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The Role Seemingly of Amorphous Silica Gel Layers in Chiral Separations by Planar Chromatography

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Abstract

In planar chromatography, silica gel appears as the most frequently used adsorbent. Its preference as planar chromatographic stationary phase is due to its high specific surface area (ca. 700 m^2 g^{-1}) and relatively simple active sites (silanol groups, ≡Si-OH). The high specific surface area of silica gel and a high density of coverage of its surface with the silanol active sites contribute jointly to an excellent separation performance of this adsorbent. In our experiments on chiral separation of the enantiomer pairs by planar chromatography, contradictory behavior of the silica gel layers versus the chiral compounds was observed. The migration tracks of chiral compounds in the ascending planar chromatographic mode were not vertical but bent on either side being a function of analyte chirality. This deviation of the analyte's migration track was noticed, when using the densitometric scanner to quantify the respective chromatograms. In order to confirm the hypothesis as to the microcrystalline nature of silica gel used in liquid chromatography, it was further investigated through circular dichroism (CD) and the data thereof confirmed that the 'chromatographic' silica gels are not amorphous but microcrystalline, contributing to the (partial) horizontal enantioseparation of the antimer pairs. This paper summarizes the results of our investigation on the microcrystalline nature of silica gels used in planar chromatography and their impact on enantioseparation of the selected pairs of antimers.

Introduction

Right from its earliest use as chromatographic stationary phase, silica gel, has so far been considered as an amorphous solid, that is, lacking a crystalline structure. On the other hand, the naturally occurring or synthetic silicon dioxide – known as quartz – often appears as a macrocrystal having the ability to rotate the plane of polarization of light.

Firstly, it was strange in our thin-layer chromatographic enantioseparations not to find though anticipated non-volatile and chemically stable chiral analytes [1]. Only after having repeatedly scanned the chromatogram with parallel scans of 1-mm intervals at a width of 1 cm on either side of the anticipated vertical migration track, it was possible to densitometrically locate the "missing" analyte. These repeatedly occurring horizontal deviations of chiral analytes led to the suspicion that this unusual phenomenon could be due to the chromatographic silica gel not being amorphous. Moreover, this deviation of the chiral analytes' migration tracks (never observed with the non-chiral analytes) was due either to the microcrystalline nature of silica gel and its crystalline chirality, or to the presence of certain microcrystalline enclaves, or "islands", in the predominantly amorphous silica gel moiety.

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Enantioseparation of ibuprofen, naproxen, and 2-phenylpropionic acid on silica gel layers impregnated with L-arginine

Paper [1], presented the results of the enantioseparation of the three antimer pairs, i.e., of *S,R*-(±)-ibuprofen, *S,R*-(±)-naproxen, and *S,R*-(±)-2-phenylpropionic acid. The analytical procedure was adapted from that earlier described by Bhushan and Parshad [2] and modified to suit the purpose of modern TLC [3]. Thus it replaced the lab-coated chromatographic plates with the commercial ones and visualisation of the chromatograms was done not in the iodine vapors, but by the densitometric detection. The following chromatographic system was used:

Stationary phase: silica gel layer impregnated with L-arginine; Mobile phase:

(a) for ibuprofen, acetonitrile (ACN) – methanol (MeOH) – water (H_2O), 5:1:1 (*v/v*);

(b) for naproxen, ACN – MeOH – H_2O, 5:1:1.5 (*v/v*);

(c) for 2-phenylpropionic acid, ACN – MeOH – H_2O, 5:1:0.75 (*v/v*).

Each mobile phase contained an extra addition of several drops of glacial acetic acid to fix its acidity at the pH < 4.8 level. The mechanism given below of ion-pair formation was proposed to explain the separation achieved:

- L-arginine$^{(cation)}$ + S-(+)-2-arylpropionic acid$^{(anion)}$ ↔ ion pair (1) (characterized by K_1)
- L-arginine$^{(cation)}$ + R-(−)-2-arylpropionic acid$^{(anion)}$ ↔ ion pair (2) (characterized by K_2)

where ($K_1 \neq K_2$).

