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*Review*

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# Oscillatory transenantiomerization of the selected 2-arylpropionic acids (2-APAs) in vitro as a spontaneous phenomenon

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**Abstract**

In this paper, we summarize the results of our earlier investigations on an attempted enantioseparation of the selected 2-arylpropionic acids (2-APAs) by means of the chiral thin layer chromatography (TLC). These results have been originally presented in a series of the research papers published in several chromatography journals. In the current article it was reminded that the prolonged storage of the investigated 2-APAs in the aqueous and the non-aqueous solutions results in an oscillatory change of the respective retardation factor ($R_F$) and the specific rotation ($[\alpha]_D$) values. An assumption is introduced as to the chemical nature of the observed phenomenon. It is assumed that the observed oscillations are due to the repeated structural inversion (in our study labelled as oscillatory transenantiomerization) of one enantiomer to its respective antimer. One attempts to at least roughly explain the molecular mechanism of transenantiomerization either by keto-enol tautomerism, or by formation of an intermediate enolic anion, any of these two reaction mechanisms possible only in the basic environment. Then one reflects on the most probable mechanism responsible for the oscillatory nature of the observed structural inversion. It is concluded that the oscillations could be due to an enhanced viscosity of the investigated 2-APA solutions (as compared with those of the respective pure solvents) and/or due to the molecular self-organization within these solutions, resulting in anisotropic properties thereof. Finally, it is concluded that an ultimate explanation of the observed oscillatory transenantiomerization of the selected 2-APAs could probably be offered by the Brusselator-type kinetic model implemented with the diffusion term. In the last section of this paper, argumentation is presented strongly in favour of this particular model and against any alternative speculation as to the supramolecular nature of the observed oscillatory phenomena.

## Introduction

The 2-arylpropionic acids (2-APAs), or profens, are an important class of nonsteroidal antiinflammatory drugs (NSAIDs) that have been in clinical use for ca. 40 years now. Widely used members of this drug class include naproxen, ibuprofen, ketoprofen, and flurbiprofen. The most important therapeutic activity of 2-APAs consists in their antiinflammatory and pain relieving action. All 2-APAs contain one asymmetric carbon atom in their molecular structure and hence, they can appear in the two enantiomeric forms, as the S-(+) and the R-(-) species. From the pharmacological investigations it comes out that the S-(+) enantiomers are incomparably more effective than their respective antimers.

In the case of any enantiomeric drug one problem always is of an ultimate importance: Should these drugs be administered to the patients as racemates, or as single and optically pure enantiomers? Since the greatest tragedy of modern pharmacology caused in the late fifties and the early sixties of the past century by Thalidomide advertised as a "miraculous" sedative and administered in almost fifty countries as a racemic mixture to pregnant women, one of the paramount threats of all racemic drugs is a possible

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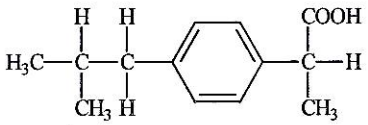
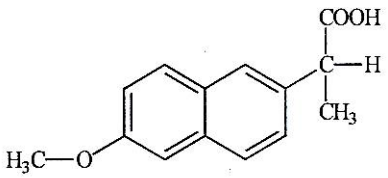
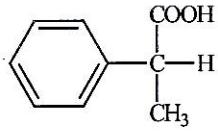
theratogenic activity of the 'ballast' antimer present in the mixture.

Modern tendency to administer enantiomeric drugs in their optically pure form can be put into practice in the two different ways, i.e., either by chromatographic enantioseparation of the racemic mixture and extraction of the curative species, or by asymmetric synthesis resulting in a single enantiomer of choice. Presently, the strategy of enantioseparations is better established, as it seems more rapid, flexible, and cost effective than asymmetric synthesis, although the latter one becomes a dynamically growing field of organic chemistry.

It has been our initial target to re-examine the performance of thin layer chromatography (TLC)–the least frequently utilized chromatographic technique–in the area of enantioseparations on an analytical scale. The first class of analytes we have focused on were 2-APAs, and more specifically ibuprofen, naproxen, and 2-phenylpropionic acid. We started our investigations from repeating and modifying an analytical procedure of enantioseparating the racemic ibuprofen mixture established by Bhushan and Parshad [1], but pretty soon we realized that our analytes of choice undergo a strange and unexplained process, when stored for a longer period of time in the solutions of the different solvents. Basic characteristics of 2-APAs employed in our study are given in Table 1.

**Table 1. Schematic representation of the chemical structures of the three 2-APAs and their specific rotation ($[\alpha]_D$), as taken from literature**

| 2-APA | Chemical structure | $[\alpha]_D$, angle degrees | |
|---|---|---|---|
| | | *S* | *R* |
| Ibuprofen |  | +53.2 [2] | -57.5 [3] |
| Naproxen |  | +64.9 [4]<br>+66.3 [5] | -67.2 [5] |
| 2-Phenylpropionic acid |  | +69.2 [6]<br>+79.2 [3] | -80.0 [3] |

***Investigations on a prolonged storage of the selected 2-APAs in solutions of different solvents***

Optically pure enantiomers are quite expensive and for the sake of economy, we decided to store the 2-APA solutions for a longer period of time, instead of preparing the fresh solutions prior to each thin layer chromatographic experiment. An encouragement to do so came from pharmaceutical literature saying that these particular compounds are practically indestructible, when dissolved in common solvents like water, alcohols, dichloromethane, tetrahydrofuran etc. In this part of our experiment we employed the following three 2-APA samples: S-(+)-ibuprofen and S-(+)-naproxen as the optically pure isomers, and S, R-(±)-2-phenylpropionic acid as a racemate. Very soon we discovered that positions of the investigated 2-APAs on the thin layer chromatograms (expressed in form of the retardation factor, $R_F$ values) change in an oscillatory manner with the time of storage of the respective samples. In Fig. 1, we present this phenomenon in a schematic way.

