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Chiral separations and quantitative analysis of optical isomers on cellulose tribenzoate plates

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ABSTRACT

In this paper new cellulose tribenzoate/gypsum layers in the ratio up to 8/1 (*w/w*) were investigated for the chiral resolution of closely related aromatic ketones (e.g. tetralones and indanones), alcohols (e.g. benzhydrols) and racemates or enantiomers of other compound classes (e.g. dinitrophenyl amino acids). Among 22 investigated compounds, 16 racemates were baseline or partially resolved by eluting with methanol or 2-propanol/water mixtures on 4/1 (*w/w*) layers. The best results were compared with those achieved on microcrystalline cellulose triacetate plates and on cellulose tribenzoate columns. The study of structurally related solutes allowed us to increase the knowledge of the retention and resolution mechanisms on this chiral stationary phase and to highlight the role of π–π interactions between cellulose tribenzoate and solutes with different substituents on the aromatic ring. However, some results were unexpected and confirmed the complexity of enantioseparation mechanisms, thus evidencing the importance of experimental tests. Densitometric scan in the visible region of cellulose tribenzoate/gypsum plates after their exposure to iodine vapours allowed us to successfully perform the quantitative analysis of the investigated compounds, thus overcoming the detection problems normally encountered with this stationary phase.

1. Introduction

It is well-known that enantiomers frequently exhibit different biological, pharmacological and toxicological properties; it is therefore of paramount importance to obtain optical isomers with high enantiomeric purity and to develop analytical methods suitable for chiral discrimination [1].

Among chiral chromatographic techniques, planar chromatography (PC) plays an important role for the real-time monitoring of chiral synthesis progress, owing to its simplicity, low cost and flexibility, and allows for obtaining results that can be usefully related to the ones achieved in columns [2]. Moreover, PC has the distinctive characteristics to permit the simultaneous study under exactly the same experimental conditions of a number of racemates and/or the quantitative determination of enantiomeric pairs, applying standard solutions and unknown samples on the same plate.

An excellent book [3], recently published within the Chromatographic Science Series, reported numerous PC chiral separations obtained until 2007, highlighting the great effectiveness of this technique for the resolution and quantitative determination of optical isomers belonging to very important classes of organic compounds, such as plant secondary metabolites or active ingredients of drugs and insecticides.

The stationary phases mainly employed for PC chiral separations are based on cellulose [4,5] and its derivatives, especially microcrystalline cellulose triacetate (MCTA) [6–15], cellulose tricarbamate (CTC) [16,17] and cellulose tribenzoate (CTB) [18,19].

Lepri and co-workers used non-commercial layers of CTB/Silica gel 60 GF_{254} mixtures for the resolution of various racemic alcohols and the quantitative determination of some of them [18,19]. These studies evidenced that enantioresolution of CTB layers increased with increasing the CTB percentage and the ratio 3/1 (*w/w*) was the maximum that allowed for obtaining layers with satisfactory compactness.

The use of CTB in chiral PC is limited to these two papers, while several applications of CTB and its alkyl derivatives in chiral column chromatography are reported in the literature [20–27]. This is probably due to the strong adsorption of CTB in the UV region below 300 nm [19] that makes impossible the quantification by UV-densitometric scanning of analytes adsorbing exclusively within the afore mentioned wavelength range.

In order to enhance the chiral resolution of CTB layers and to increase their compactness with respect to those based on CTB/Silica gel mixtures, it is herein proposed a chiral stationary phase (CSP) consisting of CTB and gypsum (CTB/G) up to a 8/1 (*w/w*) ratio. These plates have been used to resolve racemates and/or

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enantiomeric pairs never investigated on CTB, including selected optical isomers previously studied on CTB/Silica gel 3/1 (*w/w*) layers, with the following aims:

(1) to compare the chiral resolution of CTB/G mixtures with the one of CTB/Silica gel 3/1 (*w/w*);
(2) to investigate the separation of new structurally related enantiomers on CTB/G layers, thus increasing the knowledge of the mechanisms governing the retention and selectivity of this CSP, and to relate the data obtained by PC with those determined on column;
(3) to compare each other the results obtained on CTB and MCTA layers;
(4) to evaluate the feasibility of analyte quantification using densitometric scanning in the UV and, overall, in the visible region, the latter performed after exposure of the plates to iodine vapours and representing a technique of broader applicability with respect to the UV densitometry.

2. Experimental

2.1. Chemicals

Racemates and high purity (≥95%) optical isomers were purchased from Sigma–Aldrich (St. Louis, MO, USA), Alfa Aesar (Ward Hill, MA, USA), Acros Organics (Geel, Belgium) and Lancaster Synthesis (Morecambe, England).

2.2. PC analyses

Water, acetic acid, methanol, ethanol and 2-propanol used for the preparation of analyte solutions and the elution of CTB layers were all HPLC grade and were obtained from Merck (Darmstadt, Germany).

CTB for HPLC (catalogue number 02388, particle size 10–20 μm) was purchased from Fluka (Buchs, Switzerland). Gypsum ($CaSO_4 \cdot ½H_2O$), pure for analysis, was supplied by Merck. CTB/G layers with a ratio 4/1 (*w/w*) were obtained by adding 1.25 g of gypsum to 15 mL of water; the suspension obtained was magnetically stirred for about 5 min and afterwards 5 g of CTB and 25 mL of 2-propanol were added; the suspension was stirred for 5 min and finally transferred into a Camag (Muttenz, Switzerland) automatic PC-plate coater. The layers (10 cm × 20 cm or 20 cm × 20 cm, thickness 250 μm) were dried at room temperature and used within 24 h.