The developed and dried chromatograms were densitometrically evaluated and we the horizontal deviation of the analytes' migration tracks from the strict verticality was witnessed. Moreover, these deviations were not random, but systematic and the magnitude of these deviations was higher than in random cases (when it is limited to ±1 mm). This specificity has been confirmed by a wide number of the thorough preliminary experiments that preceded proper and systematic investigations. For instance, we have even checked evenness and strict horizontality of the laboratory bench top with a water level, in order to eliminate the surprise external factors, which might negatively affect the results. Thus the influence of the non-chirality-based factors have been excluded from the ultimate results. The obtained results are summarized in Table 1 and Figure 1.

It was quite evident that L-arginine and the mechanism of ion-pair formation (see Eqs (1) and (2)) were responsible for vertical enantioseparation of the three investigated racemic mixtures. It seemed equally evident that horizontal deviation of the migration tracks with each individual pair of antimers additionally improved the obtained enantioseparation, although at the beginning the mechanism of this horizontal deviation seemed rather obscure.

Table 1. **Deviation (in terms of direction and magnitude) from the strict verticality of the enantiomer migration tracks with the two antimers of ibuprofen, naproxen, and 2-ph enylpropionic acid[*] [1]**

Analyte	Chiral configu ration	R_F[**]	Deviation From verticality[**] [mm]	Direction of the deviation
Ibuprofen	*S*-(+)	0.91 (±0.02)	2 (±1)	Right
	R-(-)	0.88 (±0.02)	2 (±1)	Left
Naproxen	*S*-(+)	0.89 (±0.02)	3 (±1)	Left
	R-(-)	0.85 (±0.02)	3 (±1)	Right
2-Pheny-lpropionic acid	*S*-(+)	0.90 (±0.02)	5 (±1)	Right
	R-(-)	0.80 (±0.02)	2 (±1)	Left

[*]Stationary phase: silica gel 60 F_{254} (precoated plates, Merck, cat. # 1.05715). Mobile phase: ACN - MeOH - H_2O; quantitative composition for ibuprofen, 5:1:1 (*v/v*); naproxen, 5:1:1.5 (*v/v*); and 2-phenylpropionic acid, 5:1:0.75 (*v/v*).The migration distance of mobile phase: 15 cm.

[**]The presented numerical results were derived from 27 individual enantiomer migration tracks (i.e. from three chromatographic plates, nine separate development lanes per plate)

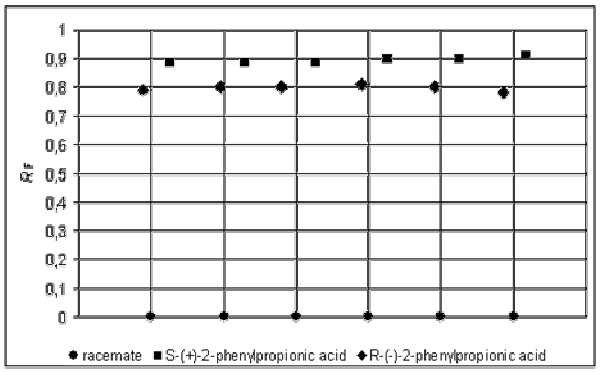

Figure1. **Schematic presentation of the direction-wise deviation from verticality of the migration tracks with the antimers of 2-phenylpropionic acid. Stationary phase: silica gel 60 F_{254} (precoated plates, Merck, cat. # 1.05715), impregnated with L-arginine. Mobile phase: ACN - MeOH - H_2O, 5:1:0.75 (*v/v*) [1]**

Enantioseparation of S,R-(±)-ketoprofen on silica gel layers impregnated with L-arginine

The investigation analogous to that described in the preceding Section 1 was performed with the racemic mixture of ketoprofen [4]. Again, the racemic mixture was enantioseparated on the silica gel layer impregnated with L-arginine and the systematic deviation of the analytes' migration tracks from verticality was observed. In this experiment, the following mobile phase was used: ACN + H_2O, 5:1 (*v/v*) acidified with glacial acetic acid to fix the pH < 4.8. The obtained results are shown in Figure 2.