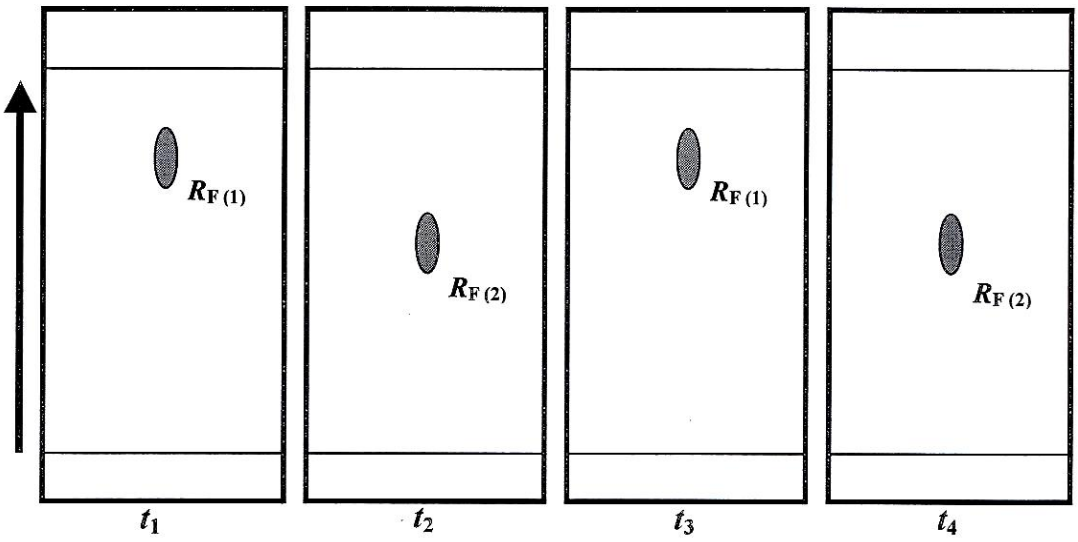
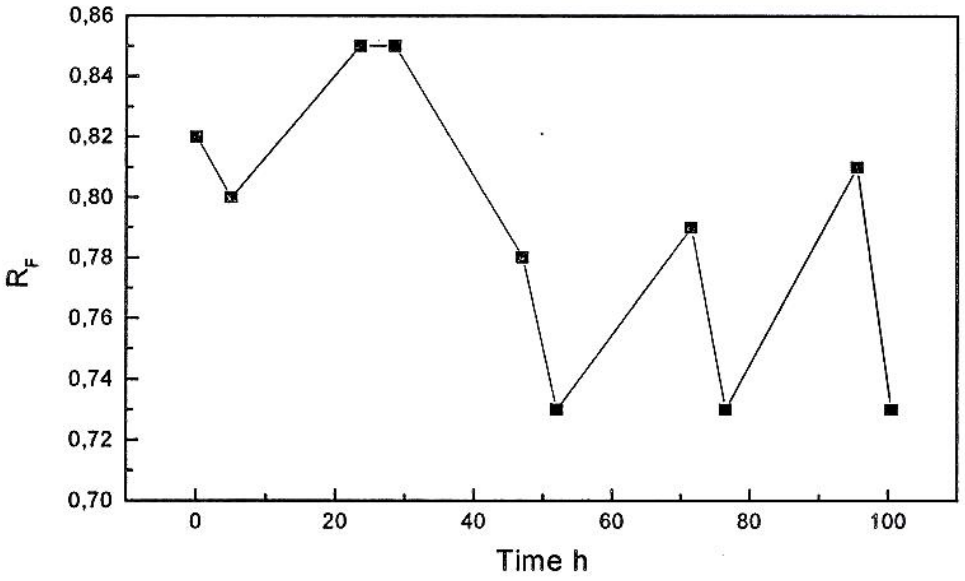


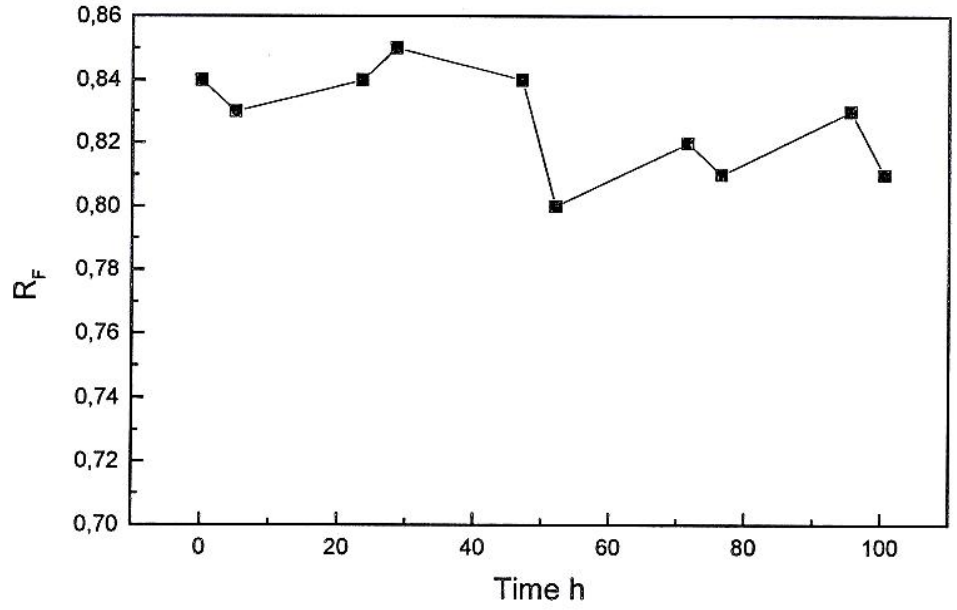

**Figure 1. Schematic presentation of the oscillatory changes of the $R_F$ values (valid for each investigated 2-APA) as a function of storage time in the solutions. Stationary phase: silica gel impregnated with L-arginine; mobile phase: ternary liquid mixture composed of ACN, MeOH, and $H_20$, plus several drops of glacial acetic acid (originally published in [7]).**

The experiments on measuring the retardation factor ($R_F$) values of the three 2-APAs as a function of their storage time were carried out for the low-concentration solutions of these compounds in the following solvents: 70% ethanol, dichloromethane, and physiological salt. The obtained results were abundant and many of them are shown in papers [7-9]. For the sake of illustration, in Fig. 2 we present the example of the oscillating retardation factor ($R_F$) values, taken from paper [8].

Oscillations of the retardation factor ($R_F$) values with the three investigated 2-APAs were accompanied by the strongly pronounced changes of the concentration profiles of these compounds on the chromatograms, as registered by use of densitometric detection. In Fig. 3, we present the sequence of the changing concentration profiles with S, R-(±)-2-phenylpropionic acid dissolved in 70% ethanol and stored at 22±2°C, in the form of the "movie pictures".




