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Journal of Chromatography A, 718 (1995) 257–266

JOURNAL OF CHROMATOGRAPHY A

Supramolecular effects in the chiral discrimination of *meta*-methylbenzoyl cellulose in high-performance liquid chromatography

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First received 4 April 1995; revised manuscript received 15 June 1995; accepted 15 June 1995

Abstract

A series of chiral stationary phases (CSPs) were prepared from *meta*-methylbenzoyl cellulose (MMBC) by deposition on silica using different methods (evaporation or precipitation) and various solvating agents. The chromatographic properties of these CSPs were evaluated using a set of racemic compounds. Both the method of deposition and the solvating agent have a profound influence on the optical resolving power of the MMBC CSPs. Surprisingly, variations were observed not only in the enantioselectivities, but also in the enantiomeric elution order of some of the substances. The precipitation method of deposition is preferable to coating by evaporation. The highest enantioselectivities were obtained with the phases prepared by precipitation of MMBC, either from pure methylene chloride (MC-P), or from methylene chloride solution in the presence of phenol (MC-PHN). These two CSPs show complementary properties in terms of resolution for most compounds investigated and in some cases exhibit opposite optical discrimination patterns. This phenomenon points to the importance of the supramolecular structure of the chiral polymer MMBC on its chiral recognition capability.

1. Introduction

A wide range of cellulose-based CSPs are now available for the separation of enantiomers by HPLC and they have proved to be very useful in the chromatographic resolution of racemic compounds. Various derivatives, such as the acetyl, benzoyl, and phenylcarbamoyl derivatives of cellulose, have been developed for this purpose, either in the pure polymeric form [1,2] or in the coated form [3–5] (i.e., supported on silica gel). Earlier studies of the optical resolution of enantiomers on these CSPs have demonstrated that their chiral discrimination ability strongly depends on the type of derivatizing group, which obviously causes a change in the structure at the molecular level. Minor alterations in the molecular structure, even at positions remote from the chiral centers in the glucopyranose moieties of the cellulose, can lead to considerable changes in selectivity [2].

Changes in selectivity can, however, also be caused by the supramolecular structure. It is a well-established fact that the supramolecular organization of molecular systems influences their physicochemical properties. It gives origin to various properties encountered both in biological systems and in material science. In poly-

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0021-9673/95/$09.50
SSDI 0021-9673(95)00686-9

mers, a change in supramolecular structure refers to a change at a level that does not affect the molecular structure per se, such as a change in the conformation (secondary structure) or a change in the order of the polymer chains (agglomeration, crystal structure). This phenomenon is particularly interesting when it occurs in asymmetric systems capable of chiral discrimination with respect to enantiomeric molecules. Evidence of such a relationship between chiral recognition ability and supramolecular structure was already brought to light some years ago, during our study on enantiomeric separations by liquid chromatography on cellulose triacetate (CTA) [7]. In that paper, we called attention to the importance of the crystal (supramolecular) structure of CTA on its chiral recognition ability. The two crystal forms of cellulose triacetate, CTA I and CTA II, respectively prepared under heterogeneous and homogeneous conditions, have intrinsically the same chemical structure, but they behave in completely different ways when used as CSPs [6,7]. Using a series of chiral compounds as a probe, we investigated the possible existence of this phenomenon in other cellulose derivatives.

In the present study, we were particularly interested in the CSPs based on *meta*-methylbenzoyl cellulose (MMBC). In the course of our investigations of the interaction mechanism exerted by this chiral material, we observed that the preparation conditions could dramatically alter the chiral recognition ability of the CSP.

2. Experimental

2.1. Preparation of MMBC

The cellulose used as starting material for the synthesis of MMBC was prepared by degradation of a commercially available product (Linters from Schleicher and Schüll, Germany) so as to obtain a cellulose of appropriate molecular mass. Details on the degradation procedure were described previously [8]. The average molecular mass of the resultant cellulose was estimated as 4500 (degree of polymerization: 27.7) according to vapor pressure osmometry measurements performed on the corresponding CTA in chloroform. Determination of the molecular-mass distribution of the cellulose was also made by MALDI-TOF (matrix-assisted laser desorption ionization/time of flight). The spectrum (Fig. 1) shows a number-average degree of polymerization of about 14. This is only half the value determined by osmometry, showing that there is a discrepancy between the two methods of determination. It is possible that the data obtained by MALDI-TOF were underevaluated due to inhomogeneous crystallization of the cellulose with the matrix during sample preparation. It is known that this co-crystallization step of the matrix with the sample is essential for successful application of MALDI-TOF. Preferential crystallization of the low-molecular-mass chains of cellulose in the matrix during the sample preparation cannot be excluded. A similar discrepancy has recently also been observed in a poly(methylmethacrylate) sample for which the molecular mass, according to the GPC results, was underevaluated by MALDI [9].