Layers with a CTB/G ratio 8/1 (*w/w*) were also prepared as described above, using 0.50 g of gypsum, 10.0 mL of water, 4 g of CTB and 20 mL of 2-propanol.

Solutions (10–15 mg mL^{-1}) of racemates and pure optical isomers were prepared in either methanol or aqueous ethanol (80%). These solutions (0.5–1 μL) were applied 1 cm from the bottom and at least 1.5 cm from the sides of the plates, using a MICROLITER™ syringe (Hamilton, Reno, NV, USA). The plates were developed via the ascending technique in a Desaga (Wieslock, Germany) thermostatic chamber (22 cm × 22 cm × 6 cm), at 23 °C, after saturation for 1 h with the elution mixture.

Detection was carried out by exposure to iodine vapours at 22 °C for 24 h. Densitometric measurements were performed in the reflection mode and with a zigzag scan (4 mm) using a Shimadzu (Kyoto, Japan) CS-9001PC densitometer coupled to a Pentium 1 IBM-compatible PC. Both UV and visible wavelengths were used; in particular, plates previously exposed to iodine were scanned at λ = 410 nm, while not exposed plates were scanned in the UV region at λ = 300 nm. All functions of the scanner were controlled and data were processed with PC-specific software manufactured by Shimadzu. Real time background correction was automatically performed in the zigzag scanning mode.

3. Results and discussion

3.1. Chiral resolution power of CTB/G and CTB/Silica gel layers

In order to compare the chiral resolution power of CTB/G layers with the one of CTB/Silica gel, the α-values of Troger's base, which is widely adopted as the reference racemate for this purpose, should be evaluated. In this research the best separation factor was obtained with CTB/G 4/1 (*w/w*) ratio (α = 2.08); this value was significantly higher than that observed on CTB/Silica gel 3/1 (*w/w*) (α = 1.80) [18]. This finding indicated

Table 1
Retention (hR_{f1}, hR_{f2}) and separation (α, R_S) factors for the best enantioresolutions obtained for several racemic compounds under different elution conditions on non commercial CTB/Gypsum 4/1 (*w/w*) plates. Migration distance ≅ 16 cm.

Racemate	hR_{f1} [a]	hR_{f2} [a]	α [b]	R_S [c]	Eluent
Troger's base	19 (+)	31 (−)	2.08	3.3	Methanol
4-Chlorobenzhydrol (σ = 0.227) [d]	41	50	1.44	1.9	Methanol
	17	23	1.46	1.8	2-Propanol/water 80/20 (*v/v*)
3-(Trifluoromethyl)benzhydrol (σ = 0.430) [d]	66	75	1.55	2.3	Methanol
4-(Trifluoromethyl)benzhydrol (σ = 0.540) [d]	69	75	1.35	1.6	Methanol
	39	49	1.50	2.4	2-Propanol/water 80/20 (*v/v*)
2-Methylbenzhydrol	51	57	1.27	1.8	Methanol
4-Methylbenzhydrol (σ = −0.170) [d]	48	53	1.22	1.4	Methanol
4-Methoxybenzhydrol (σ = −0.268) [d]	35	38	1.14	0.9	Methanol
1-Methyl-2-tetralone	38	42	1.18	1.0	Methanol
3-Phenyl-1-indanone	46	49	1.13	1.0	Methanol
4-Phenyl-1,3-dioxane	33	39	1.30	1.6	Methanol
cis-3-Phenyltetrahydropyrrolo-[2,1-b]oxazol-5(6H)-one	36 (3R)-(−)	40 (3S)-(+)	1.18	1.0	Methanol
1,4-Dibenzyloxy-2,3-butanediol	32 (2S,3S)-(−)	36 (2R,3R)-(+)	1.20	0.9	Methanol/water 96/4 (*v/v*)
4-Chromanol	67	72	1.27	1.2	Methanol
	54	65	1.58	2.1	2-Propanol/water 80/20 (*v/v*)
cis-2-Benzylaminocyclohexanemethanol	48 (1S,2R)-(−)	58 (1R,2S)-(+)	1.50	1.7	2-Propanol/water 80/20 (*v/v*)
N-2,4-Dinitrophenyl-DL-ethionine	19	23	1.27	0.9	Methanol/water/acetic acid 95/4/1 (*v/v*)
N-2,4-Dinitrophenyl-DL-α-amino butyric acid	29	34	1.26	1.0	Methanol/water/acetic acid 95/4/1 (*v/v*)

[a] $hR_f = R_f \times 100$.
[b] $\alpha = [(1/R_{f1}) - 1]/[(1/R_{f2}) - 1]$.
[c] $R_S = 2 \times$ (distance between the centres of two adjacent spots)/(sum of the widths of the two spots in the direction of development).
[d] σ = Hammett constant [28].

the good enantioselectivity of the former layers, in agreement with the larger amount of CTB present in the mixture. In addition, the occurrence of significant amount of gypsum as binder allowed for obtaining very compact layers that can be used with eluents having a wider range of polarity, including aqueous/alcoholic and *n*-hexane/2-propanol mixtures. It should also be noted that the α-value achieved for Troger's base with CTB/G 4/1 (*w*/*w*) was similar to that obtained on CTB column ($\alpha = 2.0$) [2].