(a)




(b)

**Figure 2. Dependence of the retention parameter ($R_F$) on the sample storage time ($R_F$ = f(t)) for the S-(+)-ibuprofen solution in dichloromethane (a) at ambient temperature (22±2°C) and (b) in refrigerator (6±2°C) (originally published in [8]) .**

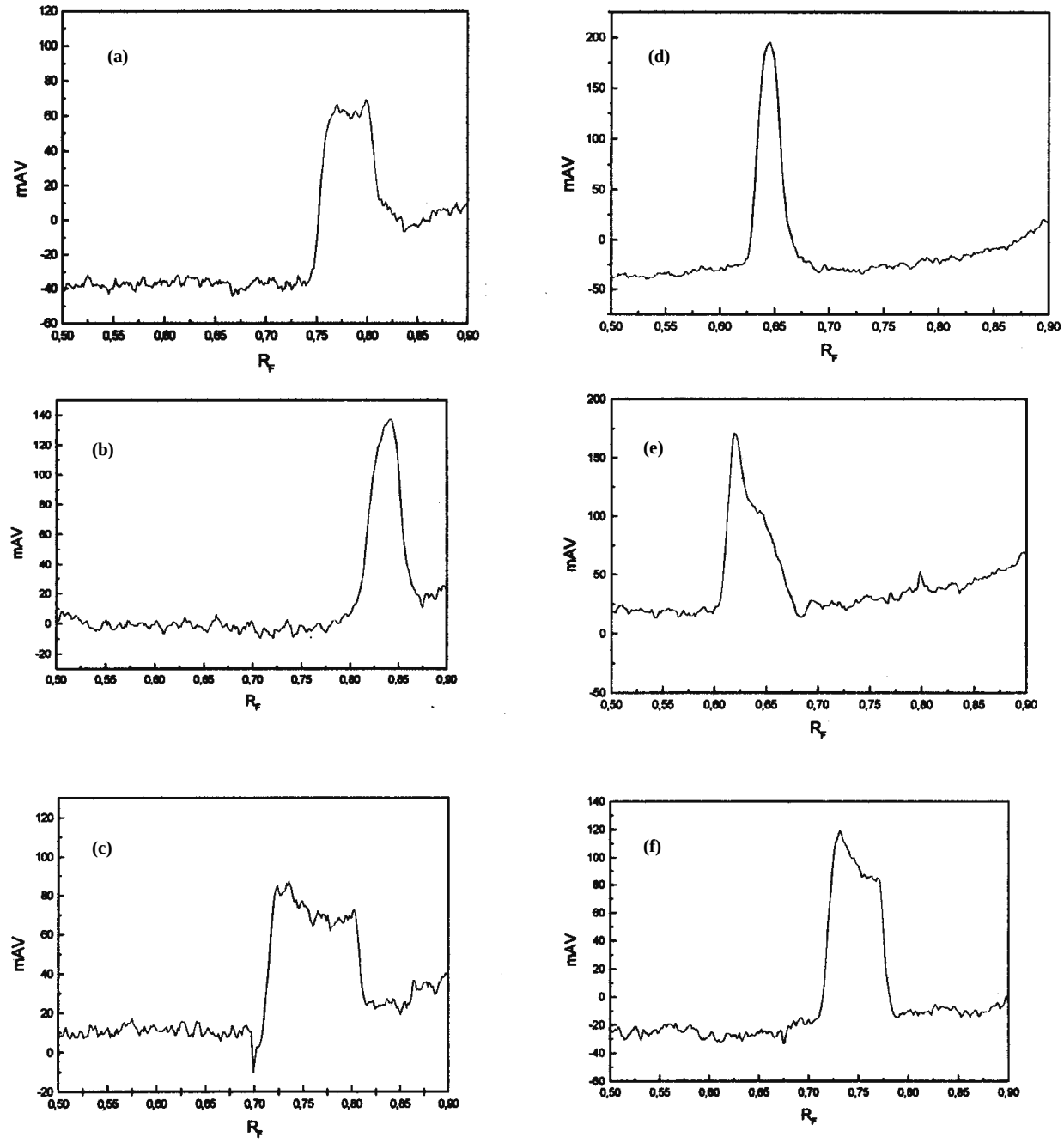



**Figure 3. Sequence of the densitometric concentration profiles of S,R-(±)-2-phenylpropionic acid after: (a) 0 h; (b) 22.5 h; (c) 27.5 h; (d) 46.5 h; (e) 51.5 h; and (f) 70.5 h storage time in 70% ethanol at 22 ± 2°C. Changes of the concentration profiles are accompanied by the changing $R_F$ values (originally published in [7])**

From the results of our thin-layer chromatographic experiments given in papers [7-9] it became apparent that the optically pure 2-APA enantiomers (S-(+)-ibuprofen and S-(+)-naproxen), and also the racemic sample (S,R-(±)-2-phenylpropionic acid), were undergoing the oscillatory changes of their respective retardation factor ($R_F$) values, when dissolved in 70% ethanol, dichloromethane, and physiological salt. These changes were most vigorous (in terms of the highest amplitude and the shortest period) in 70% ethanol and the least pronounced in the non-

aqueous solvent, i.e. dichloromethane. Moreover, it was experimentally confirmed that in spite of the oscillatory changes of the analytes' retardation factor ($R_F$) values, the prolonged storage of the samples did not result in any molecular destruction or transformation. In these circumstances, the only possible explanation of the observed oscillations seemed a continuous (and also oscillatory) structural inversion of the S-(+) species to their respective R-(-) antimers and vice verse. The phenomenon of structural inversion of the selected enantiomeric profen drugs running in vivo had been described in multiple pharmacological articles prior to our own study (e.g., in [10-12]), but there was no information in the available literature as to the structural inversion of these compounds in vitro. Thus the most direct way to check the hypothesis as to such structural inversion of 2-APAs (called in our articles the 'oscillatory transenantiomerization') was by use of polarimetry.

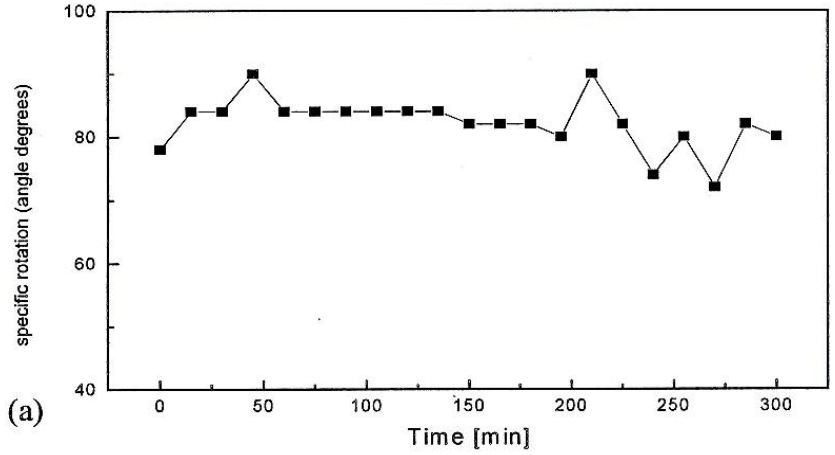
***Polarimetric investigation of the selected 2-APAs stored in solutions of different solvents***

