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Functionalized polymer particles for chiral separation

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ABSTRACT

The synthetic chiral polymer poly(N-acryloyl-S-phenylalanine ethyl ester) was immobilized by grafting to macroporous polymer particles of various composition and structure in a process involving copolymerization of the chiral monomer with residual double bonds present in the macroporous support particles. The support particles were prepared by suspension or micro-suspension polymerization of trimethylolpropane trimethacrylate (TRIM), divinylbenzene or by copolymerization of styrene and TRIM. The maximum amount of immobilized chiral polymer and the mechanical properties of the resulting materials varied with the swelling capacity of the parent support particles. Up to 60% (w/w) of chiral polymer could be immobilized to the pore system of highly cross-linked TRIM particles. The enantioselectivity of the chiral stationary phases increased with increase in the amount of immobilized chiral polymer. The results of studies of porosity and particle size variation during grafting form the basis for a discussion of the structure of the final materials.

INTRODUCTION

Recently we reported [1] the preparation of and enantiomer separation on a new kind of chiral stationary phase (CSP) based on a chiral polymer, poly(N-acryloyl-S-phenylalanine ethyl ester), anchored in the pore system of macroporous polymer support particles composed of poly(trimethylolpropane trimethacrylate) (TRIM). Our preliminary results of enantiomer separations on these CSPs were very encouraging and prompted further studies. We now report on the preparation, characterization and chromatographic evalution of CSPs based on the principles outlined above. The chiral polymer poly(N-acryloyl-S-phenylalanine ethyl ester) was immobilized to macroporous support particles prepared by suspension or micro-suspension polymerization of TRIM, divinylbenzene (DVB) or by copolymerization of styrene (S) and TRIM. An extensive study was made to characterize the support particles and the CSPs, especially for the TRIM-based materials, in order to understand the functionalization process and the chromatographic performance of the materials. The materials were characterized with respect to their swelling capacity, porosity and compressibility, which are important determinants of the chromatographic performance of the CSPs. The variation of enantioselectivity, with chiral polymer loading, and the localization of the chiral polymer in the porous carrier particles are discussed.

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EXPERIMENTAL

Chemicals

N-Acryloyl-S-phenylalanine ethyl ester (chiral

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monomer) was prepared according to the method described by Backmann [2]. The initiator α,α′-azo-isobutyronitrile (AIBN) and ethylene glycol dimethacrylate (EDMA) were purchased from Merck. Poly(vinyl acetate) (PVAc) with a relative molecular mass (M_r) of 500 000 was obtained from BDH. 1,3,5-Tri-*tert*.-butylbenzene (TTB), *p*-nitrotoluene, acetanilide and hydroquinone were obtained from Fluka. Chlorthalidone was purchased from Sigma, and the racemates 7-methoxycoumarin dimer and coumarin dimer were a gift from Jan Sandström (Lund, Sweden) and Kailasam Venkatesan (Bangalore, India), respectively. Ammonium laurate was prepared by mixing 1.0 g of lauric acid (BHD) and ammonia to a pH of 9.6–9.8.

Two suspension stabilizers of poly(vinyl alcohol) (PVAI) were used: Rhodoviol (Rhone-Poulenc) with M_r 72 000 and a degree of hydrolysis of 71.5 mol%, and PVAI (Fluka) with M_r = 72 000 and a degree of hydrolysis of 97.5-99.5 mol%. All other chemicals were of analytical-reagent grade or better, and used as received.

The structures of the racemates chlorthalidone, 7-methoxycoumarin dimer and coumarin dimer are given in Fig. 1, together with the structures of the chiral monomer N-acryloyl-S-phenylalanine ethyl ester and the monomer trimethylolpropane trimethacrylate (TRIM).

Support particles

The support materials TRIM particles, poly(divinylbenzene) (DVB) particles and poly(styrene–TRIM) (S/TRIM) particles were prepared in cooperation with Casco Nobel (Sundsvall, Sweden), by suspension or micro-suspension polymerization. The silica particles, LiChrosorb (diol phase, 10 μm, 300 A), were a gift from Perstorp Biolytica (Lund, Sweden). Experimental details concerning the preparation of support particles are presented in Table 1.

Preparation of chiral stationary phases based on porous carrier particles

The support particles, materials A-E in Table I, were functionalized according to the following procedure.

Porous polymer particles were first dispersed in water in a 200-ml reactor described elsewhere [I]. The monomer solution, containing the chiral monomer, initiator (AIBN) and solvent (toluene),

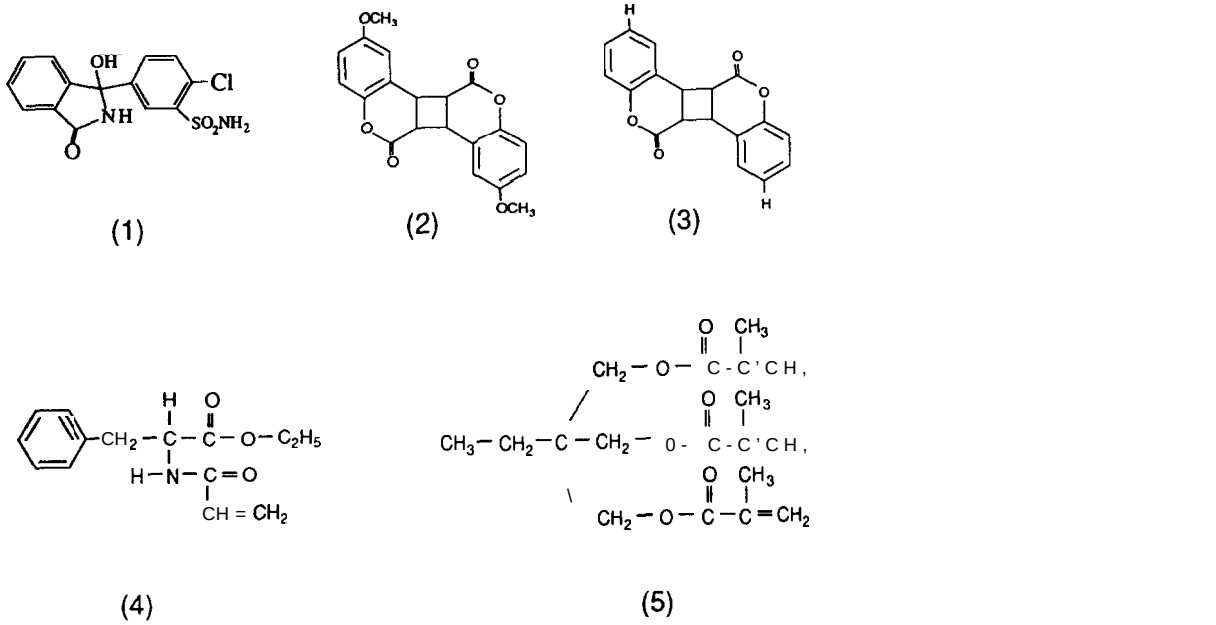

Fig. 1. Structure of the analytes and monomers: 1 = chlorthalidone: 2 = 7-methoxycoumarin dimer; 3 = coumarin dimer; 4 = N-acryloyl-S-phenylalanine ethyl ester; 5 = trimethylolpropane trimethacrylate (TRIM).