The further increase of CTB, up to 8/1 (*w*/*w*) ratio, gave rise to a generally higher retention of analytes but did not produce any significant variation of the selectivity coefficients. Moreover, the background adsorption of the layer, either in the UV or VIS region (the latter after exposure to iodine vapours), increased with the rise in CTB percentage, thus making much more difficult the analyte detection and quantification.

Based on the aforementioned considerations, the present study was conducted on CTB/G 4/1 (*w*/*w*) layers.

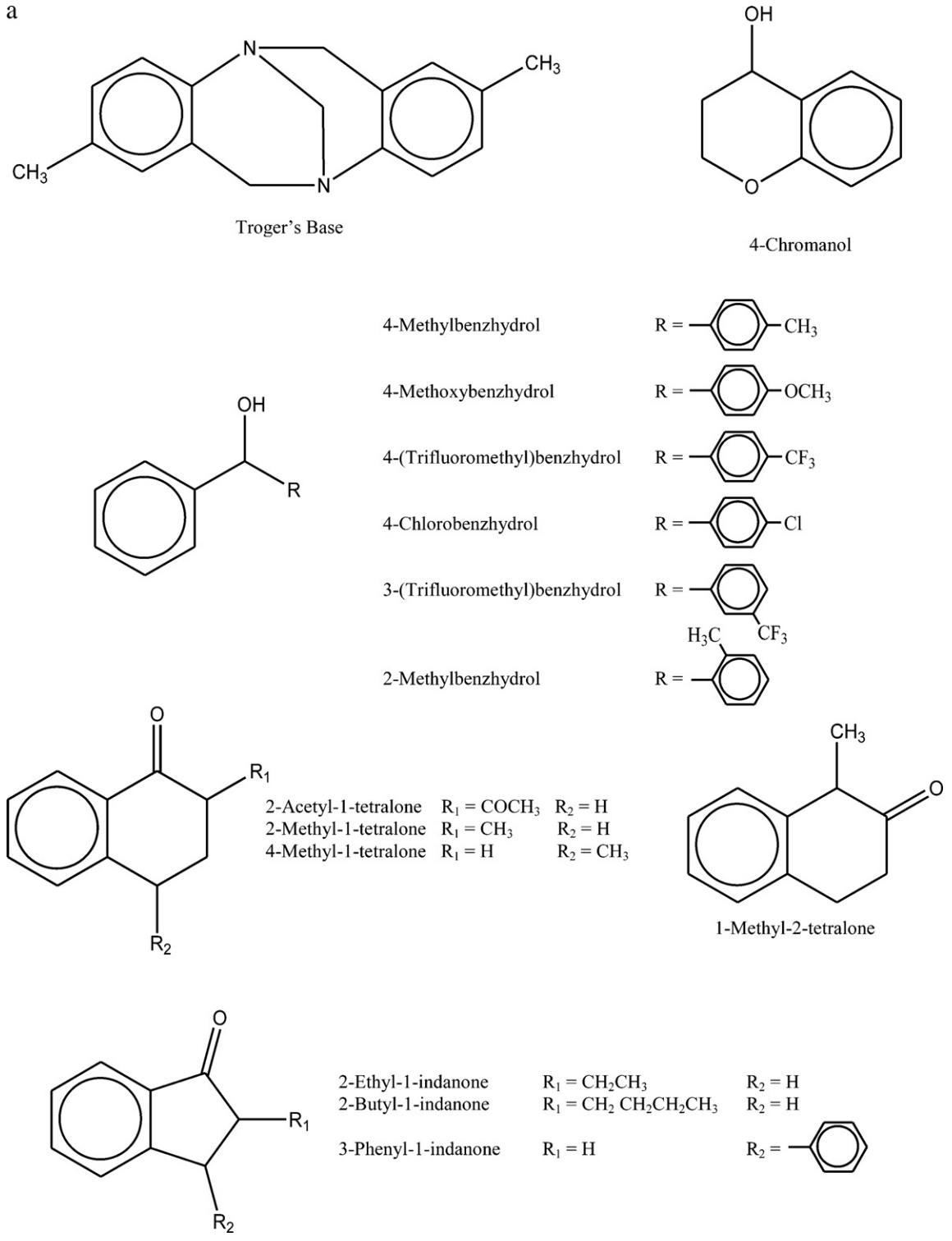
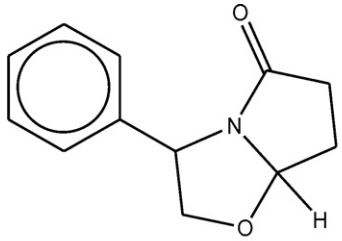
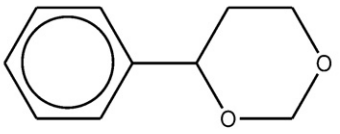


Fig. 1. Structures of the test solutes.

