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ON THE GELATION OF PROFENS AS A PROPERTY CAUSING THEIR OSCILLATORY TRANSENANTIOMERIZATION

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SUMMARY

We have previously revealed, for the first time, the tendency of some profens (*S*-(+)-ibuprofen, *S*-(+)-naproxen, and *S*,*R*-(±)-2-phenylpropionic acid) to undergo oscillatory transenantiomerization when dissolved in some low-molecular-weight solvents. Such oscillatory reactions, which are relatively rare among chemical reactions, can be stimulated by several different physical and chemical conditions. It was the objective of this study to reveal the reason for the oscillatory transenantiomerization of profens. The analytical techniques used in our study embraced measurement of the viscosity of the profen solutions and acquiring their HPLC chromatograms and ^{1}H NMR spectra.

It has been firmly established that the physical property leading to oscillatory transenantiomerization of the profens is their ability to act as low-molecular-weight gelators, which results in substantial differences between the viscosity of their solutions and that of the pure solvents. One well-known cause of oscillatory reactions is diffusion-driven instability which appears in the form of oscillations. This occurs when, because of high solution viscosity, the rate of diffusion of an intermediate reaction product becomes less its rate of formation. For the profens investigated it seems most likely that their ability to substantially increase the viscosity of the solution results in the rate of diffusion of an intermediate keto–enol product being lower than its rate of formation from the respective profen enantiomer.

INTRODUCTION

We have previously reported the oscillatory change of the specific rotation $[\alpha]_D$ of solutions of selected profens (*S*-(+)-ibuprofen, *S*-(+)-naproxen, and *S*,*R*-(±)-2-phenylpropionic acid) in a variety of the monocom-

ponent and mixed solvents [1–3]. This oscillatory change of the specific rotation ($[\alpha]_D$) was established by polarimetry and suggests a sequence of oscillatory chemical reactions is responsible for the behaviour. In the same work another oscillatory property of solutions of these profens was observed, an oscillatory change of their thin-layer chromatographic retention (R_F) during prolonged storage of their solutions. Bearing in mind the long-recognized and substantial chemical stability of the profens under environmental conditions (i.e. stability toward oxygen, temperature, and irradiation with visible light), we came to the conclusion that this behaviour of the profens most probably originated from their oscillatory transenantiomerization.

A simple schematic representation of the base-catalyzed transenantiomerization of the profens (a single and not repeatable, i.e. oscillatory, reaction) was first introduced by Xie et al. [4] and is given in Fig. 1. It is apparent from this figure that transenantiomerization of the profens occurs via keto–enol tautomerism.