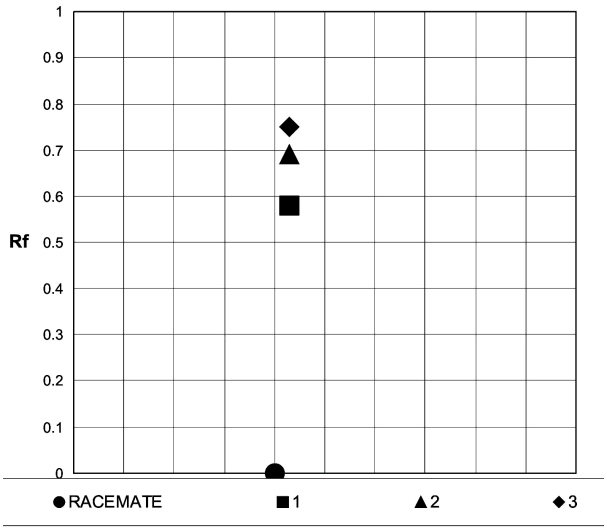

Figure 2. **Schematic presentation of the right-handed deviation from verticality of the migration tracks with the three chromatographic peaks derived from the same *S,R*-(±)-ketoprofen sample. The respective R_F values in ascending order were: 0.58±0.02, 0.69±0.02, and 0.75±0.02. Stationary phase: Merck silica gel 60 F_{254} precoated plates, impregnated with L-arginine. Mobile phase: ACN - H_2O (5:1, v/v) [4].**

As shown in Figure 2, in this case enantioseparation resulted in three chromatographic bands instead of the expected two bands, one for each antimer, all three bands horizontally deviated from verticality and in each case this deviation was right-handed. In Figure 3, the chromatogram is presented, obtained in the two-dimensional (2D) development mode and clearly showing the three separated bands. It seems highly probable that the separated species were the following: H-bonded ketoprofen dimers: *SR* (the quantitatively predominant peak 2 showing the medium R_F value), *SS* (peak 3 of relatively low intensity showing the highest R_F value) and peak 1, also of relatively low intensity, showing the lowest R_F value and most probably representing the *RR* dimer.

Enantioseparation of S,R-(±)-ketoprofen, S-(+)-ibuprofen, and S-(+)-naproxen on the plain (i.e., non-impregnated) silica gel layers

The results presented in Sections 1 and 2 and showing systematic deviation from verticality of the migration tracks with the four pairs of 2-arylpropionic acids antimers (i.e., ibuprofen, naproxen, 2-phenylpropionic acid, and ketoprofen) led to the suspicion that L-arginine acting as chiral selector cannot be responsible for this phenomenon and it is certainly responsible for the enantioseparation in the vertical direction. The next candidate possibly responsible for the enantioseparation in the horizontal direction seemed to us silica gel. In order to support this hypothesis, it was decided to attempt the enantioseparation of *S,R*-(±)-ketoprofen on the plain (i.e., non-impregnated with L-arginine) silica gel layer and using the same mobile phase as that reported in paper [4], i.e., ACN + H_2O, 5:1 (*v*/*v*) acidified with glacial acetic acid. The obtained results were presented in paper [5] and they are now summarized in Figure 4.

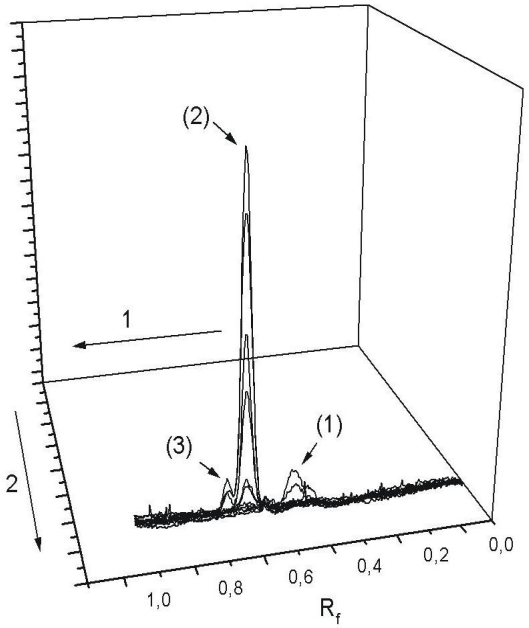

Figure 3. **Three-dimensional presentation of the *S,R*-(±)-ketoprofen chromatogram with two development directions, 1 and 2, indicated. Densitometric scanning (at parallel 1.5-mm intervals) of the 30-mm wide track perpendicular to the second direction of the development was performed to better illustrate the separation performance and the skewed arrangement of the three separated species, indicated by arrows [4].**

