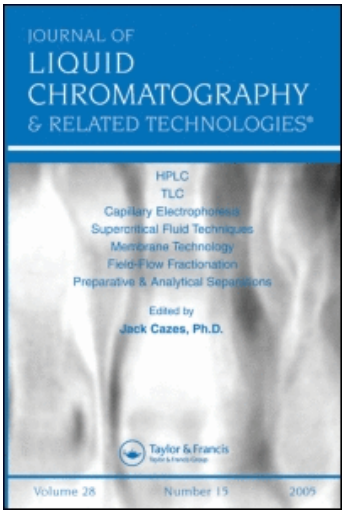

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Enantioseparation and Oscillatory Transenantiomerization of *S*,*R*-(±)-Ketoprofen, as Investigated by Means of Thin Layer Chromatography with Densitometric Detection

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Abstract: In the course of our earlier and rather extensive investigations, we discovered a striking ability of the two non-steroidal anti-inflammatory drugs (NSAIDs) from the group of 2-arylpropionic acids (2-APAs), i.e., ibuprofen and naproxen, and also of 2-phenylpropionic acid (which is not a drug) to, in vitro, undergo a repeated structural conversion from one chiral configuration to the opposite one. We labelled the discovered phenomenon 'oscillatory transenantiomerization' and formulated a hypothesis that all the 2-APAs can behave in a similar manner when dissolved in certain low-molecular-weight (aqueous or non-aqueous) solvents. Furthermore, we assumed that structural differences among the various 2-APAs can result in differentiated dynamics of oscillatory transenantiomerization, which is omnipresent with this class of compounds. In this paper,we present the results of an analogous study, this time devoted to still another 2-APA, *S*,*R*-(±)-ketoprofen. The chemical structure of ketoprofen is, in a sense, unique as a keto-enol tautomer (a transition form between *S*-(+)-ketoprofen and its *R*-(-) antimer) that contains a long assembly of eight conjugated π-electron pairs. Such a peculiar electron structure seems to particularly well stabilize the keto-enol tautomer derived from ketoprofen and, consequently, to efficiently promote oscillatory transenantiomerization of this compound. The results of our investigations fully confirmed this intuition, and in our thin-layer chromatographic experiment, we managed to demonstrate the ability of *S*,*R*-(±)-ketoprofen to undergo oscillatory transenantiomerization, in a manner similar to that of the other already examined 2-APAs. Moreover, we performed a successful densitometric scrutiny of the chromatograms of *S*,*R*-(±)-ketoprofen, proved to have separated three different

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species, to run, in situ, their respective UV spectra (all three of them practically identical), and to speculate about their chemical nature. In the HPLC experiment we produced evidence of a considerable viscosity of *S*,*R*-(±)-ketoprofen when chromatographed with pure acetonitrile and with the acetonitrile + water mixtures (containing very low amounts of water). This viscosity seems to be the main factor that contributes to the oscillatory (i.e., repeated) nature of the observed transenantiomerization.

Keywords: *S*,*R*-(±)-Ketoprofen, TLC, Chirality, Oscillatory transenantiomerization, UV absorption spectra, Viscosity

INTRODUCTION

In our earlier articles[1–3] we presented the results of our discovery that the selected 2-arylpropionic acids (2-APAs), when dissolved in low-molecular-weight aqueous and non-aqueous solvents, can undergo a repeated conversion of their chiral configuration, labelled by us 'oscillatory transenantiomerization'. Two facts were striking about these results: (i) that 2-APAs can undergo chiral conversion not only in vivo (which is a widely described phenomenon in biochemical and pharmaceutical papers), but also in vitro; and (ii) that this conversion is of an oscillatory nature. Schematically, such conversion is shown in the following scheme:

$$\begin{aligned} &S\text{-}(+)\text{-2-arylpropionic acid} \longleftrightarrow \text{keto-enol tautomer} \\ &\longleftrightarrow R\text{-}(-)\text{-2-arylpropionic acid} \end{aligned} \tag{1}$$

Obviously, oscillatory transenantiomerization of an optically pure enantiomer is a driving force pushing the whole system toward racemization, as schematically shown in Fig. 1. Upon obtaining these initial results, two important questions arose, namely: (i) one referring to the molecular mechanism of transenantiomerization, and (ii) the second referring to the factor generating the oscillatory (i.e., repeated) mechanism thereof.

From the theory of molecular mechanisms in organic reactions, it is well known that a basic environment can catalyze formation of the keto-enol tautomers, whereas an acidic one usually tends to hamper this process. We performed an experiment in which we stored the selected optically pure 2-APAs both in a basic and an acidic environment. In the basic environment. the racemization process went quite rapidly, while in the acidic environment, it did not take place at all.[4] Thus, we made sure that the mechanism responsible for conversion of the steric configuration of the investigated 2-APAs is via the keto-enol tautomerism.