The easiest way to scrutinize our hypothesis as to the oscillatory structural inversion of the three investigated 2-APAs could be by use of polarimetry. Although the oscillatory changes of the specific rotation ($[\alpha]_D$) with the aqueous and non-aqueous solutions of these compounds could not be considered as a sufficient proof in favour of this hypothesis, they certainly can act as a significant hint. In papers [7-9], we presented the experimental results of polarimetric measurements of the specific rotation ($[\alpha]_D$) with the solutions of S-(+)-ibuprofen, S-(+)-naproxen, and S,R-(±)-2-phenylpropionic acid, stored for the longer periods of time. In fact, one observed quite vigorous oscillatory changes of the specific rotation values with the optically pure 2-APAs (ibuprofen and naproxen), and moderate oscillations in the case of the racemate (2-phenylpropionic acid), when dissolved in 70% ethanol. In the case of dichloromethane, the oscillatory changes of the specific rotation values for all three 2-APAs were also observed, although quite weak in comparison with 70% ethanol used as a solvent. In Fig. 4, the selected examples of the obtained results are given.

The results of our polarimetric measurements supported the hypothesis as to the possible oscillatory transenantiomerization of ibuprofen, naproxen, and 2-phenylpropionic acid, when stored for the longer periods of time in the selected solvents. It seemed apparent that the oscillatory transenantiomerization is running vigorously in the aqueous medium (70% ethanol), but to a modest extent it can also take place in dichloromethane. These results gave rise to the following two questions:

(i) What is the mechanism of the observed chiral inversion of 2-APAs?
(ii) What is the mechanism that generates oscillations of this chiral inversion?

We attempted to find the relevant answers to these two questions.



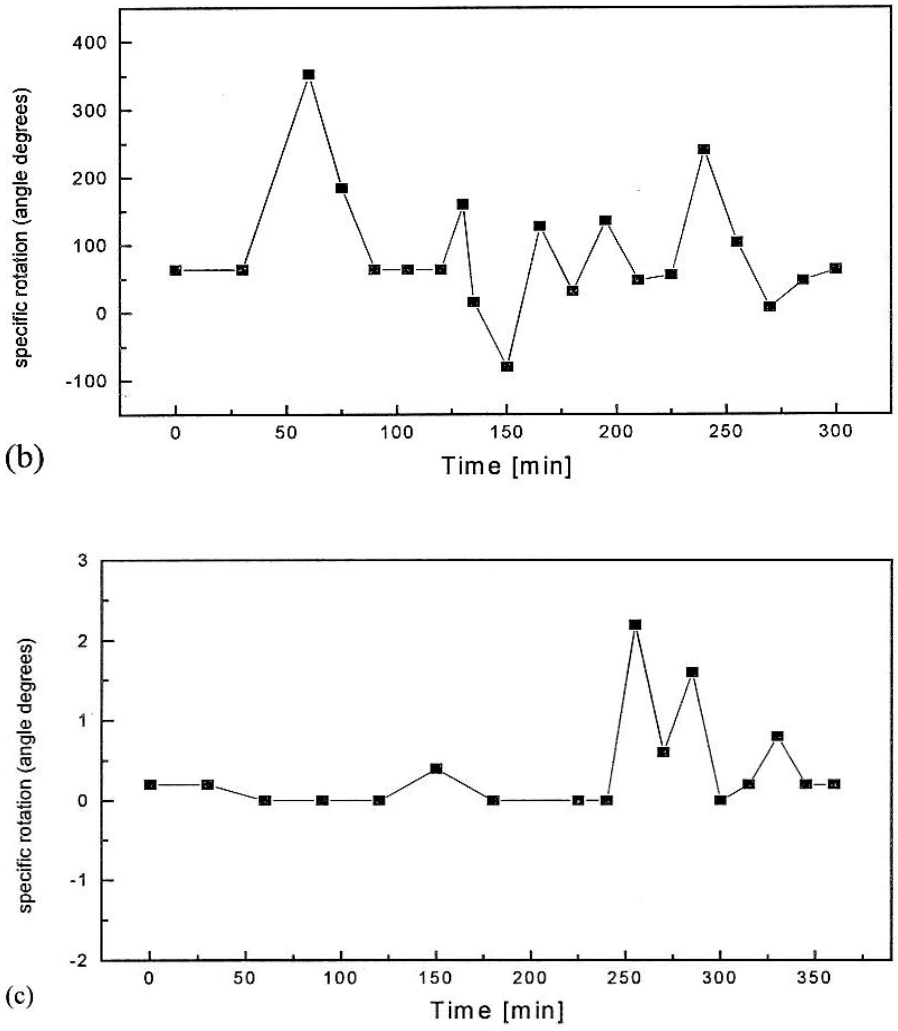



**Figure 4. Dependence of the specific retention ($[\alpha]_D$) of S-(+)-ibuprofen (a), S-(+)-naproxen (b), and S,R-(±)-2-phenylpropionic acid (c) dissolved in 70% ethanol on the sample storage time ($[\alpha] = f(t)$) at 6±2 °C (originally published in [7])**

### *Possible mechanisms of transenanti-omerization*

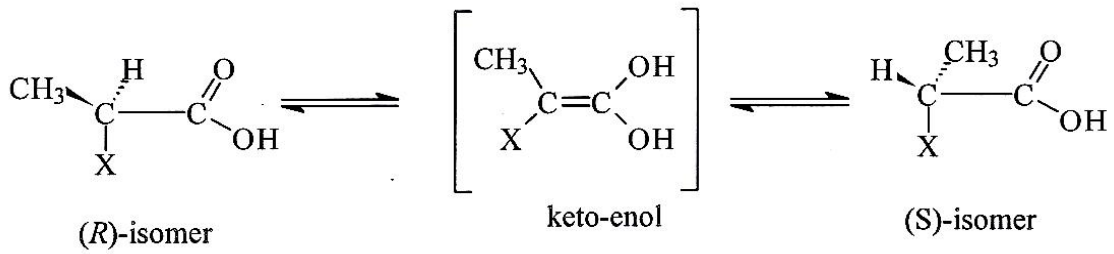
As mentioned in the preceding sections of this paper, no report had been available in the literature prior to our papers [7-9] on a possibility of the in vitro racemization of the 2-APAs. However, many reports are available on the analogous racemization processes running in vivo. In paper [13], a suggestion was made that racemization of 2-APAs is possible in the basic environment, involving keto-enol tautomerism as an intermediate reaction step. Schematic presentation of this process is given below.