TABLE 1

EXPERIMENTAL DATA FOR THE PREPARATION OF DIFFERENT SUPPORT PARTICLES

Material	Monomer (v/v)	Porogenic solvent (v/v)	Solvent-to-monomer ratio (v/v)	Polymerization		Particle size (μm)
				Temperature (°C)	Time (h)	
A	TRIM	Toluene	4:1	70	12	1.2
B	TRIM	Toluene-isooctane (1:1)	1:1	(1) 65	6	8.3
				(2) 85	10	
				(3) 120	4	
C	DVB	Toluene	3:1	70	12	9.4
D	DVB	Toluene	1:1	70	12	2s
E(50/50)	S/TRIM (50:50)	Toluene	4:1	(1) 60	12	5-20
				(2) 75	3	
E(40/60)	S/TRIM (40:60)	Toluene	4:1	60	12	s-20
E(20/80)	S/TRIM (20:80)	Toluene	4:1	60	12	5-20

was then added to the particle dispersion. This solution was spontaneously drawn into the pore system of the particles by capillary pressure. The volume of the monomer solution used in the experiments was equal to the total swelling capacity of the particles with toluene, as determined experimentally. The concentration of the initiator was between 1 and 2.5 mol% of the chiral monomer. Prior to polymerization, the support particles containing the monomer solution were allowed to swell for about 2 h at 30–40°C. The polymerization was performed at 80°C for about 4 h. The product was filtered off (sintered-glass filter) and washed, first with an excess of toluene to rid it of non-immobilized chiral polymer and monomer, then with dioxane, acetone and diethyl ether. The washed particles were dried overnight at 60°C.

Preparation of soft unreinforced chiral gels

Soft unreinforced gels, material F in Table I were prepared by means of suspension polymerization in the following way. A toluene solution of the chiral monomer, 1 mol% of the initiator and 10 mol% of the cross-linker (EDMA) with respect to chiral monomer, was suspended in water (stirrer rate 600 rpm) containing various suspension stabilizer. The polymerization experiments were performed in a nitrogen atmosphere in a stirred reaction vessel kept at 80°C for 4–6 h. The gel particles formed were filtered off (sintered-glass filter) and washed, first with hot water to rid them of suspension stabilizer, then with ethanol, acetone and toluene to eliminate monomers and uncross-linked polymers. No further preparation of the materials was done before the chromatographic evaluations.

Elemental analysis of CSPs

The amount of immobilized chiral polymer in the particles was calculated from the nitrogen content of the samples as determined by elemental analyses. The results of these analyses were in good agreement with those of gravimetric analyses, ***i.e.,*** from the mass difference of dry particles before and after functionalization.

Chromatographic evaluations

The chromatographic set-up used for chiral separations consisted of a Beckman Model 110 B pump, an LKB Model 2158 Uvicord SD UV detector and a BBC SE 120 dual-channel potentiometric recorder. The specific rotation was monitored with a Perkin-Elmer Model 141 M polarimeter equipped with an 80-μl (1 dm long) flow cell to establish the elution order between the enantiomers. The samples were injected by means of a Rheodyne Model 7120 loop injector.

Analytical columns were prepared by packing the CSPs (A-E) into stainless-steel columns (100 or 250

× 4.6 mm I.D.) with a descending slurry-packing technique. Prior to packing the CSPs were allowed to swell in the packing solvent for 2–4 h. Unreinforced, soft chiral gels (material F), used for the purpose of comparison, were packed into 500 × 10 mm I.D. glass columns by pouring a swollen, homogeneous and fairly thick gel suspension into the column. Before the chromatografic experiments, two or three column volumes of the eluent were pumped through the bed to equilibrate it. All CSPs were evaluated with an organic mobile phase, usually n-hexane-dioxane (55:45, v/v).

Swelling

The swelling of the particles in various solvents was measured using a method described previously [3]. About 0.2 g of dry particles was weighed into a tube, containing a 17–40-μm, sintered-glass filter disc at the bottom. The sample in its tube was immersed in the swelling agent at 25°C for 24 h. The amount of swelling agent taken up by the particles was determined by weighing the glass filter tube after removing excess of solvent by centrifugation for 15 min at 600 g. Some of the experiments were repeated with a reduced swelling time (1 h).

Size-exclusion chromatography (SEC)

The SEC experiments were performed with the same equipment as used for the chromatographic experiments described above. The solutes were TTB and polystyrene (PS) standards with M_r ranging from 600 to 2 700 000 and with a polydispersity ratio (M_w/M_n, where M_w = mass-average molecular mass and M_n = number-average molecular mass) of less than 1.1. Hexane-dioxane (55:45, v/v) was used as the eluent. The apparent radius (r) of the PS standards were calculated with the use of the following equation, due to Knox and Scott [4]:

$$r\ (\text{A}) = 0.0123\ M_r^{0.588} \quad (1)$$

where M_r is the relative molecular mass of the PS standard.

Pressure stability

Analytical columns (100 × 4.6 mm I.D.) in standard chromatographic equipment (Varian VISTA 5500) were used to determine the relationship between flow-rate and back-pressure for the CSPs and the original support particles. Four different materials, three based on TRIM and one on silica particles, were tested. The swollen materials had similar particle sizes, 9-11 μm. The flow-rate was continuously increased to a maximum of 1.0 or 10.0 ml/min over 10 min, and then continuously decreased to zero also over 10 min. The pressure drop over the column was recorded continously as the flow-rate changed.

Characterization of support particles and CSPs

The pore size distribution and pore volume of small pores (20-60 Å in diameter) were measured by nitrogen adsorption and desorption according to the method of Emig and Hofmann [5], whereas the pore size distribution and pore volume of large pores (> 60 Å in diameter) were measured by mercury porosimetry on a Micromeritics Model 9310 pore sizer. Specific surface area was measured with a Micromeritics Flowsorber 2500.

The particle size distribution of dry particles was determined from scanning electron microscopy (SEM) micrographs on an ISI 100 U instrument, and of swollen particles from light micrographs. The number-average particle size (D_n) was calculated from measurements on 1000 particles.

The amount of unreacted double bonds in the materials was measured by the bromine addition method, as reported previously [6].

RESULTS AND DISCUSSION

Chiral stationary phases (CSPs) were prepared by polymerization of the chiral monomer, N-acryloyl-S-phenylalanine ethyl ester, in the pore system of pre-made macroporous support particles. Immobilization of the chiral polymer to the support particles affects the prepared CSPs particle size and porosity (Table II). To clarify the properties of the support particles and the observed changes in the properties of the CSPs, a brief description of particle structure and of the proposed mechanism of particle formation [7] will be given. In the present discussion, the main emphasis is on experiments with the TRIM-based support particles A and B (Table I).

Particle structure formation of TRIM particles

Polimerization of TRIM monomer is thought to result initially in the formation of linear TRIM

TABLE II

CHARACTERIZATION OF TRIM-BASED MATERIALS

Material	Chiral polymer (%, w/w)	Particle size (μm)	Toluene swelling (g/g)	Surface area (m^2/g)	Pore volume (cm^3/g)	
					20–60 Å	>60 Å
A	0	1.2	4.0	494	0.22	0.50
A	24	9.1	2.5	342	0.13	1.20
A	37	9.2	1.9	181	0.06	0.88
A	50	–	1.7	40	0.01	0.20
B	0	8.3	1.5	490	0.21	0.88
B	6	–	1.2	514	0.19	0.83
B	11	8.3	1.1	461	0.17	0.76
B	21	8.4	1.1	321	0.12	0.58

polymer molecules, as methacrylic groups on adjacent TRIM residues in a linear TRIM oligomer molecule cannot add to each other owing to steric restrictions [8]. In media of good solvency, as with toluene, the linear TRIM polymer would be soluble. Such linear TRIM polymer molecules will copolymerize with growing chains, giving rise to branched molecules. Particle formation probably occurred via a phase separation process that first involved intermolecular cross-linking of branched molecules, leading to the formation of small swollen microgel particles (200-500 A). Larger structural units, referred to as grains, having a size in the range 1000-1500 A, were then formed by succesive agglomeration of microgel particles. The interstices between the microgel particles and other packing defects in the grains may account for the presence of a fairly open network of fine pores (< 60 Å in diameter) in the final particles. The interstices between the grains form a continuous macroporous network (> 60 Å in diameter). This type of particle formation mechanism [7] would be relevant for material A particles, which were prepared using toluene as the porogenic agent. As will be discussed more fully below, the toluene swelling of A particles was about five times the total pore volume of the dry particles (Table II). This behaviour can be explained by re-expansion of micropore domains that collapsed during drying.