Among the known factors responsible for generating oscillatory chemical reactions, the effect of gelation (i.e., of an increased viscosity of a given solution, as compared with that of the pure solvent) is usually placed first. Once the rate of formation of an intermediate product in a given multi-step reaction surpasses that of its diffusion in a given system, the

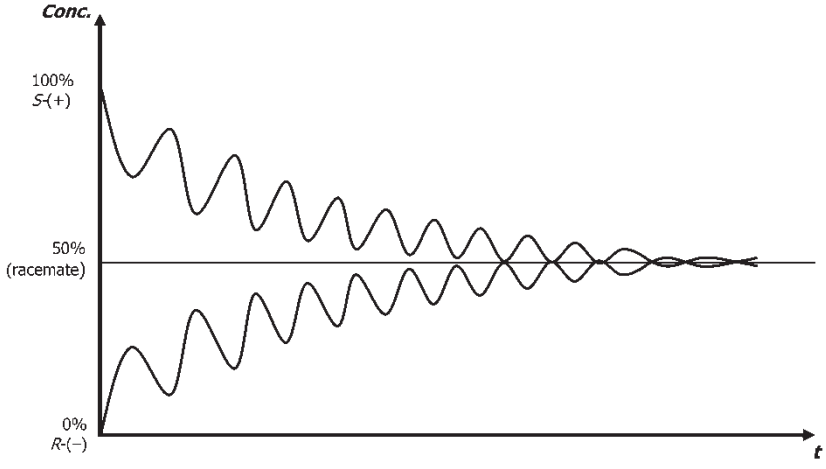

Figure 1. Schematic presentation of the oscillatory transenantiomerization of a given *S*-(+)-2-APA to the *R*-(-) antipode form; the oscillatory plots mirror fluctuations in the decrease of concentration of the *S*-(+) species and the corresponding fluctuations in the increase of concentration of the *R*-(-)-species.

mandatory precondition of an oscillatory reaction is fulfilled. In a study reported earlier,[5] we managed to demonstrate the gelating ability of the selected 2-APAs.

Even if we anticipate that oscillatory transenantiomerization is a common phenomenon among all 2-APA solutions, we are well aware of the fact that the dynamics of this process can considerably differ from one compound to another, depending on the respective molecular structures. For this reason, we decided to focus our attention in this study on ketoprofen, a very popular NSAID having the molecular structure shown in Figure 2.

The chemical structure of ketoprofen is, in a sense, unique, as its keto-enol tautomer (an anticipated transition form between *S*-(+)-ketoprofen and its *R*-(-) antimer) contains a long assembly of eight conjugated π-electron pairs (see Figure 3). Such a peculiar electron structure seems to considerably stabilize the keto-enol tautomer derived from ketoprofen and,

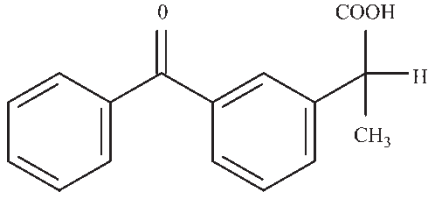

Figure 2. Chemical structure of the ketoprofen molecule.

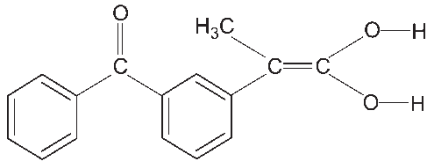

Figure 3. Chemical structure of the keto-enol tautomer derived from ketoprofen.

consequently, to efficiently promote the oscillatory transenantiomerization of this compound.

In the following sections of this paper, we present the results of our study on the ability of *S*,*R*-(±)-ketoprofen (the commercially available racemate) to undergo oscillatory transenantiomerization. The results obtained clearly point out that, in spite of the racemic form, ketoprofen solution remains in the state of dynamic equilibrium with continuously changing quantitative proportions between the two antimers of this compound.

EXPERIMENTAL

S,*R*-(±)-Ketoprofen

In our study, we used *S*,*R*-(±)-ketoprofen, manufactured by Sigma-Aldrich (St Louis, MO, USA; cat. # K1751-5G). In the thin-layer chromatographic experiments we used a solution of *S*,*R*-(±)-ketoprofen in 70% ethanol, its concentration equal to 0.1 mg mL^{-1} (i.e., ca. 3×10^{-4} mol L^{-1}). In the high performance liquid chromatographic experiments, we used a solution of *S*,*R*-(±)-ketoprofen in acetonitrile (ACN), its concentration equal to 1 mg mL^{-1} (i.e., ca. 3×10^{-3} mol L^{-1}). In the polarimetric experiment we used a solution of *S*,*R*-(±)-ketoprofen in 70% ethanol, its concentration being equal to 0.05 g mL^{-1}.

Polarimetric Measurements of the Specific Rotation $[\alpha]_D$

Measurements of the specific rotation ($[\alpha]_D$) of the *S*,*R*-(±)-ketoprofen solution in 70% ethanol were carried out at 22 ± 2°C for 180 min (in 15-min intervals) with use of a Polamat A model polarimeter (manufactured by Carl Zeiss, Jena, Germany). The optical path length of the employed measuring cell was exactly 10 cm (=1 dm), and its volume was ca. 1 mL. Specific rotation $[\alpha]_D$ was calculated, using the following standard equation:

$$[\alpha]_D = 100\alpha/cd \tag{2}$$

where α is the measured rotation (in the angle degrees); D is the employed wavelength $\lambda = 589$ nm, which corresponds with the sodium D line; c is the concentration of a given compound in g 100 mL^{-1} solution; and d is the measured sample thickness in dm.

