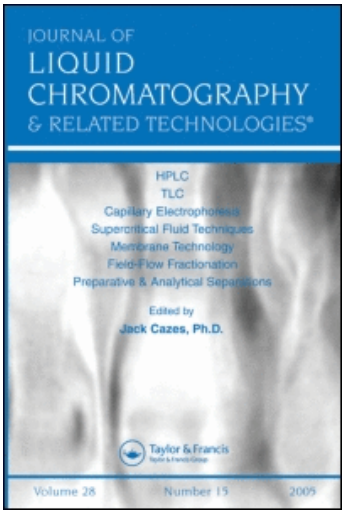

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Sajewicz, Mieczysław , Wrzalik, Roman , Gontarska, Monika , Kronenbach, Dorota , Leda, Marcin , Epstein, Irving R. and Kowalska, Teresa(2009) '*In vitro* Chiral Conversion, Phase Separation, and Wave Propagation in Aged Profen Solutions', Journal of Liquid Chromatography & Related Technologies, 32: 9, 1359 — 1372

To link to this Article: DOI: 10.1080/10826070902854920

URL: http://dx.doi.org/10.1080/10826070902854920

Journal of Liquid Chromatography & Related Technologies®, 32: 1359–1372, 2009

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070902854920

In vitro Chiral Conversion, Phase Separation, and Wave Propagation in Aged Profen Solutions

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Abstract: We have shown, earlier, that profens, i.e., 2-arylpropionic acids can undergo oscillatory *in vitro* chiral conversion, exhibiting oscillatory changes in the chromatographic retention parameter (R_F) and the specific rotation ($[\alpha]_D$) for periods of up to two weeks after preparation of the solutions in aqueous ethanol. Here, we examine the oscillatory chiral conversion of *S*-(+)-ibuprofen and *S,R*-(±)-ketoprofen dissolved in 70% aqueous ethanol and stored in tightly closed colorless glass vials for one year. During that time, each initially homogenous, transparent profen solution separates into two immiscible layers, one clear and the other turbid. Employing chiral thin layer chromatography, high performance liquid chromatography with diode-array detection, gas chromatography, and Raman spectroscopy, we find that both samples undergo chiral conversion to yield a strong predominance of the *R*-(−) antimer. Additionally, microscopy reveals characteristic spatial patterns in both layers of the demixed ibuprofen and ketoprofen solutions. We use a model proposed earlier for the homogeneous oscillatory interconversion of the *S* and *R* profens involving two linked templators to simulate microscopic spatial patterns analogous to those observed in the solutions investigated here.

Keywords: Chiral conversion, Enantioseparation of profens, Microscopy, Polarimetry, Raman spectroscopy, Simulation of chiral conversion, TLC

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INTRODUCTION

The remarkable phenomenon of spontaneous *in vitro* oscillatory chiral conversion of selected profens and amino acids has been the focus of our attention for some time. In papers,[1–3] we reported on the chiral conversion of *S*-(+)-ibuprofen, *S*-(+)-naproxen, *S,R*-(±)-2-phenylpropionic acid, *S*-(+)-flurbiprofen, *R*-(−)-flurbiprofen, and *S,R*-(±)-ketoprofen. Our investigations were carried out mainly by means of thin-layer chromatography (TLC) and polarimetry, and occasionally with the aid of such complementary measuring techniques as viscosimetry, high performance liquid chromatography (HPLC), and ^{1}H NMR spectrometry. In a paper,[4] we reported the analogous behavior of L-α-phenylalanine. There, we introduced a kinetic-diffusive model of the oscillatory chiral conversion, derived from the templator model of Peacock López and collaborators,[5,6] which can be applied to amino acids structurally derived from propionic acid as well as to the profens. All our investigations were carried out with dilute solutions (10^{-2}–10^{-3} M in 70% aqueous ethanol) for periods of several hours or several days, depending on the measuring technique employed. The ability of all investigated chiral compounds to undergo immediate and spontaneous *in vitro* chiral conversion has thus been reliably established.