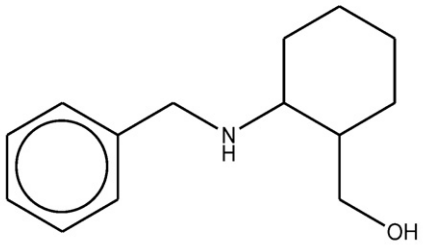
b



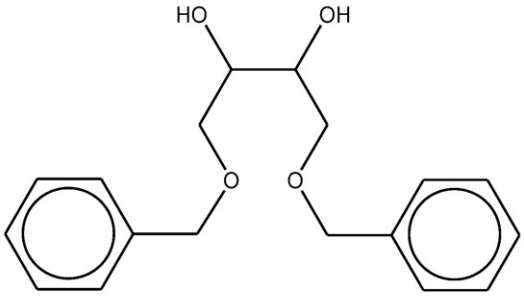
cis-3-Phenyltetrahydropyrrolo-[2,1-b]oxazol-5(6H)-one



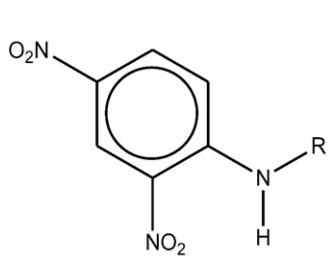
4-Phenyl-1,3-dioxane

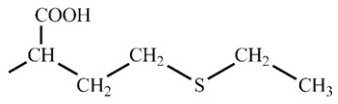


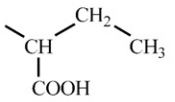
cis-(1S,2R;1R,2S)-2-Benzylaminocyclohexanemethanol



1,4-Dibenzyloxy-2,3-butanediol



N-2,4-dinitrophenyl-DL-ethionine R = 

N-2,4-dinitrophenyl-DL-α-amino-n-butyric acid R = 

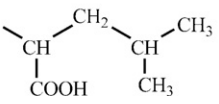
N-2,4-dinitrophenyl-DL-leucine R = 

Fig. 1. (*Continued*).

3.2. Enantioresolution power of CTB/G layers

Even though the use of low polarity eluents like *n*-hexane/2-propanol 80/20 (*v*/*v*) is allowed, our best results were obtained by eluting with aqueous/alcoholic mixtures or alcohols alone (see Table 1).

Among the 22 compounds investigated (see Fig. 1), 16 racemates and enantiomeric pairs were baseline or partially resolved under the different experimental conditions adopted. The resolved analytes belonged to alcohols, ketones, heterocyclic compounds and dinitrophenyl (DNP) amino acids, highlighting the great potential of this CSP for the enantioresolution of a broad range of analytes.

Most racemates and enantiomeric pairs were resolved with methanol, even though, in some cases, the use of 2-propanol/water 80/20 (*v*/*v*) allowed us to obtain comparable or better results (see Table 1); furthermore, for DNP-amino acids, the addition of acetic acid was necessary in order to prevent the dissociation of the carboxylic group and to increase the chance of chiral resolution.

The largest homogeneous group investigated was represented by α-substituted benzyl alcohols that differed one from each other for the substituent group and/or its position in the aromatic ring (see Fig. 1). A chromatogram representative of benzhydrols behaviour by eluting with methanol is reported in Fig. 2 (lines a–f). Within this group, 2- and 4-methyl, 4-chloro, 3- and 4-trifluoromethyl derivatives were baseline resolved, whereas for 4-methoxybenzhydrol only a partial resolution was achieved, with α and R_S values of 1.14 and 0.9, respectively.

For the benzhydrols substituted in the para or meta position the retention can be interpreted on the basis of π-basicity of the aromatic portion of the molecule underwent to substitution, as measured by the Hammett constant (σ) [28]; in fact, when σ values were plotted as a function of $(1/R_f) - 1$ data, an inverse linear trend was obtained for both the enantiomers, with *r*-values of 0.814 ($P = 0.093$) and 0.869 ($P = 0.056$) for the most and less retained optical antipodes, respectively. This result evidenced that the π–π interactions between the aromatic rings of CTB and solutes are an important aspect of the retention mechanism. In addition, the comparison of the σ constants of the substituted benzhydrols and α-values evidenced a positive linear correlation ($r = 0.815$; $P = 0.093$), indicating that an increase of π basicity results in a decrease of chiral resolution. When the regression analysis was run without the point relative to the 4-(trifluoromethyl)benzhydrol,