This cellulose was reacted with *meta*-toluoyl chloride at 90°C for 20 h in a mixture of pyridine and triethylamine in the presence of a catalytic amount of N,N-dimethylaminopyridine, as previously described [2]. MMBC was then isolated by precipitation in methanol and purified by reprecipitation twice from a methylene chloride solution.

2.2. Coating and column packing

In our study, two depositing methods were used to prepare the MMBC-CSPs.

(a) Coating by evaporation: 0.75 g MMBC was dissolved in 10 ml solvent and mixed with 3.0 g silica gel (Nucleosil 4000-10, from Macherey-Nagel, Germany) previously treated with 3-aminopropyl triethoxysilane. The solvent was then removed under reduced pressure at ambient temperature by means of a rotavapor.

(b) Covering by precipitation: 3.0 g silica gel, modified as above, was suspended in 40 ml of solvating agent containing 0.75 g MMBC. Under moderate magnetic stirring, 240 ml hexane was

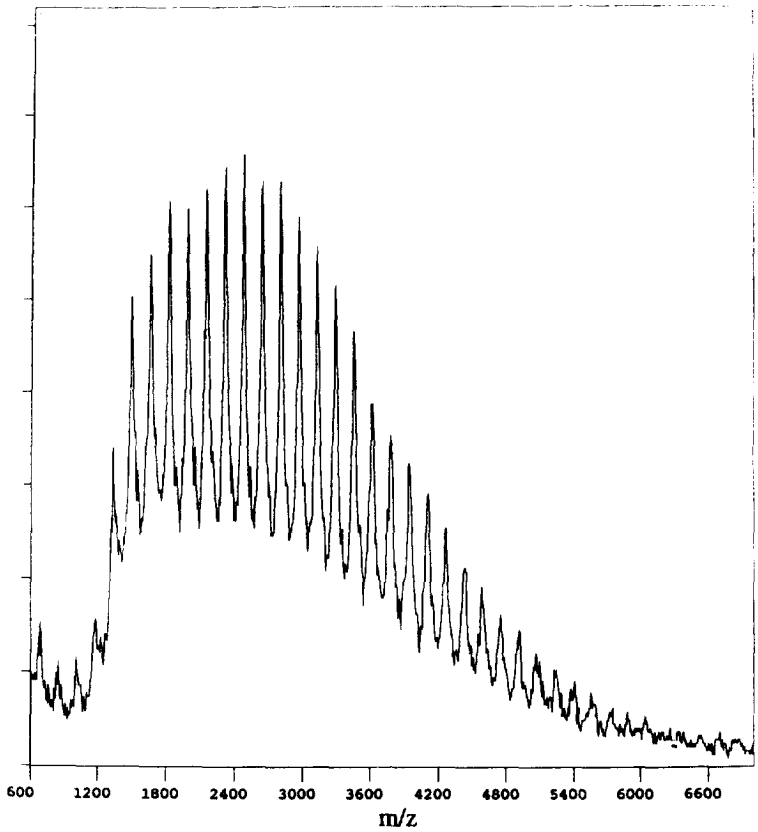

Fig. 1. MALDI-TOF mass spectrum of low-molecular-mass cellulose (matrix: 2,5-dihydrobenzoic acid. Polarity: positive).

then added dropwise (0.8 ml/min.) as precipitating agent.

Materials obtained by both depositing methods were mixed with hexane–isopropanol (90:10, v/v) to form a slurry and packed into stainless-steel columns (250 × 4.0 mm I.D., Macherey-Nagel).

2.3. X-ray measurement

X-ray measurements of the samples prepared in the absence of silica gel were performed on a Philips powder diffractometer, as described previously [7].

2.4. Equipment and running conditions

The HPLC system consisted of a Shimadzu SPD-6AV pump, a variable-wavelength Shimadzu LC-6A UV–Vis detector connected to a Perkin-Elmer 241 polarimeter, and a Reodyne injector fitted with a 20-μl sample loop. The apparatus was connected to an IBM (PC-AT) computer running the Maxima 820 chromatographic software program.