Polymerization of TRIM in the presence of a porogenic agent of poor solvency, toluene-isooctane (1:1), resulted in material B particles (Table I). These had a low swelling capacity, which was only slightly (about 30%) larger than the total pore volume of the dry particles. In this instance, phase separation was probably predominantly due to a precipitation mechanism resulting in the formation of bundles of tightly packed linear polymers of TRIM [7]. Further agglomeration resulted in the formation of fine microsphere particles. The final particles consisted of coagulated microspheres. Owing to packing defects, the latter particles contained small pores (Table II). However, these small diameter pores did not shrink noticeably during drying or expand on immersion in toluene.

Swelling of TRIM particles

As support materials A and B both contain highly cross-linked material, their difference in swelling capacity (Table II), can largely be explained by the difference in the way their structural elements are joined together, rather than by a difference in the degree of cross-linking.

The toluene swelling of material A (Table II) corresponded fairly well with the toluene to TRIM ratio used in its preparation (Table I). This means that the swelling resulted in a reversal of the particle contraction that occurred during drying. The presence of bundles of flexible, crosslink-deficient molecules in the contact zones between intraparticle substructures is probably responsible for the reversible contraction and expansion of the particles during drying and swelling. Interestingly, support material A showed almost the same swelling capacity, 4.0–4.5 ml of swelling agent per gram of dry particles, in all organic solvents tested (Fig. 2), irrespective of

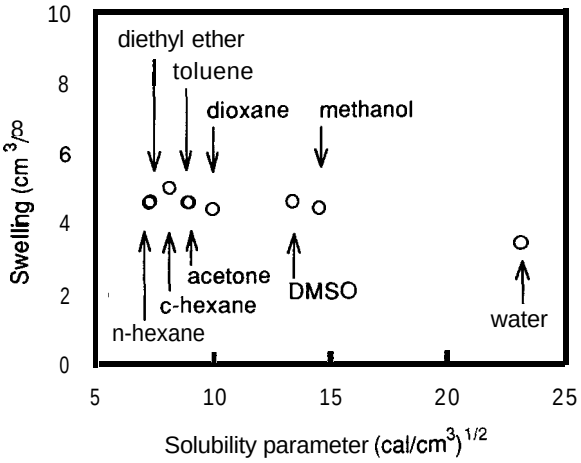

Fig. 2. Solvent uptake (swelling capacity) of support material A for different solvents.

their polarity. This suggests that the internal compression stress, built up during the drying process, relaxed as a solvent was introduced into the pore system. Similar swelling results have been reported for other highly cross-linked polymer particles [9]. Except with water, only a minor increase in particle swelling was observed as the swelling time was increased from 1 h to 24 h, whereas the water swelling doubled, approaching that of organic solvents after 24 h. This probably reflects the low plasticizing ability of water towards the TRIM polymer.

For B particles, the total pore volume of the dry particles corresponded fairly well with the volume of porogenic solvent used in their preparation (Table I). As discussed above, the toluene swelling was poor. These observations suggest that, in this instance, collapse and re-expansion of the small pores did not occur, or occurred to only a very limited extent. This supports the suggestion that the phase separation mechanism differed between porogenic media of high versus low solvency towards TRIM polymers.

TRIM-based chiral stationary phases

The functionalization process. Functionalization of TRIM particles involved polymerization of the chiral monomer in the pore system of the support particles. This was done in the presence of a solvent for the functional polymer and its monomer. Immobilization probably occurred by copolymerization of the chiral monomer with residual double bonds present in the support particles. Continuous overnight extraction of the CSPs with organic solvents and their long-term stability in the chromatographic experiments strongly indicate that the chiral polymer was bound to the support matrix by covalent bonds. The consumption of residual double bonds during the grafting process to material A was verified by analysis based on bromine addition (Fig. 3) and by ^{13}C cross-polarization magic angle spinning NMR analysis [6].

The maximum amount of chiral polymer that could be bound to the particles was found to vary with their swelling capacity and with the amount of residual double bonds. Up to 60% (w/w) chiral polymer could be immobilized to the highly swelling support material A, whereas a maximum of 21% (w/w) of chiral polymer could be immobilized to the poorer swelling support material B. A similar relationship between support swelling and the amount of grafted polymer has been reported previously with other kinds of polymeric support particles [10,11]. The higher grafting capacity of A particles compared with B particles can probably be explained as follows. The average number of unreacted double bonds in A and B particles was 9 and 6 mol%, respectively, of the initial amount of double bonds of TRIM monomer. For both materials, on maximum grafting, the amount of unreacted double bonds decreased to about 3 mol%. The remaining unreacted double bonds were obviously inaccessible to further grafting reactions. As a result, material A contained about twice as many accessible double bonds as did material B, or about 0.5 mmol/g TRIM particle. The difference in structure and its effect on swelling can explain the higher relative accessibility of the double bonds on material A.

The high yield of grafting and the simplicity of the functionalization method represent great advantages of the present support particles. As outlined under Experimental, the functionalization technique involved first the preparation of a suspension of porous support particles in water. To this suspension was then added a monomer-toluene solution containing a thermal initiator, which was sucked into the porous particles by capillary forces. The volume of the monomer solution added corresponded to the swelling capacity of the particles as determined in experiments with toluene. As the

polymerization and grafting reactions are almost complete, predetermined amounts of grafted functional polymer can be introduced in the particles simply by changing the concentration of the functional monomer in the added monomer solution. The functionalization method described is thus very suitable for work with expensive monomers. The concentration of chiral monomer in the monomer solution during preparation of CSPs was between 0.3 and 5.0 mol/l.

Chain length of grafted chiral polymer. The grafted chiral polymer may be attached to the support by one or several bonds, resulting in the formation of polymer loops. If the distribution of unreacted double bonds over the pore surface is assumed to be uniform, the amount of unreacted double bonds per unit volume of pores is much higher in the micropores than the macropores. Hence smaller loops would be formed in the micropores than the macropores.

An estimation of the loop size or chain length of the grafted chiral polymer, based on the consumption of double bonds (Fig. 3), predicts a very small average loop size, only about 2–4 monomer units. Such a small loop size would seriously restrict the conformational freedom of the chiral polymer and result in a low enantioselectivity [1]. In all likelihood, the distribution of loop size was broad. In the micropores, where the concentration of residual double bonds would be high, probably very small loops are formed, whereas in the macropores the loops might be long. This may be understood as follows. Assume functionalization of support particles A with 0.25 g (1 mmol) of chiral monomer/g TRIM. This would result in a decrease in the amount of unreacted double bonds in the TRIM particles by 3 mol% (0.26 mmol)/g TRIM (Fig. 3). If two thirds of the consumed double bonds in the TRIM particles react with only one monomer unit each, the remaining amount of chiral monomer (84% of the added amount) would form a grafted chiral polymer with an average chain length of ten monomer units (M_r = 2500). This loop size corresponds to the distance between cross-links in material F and should, as will be discussed further below, give the same enantioselectivity as the latter material.

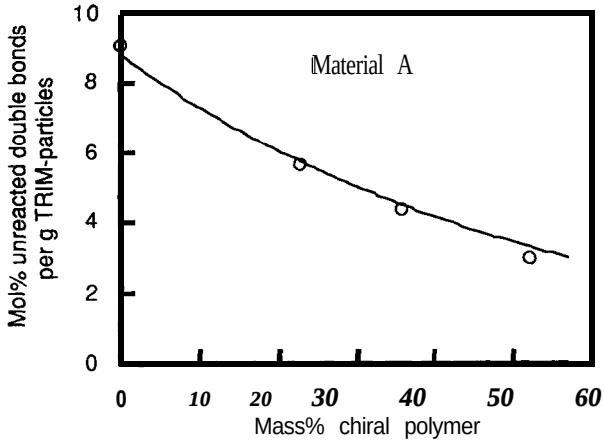

Fig. 3. Amount of unreacted residual double bonds of material A as a function of the amount of immobilized chiral polymer. The amount of unreacted double bonds is expressed in mol% of the initial amount of unreacted double bonds per unit mass of TRIM monomer. The bromine addition method was used for the determination of the unreacted double bonds.