From the literature[6] it is known that the specific rotation of *S*-(+)-ketoprofen equals $+57.1^{\circ}$, and that of the *R*-(-) species equals -57.4°.

Commercial TLC Silica Gel Layers and Their Pretreatment

TLC was performed on commercial glass plates (20 cm × 20 cm) precoated with 0.25 mm layers of silica gel 60 F_{254} (Merck KGaA, Darmstadt, Germany; cat. # 1.05715). Before use, the plates were carefully washed by predevelopment with methanol-water, 9:1 (v/v), and then dried at ambient temperature for 3 h.

The washed and dried plates were then impregnated with a 3×10^{-2} mol L^{-1} solution of L-arginine in methanol by conventional dipping for 2 s. The concentration of the impregnating solution was calculated as that depositing 0.5 g of L-arginine per 50 g of the dry silica gel adsorbent layer. Finally, the washed and impregnated adsorbent layers were ready for chromatography.

Mobile Phase and Development of Thin Layer Chromatograms

Development of the *S*,*R*-(±)-ketoprofen samples was carried out at 22 ± 2°C for a distance of 15 cm using the binary mobile phase composed of ACN and water (H_2O) in the quantitative proportion of 5:1 (v/v), and it contained an extra addition of several drops of glacial acetic acid to fix the pH < 4.8. It was carried out in the one-dimensional and the two-dimensional development mode, in both directions of the development using the same composition of mobile phase. The anticipated mechanism of retention with each of the two ketoprofen antipodes is given by Equation (1).

Sample application to the plates was with the use of an autosampler (the AS 30 model autosampler manufactured by Desaga, Heidelberg, Germany). The *S*,*R*-(±)-ketoprofen solutions were applied to the plate 1.5 cm above the lower edge of the plate in aliquots of 5 mL $spot^{-1}$ (the one-dimensional development mode). Nine samples in the equal distance of 2 cm from one another were applied per one plate, and then the chromatogram was developed in the one-dimensional mode. After development, the plates were dried at ambient temperature for 3 h, and the surface of each plate was densitometrically scanned in 1-mm intervals in the direction of development. Each experiment was carried out on at least three plates. Thus the numerical results given in this paper originate from at least 27 individual development lanes, as

it was our aim to reliably assess the maximum deviation from verticality of the migration tracks with the separated pair of chiral antimers.

As mentioned earlier, development of the chromatograms was also carried out in the two-dimensional mode. Plates with a single 10-μL spot of the *S*,*R*-(±)-ketoprofen solution at a corner were developed to a distance of 15 cm in the first direction. Then, the plates were first dried in an ambient atmosphere for 3 h and then developed to a distance of 15 cm in the second direction (perpendicular to the first one). After development the plates were again dried in an ambient atmosphere, and tracks 30 mm wide, in the first and the second direction of development, were scanned densitometrically in 1.5-mm intervals. This experiment was repeated three times.

Densitometric Assessment of the Thin Layer Chromatograms

Densitograms were acquired with a Desaga (Heidelberg, Germany) Model CD 60 densitometer equipped with Windows-compatible ProQuant software. Concentration profiles of the development lanes for *S*,*R*-(±)-ketoprofen were recorded in ultraviolet (UV) light from the deuterium lamp (in the reflectance mode) at 252 nm (this was the approximate maximum of the UV absorption spectrum of ketoprofen, as densitometrically recorded in situ from the ketoprofen chromatograms). The dimensions of the rectangular light beam were 2.0 mm × 0.1 mm. The maxima of the concentration profiles were used for calculation of R_F values.

High Performance Column Liquid Chromatography (HPLC)

The HPLC investigations were carried out with aid of the P580A LPG model liquid chromatograph, equipped with the Gina 50 model autosampler; UVD340V DAD model detector (Gynkotek/Dionex, Germering, Germany); and RP-18 column (LichroCART® cartrige with the LiChrospher® 100, 5 μm; 250 mm, 4 mm i.d., cat.# 1.50983.0001, Merck KGaA, Darmstadt, Germany).

Samples of *S*,*R*-(±)-ketoprofen in 70% ethanol were injected by the autosampler from the same stock solution in the 10-mL aliquots, in 30 min intervals for up to 5 h. The mobile phase flow rate was with all mobile phases equal to 0.6 mL min^{-1} The investigated HPLC systems can be divided into the two categories:

i. ACN as the solvent of the *S*,*R*-(±)-ketoprofen sample and ACN as the monocomponent mobile phase (see Figure 4); and
ii. ACN as the solvent of the *S*,*R*-(±)-ketoprofen sample and the ACN + H_2O binary mobile phases. The applied volume percentages of