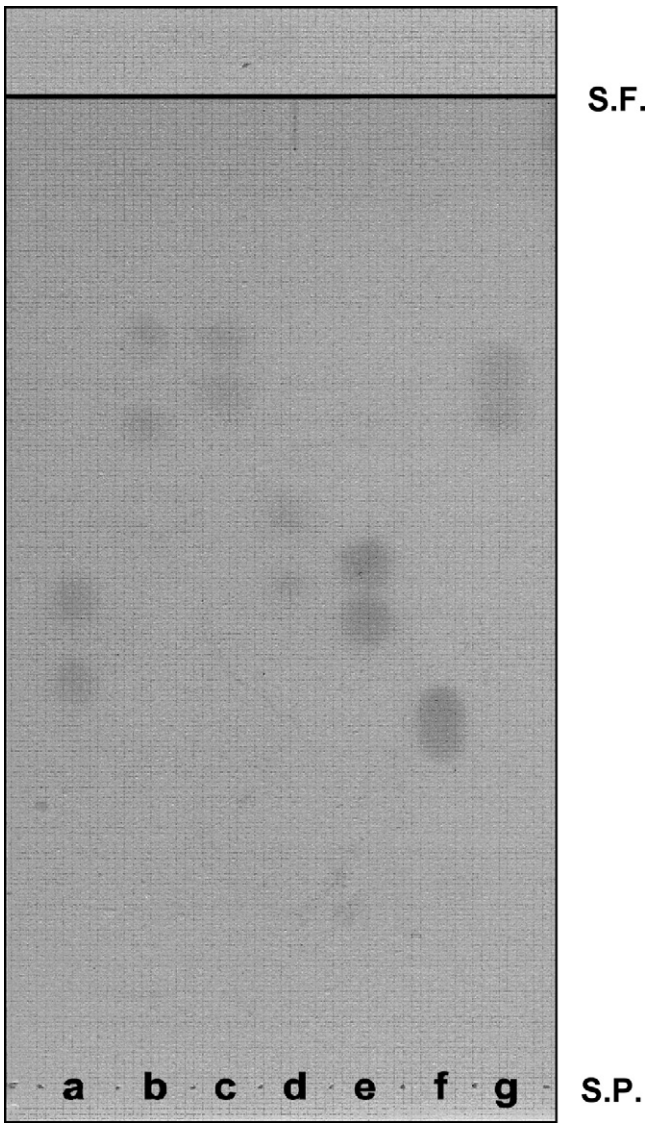


Fig. 2. Thin layer chromatogram of racemates on CTB/Gypsum 4/1 (*w/w*) layers, eluted with methanol. The migration distance was 16 cm. 1.0 μL of a 15 mg mL^{-1} solution was applied to the plates; (a) 4-chlorobenzhydrol; (b) 3-(trifluoromethyl)benzhydrol; (c) 4-(trifluoromethyl)benzhydrol, (d) 2-methylbenzhydrol; (e) 4-methylbenzhydrol; (f) 4-methoxybenzhydrol; (g) 4-chromanol. S.P. = starting point. S.F. = solvent front. Detection: iodine vapours.

the compound which deviated the greatest from the linear correlation, an increase of the *r*-value to 0.998 ($P = 0.001$) was observed.

Contrasting results have been obtained by Wainer et al. on column of CTB adsorbed on macroporous silica, by eluting with *n*-hexane/propanol, for a series of enantiomeric amides [21] and aromatic alcohols [22]. In fact, for amides an increase in the π-basicity of the aromatic substituent gave rise to an increment of the chiral resolution, while an opposite result was observed by comparing 1-phenylethanol and 1-*p*-tolylethanol, in agreement with our findings for benzhydrols.

The use of 2-propanol/water 80/20 (*v/v*) as eluent allowed for significantly improving the α value of 4-trifluoromethylbenzhydrol, which increased from 1.35 to 1.50; moreover, with this eluent, a separation factor comparable with the one obtained with methanol was achieved for 4-chlorobenzhydrol (see Table 1). Conversely, 2-methylbenzhydrol ($R_f = 0.31$), 4-methylbenzhydrol ($R_f = 0.19$) and 4-methoxybenzhydrol ($R_f = 0.12$) were not resolved. Even with this eluent mixture, the increase of π-basicity, as measured by Hammett constants, resulted in an increment of analyte retention and, with regard to the resolved benzhydrols, a decrease of selectivity.

The lack in resolution of methyl and methoxy derivatives can therefore be attributed to the stronger achiral π–π interactions between the aromatic rings of solutes and CTB.

A group of benzhydrols structurally analogous to those herein investigated and consisting of the three chloro and the three metoxy derivatives, has been studied by Francotte and Wolf [23] on CTB column, by eluting with *n*-hexane/2-propanol 90/10 (*v/v*). Under these experimental conditions, the resolution of only 2-methoxybenxhydrol ($\alpha = 1.21$) and 3-chlorobenzhydrol ($\alpha = 1.62$) was obtained.

These findings suggested that, even for CTB column, the use of methanol and/or 2-propanol/water as eluents could give rise to a better resolution compared to the hexane/alcohol mobile phases.

The results obtained with CTB plates are itself of paramount importance, as well as for the prediction of the chromatographic behaviour on CTB column, since it is not possible to theoretically know with any certainty whether two enantiomers will be separated on cellulose derivatives. In fact, even small variations of the chemical structure of solutes might have adverse effects on enantioseparations.

The chromatographic behaviour of 4-chromanol by eluting with methanol is reported in Fig. 2 (line g). Under these conditions, ΔR_f value of 0.05 was achieved, whereas a significant improvement of the resolution ($\Delta R_f = 0.11$) could be obtained with 2-propanol/water 80/20 (*v/v*). With this eluent a good separation was achieved also for cis-2-benzylaminocyclohexanemethanol enantiomers ($\alpha = 1.50$, $R_S = 1.7$). Even 1,4-dibenziloxy-2,3-butanediol was resolved, confirming the good selectivity of CTB for aromatic alcohols.

With regard to ketones, some tetralones, indanones and the enantiomers of cis-3-phenyltetrahydropyrrolo-[2,1-b]oxazol-5(6H)-one were studied (see Fig. 1).

Within tetralone group, only 1-methyl-2-tetralone was resolved (see Table 1), highlighting the crucial role of the presence of the carbonyl group in the 2-position of the aliphatic ring for chiral discrimination.

For indanones, only 3-phenyl-1-indanone was resolved, indicating the importance of the presence and/or the position of the aromatic ring, in agreement with the significant role of π–π interactions between CTB and solute for enantioresolution. In fact, these interactions, together with hydrogen bonds (or dipole–dipole interactions) between the ester carbonyl group of CTB and the alcoholic hydrogen (or the carbonyl group) of solutes, have been reported as important chiral discrimination factors for CTB [29].