The mobile phase was invariably a 90:10 (v/v) mixture of hexane–isopropanol. Chromatographic runs were performed at ambient temperature with a flow-rate of 0.7 ml/min. The eluates were monitored at 254, 230, or 210 nm. The dead time was determined by injecting 1,3,5-tri-*tert*.-butylbenzene onto the column.

3. Results and discussion

High enantioselectivities have already been observed with MMBC used in the pure form [2],

and a few enantiomeric separations have also been reported using the same material coated on macroporous silica [4]. As the coating method offers greater versatility for changes in the parameters controlling the preparation of cellulose-based CSPs, we used this technique to prepare the desired CSPs, applying various conditions. Initially, two factors were varied: the method of deposition and the solvating agent from which MMBC is solidified. The preparation conditions of MMBC CSPs are summarized in Table 1. The various CSPs were tested with a series of racemates whose structures are presented in Fig. 2.

3.1. Influence of the deposition method on the chiral recognition ability of MMBC CSP

The method of preparing cellulose-based CSPs originally reported by Okamoto and co-workers produces a coating of macroporous silica by evaporation of a solution of the cellulose derivative in an organic solvent [3,4]. A few years later, Erlandsson et al. [10] reported on attempts to resolve the enantiomers of omeprazole with trisphenylcarbamoyl cellulose (TPCC) supported on silica. In that study, they also investigated the influence of the coating method on the optical discrimination ability of this TPCC CSP, using both the evaporation and the precipitation methods. However, the chromatographic results revealed no distinction between these two methods. A similar procedure also based on precipitation was successfully applied to the preparation of a series of CSPs based on amylopectin [11]. In this case, it was found that better enantioselectivities were often attained when the CSPs were prepared by the precipitation method.

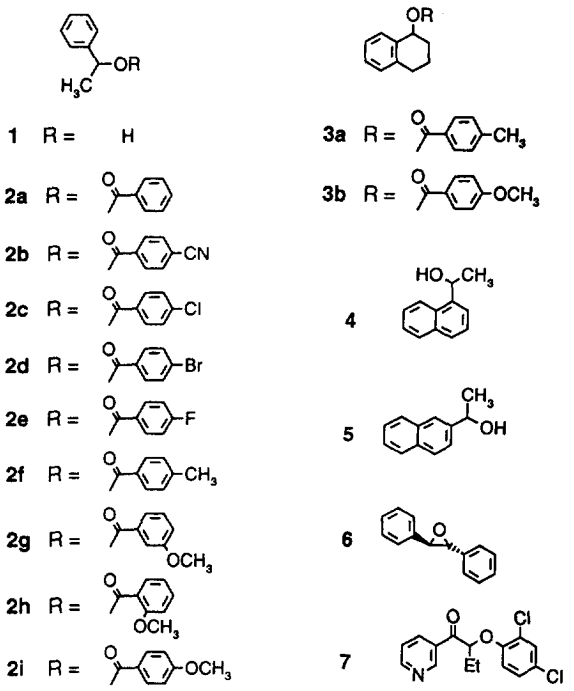

Fig. 2. Structure of the racemates.

To determine the influence of the deposition

Table 1
Preparation conditions of the MMBC CSPs

Column	Solvating agent	Deposition method
MC-E	MC (10.0 ml)	E
MC-P	MC (40.0 ml)	P
MC-NB33	NB (13.3 ml)/MC (26.7 ml) 33% NB (in volume)	P
MC-NB50	NB (20.0 ml)/MC (20.0ml) 50% NB (in volume)	P
MC-NB85	NB (34.0 ml)/MC (6.0 ml) 85% NB (in volume)	P
NB	NB (40.0 ml)	P
MC-THF	THF (13.3 ml)/MC (26.7ml) 1.00/2.54 (mole/mole)	P
MC-TFA	TFA (14.3 ml)/MC (25.7 ml) 1.00/1.80 (mole/mole)	P
MC-PHN	PHN (7.3 g)/MC (40 ml) 1.00/8.00 (mole/mole)	P
NB-PHN	PHN (4.6 g)/NB (40 ml) 1.00/8.00 (mole/mole)	P

E: evaporation; P: precipitation; MC: methylene chloride; NB: nitrobenzene; THF: tetrahydrofuran; TFA: trifluoroacetic acid; PHN: phenol.

procedure on the chromatographic properties of MMBC, we first prepared two CSPs, using methylene chloride as a solvent. The evaporation and precipitation methods gave two different CSPs, MC-E and MC-P, respectively. Upon examination of the chromatographic results of the 1–7 series of racemates on both CSPs (Table 2), we found that the optical resolving power of the MMBC CSPs depended to a great extent on the deposition method. Precipitation actually produces a more efficient CSP than evaporation. High enantioselectivities were generally obtained with MC-P CSP, whereas MC-E CSP exhibited practically no optical discrimination for the majority of the tested solutes. One example is shown in Fig. 3; compound 3a is not resolved on MC-E (Fig. 3a), while it is well resolved on MC-P (Fig. 3b).