By chance, two samples, *S*-(+)-ibuprofen and *S,R*-(±)-ketoprofen, dissolved in 70% aqueous ethanol in tightly stoppered vials, were stored in our laboratory at $22 \pm 2^{\circ}$C for one year. By means of HPLC/DAD, we confirmed that, in the course of this storage period, neither ibuprofen nor ketoprofen decomposed or transformed to another product in chromatographically measurable amounts. This result is in agreement with numerous claims in the pharmaceutical literature as to the long-term durability of profen solutions. This stability allows commercial forms of many profens to be sold not only as tablets, but also as ready-made injections, ointments, and syrups. However, in the course of the one-year storage period, the colorless, transparent, and initially homogenous solutions of *S*-(+)-ibuprofen and *S,R*-(±)-ketoprofen in 70% ethanol demixed to give two immiscible layers which form an opalescent emulsion upon shaking. The aim of this study is to determine the conversion yields after the one-year storage period and to expand our earlier model of the oscillatory chiral conversion of profens and amino acids[4] based on the new experimental data.

EXPERIMENTAL

Analytes

Samples of *S*-(+)-ibuprofen (Sigma–Aldrich, St Louis, MO, USA; cat. # I-106) and *S,R*-(±)-ketoprofen (Sigma–Aldrich; cat. # K1751) were

prepared in 70% aqueous ethanol at concentrations of 5 mg mL^{-1} (ca. 2.4×10^{-2} M) and 0.1 mg mL^{-1} (ca. 3.9×10^{-4} M), respectively. Both profens and ethanol were of analytical purity, and the water was double distilled and de-ionized. Solutions were stored on the laboratory shelf in tightly closed colorless glass vials at $22 \pm 2°C$ for one year.

Thin-Layer Chromatography (TLC)

We used chiral TLC with densitometric detection to quantify the proportions of the two ibuprofen and ketoprofen antimers in the upper and lower layers of the demixed solutions. The chromatographic conditions are described in detail in earlier papers.[1,2] As stationary phase, we used commercial glass plates precoated with silica gel (Merck KGaA, Darmstadt, Germany; cat. # 1.05715) and impregnated with L-arginine. As mobile phases, we used acetonitrile–methanol–water (5:1:1, v/v) for ibuprofen and acetonitrile–water (5:1, v/v) for ketoprofen. In both cases, the mobile phases were acidified with a few drops of glacial acetic acid to keep their pH below 4.8. 5-μL aliquots of the upper and lower layers of each profen solution were applied to the chromatographic plate, and then the chromatograms were developed.

The densitograms were acquired with a Desaga (Heidelberg, Germany) Model CD 60 densitometer equipped with Windows-compatible ProQuant software. Scanning of the chromatograms was carried out at wavelengths $\lambda = 210$ nm (ibuprofen) and 252 nm (ketoprofen). With each profen, 30 mm wide tracks were scanned in 1-mm intervals.

High Performance Liquid Chromatography with Diode Array Detection (HPLC/DAD)

HPLC/DAD analysis of the ibuprofen and ketoprofen solutions after one year of storage was carried out with a P580A LPG model liquid chromatograph, equipped with a Gina 50 model autosampler and a UVD340 V DAD model detector (Gynkotek/Dionex, Germering, Germany); column: RP-18 (LichroCART cartridge with LiChrospher 100, 5 μm; 250 mm, 4 mm i.d.), cat.# 1.50983.0001, Merck. As mobile phase, we used methanol–water (8:2, v/v; flow rate: 0.8 mL min^{-1}). The analyses were run in the isocratic mode.

Gas Chromatography (GC)

We used GC to assess the difference in ethanol concentrations for the mixed solvent (ethanol–water) in the upper and lower layers of the

ibuprofen solution. In the case of ketoprofen, the lower layer was too viscous to perform a quantitatively accurate comparison.