Another mechanism is also reported in literature [14] as highly probable in the basic environment. This mechanism is particularly applicable to the dissociated form of the substituted propionic acids and involves the enolic anion, as shown in Scheme 2:

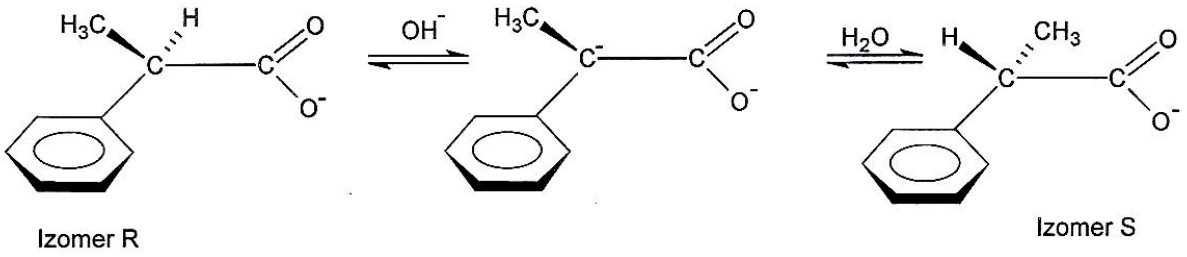
It seems rather difficult to decide, which one of the two transenantiomerization mechanisms (given by **Schemes 1** and **2**) is more probable in the case of the phenomena observed in our study and for this reason we decided to consider them as parallel.

To additionally confirm the correctness of the reports known from the literature and stating that the basic environment catalyzes the keto-enol tautomerism (and also the structural inversion via

the enolic anion), whereas the acidic environment inhibits this process, we performed a study on the storage of S-(+)-naproxen both in the basic and the acidic environment. The results of the performed experiment are extensively discussed in paper [15]. It was clearly demonstrated that the prolonged storage of S-(+)-naproxen in the basic solution (with the solvent composed of ethanol and the buffer, pH = 9, 7:3, v/v) resulted in partial conversion of S-(+)-naproxen to its R-(-) antimer. The process of conversion was rapid and it was not accompanied by any oscillatory changes of the specific rotation $[\alpha]_D$ with the investigated solution.




**Scheme 1. The mechanism of transenantiomerization of 2-APAs via the keto-enol tautomerism (X: aryl substituent of propionic acid in position 2 of the carbon chain)**




**Scheme 2. The mechanism of transenantiomerization of 2-phenylpropionic acid via the enolic anion**

S-(+)-naproxen was also stored in the acidic solution (with the solvent composed of ethanol and glacial acetic acid, 7:3, v/v). In this case, even a prolonged storage did not result in a measurable structural inversion of the original enantiomer and no oscillatory changes of the specific rotation $[\alpha]_D$ or the retardation factor ($R_F$) with the investigated enantiomer were observed. In Figs 5 and 6, we present the two three-dimensional chromatograms originating from a two-dimensional TLC development of the samples of S-(+)-naproxen dissolved, respectively, in the basic and the acidic medium, and then stored for 5 hours at 22±2°C.

Thus it seems justified to assume that the structural inversion of the three investigated 2-APAs occured via the keto-enol tautomerism and/or via the formation of the enolic anion. In the case of the aqueous solvent (70% ethanol), oscillations of the $R_F$ and the $[\alpha]_D$ values were incomparably more vigorous than in the organic one (dichloromethane). This observation remains in good agreement with the general knowledge about the mechanisms of this type of structural inversions. Namely, water and ethanol are the recognized amphiprotic solvents, apparently able to catalyze transenantiomerization of 2-APAs. Moreover, migration of the proton (which is indispensable with this type of inversion) occurs much easier in the aqueous medium than in the purely organic one.

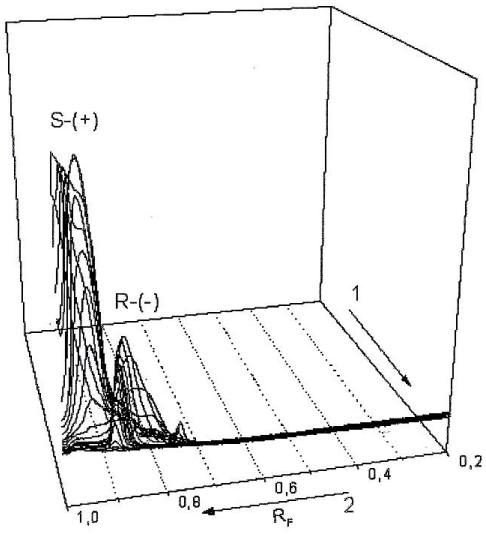



**Figure 5. Three-dimensional presentation of the naproxen chromatogram with two development directions, 1 and 2, indicated. Densitometric scanning (at parallel 1.5-mm intervals) of the 35-mm wide track in the second direction of the development was performed to better illustrate the separation performance and the skewed arrangement of S-(+)-naproxen relative to its R-(−) counterpart for the S-(+)-naproxen sample stored for 5 hours in the EtOH-basic buffer mixture, pH=9; 7: 3, v/v (originally published in [15]).**

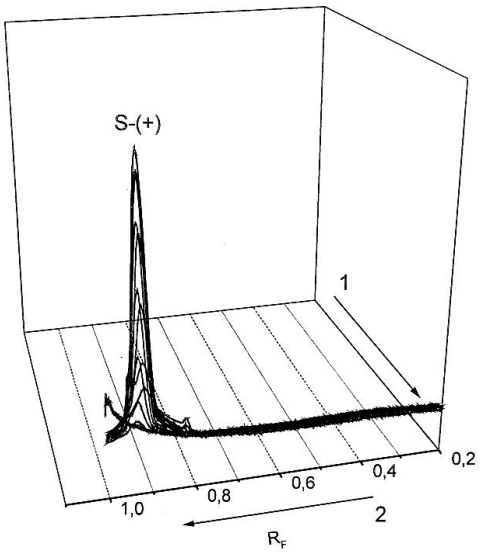



**Figure 6. Three-dimensional presentation of the naproxen chromatogram with two development directions, 1 and 2, indicated. Densitometric scanning (at parallel 1.5-mm intervals) of the 35-mm wide track in the second direction of the development was performed to better illustrate an absolute lack of transenantiomerization for the S-(+)-naproxen sample stored for 5 hours in the EtOH-glacial acetic acid mixture, 7:3, v/v (originally published in [15])**

## A possible mechanism of oscillations

It is a well established fact that the oscillatory chemical processes occur incomparably less frequently, than the non-oscillatory ones. Probably the best known among the oscillatory chemical processes is the Belousov-Zhabotinskii (B-Z) reaction. The theoretical phenomena (and also those experimentally confirmed) of the oscillatory chemical processes are discussed in multiple publications, e.g., in books [16,17].