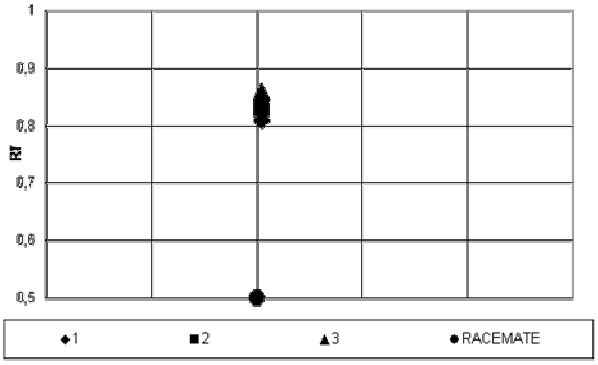

Figure 4. **Schematic presentation of the right-handed deviation from verticality of the migration tracks of the three chromatographic spots derived from the same *S,R*-(±)-ketoprofen sample. The respective R_F values were (in ascending order): 0.81±0.02, 0.83±0.02, and 0.86±0.02. Stationary phase: silica gel 60 F_{254} (precoated plates, Merck, cat. # 1.05715). Mobile phase: ACN - H_2O, 5:1 (*v*/*v*) (*v*/*v*), plus several drops of glacial acetic acid [5].**

As it comes out from Figure 4, even without the chiral selector (i.e., L-arginine) deposited on the silica gel layer, enantioseparation of the *S,R*-(±)-ketoprofen racemate in the applied planar chromatographic system was obtained. Vertical enantioseparation was much less pronounced than that obtained in presence of L-arginine (see Figure 2), but also in this case the side-wise deviation of the enantiomers' migration tracks was evident. This result certainly supported the hypothesis of microcrystalline chirality of silica gel.

We also investigated the chromatographic behavior of *S*-(+)-ibuprofen and *S*-(+)-naproxen on the plain silica gel layers, that is in the absence of L-arginine as chiral selector. In that case the mobile phases were analogous to those of ibuprofen and naproxen racemates, respectively (paper [1], Section 1) and the obtained results were given in paper [6]. Now they have been summarized in Table 2 and Figure 5.

Table 2. **Deviation (in terms of direction and magnitude) from the strict verticality of the migration tracks with *S*-(+)-ibuprofen and *S*-(+)-naproxen[*] [6]**

Analyte	R_F[**]	Deviation from verticality[**] [mm]	Direction of deviation
S-(+)-Ibuprofen	0.91 (±0.01)	5 (±1)	Right
S-(+)-Naproxen	0.90 (±0.02)	3 (±1)	Left

[*]Stationary phase: silica gel 60 F_{254} (precoated plates, Merck, cat. # 1.05715). Mobile phase: ACN - MeOH - H_2O; quantitative composition for *S*-(+)-ibuprofen, 5:1:1 (*v/v*) and for *S*-(+)-naproxen, 5:1:1.5 (*v/v*). The migration distance of mobile phase: 15 cm

[**]The presented numerical results were derived from 27 individual migration tracks per analyte (i.e. from the three chromatographic plates, nine separate development lanes per plate)

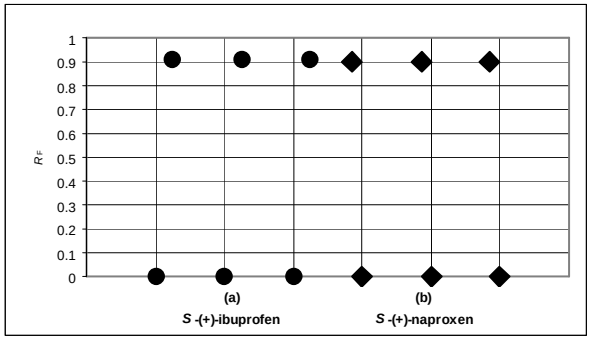

Figure 5. **Schematic presentation of the direction-wise deviation from verticality of the migration tracks with (a) *S*-(+)-ibuprofen and (b) *S*-(+)-naproxen. Stationary phase: silica gel 60 F_{254} (precoated plates, Merck, cat. # 1.05715). Mobile phase: ACN - MeOH - H_2O, (a) 5:1:1 (*v/v*); (b) 5:1:1.5 (*v/v*), acidified with glacial acetic acid in each case [6].**