Experiments were performed with a Carlo Erba Instruments (Rodano, Milan, Italy) HRGC 5300 series capillary gas chromatograph with on-column injection and flame ionization detection (FID). Isothermal chromatography was performed at 80°C.

Analyses were run on a capillary column (Agilent Technologies, Palo Alto, CA, USA) 30 m long, internal diameter 0.32 mm, with DB-Wax stationary phase and a film thickness of 1 μm. Helium was used as the carrier gas at a flow rate of 0.80 mL min^{-1}. The analyzed samples were 0.1-μL aliquots of the upper and lower layers of the demixed ibuprofen solution. Acquisition and immediate processing of the chromatographic data were achieved by use of ChromCard (Thermo Scientific, Waltham, MA, USA) software.

Raman Spectroscopy

Raman spectra were recorded using a LabRam System spectrometer (Jobin Yvon Horiba, USA/France) at room temperature. Argon-ion laser light of 514.5 nm was used to excite the spectra. The power at the sample was below 5 mW. To eliminate the Rayleigh line, the scattered light was passed through a holographic notch filter with a cutoff at 200 cm^{-1}. The Raman spectra were recorded in zero degree geometry in the range 300–3800 cm^{-1} with a spectral resolution of 3.5 cm^{-1}. A collection time of 20 s and an accumulation of 4 scans were chosen. The monochromator was calibrated using a silicone plate (520.7 cm^{-1}).

Microscopy

In the microscopic studies, we used an Olympus BX51 model research polarizing microscope in non-polarizing mode. Microliter volumes of the upper and lower layers of the demixed ibuprofen and ketoprofen solutions were sandwiched between two flat optical windows. The gap between the windows was 0.16 mm, which was the thickness of the glass spacer placed between the windows. Snapshots were taken with a Nikon Coolpix 990 model digital camera, and movies were taken with a JVC GZ-MG26E digital camera. The microscopic magnifications were 100x and 500x. The temperature was 22°C.

RESULTS AND DISCUSSION

Visual inspection of the two vials, one containing a solution of ibuprofen and the other an analogous solution of ketoprofen (both solutions

prepared in 70% aqueous ethanol), showed that, in the course of one year, both originally homogenous, colorless, transparent liquids demixed to give two colorless, immiscible layers. With ibuprofen, the upper layer was turbid and the lower layer was transparent; with ketoprofen, the reverse effect was observed (i.e., the lower layer was turbid and the upper layer was clear). Shaking of the contents of each vial resulted in a colorless emulsion, which slowly settled to reconstitute the two separate liquid moieties.

We first analyzed the upper and lower layers in each vial by HPLC/DAD in a non-chiral chromatographic system. We wanted to establish whether the profens decomposed and/or transformed to other product(s) in the course of the storage period. The chromatograms and the accompanying UV spectra of the chromatographic bands confirmed the sole presence of ibuprofen and ketoprofen, respectively, in both layers, and they did not reveal any byproducts that might originate from decomposition, oxidation, or chemical condensation of the starting material (within the detection limits of our measuring technique). Thus, we assumed that the only process occurring within the profen samples in the course of the long storage period was the spontaneous *in vitro* chiral conversion.

In order to confirm this hypothesis, we enantioseparated the antimers contained in each layer by chiral TLC, using the working conditions described earlier.[1,2] The results obtained are summarized in Table 1 and in Fig. 1. In Fig. 1 a–d, we present four 3D densitograms from the upper and lower layers, respectively, of the ibuprofen and ketoprofen solutions.

The TLC results allowed us to estimate the relative concentrations of profens in the two layers, but it was impossible to measure the respective volumes of the two layers due to the small volumes of the samples and the tendency of the layers to mix to form an emulsion. We also roughly quantified the proportions of the two antimers in each layer based on the fact that, in the TLC systems, *R*-(−) profens migrate slower than their *S*-(+) counterparts.[7] The results given in Table 1 can be considered as semiquantitative only, basically due to the sticky, viscous nature of the samples, which made it difficult to sample them precisely.