What are the necessary preconditions for the oscillatory chemical processes to occur? The indispensable precondition is that such process be running through more than one elementary step. Besides, the oscillatory chemical processes are incomparably more probable, if at least one elementary step is not the first-order reaction, but a higher order. Finally, it is a well established fact that the oscillatory chemical processes are favourized in anisotropic liquids (and/or in those with a considerable viscosity). In such liquids, the molecular diffusion coefficients of the intermediate products strongly depend on the directions distinguished by the molecular self-organization, and they can be quite different in each direction.

One of the best known theoretical models of the oscillatory chemical processes was elaborated by the two Belgian scientists, Prigogine and Lefevre, and it is known as Brusselator. It assumes four elementary steps, the first-order, the third-order, the second-order, and again the first-order. These four elementary steps do not refer to any known oscillatory chemical process and they are purely abstract chemical reactions. The original Brusselator is an exclusive kinetic model that applies to the perfectly homogenous reaction systems and it does not assume anisotropic properties of such systems. However, it can be implemented with a diffusion term that takes into the account not only the kinetics, but also the anisotropic diffusion of the intermediate reaction product(s) in the reaction vessel, as postulated by Turing [18]. This additional term directly results from the Fick's Second Law and the expanded model is known as Brusselator with diffusion.

Now let us return once again to the oscillatory transenantiomerization of the

investigated 2-APAs. In fact, the detailed mechanism of this process (in terms of the consecutive elementary steps and their respective kinetic constants) remains fully unknown, and Schemes 1 and 2 provide a rough and temporary explanation only. E.g., the crucial role played by the basic environment as a catalyst of the observed structural inversion is not reflected in any of these two schemes. Thus one can rightfully assume that the oscillatory transenantiomerization has a more complicated mechanism than the two elementary steps only and one cannot exclude that some of these steps are of the second, or even of the third order. In our study, we managed to gather a convincing enough experimental evidence in favour of the molecular self-organization in the solutions of 2-APAs in the low-molecular-weight solvents (like, e.g., 70% ethanol and dichloromethane). E.g., in paper [19] the results of the acoustic and volumetric studies of the dilute solutions of S-(+)-naproxen in acetonitrile were presented and it was firmly established, that the limiting partial compressibility of S-(+)-naproxen is close to zero and it decreases only slightly with an increasing temperature. In paper [20], we presented different results in favour of a considerably enhanced viscosity of the 2-APA solutions in certain low-molecular-weight solvents (as compared with the pure solvents) and/or of the molecular self-organization of the respective solutions. These results originate from such diverse measuring techniques, as viscosimetry, high-performance liquid chromatography (HPLC), and spectroscopy of the nuclear magnetic resonance ($^1$H NMR).

The HPLC evidence of a considerably enhanced viscosity of the 2-APA solutions in the organic solvents is in the form of the unusually tailing concentration profiles of the respective 2-APAs and of the strikingly long migration times of these compounds through the chromatographic column (in certain cases lasting even more than one hour). An illustration of this phenomenon is given in Fig. 7.

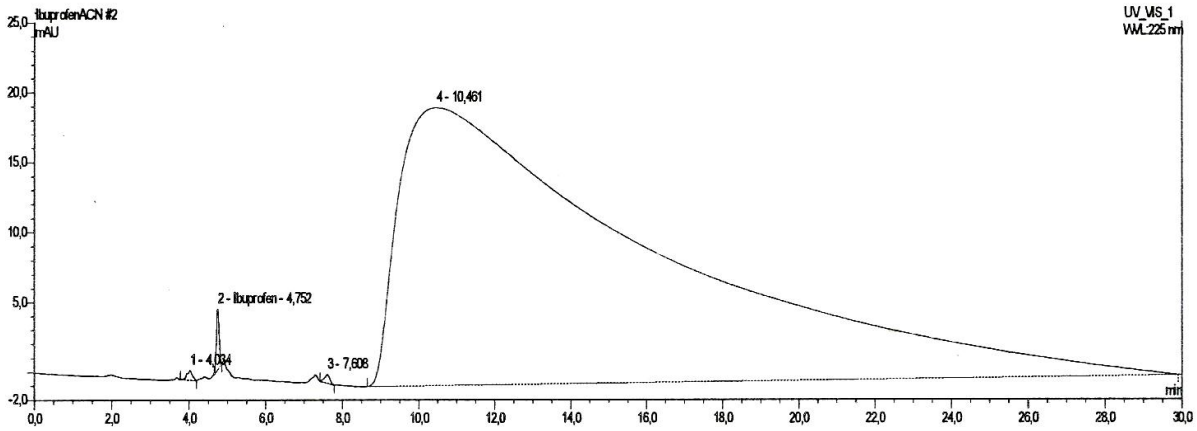
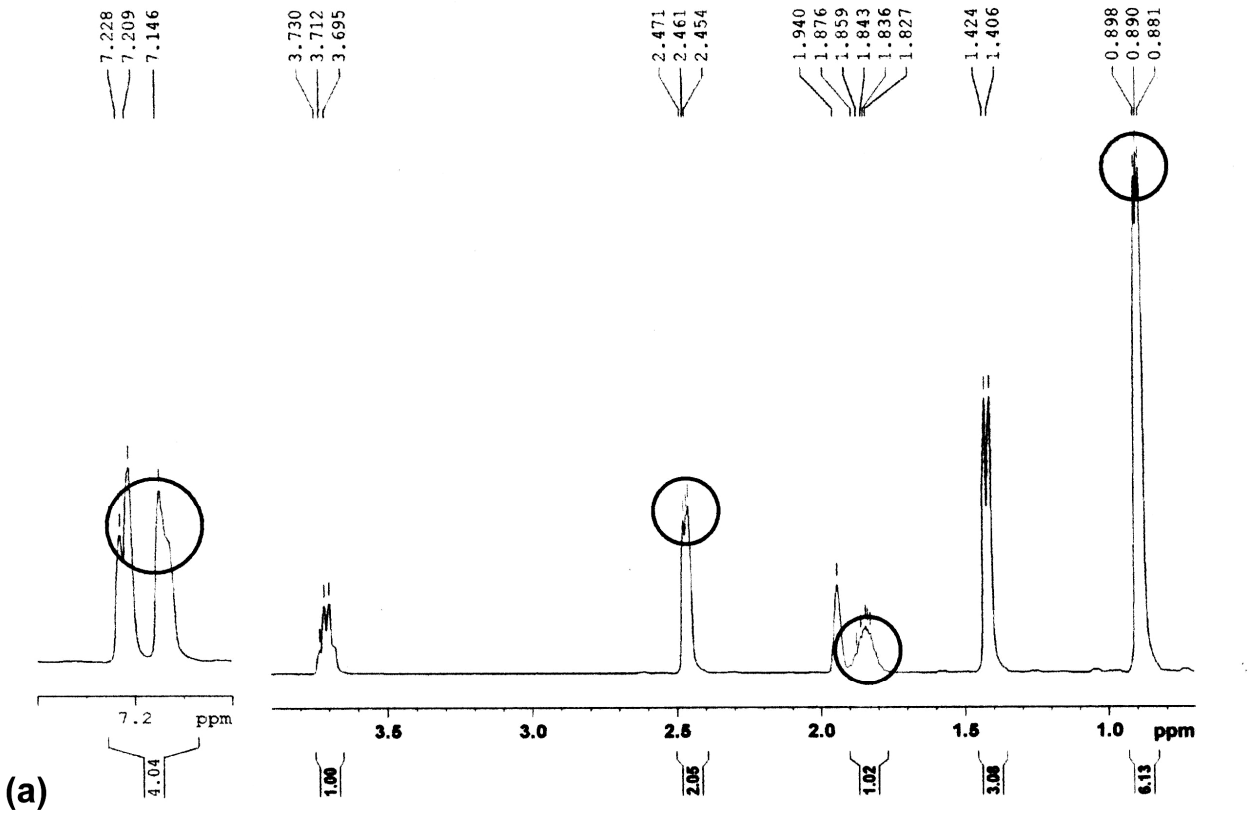