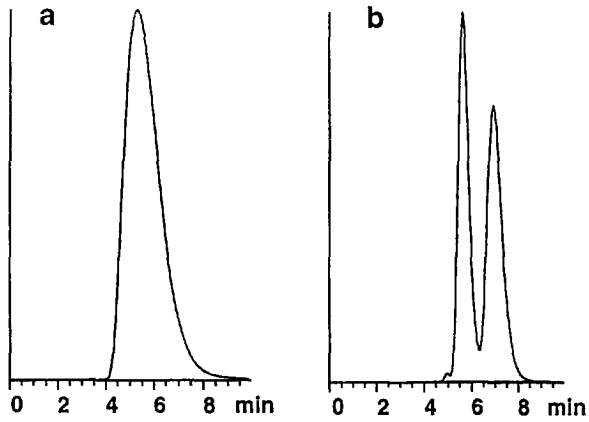

Fig. 3. Chromatographic resolution of racemate 3a on (a) MC-E, and (b) MC-P CSPs. HPLC column (250 × 4 mm I.D.); mobile phase, hexane–2-propanol (9:1, v/v); flow-rate, 0.7 ml/min.

Even more surprising is the inversion of the elution order observed for the enantiomers of some racemates from MC-E to MC-P. Indeed, although the MC-E phase failed to efficiently resolve any compounds in the 2a–i series, slight enrichment of the first and last fractions was sufficient to permit determination of the elution order by polarimetric detection. The polarimetric signal clearly indicates that 5 compounds in this series (2b, 2c, 2d, 2h, and 2i) were eluted in reverse order on the respective CSPs. This difference in the behavior of the two CSPs does

Table 2
Chromatographic results on MMBC CSPs solidified from methylene chloride, nitrobenzene, and methylene chloride–nitrobenzene solvating agents

Racemate	Column											
	MC-E		MC-P		MC-NB33		MC-NB50		MC-NB85		NB	
	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α
1	0.67(−)	1.00	0.69(−)	1.13	0.87(−)	1.15	0.81(−)	1.17	0.56(−)	1.21	0.58(−)	1.00
2a	1.13(−)	1.00	0.75(−)	1.88	1.13(−)	1.53	1.26(−)	1.34	1.16(−)	1.61	0.84(+)	1.00
2b	3.73(+)	1.00	3.04(−)	1.28	4.15(−)	1.14	4.19(−)	1.00	2.55(+)	1.00	2.83(+)	1.30
2c	0.82(+)	1.00	0.67(−)	1.22	0.95(−)	1.00	0.91(+)	1.06	0.57(+)	1.20	0.53(+)	1.32
2d	0.93(+)	1.00	0.85(−)	1.00	1.07(+)	1.00	0.94(+)	1.21	0.59(+)	1.39	0.56(+)	1.37
2e	0.95(−)	1.00	0.70(−)	1.63	1.01(−)	1.29	1.15(−)	1.13	0.83(−)	1.00	0.72(+)	1.00
2f	1.15(−)	1.00	0.75(−)	2.00	1.07(−)	1.56	1.31(−)	1.36	1.03(−)	1.00	0.83(+)	1.00
2g	1.62(−)	1.00	1.35(−)	1.52	1.80(−)	1.26	2.14(−)	1.07	1.42(−)	1.00	1.39(+)	1.00
2h	3.16(−)	1.00	2.46(+)	1.14	3.64(+)	1.00	4.05(−)	1.07	2.47(−)	1.00	1.78(−)	1.78
2i	2.57(+)	1.00	2.40(−)	1.36	3.05(−)	1.24	3.78(−)	1.00	2.34(+)	1.00	1.93(+)	1.40
3a	0.57(+)	1.00	0.44(+)	1.77	0.58(+)	1.62	0.58(+)	1.56	0.42(+)	1.00	0.47(+)	1.00
3b	1.21(+)	1.30	0.96(+)	1.68	1.34(+)	1.63	1.38(+)	1.62	0.95(+)	1.72	0.91(+)	1.44
4	1.77(−)	1.54	1.97(−)	1.59	2.33(−)	1.59	2.22(−)	1.59	1.15(−)	1.51	1.16(−)	1.43
5	2.29(−)	1.24	2.31(−)	1.29	2.78(−)	1.28	2.08(−)	1.28	3.24	1.00	1.60(−)	1.23
6	0.98(+)	1.78	0.93(+)	2.97	1.18(+)	2.70	1.15(+)	2.45	0.74(+)	2.02	0.69(+)	1.63
7	1.74(−)	1.52	1.37(−)	1.71	1.80(−)	1.81	1.74(−)	1.86	1.28(−)	1.76	1.32(−)	1.74