Particle size and volume expansion

The number-average particle size (D_n) of unmodified support material A, A(0%), and material A immobilized with 37% (w/w) of chiral polymer, A(37%), was determined in both the dry and swollen states. On immersion in toluene, the particle size of material A(0%) increased from 7.2 μm (dry material) to 10.5 μm, whereas that of material A(37%) increased from 9.2 μm (dry material) to 10.7 μm. Introduction of the chiral polymer in the pore system of the highly swelling particles thus resulted in an irreversible increase in particle diameter (Table II). This confirms the assumption that a fraction of the chiral polymer becomes grafted in the swollen micropores, which would prevent them from contracting to their original size during drying.

The volume expansion factor on grafting was 2.1, as calculted from particle size data on dry material A(O%) and A(37%) (Table II). An alternative volume expansion factor for dry particles was calculated by comparing the total volume of modified material A, per unit mass of TRIM ***(i.e.,*** volume of 1 g of TRIM matrix plus volume of chiral polymer per gram of TRIM and the total pore volume per gram of TRIM), with that of the original support material A, assuming that both TRIM and the chiral polymer have unit density. This calculation gives a particle volume expansion factor of 1.8. The discrep-

ancy between these results is probably due to minor errors in the particle size determinations. The swelling of the particles was not isotropic, however, This is obvious from the observation that per gram of TRIM, the pore volume of pores with a diameter exceeding 60 Å increased by a factor of 3.2 on going from material A(O%) to A(24%). The pores thus expanded much more than the highly cross-linked part of the TRIM matrix. With material B, measurements indicated the particle volume expansion factor and the expansion factor for macropores (>60 Å in diameter) to be close to unity, i.e., no significant matrix expansion was observed.

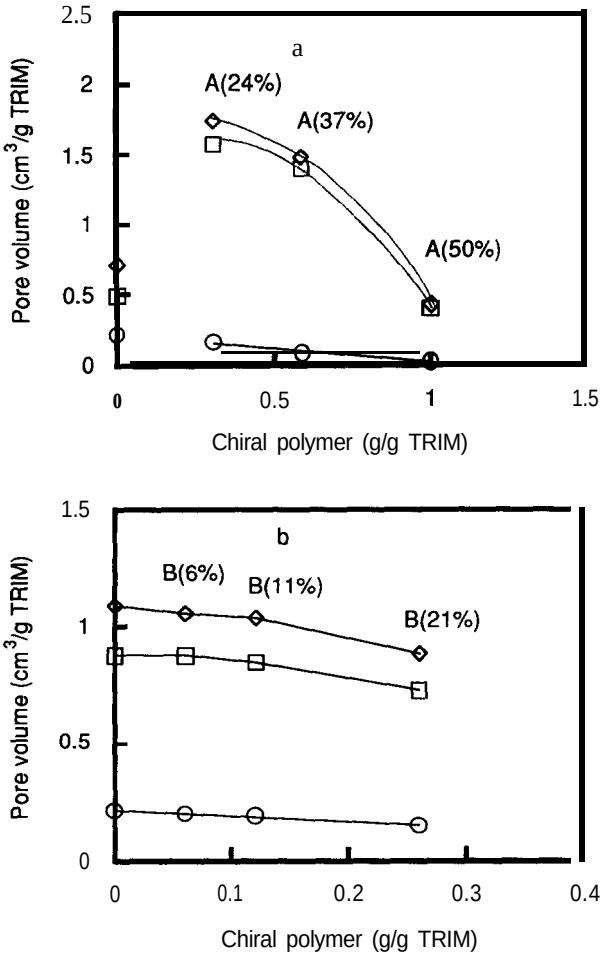

Fig.4. Effect of the amount of immobilized chiral polymer on the specific pore volume of (a) material A and (b) material B. 0 = Total pore volume; □ = pore volume of pores larger than 60 Å in diameter; 0 = pore volume of pores between 20 and 60 Å in diameter.

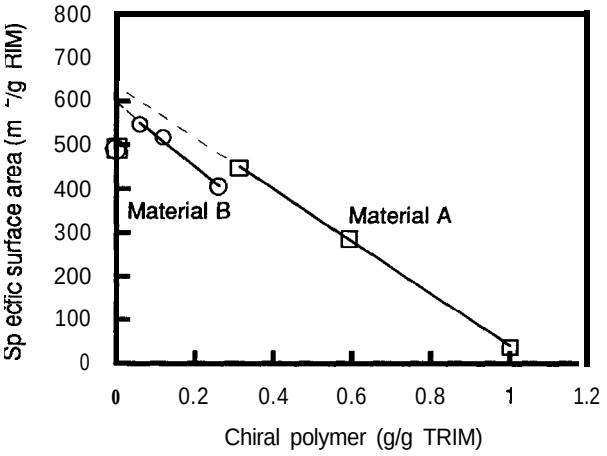

Fig. 5. Relationship between amount of immobilized chirai polymer and the specific surface area of materials A and B.

Porosity

Results from pore volume and specific surface area measurements for materials A and B before and after functionalization are presented in Table II. As shown in Fig. 4a for material A(O%) and A(24%), and suggested by particle size measurements, the total pore volume is increased after the grafting. The increase in pore volume depends on the previously discussed irreversible volume expansion of the particles due to deposition of polymer in the small pores. For the poorly swelling material B, a decrease in the total pore volume was observed at a comparable loading of chiral polymer (Fig. 4b).

The specific surface area of chiral stationary phases, based on materials A and B, decreases linearly with increasing amount of immobilized chiral polymer (Fig. 5). The decrease in surface area with increasing amounts of immobilized chiral polymer shows that grafting results in filling of the small pores which account for most of the specific surface area of the material. Extrapolation of the lines in Fig. 5 back to zero concentration of grafted polymer suggest that the specific surface area of the hypothetically unfunctionalized but expanded particles A and B would be 636 and 596 m^2/g TRIM, respectively.

The cumulative pore volume curves for pores larger than 60 Å in diameter changed, as shown in Fig. 6. Comparison of the two support materials A and B shows that material B contains a higher vol-

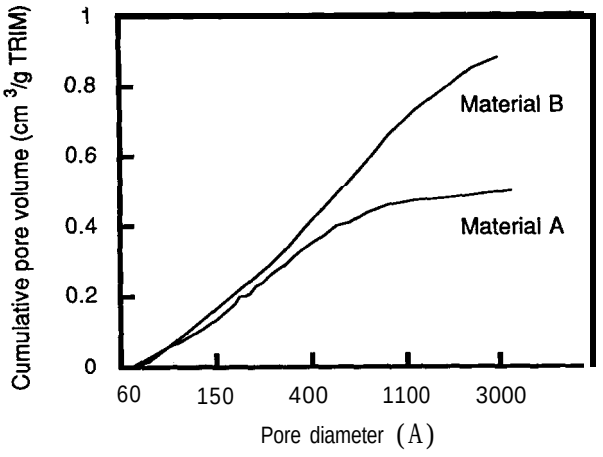

Fig. 6. Cumulative pore volume distribution curves of support materials A and B.

ume fraction of volume of material B (Table II). The cumulative pore volume curves for CSPs of material A and B are shown in Fig. 7. The irreversible volume expansion of material A(24%) is clearly observed in Fig. 7a.

Accessibility of the pore system in CSPs

A problem that arises from the introduction of the chiral polymer into the pore system of the support is whether the analytes can penetrate into the pores after this functionalization. In separate study published elsewhere [12], another batch of support material A was functionalized by polymerizing acryloyl or methacryloyl chloride in the particle pore system. It was found that, even though a large fraction of the acid chloride polymers was localized in the micropores, these groups could be reacted with amines or alcohols to yield amides and esters in almost quantitative yields.

The amount of TRIM matrix of support particles A and B per unit length of packed column was constant, 0.25 and 0.53 g per column, respectively, and independent of the amount of immobilized chiral polymer (Fig. 8). The lower amount of support material A per unit length of column, as compared with support material B, is due to the greater volume swelling of material A. A linear decrease of the elution volume of TTB with an increase in the amount of immobilized chiral polymer was observed for the CSPs. From this decrease, the excluded volume was calculated to be 1.5 cm^3 per gram of chiral polymer.