**Figure 7. The tailing concentration profile on the high performance liquid chromatogram of S-(+)-ibuprofen dissolved in acetonitrile and chromatographed in the RP-18 / acetonitrile HPLC system at ambient temperature (originally published in [20])**

An increase of viscosity and/or the molecular self-organization with a solution of S-(+)-ibuprofen in the low-molecular-weight solvent (i.e. in the deuterated acetonitrile, ACN-$D_3$) was confirmed with aid of $^1$H NMR also. Spectra were measured in the two different modes, and namely with a rotating and an immobile probe. As it can be seen from a comparison of the spectra shown in Figs 8a and 8b, rotation of the sample-containing probe (as it is usually done with the isotropic liquid samples) resulted in a low-quality spectral picture with the signals either poorly resolved, or in certain cases even unresolved. To the contrary, running of the $^1$H NMR spectrum of S-(+)-ibuprofen in the immobile probe (as is normally done with solid samples) resulted in the well resolved signals. Thus one can rightfully conclude that the solution of the investigated S-(+)-

ibuprofen in ACN-$D_3$ demonstrates the property of the anisotropic liquid and in a way resembles that of a liquid crystal. This molecular self-organization of the investigated profen-containing liquid system is inevitably accompanied by an increase of the viscosity thereof.

Summing up, it seems very likely that the well documented oscillations of the retardation factor ($R_F$) values and also of the specific rotation ($[\alpha]_D$) values with the selected 2-APAs (i.e., with S-(+)-ibuprofen, S-(+)-naproxen, and S,R-(±)-2-phenylpropionic acid) are due to the oscillatory transenantiomerization of the discussed compounds. The detailed mechanism of transenantiomerization (i.e. the respective elementary steps) for 2-APAs so far remains unknown, except for the fact that the process can be effectively catalyzed in the basic (and apparently in the amphiprotic) environment, and inhibited in the acidic one. Oscillations can be generated by the molecular self-organization and/or an enhanced viscosity of the 2-APA solutions in the selected low-molecular-weight aqueous and non-aqueous solvents. Explanation of this striking phenomenon seems to be possible by means of the Brusselator-like kinetic model (based on the knowledge of the valid elementary steps and of the kinetic constants thereof), implemented with the relevant diffusion term.



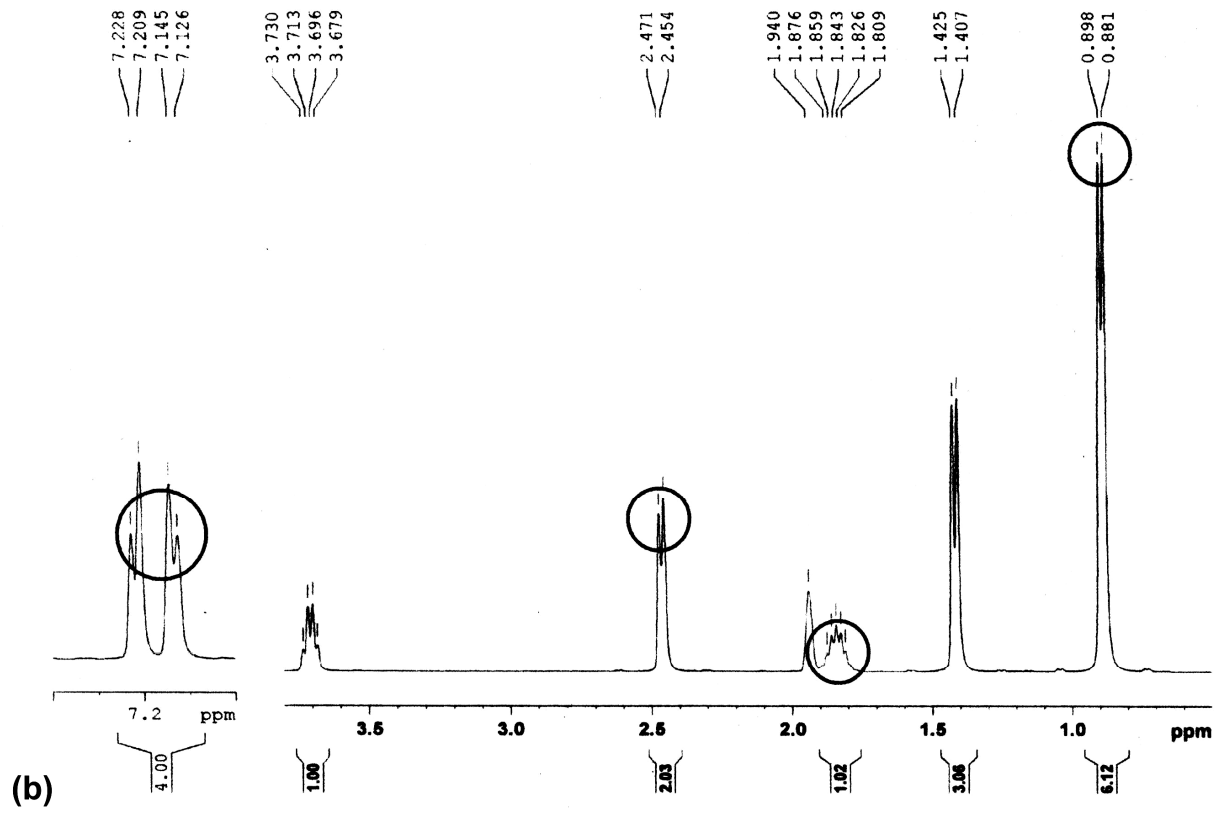



**Figure 8. The 1H NMR spectra of S-(+)-ibuprofen dissolved in ACN-$D_3$ and recorded with use of (a) the rotating probe and (b) the non-rotating probe. With circles these particular spectral regions were marked, for which recording in a rotating probe came out particularly inaccurate, i.e., the split of the respective signals was incomplete (originally published in [20])**

## Conclusions

Although it is quite difficult to state for sure that the peculiar behaviour of 2-APAs in the selected aqueous and non-aqueous solutions cannot be anything else but the oscillatory transenant-iomerization, certain facts and arguments act very strongly in support of this particular explanation. The most important argumentation is given below.