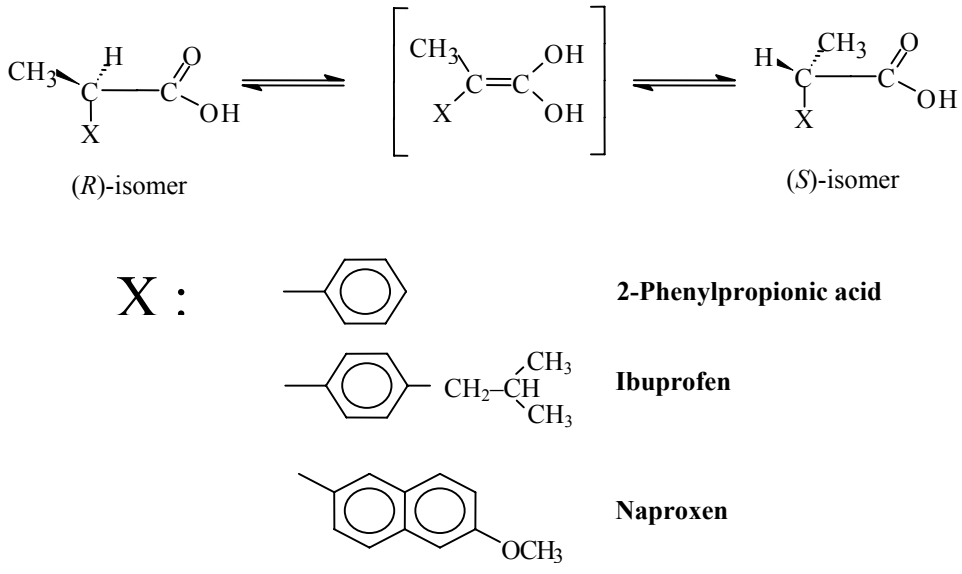

Fig. 1

Schematic representation of the transenantiomerization of profens by keto–enol tautomerism

Our task was to find answers to two important questions:

(i) Does prolonged storage of the profens in some solvents really result in spontaneous transenantiomerization of a given enantiomer to its chiral antimer?
(ii) What is the physical mechanism of the repeated (i.e. oscillatory) transenantiomerization of the profens?

We have previously confirmed our initial assumption that the mechanism of spontaneous transenantiomerization of the profens was via keto–enol tautomerism [5]. Our task in our latest research was to explain the oscillatory mechanism (or the repeated movement to and fro) of transenantiomerization of profens during their prolonged storage in different solvents.

EXPERIMENTAL

In this section we introduce a limited yet convincing selection of experiments performed in our laboratory to elucidate the mechanism of oscillatory transenantiomerization of the profens. These experiments were performed with *S*-(+)-ibuprofen and *S*-(+)-naproxen only, yet we believe these are representative of all the profens. The results from the experiments discussed in this section are persuasive enough to prove the correctness of our hypothetical mechanism for oscillatory transenantiomerization of the profens.

Measurement of Viscosity

Because we assumed that the rheological properties, i.e. high viscosity, of solutions of small amounts of the profens was responsible for the oscillatory transenantiomerization of the dissolved pure chiral isomers, we measured the viscosity of six liquids:

(i) pure acetonitrile (ACN);
(ii) a 4.3×10^{-3} mol L^{-1} (1 mg mL^{-1}) solution of naproxen in ACN;
(iii) a 2.15×10^{-2} mol L^{-1} (5 mg mL^{-1}) solution of naproxen in ACN;
(iv) 70% EtOH (i.e. 7:3 (*v*/*v*) ethanol–water);
(v) a 4.3×10^{-3} mol L^{-1} (1 mg mL^{-1}) solution of naproxen in 70% EtOH; and
(vi) a 2.15×10^{-2} mol L^{-1} (5 mg mL^{-1}) solution of naproxen in 70% EtOH.

Our measurements were performed at 25 ± 0.2°C with a Hoeppler viscosimeter, each experiment being repeated ten times ($n = 10$). The results obtained are given in Table I.

Table I

Comparison of the viscosity of solutions of *S*-(+)-naproxen at 25 ± 0.2°C with that of the respective pure solvent (n = 10)

Liquid	Concn *S*-(+)-naproxen (mol L^{-1})	Dynamic viscosity, η (cP) (± relative error)	Increase in dynamic viscosity (%)
ACN	0	0.39 (± 1.2%)	–
ACN + *S*-(+)-naproxen	4.3×10^{-3}	0.40 (± 1.4%)	2.6
ACN + *S*-(+)-naproxen	2.15×10^{-2}	0.41 (± 1.1%)	5.1
70% EtOH	0	1.14 (± 0.5%)	–
70% EtOH + *S*-(+)-naproxen	4.3×10^{-3}	1.15 (± 0.5%)	0.9
70% EtOH + *S*-(+)-naproxen	2.15×10^{-2}	1.17 (± 0.6%)	2.6

High-Performance Liquid Chromatography (HPLC)