Pore accessibility was examined by inverse size-exclusion chromatography (SEC) using commercially available polystyrene (PS) standards having relative molecular masses ranging from 600 to 2 700 000. The diameters, *d(A)*, of the PS standards were calculated according to the equation given by Van Kreveld and Van den Hoed [13]. The maximum elution volume in the chromatographic sys-

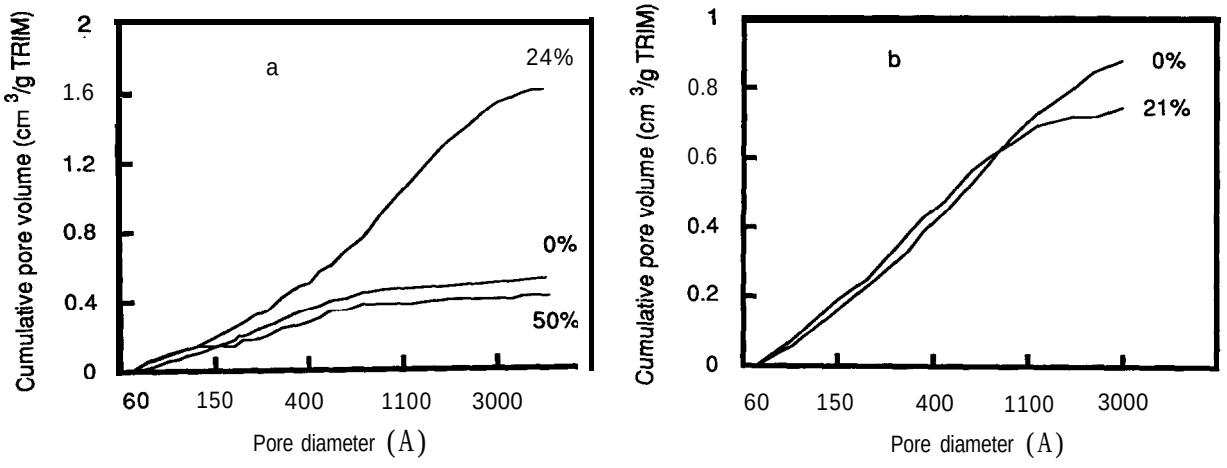

Fig. 7. Cumulative pore volume distribution curves for CSPs based on (a) support material A and (b) support material B.

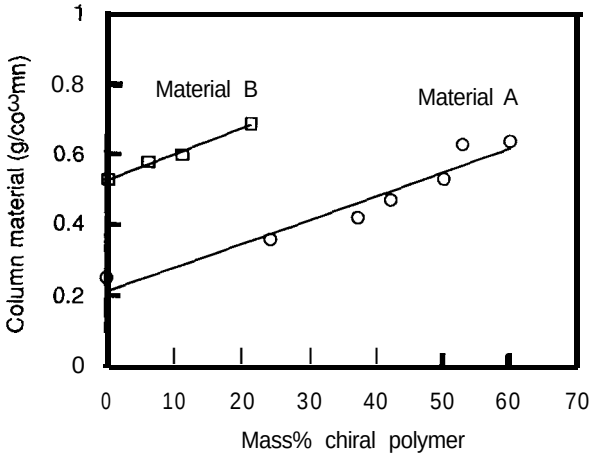

Fig. 8. Amount of dry column material as a function of the amount of immobilized chiral polymer for materials A and B. Columns, 100 × 4.6 mm I.D.

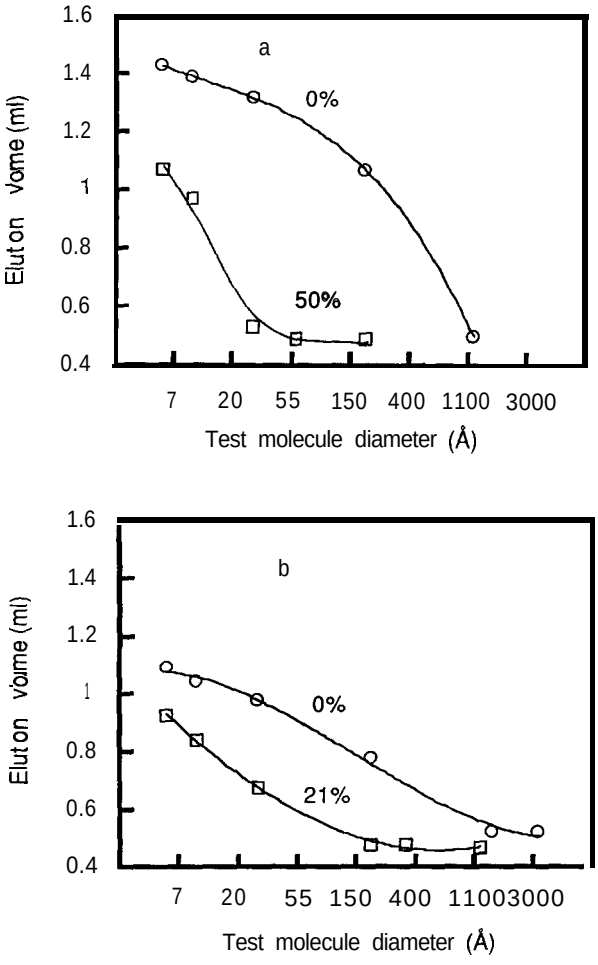

Fig. 9. Elution volume curves of (a) support material A and material A(50% chiral polymer) and (b) support material B and material B(21% chiral polymer) for TTB and different PS standards.

tern was measured with TTB, which is assumed to be non-retained by the support particles and the CSPs. The degree of permeation of the test molecules into the pore system of the polymer particles depends on both the amount of immobilized chiral polymer and the size of the test molecule. The results from the SEC experiments, presented in Fig. 9, show the decreased accessibility of the pore system due to the presence of the chiral polymer. These results confirm the presence of swollen chiral polymers in the macropores of the materials, especially for material A (50%). The porosity and separation range of TRIM particles have recently been determined from SEC experiments in toluene by injections of PS standards [14]: the total separation range for one type of TRIM particles was reported to range between M_r 250 and 2 700 000 of the PS standards, i.e., about the same separation range as for support particles A and B in the present study.

Mechanical stability

The relationship between flow-rate and pressure drop of columns containing different support particles (A, B and silica particles) is presented in Fig. 10. As expected, the pressure drop of the column packed with the highly swelling material A increased very rapidly with increased in flow-rate. The poorly swelling material B yielded a pressure drop curve similar to that of the silica material at flow-rates up to 5 mljmin, i.e., a range that is useful for chromatographic experiments. No permanent increase in flow-resistance was observed for material B or the silica material by decreasing the flow-rate continously to zero, as is demonstrated by the symmetry of the curves showing the increase and the decrease of the flow (Fig. 10). An identical result of the pressure drop curve for material B was obtained by repeating the experiment after a pause of 5 min to allow the material to subside. The high pressure drop over the column at high flow-rates

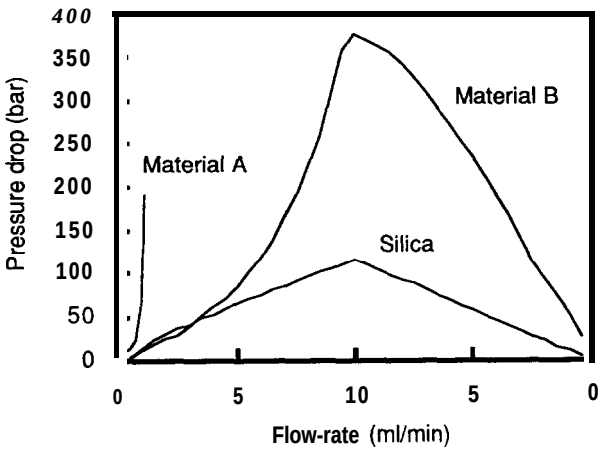

Fig. 10. Mechanical stability of different support particles, measured as the effect of the flow-rate on the column pressure drop. The linear increase and decrease in the flow-rate was 1 ml/min. Columns, 100 × 4.6 mm I.D.; mobile phase, toluene.