The sign in parentheses represents optical rotation of the first-eluted enantiomer. For column preparation, see Table 1 and for the structure of the racemates, see Fig. 2.

not simply reside in the performance of optical resolution; inversion of the elution order for the same enantiomeric pair on two CSPs prepared from the same chiral material, i.e. having the same chiral information at the molecular level, indisputably points to a change in the supramolecular structure of the CSPs. The separation mechanisms are actually different, at least for the enantiomers whose elution order was reversed. In the light of these results, we speculated that it should be possible to find conditions that could further improve the chiral recognition ability of the MMBC CSPs by favoring the formation of one supramolecular structure. Solvents interacting strongly with MMBC should in principle show the greatest effect.

3.2. *Influence of the solvating agent*

On the basis of this concept, and knowing that compounds containing a nitrobenzyl group are generally strongly retained on methylphenyl cellulose CSPs [12], we first investigated the effect of nitrobenzene as a solvating agent for MMBC. Effects of this nature on the optical resolving properties of CSPs based on CTA and TBC (tribenzoyl cellulose) were in fact already observed by Shibata et al. [13]. They reported that the admixture of some additives to the methylene chloride solution of the polymer, such as trifluoroacetic acid or phenol for CTA and benzene or nitrobenzene for TBC, could substantially improve the enantiomeric resolutions of some racemic compounds. However, no reversal of enantiomeric elution order was observed in their study.

In our investigation, nine different solvating conditions were tested to study their effects on the optical resolving power of the MMBC CSPs. The composition of each solvating mixture and the denomination of the corresponding MMBC CSP are shown in Table 1.

Effects of methylene chloride and nitrobenzene

Methylene chloride and nitrobenzene as solvating agents have the advantage of completely dissolving the polymer MMBC. While methylene chloride mainly exerts its effect through Van der Waals and dipole interactions, nitrobenzene exhibits a greater solvent strength and is a strong π-acceptor system. Therefore, when MMBC is dissolved in methylene chloride or in nitrobenzene and then solidified from them, some differences in the chromatographic properties of the resultant CSPs might be expected.

The chromatographic results obtained on the MMBC CSPs prepared from methylene chloride or nitrobenzene solutions are shown in Table 2. Comparison of the chiral recognition abilities of the two CSPs precipitated respectively from pure methylene chloride (MC-P CSP) and pure nitrobenzene (NB CSP) reveals three distinct kinds of chiral solute: (i) racemates that are much better resolved on one CSP than on the other (1, 3a-b, 6); (ii) racemates for which the resolution is similar on the two CSPs (4, 5, 7); and (iii) racemates that are resolved with inversion of the elution order of the enantiomers (2a–i). For some racemates in this last-mentioned 2a–i series, the enantiomers are only well separated on MC-P (2a, 2e–g) or on NB (2d, 2h). For racemates 2b, 2c and 2i, similar enantiomeric separations can be achieved on both CSPs, but with inversion of the elution order. Fig. 4 shows the chromatograms and the polarimetric signals