HPLC was performed with a P580A LPG liquid chromatograph equipped with a Gina 50 autosampler and UVD340V diode-array detector (DAD) (Gynkotek/Dionex, Germering, Germany). Compounds were separated on RP-18 (LichroCART cartridge with 250 mm × 4 mm i.d., 5 µm particle, LiChrospher 100 column; Merck, Darmstadt, Germany; #1.50983.0001). For chromatography of *S*-(+)-ibuprofen, 70% EtOH or ACN was used as solvent and either ACN or 6:4 (*v*/*v*) ACN–water as mobile phase. For chromatography of *S*-(+)-naproxen, ACN was used as solvent and ACN as mobile phase. Sample concentrations were 2 mg mL^{-1}, and 5-µL volumes were injected by means of the autosampler. The mobile phase flow rate was 0.6 mL min^{-1}. Oscillatory changes of the retention times (t_R) of *S*-(+)-ibuprofen as a function of sample storage time (t) were measured at 15-min intervals and are shown in Figs 2–4. Results obtained from chromatography of *S*-(+)-ibuprofen and *S*-(+)-naproxen with ACN as both solvent and mobile phase are given in Figs 5 and 6.

^{1}H NMR

^{1}H NMR spectra were acquired with a Bruker Avance 400 MHz NMR spectrometer equipped with the ^{1}H probe. A solution (5 mg mL^{-1}) of *S*-(+)-ibuprofen was prepared in acetonitrile-D_3 (ACN-D_3). Spectra were acquired in two different modes – with use of the rotating probe (a routine procedure with isotropic liquid samples) and with the probe immobile (normal conditions for solid samples). The results of these investigations are given in Fig. 7.

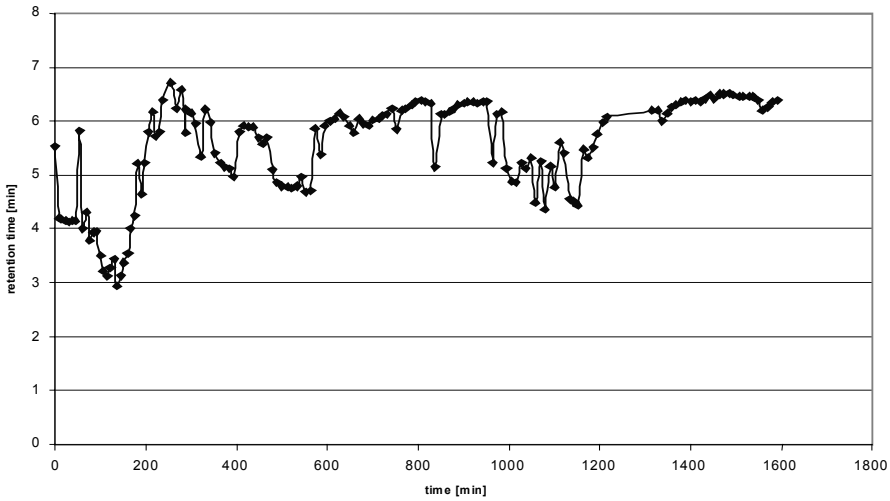

Fig. 2

Dependence of *S*-(+)-ibuprofen retention time, t_R, on sample storage time, t ($t_R = f(t)$), at ambient temperature (22 ± 2°C). The detection wavelength was 225 nm, the sample solvent 70% EtOH, and the mobile phase ACN–water, 6:4 (*v/v*)