The results obtained for the optically pure enantiomers *S*-(+)-ibuprofen and *S*-(+)-naproxen [6] were in general agreement with those presented for the racemic mixtures of these two profen drugs [1], *S*-(+)-ibuprofen confirmed the right-handed deviation of its migration track from verticality and the deviation of *S*-(+)-naproxen as left-handed. This consistency occurring both on the L-arginine impregnated and the non-impregnated silica gel layers yet again suggests that the responsible factor could be microcrystalline chirality of silica gel, normally regarded as an amorphous solid.

Circular dichroism (CD) study of silica gel used for coating thin-layer chromatographic plates

In order to verify our hypothesis the spectra of circular dichroism (CD) were recorded for the samples (courtesy Merck, Darmstadt, Germany) of the binder-free silica gel used for the coating of the commercial chromatographic plates, and separately for the binder. As expected, the binder did not show any effect pointing out to its possible chirality and in the case of the investigated silica gel sample devoid of the binder, the two well pronounced Cotton bands – one positive and one negative – were indicated in the spectrum (as shown in Figure 6).

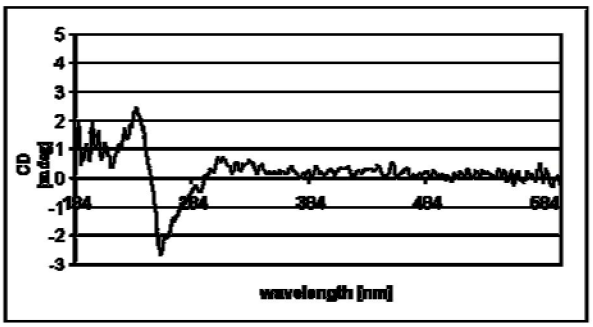

Figure 6. **The CD spectrum of the binder-free silica gel for TLC sample recorded in the nujol suspension [6,7].**

Silicon dioxide does not absorb in the UV range and hence it cannot furnish the Cotton effect either. Moreover, it is not so easy to expect that precipitation of silica gel is stereospecific. It seems quite probable though that the microcrystalline silica gel matter is constituted of the right-handed and the left-handed particles, with quantitative predominance of one enantiomeric species over its mirror image. This thermodynamically possible predominance of one asymmetric form of silica gel over its antipode seems the cause of deviation of the migration track with the investigated chiral analytes.

How then to explain the appearance of two significant Cotton bands in the investigated silica gel

samples? There exists probability of silica gel being contaminated with trace amounts of the adsorbed non-chiral organic compounds having chromophoric functional groups as carbonyl groups drawn from ambient air. Adsorbed as they are on an asymmetric substrate, their electron orbitals may be affected by its asymmetry. Consequently, these adsorbed molecules behave as asymmetric species themselves and in the case of quantitative predominance of one type of the asymmetric microcrystalline variety, the Cotton bands can appear in the CD spectrum (Figure 6).

Conclusions

From the results of our chromatographic studies on the enantioseparation of the selected 2-arylpropionic acids (i.e., ibuprofen, naproxen, 2-phenylpropionic acid, and ketoprofen) by chiral TLC it may be concluded that the two-dimensional (2D) separation of a pair of antimers is possible in the one-dimensional (1D) planar chromatographic mode. Vertical enantioseparation is due to molecular chirality of the chiral selector (in this case it was L-arginine) adsorbed on the silica gel layer. Horizontal enantioseparation is due to microcrystalline chirality of silica gel, explaining horizontal deviation of the chiral analytes' migration tracks from verticality and unexpectedly facilitating enantioseparation of the investigated pairs of antimers. Discovery of microcrystalline chirality of silica gel used as liquid chromatography stationary phase extends our understanding of the role played by this adsorbent in the separation of chirals by planar chromatography. The analogous effect is not possible in the column liquid chromatography because in that case one encounters the one-dimensional effective diffusion only (whereas in planar chromatography the effective diffusion is two-dimensional).

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