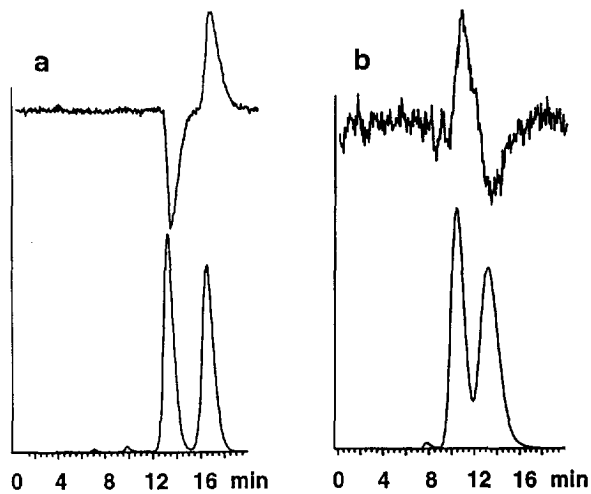

Fig. 4. Chromatographic resolution of racemate 2i on (a) MC-P, and (b) NB CSPs. HPLC column (250 × 4 mm I.D.); mobile phase, hexane–2-propanol (9:1, v/v); flow-rate, 0.7 ml/min. Detection, UV (bottom) and optical rotation (top).

of compound 2i obtained respectively on the MC-P CSP (Fig. 4a) and the NB CSP (Fig. 4b). The selectivity values obtained for these two separations are nearly identical (1.36 on MC-P and 1.40 on NB), but the (−)-enantiomer of 2i elutes first on MC-P, and second on NB. Apparently, both solvating agents, methylene chloride and nitrobenzene, favor the formation of two completely different supramolecular structures, which are referred to here as type A for MC-P and type B for NB. Again, both materials have the same molecular structure, and their opposite chiral recognition abilities can only be explained by a change in the supramolecular structure of the CSPs. Several polymeric chains are probably involved in the interaction with the chiral solute, and the relative arrangements of these chains depends on the crystal structure of the packing, which is itself dependent on the preparation conditions. It seems likely that the solvent causes a preorientation of the polymeric chains before solidification. Each crystal packing will thus provide a different chiral environment for stereoselective interactions.

Utilization of a mixture of methylene chloride and nitrobenzene as solvating agent leads to the formation of CSPs composed of a mixture of structures that exhibit selectivities intermediate between those obtained on the MC-P and the NB CSPs. For racemates showing inversion of the enantiomeric elution order, a cancelling effect is obviously observed [8]. For instance, the transition range for compound 2i lies between 50% and 85% (v/v) of nitrobenzene in methylene chloride. The MMBC CSP produced from mixtures falling in this range no longer separate the enantiomers of 2i (Fig. 5). Another example is given by the separation of compound 2c. When the capacity factors $k'_{(+)}$ and $k'_{(-)}$ of both enantiomers are plotted against the percentage of nitrobenzene in methylene chloride, the curves obtained cross at the point of 33% nitrobenzene in methylene chloride (v/v), resulting in no enantioselectivity ($\alpha = 1.00$) at this point. Below or above this point, the enantioselectivity increases, but with inversion of the elution order for both enantiomers (Fig. 6).

These results clearly indicate that the solvating

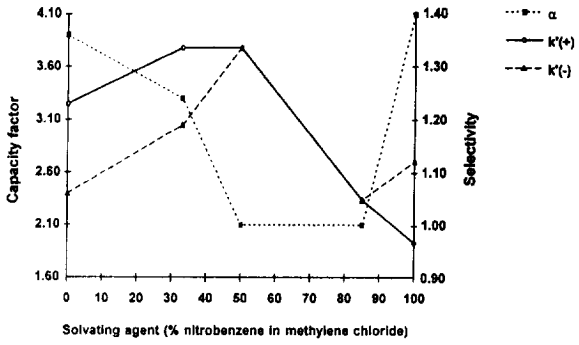

Fig. 5. Variation of the capacity factors $k'_{(-)}$ and $k'_{(+)}$, and of the selectivity for the chromatographic resolution of racemate 2i versus the composition of the solvating mixture used for dissolving MMBC before depositing on silica.

agent exerts a determinant influence on the spatial arrangement of the macromolecular chains of MMBC during solidification of the chiral material, leading to the formation of different local environments that are responsible for the interaction with the analytes.