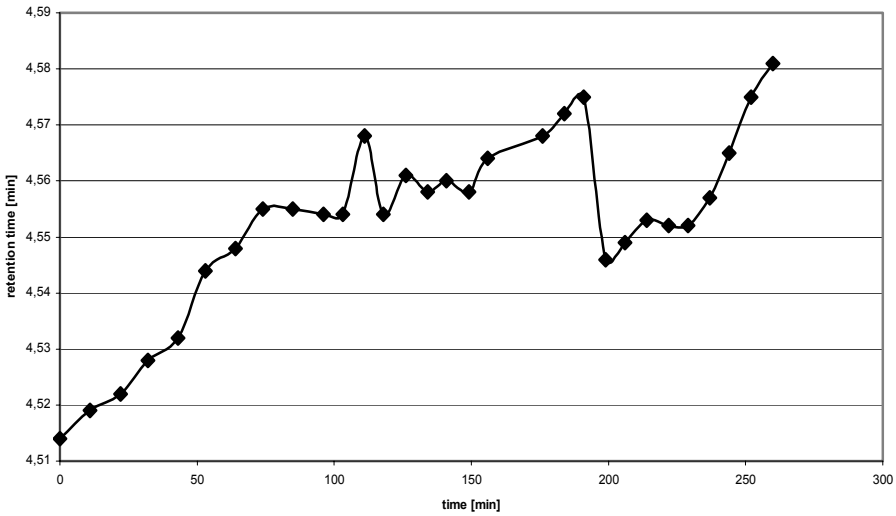

Fig. 3

Dependence of *S*-(+)-ibuprofen retention time, t_R, on sample storage time, t ($t_R = f(t)$), at ambient temperature (22 ± 2°C). The detection wavelength was 225 nm, the sample solvent 70% EtOH, and the mobile phase ACN

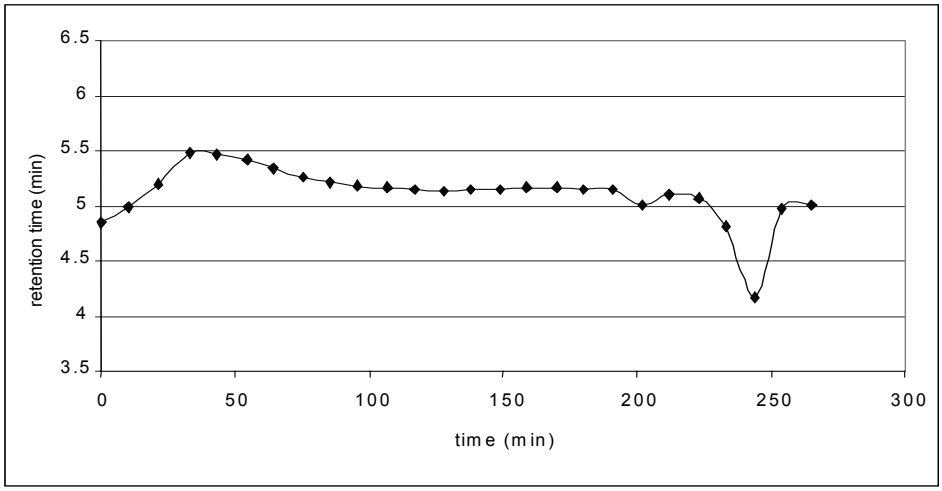

Fig. 4

Dependence of *S*-(+)-ibuprofen retention time, t_R, on sample storage time, t ($t_R = f(t)$), at ambient temperature (22 ± 2°C). The detection wavelength was 225 nm, the sample solvent ACN, and the mobile phase ACN–water, 6:4 (*v*/*v*)

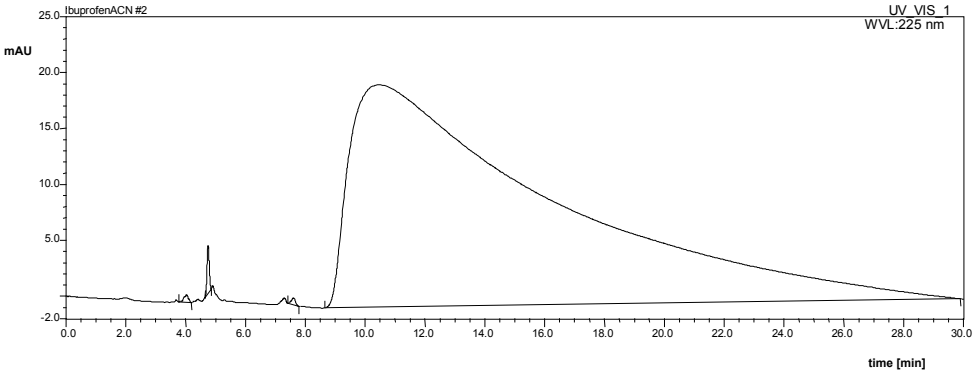

Fig. 5

Tailing chromatogram obtained from *S*-(+)-ibuprofen dissolved in ACN and chromatographed with ACN as mobile phase