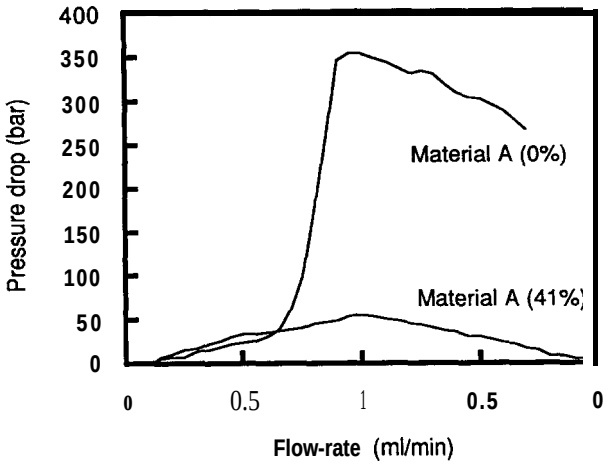

Fig. 11. Effect of the flow-rate on the column pressure drop of support material A and material A immobilized with 41% (w/w) of the chiral polymer. The linear increase and decrease in the flow-rate was 0.1 ml/min. Column, 100 × 4.6 mm I.D.; mobile phase, toluene.

(over 5 ml/min) thus seems to depend on reversible deformation (compression) of the particles. A significant increase in pressure stability was observed for material A as chiral polymer was immobilized to the particles. The column packed with material A(41 %) manifests good chromatographic performance at flow-rates up to 1 ml/min, as compared with the column containing unmodified support material A (Fig. 11).

Characterization of DVB and styrene-TRIM-based materials

Other support particles that have been used for immobilization of the chiral polymer are support particles C and D, prepared with DVB, and support particles E(50/50), E(40/60) and E(20/80), prepared by copolymerization of styrene (S) with TRIM in different ratios (S/TRIM) (Tables I and III).

The DVB-based support particles, C and D, are

TABLE III

CHARACTERIZATION OF DVB- AND S/TRIM-BASED MATERIALS

Material	Chiral polymer (%, w/w)	Particle size (μm)	Toluene swelling (g/g)	Surface area (m^2g)	Pore volume (cm^3/g)	
					20–60 Å	>60 Å
C	0	9.4	2.6	736	0.38	0.94
C	14			649		
C	44			118		0.26
D	0	25	1.0	543	0.25	0.51
D	5			483		
D	18			245	0.22	0.31
E(50/50)	0	5-20	3.3	3	0.00	0.05
E(50/50)	9		3.6	2		
E(40/60)	0	5-20	3.1	2	0.00	
E(40/60)	16		3.5	3		
E(20/80)	0	5-20	3.4	132	0.10	0.17
E(20/80)	23		3.9	4	0.00	0.14

similar to the TRIM-based support particles, A and B, both in properties and behaviour. Consequently, as with the TRIM based particles, higher amounts of chiral polymer could be immobilized to the highly swelling support material C than to the poorly swelling support material D (Table III). The surface areas of the CSPs show a linear decrease with increased amounts of immobilized chiral polymer. As with the TRIM-based materials, a linear decrease of the elution volume of TTB with an increased amount of immobilized chiral polymer was observed. The excluded volume for TTB on material D was 1.5 cm^3/g chiral polymer, i.e., comparable to that obtained on CSPs of material A and B.

The S/TRIM-based E(S/TRIM) particles (Tables I and III) differ in properties from the other support particles, A-D. The specific surface area and the total pore volumes of E particles are both very low, *i.e.*, homogeneous particles without any porosity in the dry state are obtained if a relatively large amount of styrene is copolymerized with TRIM (Table III). The swelling capacities of the three different E materials are similar and fairly high, about 3.3 g of toluene per unit mass of dry material. This indicates the presence of a pore system that collapses during drying of the material. The maximum amount of immobilized chiral polymer increases as the amount of TRIM in the support particles increases; the highest amount of immobilized chiral polymer, 23% (w/w), was obtained with the E(20/80) particles, whereas only 9% (w/w) of chiral polymer could be immobilized to E(50/50) particles (Table III). This may depend on larger amounts of available and accessible unreacted double bonds in the support particles with high amounts of TRIM, as for material E(20/80).

Chiral separations

The focus of this work has been on the characterization of the CSP, and not on screening enantiomer separations. Comparison of the materials has been based on the separation factor (α values) obtained for chlorthalidone as a model substance. A typical chromatogram of the separation of chlorthalidone has been reported previously [l].

Enantioselectivity versus amount of chiralpolymer and kind of support. All CSPs manifested an increase in enantioselectivity with increase in the amount of immobilized chiral polymer (Fig. 12).

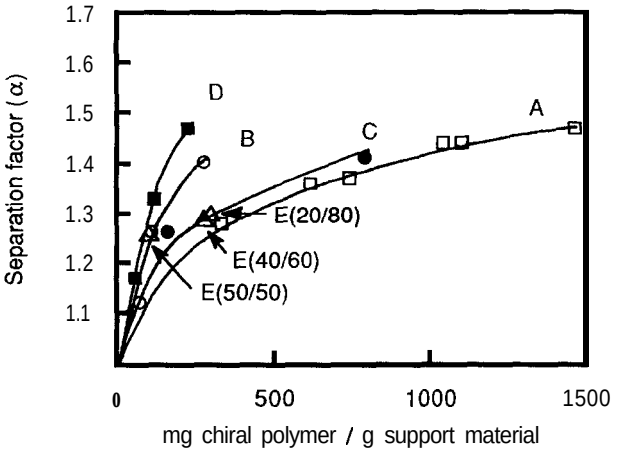

Fig. 12. Relationship between the amount of immobilized chiral polymer and the separation factor of chlorthalidone for CSPs A-E.

The highest separation factors per gram of immobilized chiral polymer were obtained for CSPs derived from the poorly swelling support particles of types B and D. However, as large amounts of chiral polymer are grafted to the highly swelling particles A and C, similar separation factors are obtained for these materials as for the poorly swelling materials B and D.

The observed difference in enantioselectivity of the TRIM-based CSPs may be explained by the chain length, or loop size, of the grafted chiral polymer. As mentioned before, the amount of polymerizable residual double bonds in support particle A is estimated to be twice that in support particle B. Owing to the large amount of unreacted double bonds in A particles and the high local concentration of double bonds in the small pores, a substantial fraction of the added chiral monomer forms only very short chains or loops (probably shorter than 2–4 monomer units), mainly located in the micropores. The results indicate that chains of such small size have little or no enantioselectivity. This is probably due to their poor capacity to form favourable enantioselective conformations. As larger amounts of chiral monomer are used in the functionalization process, the amount of grafted chiral polymer with a large loop size increases. Owing to the more compact structure of B particles, which seem to contain micropores with low swelling capacity, a larger

fraction of the added chiral monomer forms sufficiently long enantioselective chiral loops than in grafting to A particles.

The proposed explanation of the effect of chiral polymer chain length on CSP enantioselectivity is in accord with previously reported results on the effect of the degree of cross-linking on the enantioselectivity of soft chiral gels, namely that an increase in the degree of cross-linking gives a decrease in enantioselectivity [15]. The enantioselectivity of unsupported chiral gels is reported to be completely lost if as much as 50% of cross-linker is used in their preparation [15]. The soft unreinforced chiral gel used in this work, material F, was made in the presence of 10 mol% of cross-linker. This would result in an average chain length of the chiral polymer of ten chiral monomer units. As discussed above, loops of this length may well be present in the CSPs based on TRIM particles.