With ibuprofen solution, it was established that the total concentration of profen (sum of *R*-(−) and *S*-(+)) in the upper layer was ca. 1.9 times higher than in the lower layer. With ketoprofen solution, the concentration in the lower layer was ca. 2.3 times richer in total ketoprofen than the top layer. The enantiomeric excess of *R*-(−)-ibuprofen in the upper layer was ca. 80%, and in the lower layer, 50%. The enantiomeric excess of *R*-(−)-ketoprofen in the upper layer was ca. 10%, and in the lower layer ca. 92%. In Table 1, quantitative proportions of the two profen antimers in each layer are presented as a percent contribution from

Table 1. Thin-layer chromatographic quantification of the upper and lower layers of one year old solutions of ibuprofen and ketoprofen in 70% aqueous ethanol

Profen	Layer	Unitless proportions of total profen per layer volume unit	R_F		Contents of the two antimers per layer [%]		Enantiomeric excess of *R*-(−) {ee}* (%)
			R-(−)	*S*-(+)	*R*-(−)	*S*-(+)	
Ibuprofen	Upper	1.9	0.74	0.88	90	10	80
	Lower	1.0	0.72	0.88	75	25	50
Ketoprofen	Upper	1.0	0.69	0.98	55	45	10
	Lower	2.3	0.68	0.98	96	4	92

*enantiomeric excess (ee) $= ((R-S)/(R+S)) \times 100\%$.

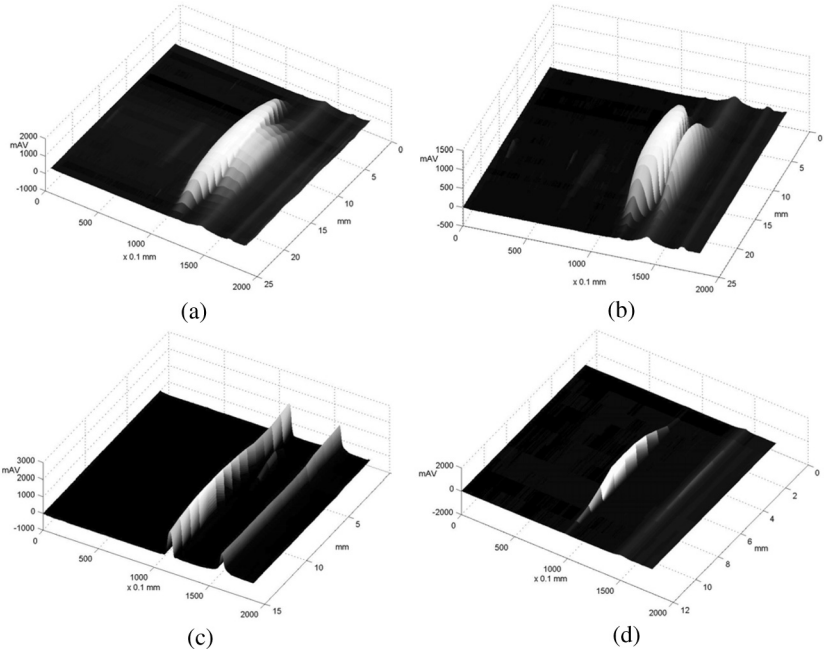


Figure 1. 3D densitogram of (a) the upper layer of the ibuprofen solution; (b) the lower layer of the ibuprofen solution; (c) the upper layer of the ketoprofen solution; and (d) the lower layer of the ketoprofen solution.

each antimer. The R_F values of the *S*-(+) and *R*-(−) antimers of the two profens coincide well with those reported in our earlier papers.[1,2]