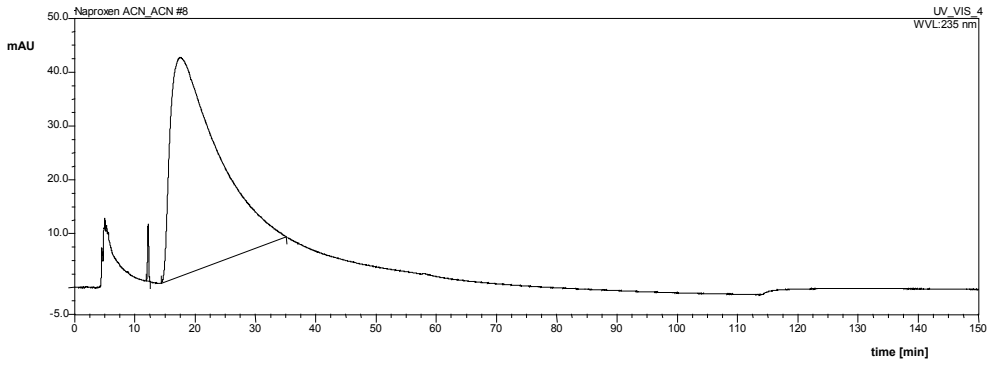

Fig. 6.

Tailing chromatogram obtained from *S*-(+)-naproxen dissolved in ACN and chromatographed with ACN as mobile phase

RESULTS AND DISCUSSION

Oscillatory reactions of the Belousov–Zhabotinskii type have a variety of different mechanisms. Among the most common reasons is high viscosity of the reaction environment. When the rate of formation of an intermediate product in a multi-step reaction exceeds its rate of diffusion in a given system, conditions for an oscillatory reaction are established. This particular mechanism seems to be responsible for oscillatory transenantiomerization of the profens, i.e. it seems most probable that the high viscosity of the profen solution means the rate of diffusion of the keto-enol intermediate in the transenantiomerization reaction is lower than its rate of formation. This change results in a local increase in the concentration of the intermediate and in its partial decomposition to the starting material, to fulfil the requirements of reaction thermodynamics.

Profens seem to behave as low-molecular-weight gelators. They can probably self-organize on the molecular level into repeating structural patterns, owing to their carboxyl groups (which self-associate by hydrogen bonding) and the aromatic moieties with high local negative charge. Self-organization of the profen molecules results in a regular steric arrangement embracing whole solution. This can incorporate solvent molecules and form inclusive host–guest type complexes. Perhaps it is worth mentioning here that one can reasonably expect anisotropy of some physical properties in self-organized liquids. This working hypothesis found convincing confirmation in the experimental results given in this paper.