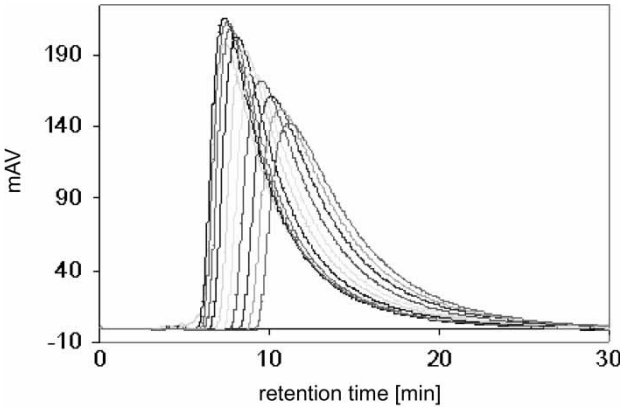

Figure 4. Tailing concentration profiles of *S*,*R*-(±)-ketoprofen dissolved in ACN and for 5 h developed with ACN by means of HPLC in 0.5 h time intervals. The longer the storage period of the *S*,*R*-(±)-ketoprofen solution in ACN, the longer is the retention time of the concentration profile maximum, and the greater is the tailing.

the added water were the following: 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, and 10.0% (see Figures 5 and 6).

These experiments were meant to clearly demonstrate an enhanced viscosity of *S*,*R*-(±)-ketoprofen, when dissolved in an organic solvent (e.g., ACN), or in an organic-aqueous solvent with a limited amount of water in it.

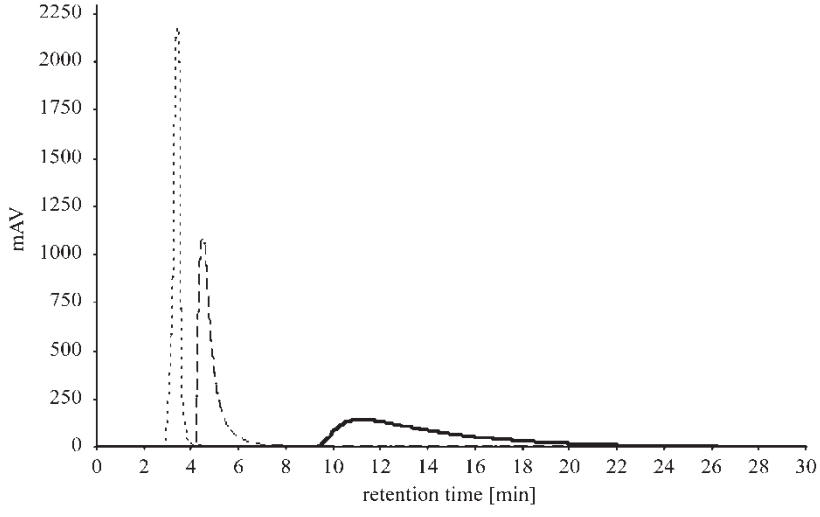

Figure 5. High performance liquid chromatograms of *S*,*R*-(±)-ketoprofen—a comparison. The mobile phases used: (i)___ pure ACN; (ii) ----- ACN + 1% H_2O; and (iii) ····· ACN + 10% H_2O.

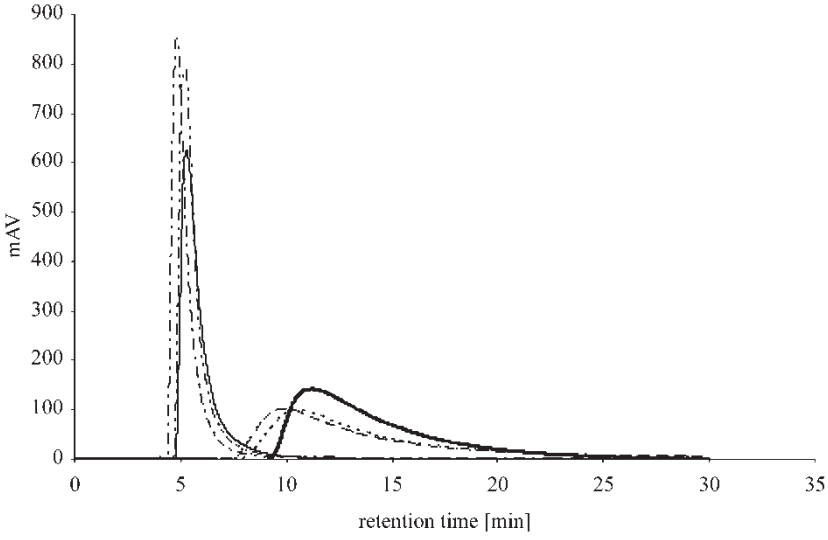

Figure 6. High performance liquid chromatograms of *S,R*-(±)-ketoprofen—a comparison. The mobile phases used: (i) __ pure ACN; (ii) ---- ACN + 0.1% H_2O; (iii) ····· ACN + 0.2% H_2O; (iv) _._._ ACN + 0.3% H_2O; (v) _.._.._ ACN + 0.4% H_2O; (vi) __ ACN + 0.5% H_2O.