By means of GC, we compared the ethanol concentrations of the upper and lower layers of the ibuprofen solution. Similar evaluation was impossible with ketoprofen, due to the high viscosity of the lower ketoprofen layer and the resultant plugging of the syringe. The ethanol concentration of the lower ibuprofen layer was ca. 1.6 times higher that of the upper layer (equivalently, the relative amount of water in the upper layer was higher than in the lower layer). We could only establish semiquantitatively that, with ketoprofen, the lower layer was richer in water. Although ibuprofen and ketoprofen are considerably more soluble in ethanol than in water, in the binary (EtOH-H_2O) solvent, we find that their concentration is higher in the layer richer in water. This surprising observation apparently has to do with hydrophilic interactions, which depend on the effectiveness of intermolecular solute–water hydrogen bonds.[8]

Thin-layer chromatographic and polarimetric evidence of the spontaneous oscillatory chiral conversion of *S*-(+)-ibuprofen and *S,R*-(±)-ketoprofen was presented in earlier papers.[1,2] In those studies, the

experimental results were collected in the course of several days (TLC) or several hours (polarimetry) after preparation of the fresh profen solutions in 70% aqueous ethanol. Our results, combined with data from the literature,[9] lead to the notion that the chiral conversion involves an enol tautomer as an intermediate. In an earlier article,[10] we demonstrated the gelation of profens, which appears to play a role in their oscillatory chiral conversion, by introducing the possibility of coupling differential diffusion to the kinetics.

We also examined each layer of the two demixed profen solutions with a microscope. In both layers of each individual solution, we noticed spatial patterns resembling those described earlier in the case of the other oscillatory reactions, e.g., in other published papers.[11,12] As examples, in Fig. 2 we present selected spatial patterns recorded in the upper ibuprofen layer, and in Fig. 3 those recorded in the upper and lower ketoprofen layers. The diameters of the circular patterns shown in these figures typically range from several dozen to over 100 μm. The layer with the lower concentration of a given profen generally showed considerably fewer

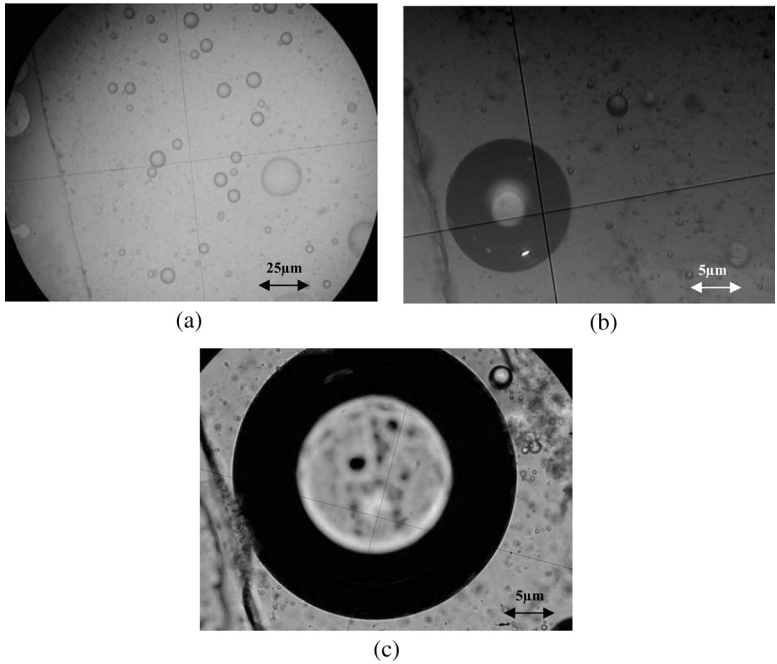


Figure 2. Patterns recorded in the upper layer of the ibuprofen solution: (a) multiple concentric patterns; (b) single concentric pattern with pure water constituting the ''white pupil''; (c) single concentric pattern with smaller microemulsion droplets visible in the ''white pupil''.