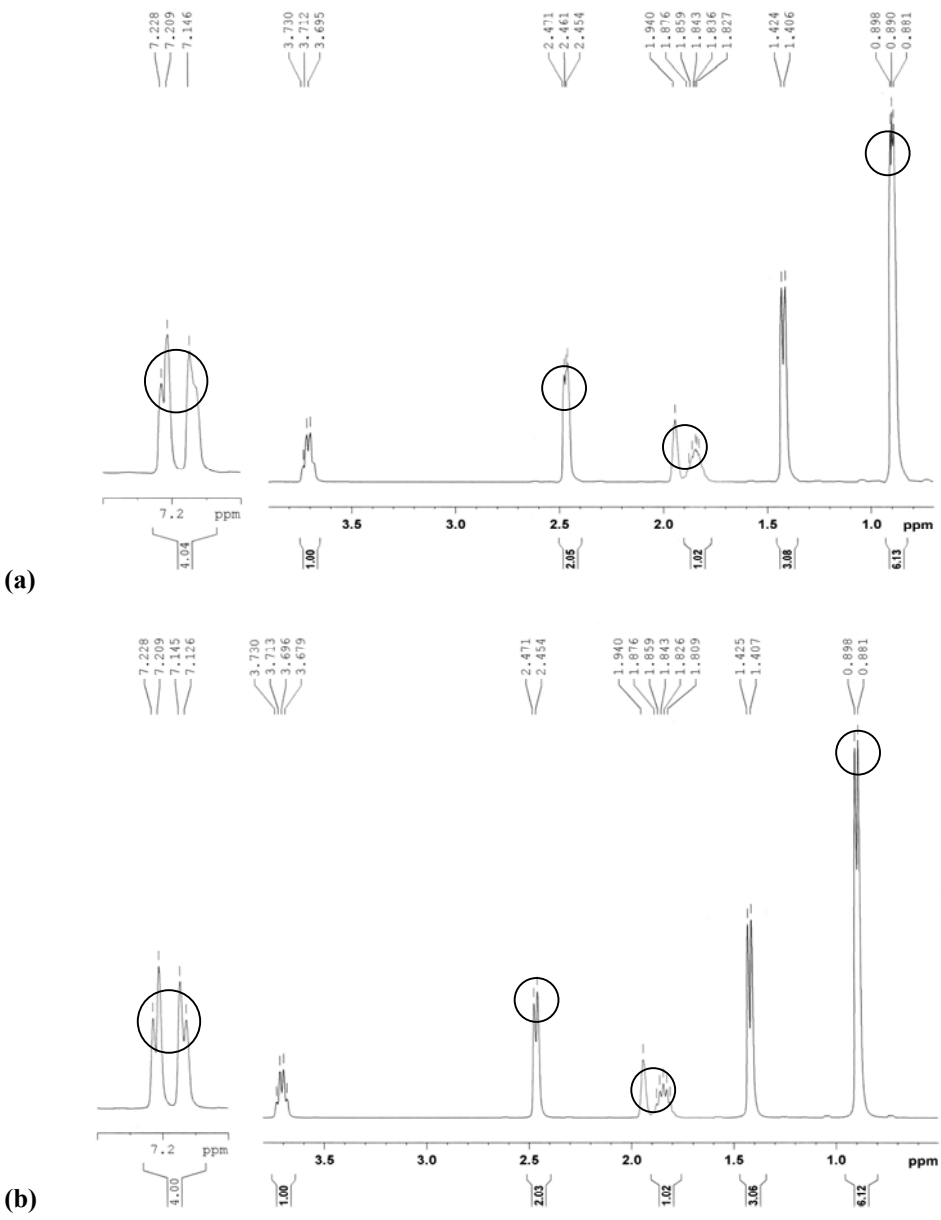

Fig. 7.

^{1}H 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. The circles denote the spectral regions for which the spectrum recorded with the rotating probe was particularly inaccurate (i.e. splitting of the signals was incomplete)

Results obtained from measurement of viscosity are shown in Table I. It is apparent from these data that dissolution of very small amounts of *S*-(+)-naproxen in ACN results in a solution with dynamic viscosity much higher (as much as 5% at 25°C) than that of pure ACN. If *S*-(+)-naproxen is dissolved in 70% EtOH the increase in viscosity is somewhat less pronounced than for ACN, an observation which emphasizes the effect of the molecular structure of the solvent on the gelating (or self-organizing) process of the profens. In summary, the measurements reveal that very low concentrations of *S*-(+)-naproxen lead to solutions with viscosity substantially higher than that of the pure solvents.