RESULTS AND DISCUSSION

Polarimetry

It was not expected to obtain any spectacular polarimetric results in the case of the racemic mixture. Nevertheless, certain effects of the changing specific rotation were observed in the course of our experiment. The first time, immediately after dissolution of *S,R*-(±)-ketoprofen in 70% ethanol, the measured specific rotation was equal to $+4^\circ$, and within a short period of approximately 15 minutes it dropped down to 0°, and then remained unchanged through the rest of our experiment. The second time, the *S,R*-(±)-ketoprofen solution in 70% ethanol showed a stable specific rotation value equal to -2°. As demonstrated in our earlier paper,[4] oscillatory transenantiomerization of 2-APAs is even better observable with the aid of TLC than polarimetry, and therefore we did not limit ourselves to polarimetric measurements only but utilized TLC as well.

Thin Layer Chromatography

From our thin layer chromatographic experiment, it is clear that the oscillatory transenantiomerization in the case of *S,R*-(±)-ketoprofen was relatively rapid.

As a matter of fact, its rate was comparable with that of automatic application of the samples of the investigated 2-APA solution to the chromatographic plate. As a result, positions of the nine *S*,*R*-(±)-ketoprofen bands on the same chromatogram give a repeated meandering pattern, as schematically shown in Figure 7. The meandering pattern of the chromatographic spots' positions corresponds well with these fragments of the two plots shown in Figure 1, which refer to the racemic mixture already obtained.

An even more persuasive proof of the oscillatory transenantiomerization occurring with ketoprofen is given in Figure 8. This plot clearly shows this striking phenomenon as a function of time, although the data points were not measured in even time intervals. However, each data point was calculated as an average of the nine R_F values originating from the same chromatographic plate, and for this reason the data presented are substantially more reliable than those originating from the individual measurements. The observed amplitude of the changing R_F values (i.e., ΔR_F) is in this case considerable and equal to ca. 0.20, which entirely excludes the effect of the measurement error (usually not higher than ± 0.02 of the dimensionless R_F units).

From our earlier investigations,[7,8] it is obvious that 2-APAs, when developed either on the impregnated silica gel layer or on the plain one, show a considerable deviation of their migration tracks from verticality (with the chromatograms developed in the normal–Stahl–tank, in the ascending mode). This phenomenon is caused both by the chirality of the impregnating agent and of the silica gel,[8] and it usually results in an enhanced separation of the two antimers in the horizontal direction (i.e., perpendicular to the analytes' migration route). In our previous studies,[7,8]

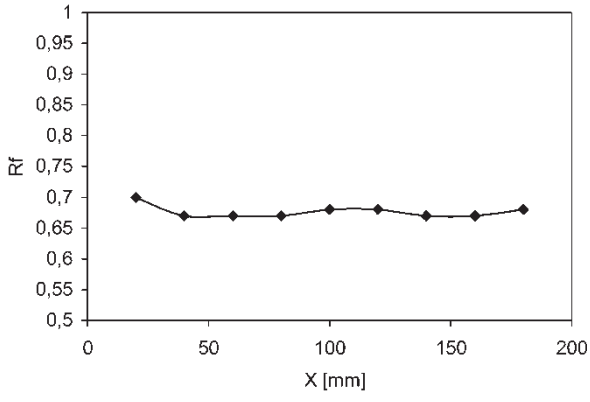

Figure 7. Oscillation of the R_F value as a function of the application position of the *S*,*R*-(±)-ketoprofen sample on the adsorbent layer [x: the distance of the sample application position from the left-hand edge of the chromatographic plate (mm)].

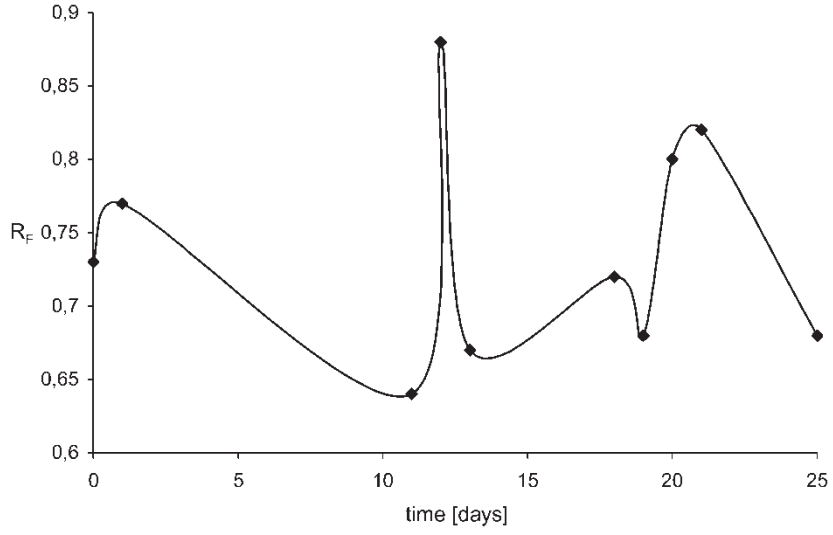

Figure 8. Oscillation of the R_F value as a function of the storage time (days) of the *S*,*R*-(±)-ketoprofen sample as the 70% ethanol solution.

we observed cases when the migration route of one enantiomer deviated from verticality to the right and that of its antipode to the left.