To confirm the change in the supramolecular structure of MMBC depending on the nature of the solvating agent, X-ray diffraction experiments were performed on two samples, respectively prepared under the same conditions as for the MC-P CSP and the NB CSP, but in the absence of silica gel as support to improve the quality of the crystallographic pattern. Although no significant differences could be detected at diffraction angles greater than 12 degrees, differences are evident in the range of small angles

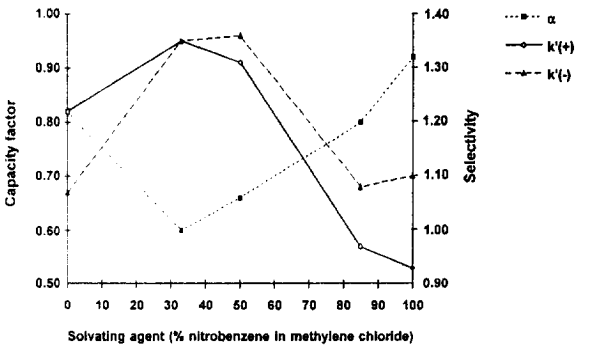

Fig. 6. Variation of the capacity factors $k'_{(-)}$ and $k'_{(+)}$, and of the selectivity for the chromatographic resolution of racemate 2c versus the composition of the solvating mixture used for dissolving MMBC before depositing on silica.

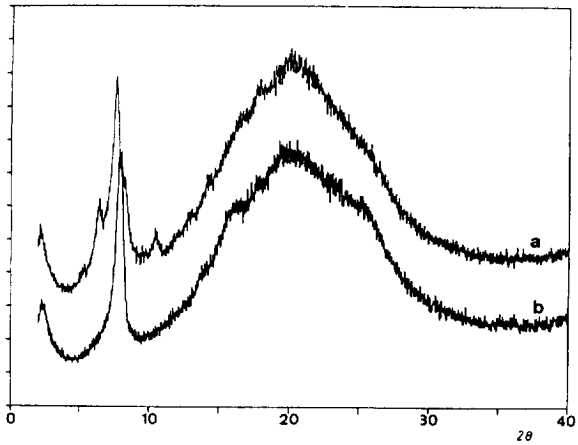

Fig. 7. X-Ray diffractograms of MMBC samples precipitated from a solution of (a) nitrobenzene and (b) methylene chloride.

(Fig. 7). A multiple peak was registered between 5° and 12° for the sample obtained from the nitrobenzene solution, while only one peak was obtained for the sample prepared from the methylene chloride solution. This differentiation becomes even more pronounced after annealing of the samples. Such a difference can be attributed to the existence of distinct crystalline forms of MMBC. Raman spectroscopic investigations were also performed but they did not afford any useful data permitting a clear distinction of the structures.

Interestingly, among the wide range of tested racemates, only the series of benzoyl esters of phenylethanol 2a–i shows this inversion of elution order. Even the structurally analogous compounds 3a and 3b do not exhibit this unexpected behavior.

Effects of polar additives

As MMBC apparently adopts different crystalline or supramolecular structures under the influence of the solvating agent methylene chloride or nitrobenzene, we were interested in investigating the effect of further solvating agents or additives. Granting that the formation of the supramolecular structure proceeds by a preorientation of the polymeric chains induced by the solvating agent, strongly interacting compounds should significantly influence the type of crystal structure obtained. To test this hypothesis, we used a cosolvent, such as tetrahydrofuran (THF), or some additives capable of interacting by hydrogen bonding with the carbonyl groups of MMBC, such as trifluoroacetic acid (TFA) and phenol. Because of their limited solubility, these compounds were applied only as additives in combination with methylene chloride or nitrobenzene. The preparation conditions of the various CSPs are summarized in Table 1. Two MMBC CSPs were prepared by adding THF or TFA in methylene chloride (MC-THF and MC-TFA, respectively). The chromatographic results obtained on these CSPs are presented in Table 3.

In fact, these two phases show similarities in their enantioselectivities and their optical discrimination patterns. Taking the MC-P phase as a reference, inversion of the elution order is observed for the enantiomers of compounds 2b, 2c, 2d, and 2h on the MC-THF CSP. Besides these four compounds, 2i was also eluted in reversed order on the MC-TFA CSP. These chromatographic results suggest that the presence of THF or TFA in methylene chloride has a tendency to transform the supramolecular structure of MMBC from type A, deriving from the preparation in pure methylene chloride (MC-P CSP), to type B, obtained from pure nitrobenzene (NB CSP). It seems, however, that neither of these additives can affect this structure strongly enough to transform it completely from type A to type B. In the light of these results, we decided to investigate the effect of phenol, which is capable, at the same time, of interacting by $\pi-\pi$ interactions and by strong hydrogen bonding. Phenol was used in combination with methylene chloride (1 mole phenol/8 mole methylene chloride) for covering macroporous silica gel with MMBC, and the chromatographic properties obtained with the resulting MC-PHN CSP are shown in Table 3. Similar to the NB CSP obtained from the pure nitrobenzene solution, the MC-PHN CSP causes inversion of the enantiomeric elution order for all nine esters