Surprisingly, high separation factors for chlorthalidone were obtained on CSPs prepared from the different support particles E (Fig. 12). Despite the materials having little or no porosity in the dry state, the grafted chiral polymer obviously has sufficient flexibility to create enantioselective conformations. The enantioselectivity of these CSPs and their physical properties, such as their low porosities and high swelling capacity, support the presence of a pore system in the materials that collapses during drying, and re-emerges in a good polymer solvent.

Although no effect of the functionality of the different polymer particle matrices (TRIM or DVB) on enantioselectivity is observed for chlorthalidone, such an effect could probably be observed for other racemates. The highest enantioselectivity obtained for chlorthalidone, on CSPs A-D ($\alpha \approx 1.5$), is similar to that obtained on unreinformed soft chiral gels, such as material F (to be discussed below), and significantly higher than that obtained on a silica-based (commercial) CSP (HIBAR Prepacked Column RT 250-4; Merck, Darmstadt, Germany) (α = 1.25) containing 16% of the chiral polymer [1].

Chromatographic properties

Irrespective of whether TRIM- or DVB-based particles were used as supports, the capacity factors for the analytes studied, both chiral and achiral, increase non-linearly with an increase in the amount of immobilized chiral polymer (Fig. 13).

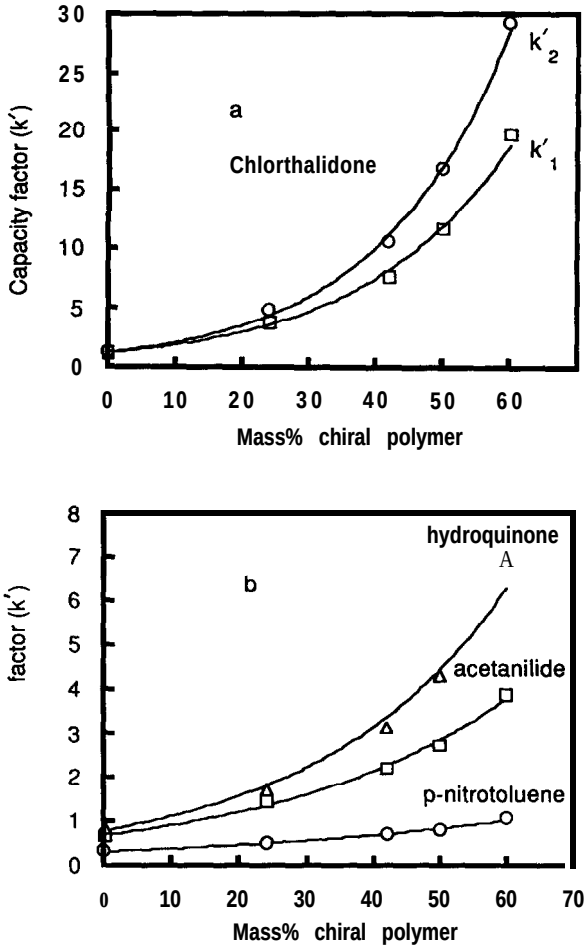

Fig. 13. Exponential dependence between the capacity factors (k') of (a) chlorthalidone and (b) three achiral analytes on the amount of immobilized chiral polymer on CSPs based on material A. Column, 100 × 4.6 mm I.D.; mobile phase, *n*-hexane–dioxane (55:45, v/v); flow-rate, 0.5 ml/min; detection, UV (254 nm).

The effects of temperature on separation factors, capacity factors and column efficiency are shown in Figs. 14–16. The capacity and selectivity factors are reduced by an increase in temperature, whereas the efficiency of the columns is improved. These results are typical of most chromatographic systems and can be explained by a reduced viscosity of the mobile phase which increases the diffusion and mass transfer rates, thus improving column efficiency. The highest column efficiency, presented in Fig. 17 as the height equivalent to a theoretical plate

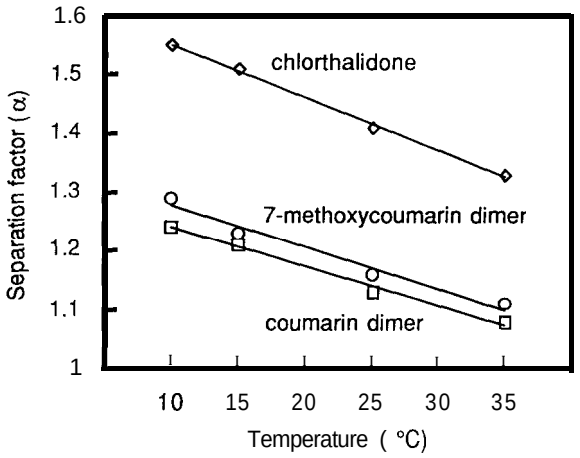

Fig. 14. Effect of column temperature on the separation factor (a) for three different racemates. CSP, material B (21% chiral polymer); column, 100 × 4.6 mm I.D.; mobile phase, *n*-hexane–dioxane (55:45, v/v); flow-rate, 0.7 ml/min; detection, UV (254 nm).

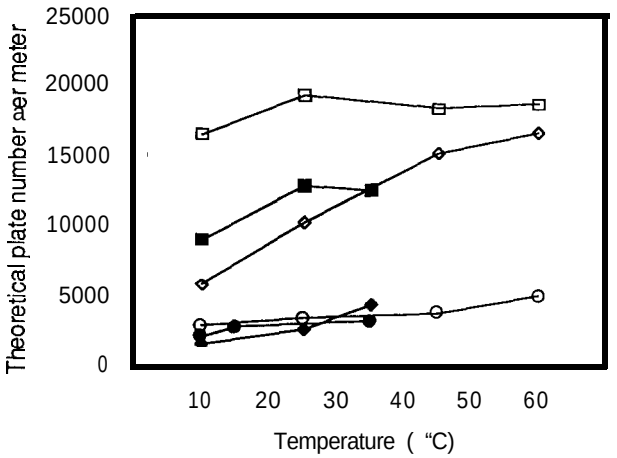

Fig. 16. Relationship between column temperature and the theoretical plate number (*N*) for three different analytes. Filled symbols denote results obtained on material B (21% chiral polymer) and unfilled symbols those on support material B. Detection, UV (254 nm); fow-rate. 0.7 ml/min; columns, 100× 4.6 mm I.D.: mobile phase, n-hexane-dioxane (55:45, v/v). Analytes: ○= chlorthalidone; 0 = p-nitrotoluene: □ = TTB.

(HETP), was obtained for achiral compounds at very low flow-rates. No increase in peak widths with an increase of the amount immobilized chiral polymer could be observed.

Long-term stability and loadability of the columns

The CSPs A-E show very good long-term stability. No change was observed in either selectivity or retention after continuous elution of more than 1000 column volumes of mobile phase (Fig. 16). Nor could any change in selectivity be observed after repacking CSPs that had been dried and stored for several months at ambient temperature. The loadability of the materials containing large

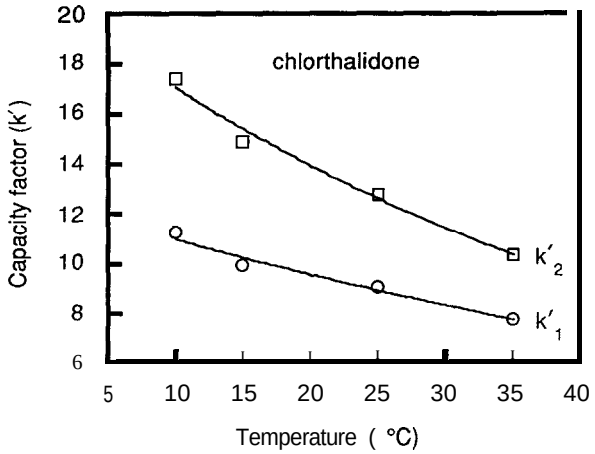

Fig. 15. Effect of column temperature on the capacity factors (k') of chlorthalidone. CSP, material B (21% chiral polymer); column, 100 × 4.6 mm I.D.; mobile phase, n-hexane-dioxane (55:45, v/v); flow-rate. 0.7 ml/min; detection, UV (254 nm).