Chiral separations are recognized as a difficult analytical task, and even more so when the antimers–like in the case of 2-APAs–are monocarboxylic acids, apt to form cyclic dimers kept together by hydrogen bonds. These hydrogen bonds apparently obstruct enantioseparation, and this negative effect is particularly annoying and acute when the separated sample is a racemic mixture (like the case with *S*,*R*-(±)-ketoprofen), i.e., it contains equal proportions of the two antipodes. The separation task seems somewhat easier when there is a sufficient enantiomeric excess of one steric form (i.e., in the case of the scalemic mixtures). However, in the case of *S*,*R*-(±)-ketoprofen, we managed to obtain chiral separations even when using the one-dimensional developing technique. The picture of a successful chromatographic separation (schematically shown in Figures 9 and 10) is rather striking.

Namely, we observed three instead of the two peaks originating from the same sample spotting, all three of them well separated and showing the same right-handed deviation from verticality of their respective migration tracks. The middle peak (in terms of the R_F value) proved predominant in quantitative terms, as demonstrated by means of two-dimensional chromatography and the subsequent scanning of the chromatogram. In order to check the chemical nature of these three peaks, the in situ UV absorption spectra were recorded for each individual peak and, to our surprise, these spectra were practically identical, both in terms of the shapes and positions of their respective maxima, and differed only in terms of the peak intensity (see Figure 11).

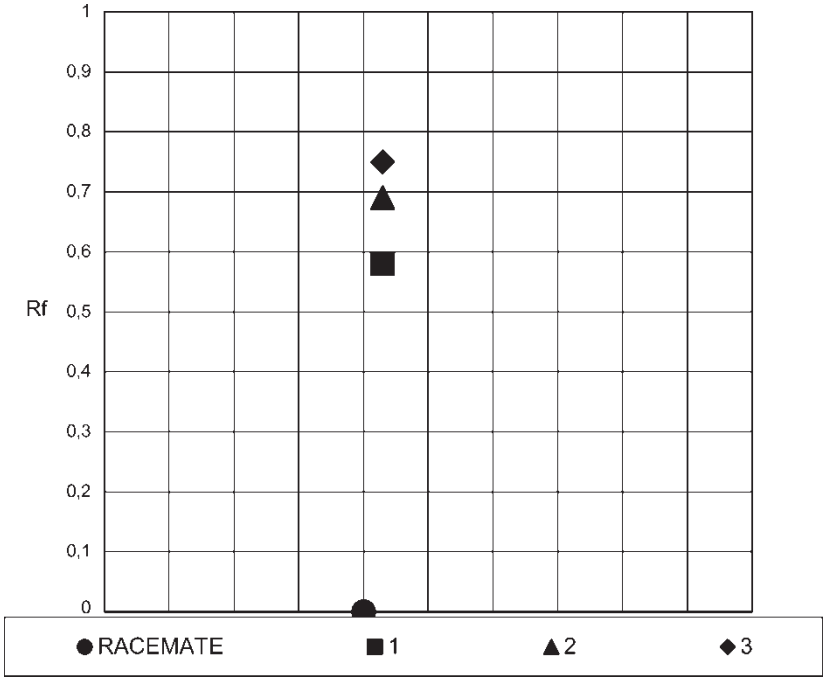

Figure 9. Schematic presentation of the right-handed deviation from verticality of the migration tracks with the three chromatographic peaks derived from the same *S*,*R*-(±)-ketoprofen sample. The respective R_F values were (in the increasing order): 0.58 ± 0.02, 0.69 ± 0.02, and 0.75 ± 0.02. Stationary phase: Merck silica gel 60 F_{254} precoated plates, impregnated with L-arginine. Mobile phase: ACN - H_2O (5:1, v/v).

The R_F values for these three separated peaks were (in an increasing order): 0.58, 0.69, and 0.75. In the same order their deviation from verticality was equal to approximately 2, 3, and 4 mm. It is very difficult to judge the chemical nature of these three well separated peaks, and we cannot think at the moment of any measuring technique that might provide a definite answer to this question. However, two optional explanations seem probable, and perhaps one of them is correct.

Option 1. It seems possible that–in the case of the non-identical amounts of the *S*-(+) and the *R*-(-) species in a given mixture (due to the progress of the oscillatory transenantiomerization)–the following three fractions of the cyclic dimers could have been separated: *RR* (the lowest R_F value), *SR* (the medium R_F value, the predominant peak), and *SS* (the highest R_F value).

Option 2. It also seems likely that the following three fractions could have been separated: keto-enol tautomer derived from ketoprofen (the lowest R_F value), *SR* (the medium R_F value, the predominant peak), and *SS* (the

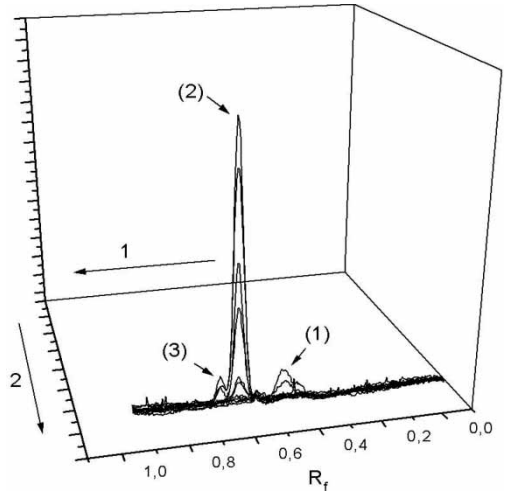

Figure 10. Three-dimensional presentation of the *S,R*-(±)-ketoprofen chromatogram with two development directions, 1 and 2, indicated. Densitometric scanning (at parallel 1.5-mm intervals) of the 30-mm wide track perpendicular to the second direction of the development was performed to better illustrate the separation performance and the skewed arrangement of the three separated species, indicated by arrows.