Table 3
Chromatographic results on MMBC CSPs solidified from methylene chloride or nitrobenzene in presence of the additives

Racemate	Column							
	MC-THF		MC-TFA		MC-PHN		NB-PHN	
	k'_1	α	k'_1	α	k'_1	α	k'_1	α
1	0.73(−)	1.14	0.54(−)	1.09	0.71(−)	1.21	0.69(−)	1.18
2a	1.12(−)	1.26	0.88(−)	1.23	1.70(+)	1.19	1.50(+)	1.09
2b	4.03(+)	1.00	2.93(+)	1.09	4.34(+)	1.45	3.80(+)	1.35
2c	0.80(+)	1.13	0.65(+)	1.15	0.90(+)	1.70	0.82(+)	1.60
2d	0.80(+)	1.19	0.67(+)	1.27	0.94(+)	1.72	0.88(+)	1.67
2e	0.96(−)	1.00	0.82(−)	1.00	1.13(+)	1.49	1.00(+)	1.39
2f	0.96(−)	1.34	0.83(−)	1.30	1.71(+)	1.13	1.68(+)	1.00
2g	1.51(−)	1.10	1.33(−)	1.00	2.12(+)	1.18	2.30(+)	1.07
2h	2.54(−)	1.12	2.06(−)	1.17	3.38(−)	1.55	3.24(−)	1.52
2i	3.20(−)	1.00	2.44(+)	1.00	3.76(+)	1.62	3.25(+)	1.52
3a	0.54(+)	1.41	0.42(+)	1.36	0.71(+)	1.23	0.69(+)	1.00
3b	1.25(+)	1.49	1.00(+)	1.42	1.63(+)	1.35	1.50(+)	1.32
4	2.00(−)	1.52	1.49(−)	1.50	1.71(−)	1.65	1.60(−)	1.65
5	2.42(−)	1.29	1.92(−)	1.28	2.50(−)	1.24	2.39(−)	1.24
6	1.05(+)	1.95	0.85(+)	1.75	1.15(+)	1.57	1.06(+)	1.60
7	1.76(−)	1.86	1.60(−)	1.88	2.00(−)	1.92	1.87(−)	1.84

The sign in parentheses represents optical rotation of the first-eluted enantiomer. For column preparation, see Table 1 and for the structure of the racemates, see Fig. 2.

2a–i of 2-phenyl ethanol. However, this last phase is generally more efficient in resolving racemic compounds than the NB CSP, as shown by the higher enantioselectivities obtained with the MC-PHN CSP for most of the reported compounds. These results show that the mere addition or non addition of phenol to the MMBC solution in methylene chloride before covering, can afford CSPs exhibiting not only markedly varying chiral discrimination power, but also reversed chiral recognition ability for several racemates.

As nitrobenzene and phenol produce materials having similar chiral recognition patterns, we also investigated the chromatographic properties of the CSP obtained from a solution of MMBC in nitrobenzene in the presence of phenol (NB-PHN). In fact, the experimental results revealed no such additive effect of the combination of nitrobenzene and phenol as might have been expected. Furthermore, the replacement of methylene chloride by nitrobenzene is rather detrimental, since in most cases lower separation factors were observed on the NB-PHN CSP than on the MC-PHN CSP.

4. Conclusion

The chiral recognition ability of MMBC is greatly affected by variations in the preparation conditions. With methylene chloride as solvating agent, covering by precipitation affords a significantly more efficient CSP in terms of chiral discrimination ability than coating by evaporation.

When nitrobenzene is used as solvating agent instead of methylene chloride, the CSP obtained exhibits completely different chromatographic properties, resulting in inversion of the elution order for the enantiomers of certain racemates. The same effect is observed when phenol is added to the methylene chloride solution before covering. The changes in the elution order observed for various racemates used as probes evidence the existence of different supramolecu-

lar structures for MMBC. The preferential formation of one or the other structure is clearly governed by the solvating agent and/or the additive, which probably favors the preorientation of the polymer chains by specific interactions before the solidification of MMBC. The overall chiral recognition ability will depend on the proportion of each structure in the CSP.

However useful the information afforded by these results may be, it does not help to predict the resolution of a particular racemate. On the contrary, these findings emphasize the difficulty of modeling and predicting chiral discrimination mechanisms with cellulose-based CSPs, and they explain the wide variations in the selectivities observed among cellulose-based CSPs prepared under different conditions.

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