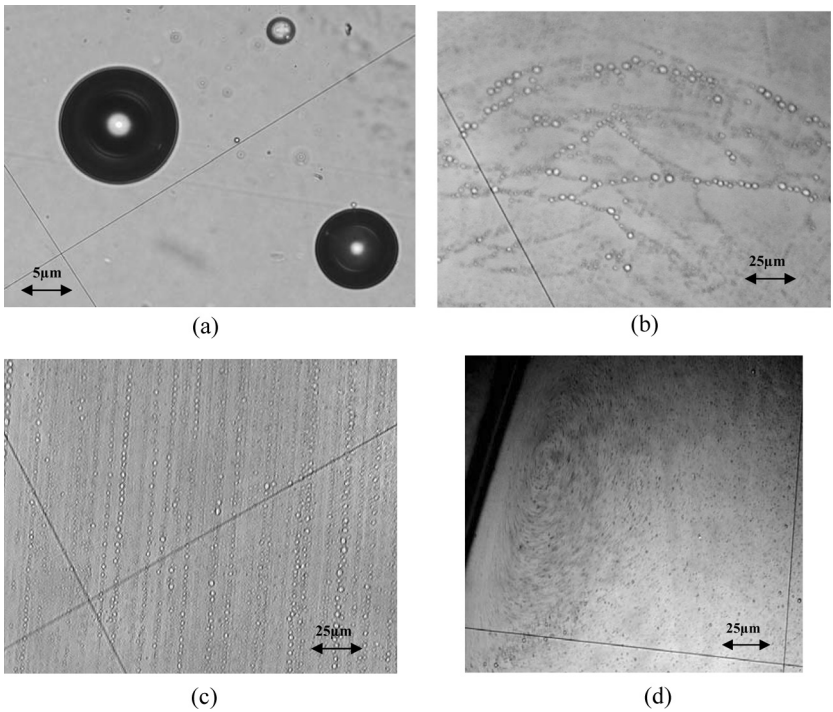
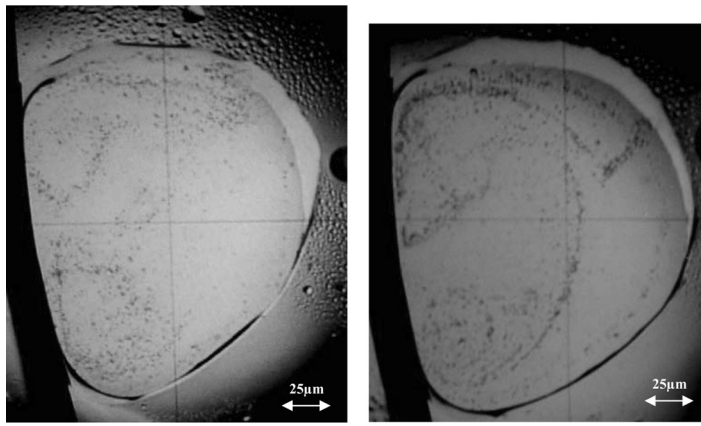


Figure 3. Patterns recorded in the upper and lower layers of ketoprofen solutions: (a) concentric patterns visible in the lower layer; (b) small white dots on a curved stripe in the upper layer; (c) small white dots on a straight stripe from the same layer; and (d) spiral pattern from the same layer.

spatial patterns than the layer with the higher concentration, but these differences were mainly quantitative.

Figure 4 contains two snapshots taken 10 s apart in the upper ibuprofen layer showing an antispiral wave, i.e., a spiral wave that moves in toward its core,[11] encapsulated in a larger droplet of microemulsion. The motion of the spatial patterns appears to result from chemical and diffusive processes rather than convection. The same behavior was observed in experiments using a water filter (a water layer ca. 0.5 cm thick in a plastic cell and placed under the sandwiched sample of the profen solution) to absorb any infrared radiation from the microscope lamp, thereby eliminating heating of the solution from below. The sandwich configuration prevents evaporative cooling of the solution from above, and the solutions are quite viscous. The typical speed of the patterns was ca. 0.25 cm min^{-1}.