1. Oscillatory changes of the retardation factor ($R_F$) values in TLC are certainly not caused by the oscillatory changes of the aggregation degree nor by the oscillatory changes of the spatial arrangement within the supramolecular structures that involve the considered 2-APA molecules.

(a) Firstly, chiral chromatographic systems are devised precisely for separation of the enantiomer pairs (for the enantioseparation), and not for structural preservation of the supramolecular aggregates, built of one optically pure enantiomer only. Such behaviour would be entirely against the principle of chromatography as a well established and an excellently well performing separation method.

(b) Secondly, in our chromatographic study we worked in the linear range of the adsorption isotherm for the investigated chiral analytes. Under such conditions, concentration of these analytes in the mobile phase filling the adsorbent pores is very low and it usually ranges from $1 \times 10^{-2}$ to $1 \times 10^{-3}$ mol $dm^{-3}$ (with several dozen nanograms of the analytes per one chromatographic spot only). Taking into the assumption low concentrations of the analytes in the chromatographic system and

additionally the presence of the adsorbent able to effectively destroy the so-called lateral (i.e., analyte-analyte) intermolecular interactions, it would be rather difficult to imagine that the oscillating $R_F$ values originate from the different supramolecular forms of a single optically pure enantiomer only that does not decompose to the separate molecules (or to the cyclic H-bonded dimers of the discussed carboxylic acids), in spite of the one hour or more lasting development of the chromatogram.

(c) Thirdly, the narrow and symmetric concentration profiles of ibuprofen, naproxen, and 2-phenylpropionic acid at the extreme positions on the chromatograms (i.e., at those with the lowest and the highest $R_F$ value) point out to the fact that in these cases we encounter the pure chemical species. Moreover, the lowest $R_F$ value corresponds well with that valid for the R-(-) species and the highest $R_F$ value with that for the S-(+) species of the investigated 2-APAs. Thus it can rightfully be concluded that the concentration profile showing the lowest numerical value of the retardation factor ($R_F$) represents optically pure R-(-) enantiomer and that showing the highest $R_F$ value is valid for the pure S-(+) enantiomer. The considerably less symmetrical concentration profiles appearing in the intermediate positions indicate the presence of the two chemical species that are not fully separated. These species most probably are the S-(+) and the R-(-) antimer.

(d) Finally, enantioseparations of the racemic 2-APA mixtures by means of TLC are not only possible, but also often carried out. Many such separations have been successfully performed under the chromatographic conditions either identical with, or very close to those employed in our study. Basic difference between the earlier successful enantioseparations and our own experimental results consists in the fact that the earlier enantioseparations had been carried out for the freshly prepared analyte solutions, while we investigated solutions stored for the longer periods of time prior to commencing the TLC experiment, and then we examined the dependence of the retention parameter ($R_F$) on the storage time (t).

2. In one of our experiments it was clearly shown that S-(+)-naproxen stored in the basic solution undergoes a relatively rapid structural inversion and yields a considerable amount of the R-(-) antimer. Apparently, the thermodynamic equilibrium of this inversion is very rapidly attained and the solution does not show any oscillations of its specific rotation, $[\alpha]_D$. In another experiment, S-(+)-naproxen was stored in the acidic solution, which neither resulted in a measurable structural inversion nor in the oscillations of the solution's specific rotation, $[\alpha]_D$. These two observations remain in agreement with the general knowledge of the reaction mechanisms in organic chemistry. Namely, it is a well established fact that the keto-enol tautomerism with the carboxylic acids (or formation of the respective enolic anions) is catalyzed by the basic environment and hampered by the acidic one. Moreover, it is well known that water and lower alcohols are the amphiprotic solvents, having the weak basic and the weak acidic properties. Oscillations of the specific rotation ($[\alpha]_D$) with solutions of the selected 2-APAs are much more vigorous in the ethanol-water mixture, than in the low-polar organic solvent. It seems that the amphiprotic nature of the ethanol-water solvent combined with a confirmed ability of 2-APAs to self-organize the molecules that constitute a solution are a sufficient precondition of the oscillatory transenantiomerization process, based on combination of the kinetic and the diffusive factor. In the case of the organic solvent that lacks amphiprotic properties, the effect of oscillations is pronounced to a perceptibly lesser degree only.

3. Apart from the amphiprotic nature of the ethanol-water mixture, one more aspect has to be underlined, related to the efficiency of the oscillatory changes of the $R_F$ and the $[\alpha]_D$ values in this particular solvent. Apparently, structural inversion of 2-APAs does not occur immediately, but via the intermediate elementary steps (like, e.g., formation of keto-enol or the enolic anion). The elementary steps

are indisputably of the ionic nature and for this particular reason the aqueous medium promotes them much better than the organic one.

4. In one experiment, S-(+)-naproxen was dissolved and stored for the longer periods of time in three different solvents having the same quantitative (and similar qualitative) composition: (i) ethanol – buffer, pH=9 (7 : 3, v/v); (ii) ethanol – water (7 : 3, v/v); and (iii) ethanol – glacial acetic acid (7 : 3, v/v). If the spontaneous oscillations of the specific rotation ($[\alpha]_D$) were the result of structural changes on a supramolecular level (i.e., due to formation of the molecular aggregates with a changing structure and a changing number of the associated molecules), it could logically be awaited that in each of these three solutions similar oscillations of the specific rotation ($[\alpha]_D$) would be observed. However, oscillations were observed exclusively in one case, i.e., in the ethanol – water (7 : 3, v/v) mixture. This outcome seems to deny any supposals as to the supramolecular nature of the observed oscillations.

5. The ability of the selected 2-APAs – ibuprofen and naproxen – to the in vivo structural inversion (i.e., transenantiomerization) has already been reported in the numerous papers from the fields of pharmacology and biomedicine. In our study, we have for the first time demonstrated that the structural inversion of 2-APAs can also take place in vitro. Recently, the in vitro transenantiomerization of S-(+)-ibuprofen was confirmed by another research team [21].

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