highest R_F value). Option no. 2 seems slightly less possible due to the fact that one should expect the identical UV absorption spectra of the two antimers of ketoprofen, yet a perceptibly shifted one in the case of the respective keto-enol tautomer. On the other hand, the in situ measured UV spectra of the separated species automatically mirror the fact that these compounds are adsorbed on the silica gel surface, thus their respective molecular orbitals are affected by the adsorption (as compared to the analogous UV spectra run in the solution).

HPLC

When we encounter any given oscillatory chemical process (these processes are not very popular, although not an absolute rarity either), our immediate interest focuses on the nature of the mechanism powerful enough to generate this exceptional phenomenon. As has been mentioned before, the most common source of oscillations is viscosity of the reaction medium, due to which the rate of diffusion of an intermediate reaction product (in our case the keto-enol tautomer) is lower than the rate of its cumulation. This kind of relation between the two rates (of diffusion and cumulation) can sometimes happen with certain multi-step reactions carried out in relatively viscous media.

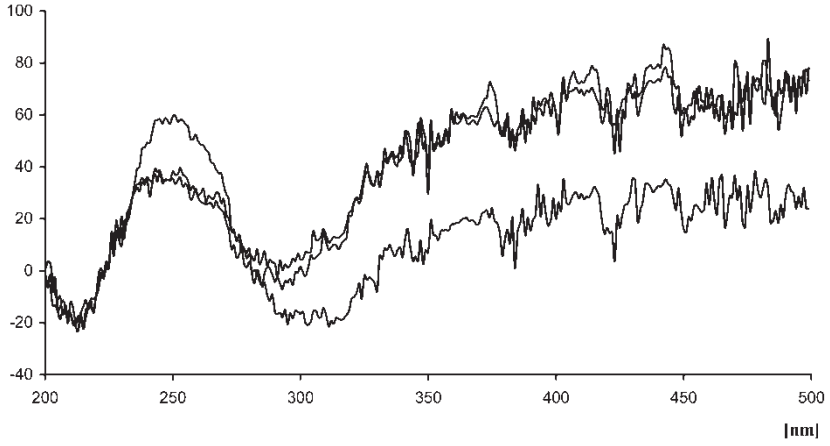

Figure 11. The UV spectra (recorded in situ on the chromatographic plate) of the three chromatographic peaks derived from the same *S*,*R*-(±)-ketoprofen sample, all showing the right-handed deviation from verticality of their respective migration tracks (see Figures 8 and 9).

In our earlier publication[5] we pointed out to the gelating ability of 2-APAs, which is most obviously responsible for the oscillatory transenantiomerization of these chiral compounds. As a result, from the low-concentrated solutions of the 2-APAs we do not obtain the proper physical gels (i.e., highly elastic solids or quasi-solids), but solutions with their viscosity markedly higher than that of the pure solvents. In the same paper[5] we proposed HPLC as a technique of choice to investigate the gelating ability of 2-APAs. If we dissolve a 2-APA in a low-molecular-weight solvent (e.g., 70% ethanol, ACN, THF, dichloromethane, etc.), inject this solution on the chromatographic column, and then use a low-molecular-weight solvent as the mobile phase, we are going to obtain a severely tailing concentration profile of an analyte as evidence of the enhanced viscosity of the injected solution. Looking at the chromatogram, one gets an impression that the "sticky" solution of the analyte considerably clogs the pores of stationary phase, perceptibly obstructing the eluent flow through the column and resulting in this enormous tailing.

In a Ph.D. thesis entirely devoted to physical gelation of low-molecular-weight organic solvents[9] with low-molecular-weight organic gelators, a statement can be found that in the case of chiral gelators only optically pure species can play this particular role, and not racemic mixtures. From our earlier HPLC investigations, a similar observation could be derived[10] (i.e., the concentration profile of *S*,*R*-(±)-2-phenylpropionic acid was not tailing, whereas the concentration profiles of *S*-(+)-ibuprofen and *S*-(+)-naproxen were heavily tailing). However, the polarimetric and the thin-layer chromatographic measurements of the *S*,*R*-(±)-2-phenylpropionic acid racemic

mixture clearly showed its continuous oscillations occuring within the relatively flat range of the two plots shown in Figure 1 and ascribed to the presence of the racemate in a dynamic equilibrium (and even more vigorous oscillations with the two optically pure species, i.e., with *S*-(+)-ibuprofen and *S*-(+)-naproxen). Two important conclusions could be drawn from our previous experiments, namely (i) that HPLC cannot be a fully reliable assessment technique, most probably showing the strongly pronounced gelating effects only and missing those that are less strong; and (ii) that the statement contained in the Ph.D. thesis[9] as to the inability of the racemates to act as gelators may not always prove true.