The separation of the enantiomers of cis-3-phenyltetrahydropyrrolo-[2,1-b]oxazol-5(6H)-one that exhibits a chiral centre in β position with respect to the carbonyl group, was also achieved.

When methanol was used as eluent, high selectivity was observed for 4-phenyl-1,3-dioxane ($\alpha = 1.30$, $R_S = 1.6$), confirming the versatility of this CSP towards structurally different classes of compounds.

Among DNP-amino acids, N-2,4-dinitrophenyl-DL-ethionine and N-2,4-dinitrophenyl-DL-α-amino butyric acid were resolved by eluting with methanol/water/acetic acid 95/4/1 (*v/v/v*), whereas for the N-2,4-dinitrophenyl-DL-leucine the complete co-elution of the two enantiomers was observed under the different experimental conditions used. DNP-amino acids were never studied before on this CSP and the obtained results highlighted new possibilities of chiral separations for these compounds, which play an important role in the primary structure determination of peptides and proteins [30]. In addition, it should be also considered that

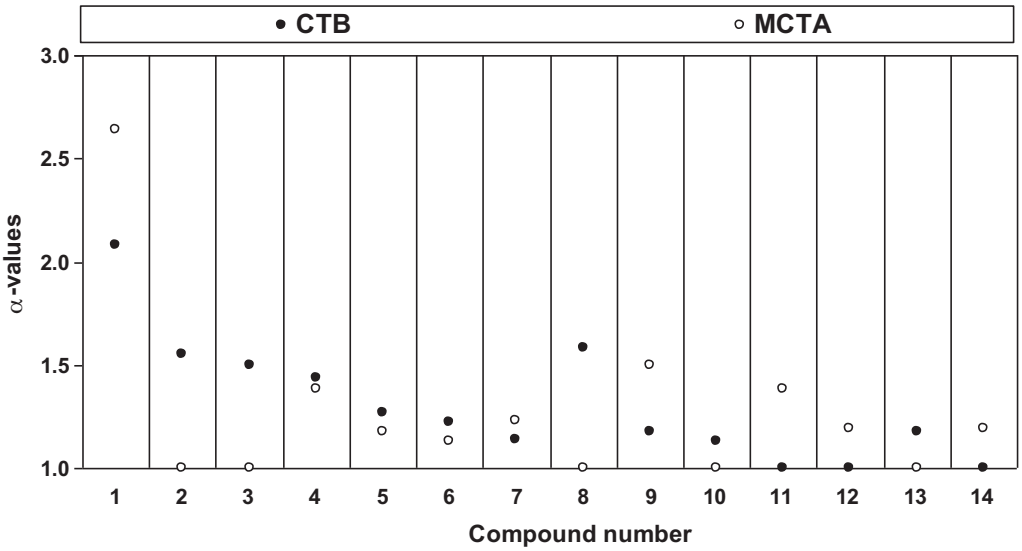


Fig. 3. Comparison of the best α values for racemates and enantiomeric pairs resolved on CTB/Gypsum 4/1 (*w*/*w*) and/or MCTA/Silica gel 4/1 (*w*/*w*) or 3/1 (*w*/*w*) layers. Compound numbers: (1) Troger's base; (2) 3-(trifluoromethyl)benzhydrols; (3) 4-(trifluoromethyl)benzhydrols; (4) 4-chlorobenzhydrol; (5) 2-methylbenzhydrol; (6) 4-methylbenzhydrol; (7) 4-methoxybenzydrol; (8) 4-chromanol; (9) cis-3-phenyltetrahydropyrrolo-[2,1-*b*]oxazol-5(6H)-one; (10) 3-phenyl-1-indanone; (11) 2-ethyl-1-indanone; (12) 2-butyl-1-indanone; (13) 1-methyl-2-tetralone; (14) 2-methyl-1-tetralone.

these analytes are yellow coloured and can be easily detected in the visible region, thus overcoming the well-known detection and quantitative determination problems of CTB plates.

3.3. Comparison of CTB and MCTA layers

Among the 22 racemates and enantiomeric pairs investigated in this study, 16 were also studied on MCTA/Silica gel plates [10–15], thus allowing us to compare their chromatographic behaviour on these CSPs.

Within the group of molecules investigated on both the CSPs, 2-acetyl and 4-methyl-1-tetralone were not resolved neither on CTB nor on MCTA. In Fig. 3 are shown the best α values obtained for racemates and enantiomeric pairs, which were resolved on CTB/G 4/1 (*w*/*w*) and/or MCTA/Silica gel 4/1 or 3/1 (*w*/*w*).

Troger's base (Fig. 3, compound 1) showed a higher separation factor on MCTA layers than on CTB, probably owing to the presence of stereogenic centres (the tertiary amine nitrogens) on a rigidly locked structure, which is an important chiral discrimination factor for MCTA [25].

For benzhydrols investigated both on CTB and MCTA, a general higher enantioresolution of the former CSP can be pinpointed from the comparison of data reported in Fig. 3. More in detail, trifluoromethyl derivatives (compounds 2–3) were resolved on CTB but not on MCTA, whereas for 4-chlorobenzhydrol (compound 4) and methyl benzhydrols (compounds 5–6) higher separation factors were obtained on CTB. Conversely, for 4-methoxybenzhydrol (compound 7), a higher α value was achieved on MCTA. Even for 4-chromanol (compound 8) an opposite behaviour of the two CSPs was observed, being the analyte baseline resolved on CTB and not separated on MCTA [15].