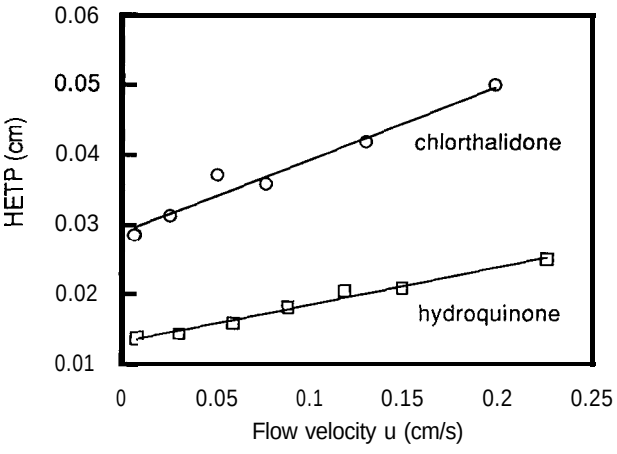

Fig. 17. Effect of flow-rate on the height equivalent to a theoretical plate (HETP) on support material B. Column, 100 × 4.6 mm I.D.; mobile phase, n-hexane-dioxane (55:45, v/v); detection. UV (254 nm).

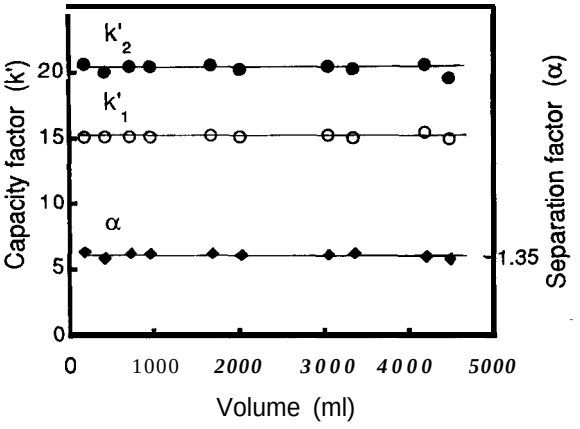

Fig. 18. Long-term stability of material A (33% chiral polymer). Analyte: chlorthalidone. Column, 250 × 4.6 mm I.D.; mobile phase, n-hexanedioxane (50:50, v/v); flow-rate, 0.8 ml/min; detection, UV (254 nm).

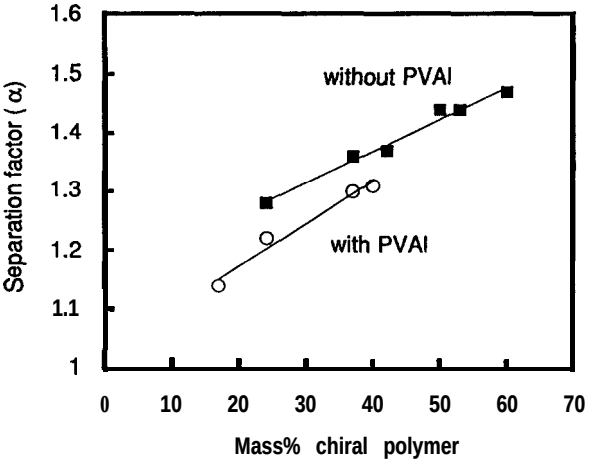

Fig. 19. Effect of the suspension stabilizer PVAl on CSP enantioselectivity for chlorthalidone. The CSPs are based on support material A. Columns, 100 × 4.6 mm I.D.; mobile phase, *n*-hexane-diozane (55:45, v/v); flow-rate, 0.7 ml/min; detection, UV (254 nm).

amounts of chiral polymer seems to be fairly good. On an analytical column (250 × 4.6 mm I.D.), material A(55%), 1.2 mg of 7-methoxycoumarin dimer [16] or 5 mg of chlorthalidone could be completely separated in one run. The CSPs should therefore be of interest for preparative or semi-preparative applications.

TABLE IV

EFFECT OF SUSPENSION AND CO-STABILIZERS ON ENANTIOSELECTIVITY OF SOFT CHIRAL GELS

Between 9 and 10 mol% of the cross-linker EDMA, calculated on the amount of added chiral monomer, were used in the preparation of the CSPs

CSP	Suspension stabilizer		Separation factor (a)
	Type	Content (%, w/w)	
F1	PVAl	5.00	1.5
F2	PVAl	0.06	1.5
F3	without PVAl		1.5
F4	Rhodoviol	0.1	1.6
	Ammonium laurate	5.0	
F5	Rhodoviol	0.1	1.5
	PVAc[b]	0.25	

[a] Amount (g) of suspension stabilizer per 100 g of water.
[b] Added to the organic phase.

Effect of suspension stabilizer on enantioselectivity

Blaschke and co-workers [15,17] found the enantioselectivity of soft chiral gels to depend not only on the structure of the chiral monomer and the degree of cross-linking, but also on the amount and kind of the suspension stabilizer used in the preparation of the CSPs. An increase in enantioselectivity with respect to mandelic acid was obtained when the amount of suspension stabilizer poly(vinyl alcohol) (PVAl) in water was increased from 0.25 to 2.5% (w/w), whereas enantioselectivity was completely lost on changing the suspension stabilizer to polyvinylpyrrolidone (PVP).

The role of the suspension stabilizer was taken into consideration in the present study, where the preparation of soft unreinforced chiral gels, material F, using various kinds and amounts of suspension stabilizers, was included with a view to comparing the effect of suspension stabilizer on enantioselectivity (Table IV). In our work, the amount and type of suspension stabilizers were found to have only a minor effect on the enantioselectivity of the soft giral gels (F). These findings are inconsistent with those reported by Blaschke and Donow [15].

Preparation of CSPs based on support material A in the presence of the suspension stabilizer PVAl [5% (w/w) in the water phase] did, however, affect the CSP enantioselectivity with respect to chlorthal-

idone. On comparable CSPs, the selectivity factors were significantly smaller for materials prepared in the presence of the suspension stabilizer (Fig. 19). The formation of non-enantioselective aggregates between the chiral polymer and PVAl would seem to be the reason for the lower enantioselectivity observed. The aggregate formation was confirmed in an experiment in which the chiral monomer was polymerized from a hot (80°C) homogeneous, aqueous solution containing 5% (w/w) of PVAl. A colloidal dispersion was obtained. The isolated product (a slimy mass) was insoluble in water, 6 *M* urea and all commonly used organic solvents, and no swelling of the material was observed in any of these solvents. According to elemental analyses, the material consisted of about equal amounts of chiral polymer and suspension stabilizer (PVAl). Attempts were made to use this material as a CSP, but without success. In another experiment, it was found that a solid aggregate was formed at the interface between a 5% (w/w) aqueous PVAl solution and a toluene solution of the chiral polymer. This interface aggregate manifested the same properties (of solubility, swelling and enantioselectivity) as the above-mentioned material obtained on polymerization. These observations clearly show the presence of fairly strong interactions between PVAl and the chiral polymer used, probably ascribable to a multiplicity of acid-base interactions. Further studies of the role of these interactions in the present context have not been made.

CONCLUSIONS

Large amounts of chiral polymer can be irreversibly immobilized to support particles by using residual double bonds in macroporous support particles as "handles" for the grafted polymer. The amount of immobilized chiral polymer can easily be varied, up to a maximum, by simply changing the monomer concentration in the added monomer solution, as the conversion of polymerization and grafting reactions are complete. The swelling capacity of the support particles and their content of residual double bonds are determinants of the maximum amount of immobilized chiral polymer. The enantioselectivity of the CSPs is comparable to that of unreinforced soft chiral gels and higher than that of a comparable commercial CSP based on silica particles. The simple and straightforward method of introducing flexible chiral polymers to porous polymer particles presented in this paper should be useful for the preparation of stationary phases for analytical and preparative chiral separations, and possibly also for other kinds of chromatographic applications.

ACKNOWLEDGEMENTS

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