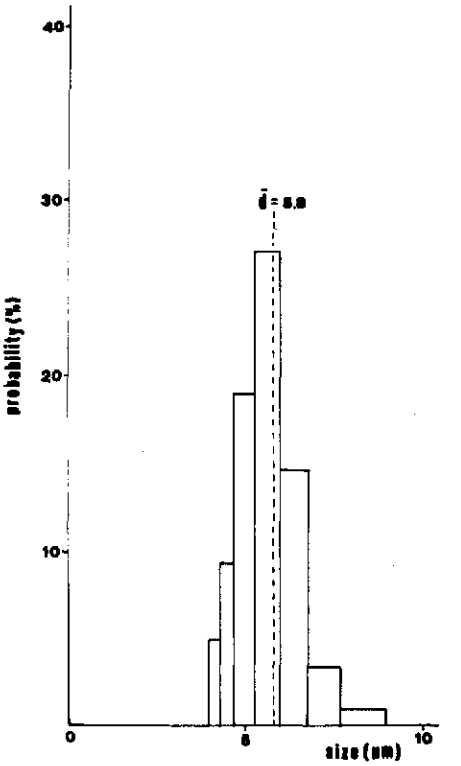

Fig. 2. Particle size distribution of the chiral gel.

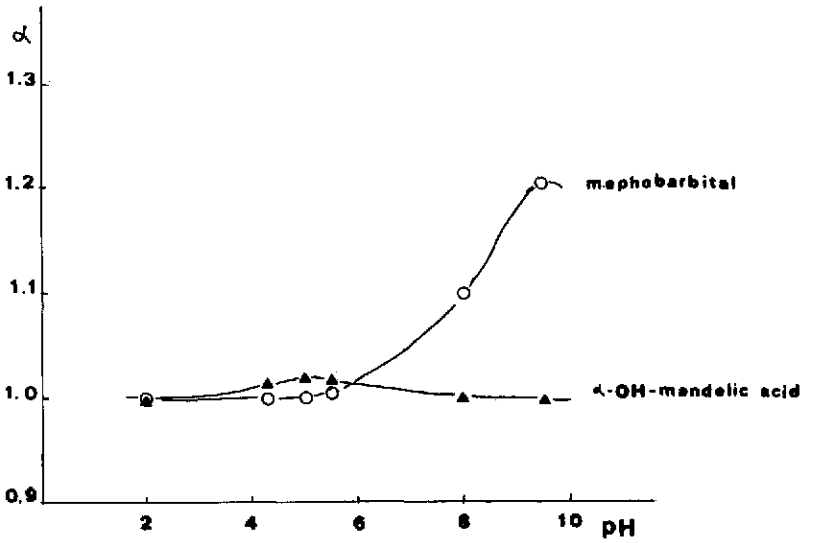

Fig. 3. Plots of α *vs.* pH of the mobile phase containing (▲) 20% or (○) 80% methanol and 0.01 *M* acetate buffer.