With regard to ketones, cis-3-phenyltetrahydropyrrolo-[2,1-b]oxazol-5(6H)-one (compound 9) showed much higher selectivity values on MCTA than on CTB, in agreement with the importance of the presence of a stereogenic centre on a rigid structure, previously mentioned for Troger's base. Moreover, an inversion of the elution order of the two enantiomers on CTB with respect to MCTA was observed, confirming that the chromatographic behaviour of optically active compounds is not easily predictable.

Within the investigated series of indanones and tetralones, an interesting behaviour of the two CSPs was highlighted, being them thoroughly complementary. In fact, 2-ethyl and 2-butyl-1-indanone (compounds 10–11) were resolved only on MCTA, whereas the optical antipodes of 3-phenyl-1-indanone (compound 12) were separated solely on CTB; moreover, 1-methyl-2-tetralone (compound 13) was resolved only on CTB and 2-methyl-1-tetralone (compound 14) solely on MCTA.

3.4. Applications of CTB/G layers for quantitative determinations

The use of CTB/G stationary phase makes difficult the identification and quantitative determination of analytes by UV densitometry, owing to the strong adsorption of tribenzoyl cellulose, approximately between 200 and 290 nm [19]. Nevertheless these layers could be employed for the determination of those molecules that strongly adsorb in the UV region, especially at wavelengths $\geq$300 nm. This is the case of Troger's base that showed a strong adsorption peak between 280 and 330 nm, with

Table 2
Slopes (*m*) and intercepts (*q*) of the least square regression equations,[a] correlation coefficients (*r*) and linearity range (μg applied to the plate) for the five-point calibration lines of enantiomers of Troger's base in the UV (λ = 300 nm) and visible (λ = 410 nm) regions, and of 4-chlorobenzhydrol in the visible region (λ = 410 nm).

Enantiomer	Acquisition Wavelength (nm)	*m*	*q*	*r*	Linearity Range (μg)
(+)-Troger's base	300	10,988	3587	0.999	1.25–6.25
	410	7871	5895	0.999	
(−)-Troger's base	300	11,444	1932	0.999	
	410	8082	5295	0.997	
4-Chlorobenzhydrol (most retained enantiomer)	410	20.0	317	0.997	4.0–20
4-Chlorobenzhydrol (less retained enantiomer)	410	22.7	372	0.998	

[a] $y = mx + q$, where y is the chromatographic area and x is the amount of analyte applied to the plate.