The two conclusions above proved correct in view of the results obtained in our present study. Namely, from the strongly tailing shape of the concentration profiles of *S*,*R*-(±)-ketoprofen (see Figure 4) it is clear that this racemate has a pronounced gelating property. Additionally, the longer the storage period of the *S*,*R*-(±)-ketoprofen solution in ACN, the longer was the retention time of the profile's maximum, and the more it tailed. In the other words, the longer the storage period with the investigated solution, the more pronounced became the effect of its gelation.

From the *S*,*R*-(±)-ketoprofen peak profiles shown in Figures 5 and 6, it is evident that the stepwise addition of the increased amounts of water to the *S*,*R*-(±)-ketoprofen—ACN system results in gradual destruction of the gelated mixture, as confirmed by the increasingly symmetric concentration profiles of the investigated ketoprofen. Once again, the results of the presented HPLC experiment confirmed our earlier finding as to the gelating effect of the 2-APAs as an indispensable precondition of their oscillatory transenantiomerization.

CONCLUSIONS

The results presented in this paper fully confirm our earlier findings with respect to the oscillatory transenantiomerization of the chiral 2-APAs. It was demonstrated that—similar to *S*-(+)-ibuprofen, *S*-(+)-naproxen, and *S*,*R*-(±)-2-phenylpropionic acid—*S*,*R*-(±)-ketoprofen also undergoes the oscillatory transenantiomerization, in an oscillatory manner repeatedly abandoning its racemic (i.e., 1:1) molar compostion. Simultaneously, the specificity of the behavior of each individual 2-APA, inherent of its unique molecular structure, was confirmed through our current results.

Among the most important findings reported in this paper was that the racemic mixture (in this case *S*,*R*-(±)-ketoprofen) can prove to be a low-molecular-weight gelating agent when dissolved in low-molecular-weight solvents. Earlier, it has been quite firmly believed that the racemates can by no means act as gelators, the property demonstrated by the optically pure enantiomers only.

Another equally important finding is the successful separation of the racemic *S*,*R*-(±)-ketoprofen sample into the three species, all of them showing the same UV absorption spectrum, registered in situ with aid of densitometer. It seems highly probable that the separated species are the following H-bonded ketoprofen dimers: *SR* (the quantitatively predominant peak showing the medium R_F value), *SS* (the peak of relatively low intensity showing the highest R_F value), and either *RR* or the keto-enol tautomer (the peak of relatively low intensity showing the lowest R_F value).

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REFERENCES

1. Sajewicz, M.; Piętka, R.; Pieniak, A.; Kowalska, T. Application of thin-layer chromatography (TLC) to investigating oscillatory instability of the selected profen enantiomers. Acta Chromatogr. **2005**, *15*, 131–149.
2. Sajewicz, M.; Piętka, R.; Pieniak, A.; Kowalska, T. Application of thin-layer chromatography (TLC) to investigate oscillatory instability of the selected profen enantiomers in dichloromethane. J. Chromatogr. Sci. **2005**, *43*, 542–548.
3. Sajewicz, M.; Piętka, R.; Pieniak, A.; Kowalska, T. Application of thin-layer chromatography to the investigation of oscillatory instability of selected profen enantiomers in physiological salt. J. Liq. Chromatogr. Rel. Technol. **2006**, *29*, 2059–2069.
4. Sajewicz, M.; Piętka, R.; Drabik, G.; Kowalska, T. On the mechanism of oscillatory changes of the retardation factor (R_F) and the specific rotation $[\alpha]_D$ with selected solutions of S-(+)-naproxen. J. Liq. Chromatogr. Rel. Technol. **2006**, *29*, 2071–2082.
5. Sajewicz, M.; Piętka, R.; Kuś, P.; Kowalska, T. On the gelation of profens as a property causing their oscillatory transenantiomerization. Acta Chromatogr. **2006**, *16*, 181–191.
6. Liversidge, G.G.; Ketoprofen. In *Analytical Profiles of Drug Substances*; Florey, K., Ed.; Academic Press: London, 1981; 443–471.
7. Sajewicz, M.; Piętka, R.; Drabik, G.; Namysło, E.; Kowalska, T. On the stereochemically peculiar two-dimensional separation of 2-arylpropionic acids by chiral TLC. J. Planar Chromatogr.-Mod. TLC **2006**, *19*, 273–277.
8. Sajewicz, M.; Hauck, H-E.; Drabik, G.; Namysło, E.; Głód, B.; Kowalska, T. Tracing possible structural asymmetry of silica gel used for precoating thin-layer chromatography plates. J. Planar Chromatogr.-Mod. TLC. **2006**, *19*, 278–281.
9. Schoonbeek, F. Making it all stick together. *The Gelation of Organic Liquids by Small Organic Molecules*; Ph.D. Dissertation, University of Groningen: Groningen, The Netherlands, 2001.

10. Sajewicz, M.; Kowalska, T. On the problems with liquid chromatographic quantification of the chiral 2-arylpropionic acids (2-APAs) by means of the UV-absorption-based detection. Acta Chromatogr. **2006**, *17*, 292–301.

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