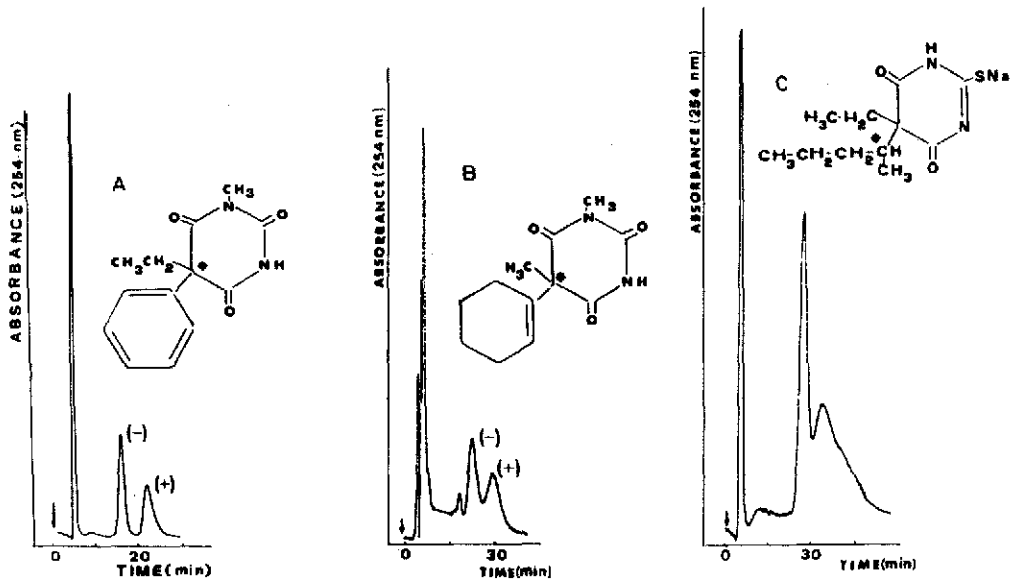

Fig. 4. Resolution of barbiturates on CSP. (A) Mephobarbital; (B) hexobarbital; (C) thiopental. Column temperature: 70°C. Eluent: (A) and (C) methanol–0.01 *M* ammonium acetate buffer (pH 9.5) (85:15, v/v), flow-rate 0.5 ml/min; (B) methanol–0.01 *M* ammonium acetate buffer (pH 9.5) (90:10, v/v), flow-rate 0.7 ml/min.

pyrimidine ring. The chromatographic results are summarized in Table I. A considerable improvement in the chromatographic pattern is brought about by increasing the column temperature. The effect of temperature on the enantioselectivity and the efficiency of the column with mephobarbital as reference is reported in Table II.

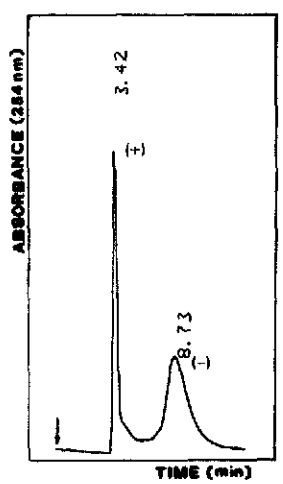

Fig. 5. Resolution of phenyllactic acid enantiomers. Eluent, methanol–0.01 *M* acetate buffer (pH 5.0) (20:80, v/v); flow-rate, 0.5 ml/min; column temperature, 60°C.

TABLE I

CHROMATOGRAPHIC CHARACTERISTICS OF ORGANIC ACID RACEMATES ON UREA–FORMALDEHYDE–L-LEUCINAMIDE POLYMER GEL

Column temperature, 70°C.

Compound	k'^a	α^b
Hexobarbital[c]	9.10	1.18
Mephobarbital[c]	7.42	1.32
Thiopental[c]	8.60	1.09
Phenyllactic acid[d]	0.44	1.36
Hydroxymandelic acid[d]	5.31	1.02
α-Hydroxycaproic acid[d]	6.54	1.00

[a] Capacity factor of the first-eluted enantiomer.
[b] Separation factor, k'_2/k'_1.
[c] Chromatographic conditions as in Fig. 4.
[d] Chromatographic conditions as in Fig. 5.

TABLE II

EFFECT OF TEMPERATURE ON THE ENANTIOSELECTIVITY (α) AND EFFICIENCY (N) USING MEPHOBARBITAL AS REFERENCE

Flow-rate, 0.6 ml/min; other conditions as in Fig. 4.

Temperature (°C)	α^a	N
23	1.26	140
50	1.30	372
70	1.32	405

[a] Separation factor, k'_2/k'_1.

CONCLUSIONS

It has been demonstrated that urea and formaldehyde-based gels copolymerized with L-leucinamide combine chemical stability with enantioselectivity for a series of organic acid racemates. Characteristic enantiodiscrimination was shown for cyclic compounds. Because a large number of optically active mono- and polyfunctional amides are commercially available or can easily be synthesized, several new chiral packings with a wide range of resolution could be produced.

ACKNOWLEDGEMENTS

The authors are greatly indebted to Dr. D. Ferro, Centro di Studio per la Termodinamica Chimica alle Alte Temperature, CNR (Rome), for the microscopic investigations. Thanks are due to Mr. F. Dianetti and Mr. L. Petrilli for the elemental analyses. This work was supported by the Consiglio Nazionale delle Ricerche (CNR, Rome).

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