HPLC was performed with the two different objectives in view. First, we were attempting to demonstrate oscillatory changes in the retention times, t_R, of the profens as a function of time, t, by use of a column chromatographic technique (our initial results, reported elsewhere [1–3], had been obtained from thin-layer chromatography only). The results obtained are shown in Figs 2–4. From these figures oscillatory changes of the retention times of *S*-(+)-ibuprofen are clearly apparent. It must be stressed that these changes occur only when either the solvent of the profen sample, or the applied mobile phase, or both, contain water as a component. Evidently, the local increase of viscosity with this type of liquid chromatographic system is somewhat lower than for non-aqueous systems (Table I), but it proves sufficient for oscillatory transenantiomerization. It is also evident from this part of our research that the most vigorous oscillations of the retention time were observed when an aqueous solvent and an aqueous mobile phase were used (Fig. 2). Somewhat less vigorous oscillations were observed for ACN as sample solvent and an aqueous mobile phase (Fig. 4). The least pronounced oscillations (almost bordering on experimental error) were observed for use of the aqueous solvent and ACN as monocomponent mobile phase (Fig. 3). These results – and particularly the last – lead to the conclusion that oscillations can be generated rapidly during retention (i.e. on-column) and not only during prolonged storage of the profen samples. The same rapidity was also observed in our polarimetric studies, reported elsewhere [1–3].

The chromatographic behaviour of the profen samples dissolved in a non-aqueous medium and chromatographed with a non-aqueous mobile phase is completely different. Demonstration of this striking phenomenon was the second objective of our HPLC experiment. Under these conditions the local increase of the viscosity in a given system is too high to enable transenantiomerization of profens. Instead, highly viscous profen samples

migrate along the chromatographic column with evident difficulty and elution of analytical (i.e. low-concentration) profen samples can take dozens of minutes or even more than an hour. Illustrative examples of the chromatograms are shown in Figs 5 and 6. Even after prolonged elution of acetonitrile solutions of profen samples with pure ACN as mobile phase, the separation quality of the RP-18 chromatographic column has remained strongly and negatively affected, as if the entire outer surface area of the adsorbent particles, and maybe some of their inner surface also, were coated with very viscous profen gel, strongly elevating the pressure of the ACN mobile phase (and most probably this was really occurring). Full recovery of the column's working performance was possible only after rinsing with aqueous mobile phase.

The objective of the third (and last) experiment was to demonstrate the gelating property of the profens. The high viscosity of a solution of *S*-(+)-ibuprofen in the low-molecular-weight solvent (ACN-D_3) was checked by ^{1}H NMR. Spectra were run in two different modes, with rotating and immobile probes (Fig. 7). It is apparent from comparison of the spectra shown in Figs 7a and 7b that rotation of the sample-containing probe (as is usually performed with isotropic liquid samples) resulted in a poor-quality spectrum with the signals either poorly resolved or sometimes unresolved. In contrast, acquiring the ^{1}H NMR spectrum of *S*-(+)-ibuprofen with the probe immobile (as is normally performed with solid samples) resulted in well-resolved signals. Thus one can reasonably conclude that the solution of the investigated profen in ACN-D_3 has the properties of a self-organized (i.e. anisotropic) liquid and thus resembles a liquid crystal. This molecular self-organization of the profen-containing liquid system is inevitably accompanied by an increase in viscosity.

CONCLUSIONS

Convincing experimental evidence of the gelating behaviour of the profens when dissolved in low-molecular-weight solvents has been obtained by means of classical viscosimetric measurements and by HPLC and ^{1}H NMR, which is certainly an unconventional approach to rheology.

Profens are important non-steroidal anti-inflammatory drugs (NSAID); many are available commercially without a medical prescription. The international pharmaceutical community is well aware of the many warnings in the medical and pharmaceutical literature about the possibility of developing hypertension as a result of frequent use of ibuprofen and the other

profens. The results and problems discussed in this paper might be the first clue enabling elucidation of a possible physiological mechanism of development of NSAID-induced hypertension in patients – in-vivo gelation of profens in the blood vessels.

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