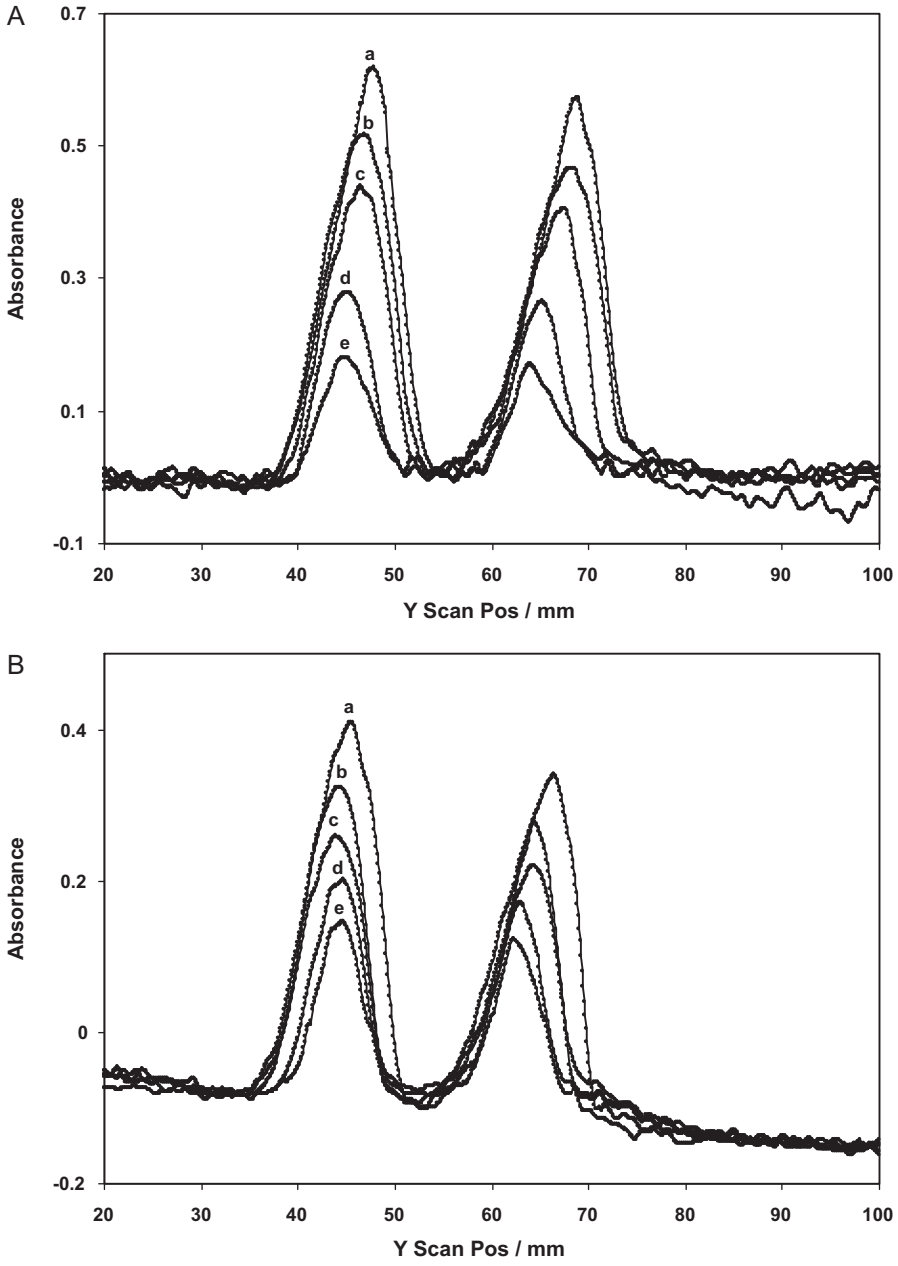


Fig. 4. Densitograms of racemic Troger's base on CTB/Gypsum 4/1 (*w/w*) layers eluted with methanol and acquired at λ = 300 nm (A) and at λ = 410 nm after plate exposure to iodine vapour (B). The migration distance was 16 cm. Amounts of racemate applied to the plate: 12.5 μg (a), 10.0 μg (b), 7.5 μg (c), 5.0 μg (d), 2.5 μg (e).

a maximum at about 300 nm, when it was applied to CTB/G plates, even at amounts of a few μg. Fig. 4A reports representative UV-densitograms (λ = 300 nm) acquired for Troger's base after deposition of different amounts (2.5–12.5 μg) of the racemate and elution with methanol; for both the enantiomers a very good linear correlation (r = 0.999) was obtained (see Table 2), indicating that this CSP is suitable for quantitative applications.

In order to overcome the detection problems due to the UV adsorption of CTB layers, the feasibility of employing densitometry in the visible region, after plate exposure to iodine vapours, was investigated for the first time. Fig. 4B shows the chromatographic profile of Troger's base obtained by exposure to iodine vapours and visible densitometry (λ = 410 nm) of the same plate previously described in Fig. 4A. When the amount of the two enantiomers was plotted as a function of the chromatographic area, a very good linear correlation was observed, under these experimental conditions. This finding evidenced the great potential of such technique, which makes possible the use of CTB also for molecules that cannot be revealed with UV densitometry. As an example, 4-chlorobenzhydrol showed a very good linear correlation for both enantiomers in the range of 4–20 μg applied to the plate (see Table 2).

The high sensitivity (about 0.5 μg for each enantiomer) and selectivity (ΔR_f = 0.12) achieved for Troger's base make possible the determination of its enantiomeric purity with both UV and visible detection techniques, while for the other investigated molecules, which showed a lower sensitivity (about 2 μg for each enantiomer) and selectivity (ΔR_f = 0.09–0.10), only the real-time monitoring of stereoselective synthesis can be performed.

4. Conclusions

CTB/G plates have been successfully used for the resolution of a wide range of aromatic racemic compounds, including alcohols, ketones, heterocyclic compounds and DNP-aminoacids.

The several and original enantioseparations herein reported provided useful information for column analytical applications.

The results obtained for the aromatic alcohols, which were all resolved on CTB, highlighted the key-role of π–π interactions and, above all, of hydrogen bonds, within the multiple interaction model proposed by Francotte et al. [23] for the enantioseparation on this CSP. In this regard, it should be noted that a left-handed 3/2-helical structure for cellulose tribenzoate and cellulose triphenylcarbamate was proposed by X-ray structural analysis [31] and this high-ordered helical structure is responsible for providing the strong chiral recognition power of these CSPs [24].

Similar to the chiral resolution factors proposed for cellulose triphenylcarbamate [32], the following interactions can be proposed also for CTB: (i) polar interactions between carbonyl or hydroxyl moieties of solute and the ester carbonyl group of CTB, (ii) π–π interactions between the aromatic rings of solute and CTB and (iii) inclusion of the molecule into the chiral helical groove of polysaccharide. With regard to the latter factors, as also reported by Wainer et al. [22], the inclusion of the aromatic portion of the molecule into the chiral cavity of CTB produces chiral π–π interactions that stabilize the diastereomeric solute–CTB complex. Conversely, aromatic portions that are excluded from the helical groove of CSP will give rise to achiral interactions that increase retention and, at the same time, decrease selectivity. In agreement with this hypothesis benzhydrols characterized by high π-basicity, are not resolved owing to the strong achiral interactions between solute and aromatic rings of CTB located outside of the chiral cavity.

However, the results obtained for the various racemates were in some cases unexpected and highlighted the need to experimentally evaluate the chromatographic behaviour of optically active compounds on polysaccharide CSPs. In fact, the above-mentioned mechanisms are still not satisfactorily elucidated at a molecular level, because of the several different interaction sites with a different affinity for enantiomers on chiral polymers and the difficulty to determine their precise structures in the presence of the solvent.

Iodine exposure followed by densitometry in the visible region allowed us to overcome the detection problems usually observed with stationary phases based on aromatic derivatives of polysaccharide [29], thus making CTB of much larger applicability even for quantitative analysis.

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