We attempted to assess the chemical composition of the circular target patterns like those shown in Figs 2b and 3a. We used Raman



(a) (b)

Figure 4. An inwardly rotating antispiral wave encapsulated in a microemulsion drop from the upper layer of ibuprofen solution after (a) 0 s, and (b) 10 s.

spectroscopy to measure the chemical composition in the white inner circle, outside the concentric annulus, and also in the dark rim between these two moieties in Fig. 2b. The spectra obtained are shown in Fig. 5. The outside of the dark rim gave a well-shaped Raman spectrum composed of superposed signals originating from ethanol and ibuprofen.

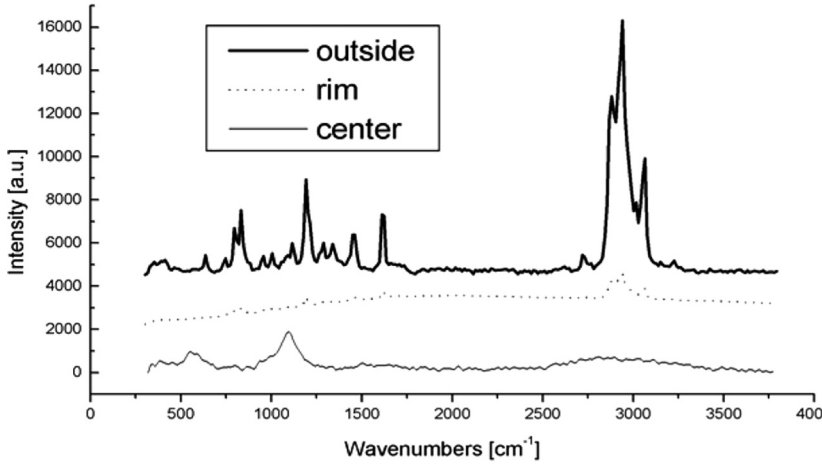


Figure 5. Raman spectra collected from the center (“white pupil”) of the concentric pattern shown in Fig. 2b, the dark rim of the pattern, and outside the pattern.

The contribution of ibuprofen to this spectrum is best documented by the presence of stretching vibrations of the carboxylic C=O group ($\nu_{C=O} \cong 1625\,cm^{-1}$) and the stretching vibrations of the aromatic C-H bonds ($\nu_{C\text{-}H} \cong 3100\,cm^{-1}$). The spectrum measured from the dark rim also revealed the presence of ethanol and ibuprofen although at much lower concentrations. The spectrum run from the white inner circle showed low Raman intensity, which suggests that, in the target center, one encounters either pure water (a very poor Raman scatterer), or a void. The void (possibly an air bubble captured in the sticky medium) seems unlikely in view of the many other targets with traces of ethanol and ibuprofen inside the "white pupil" as well (e.g., the target shown in Fig. 2c).

The combined TLC, Raman spectroscopic, and microscopic results suggest that, in the course of the spontaneous oscillatory *in vitro* chiral conversion, partial demixing of the ethanol–water binary solvent takes place, probably due to the density anisotropy caused by the gelation of the profens, which affects the molecular diffusion. As a result, a microemulsion is formed and the "ripest" emulsion droplets (like that shown in Fig. 2b) containing only water in the inner circle, while profen is dissolved in the more concentrated aqueous-ethanol solution outside the target.

Modeling

Our efforts to model this complex behavior are based on a simple model[5,6] introduced to describe oscillations in a self-replicating dimerization reaction. The core of this templator model consists of three reactions: inflow of monomer A (reaction 1) at rate $k_0 a_0$, where k_0 and a_0 represent the inflow rate and reservoir concentration, respectively; dimerization of A to form B, with another B molecule serving as a template (reaction 2) at rate $k_d a^2 b$; and removal of B at a surface (reaction 3) at rate $k_2 B/(B+K)$. Species are represented by capital letters, and their concentrations by small letters.

$$A_0 \rightarrow A \tag{1}$$

$$2A + B \rightarrow 2B \tag{2}$$

$$B \rightarrow P \tag{3}$$

In our system, A represents one antimer, say S-(+)-ibuprofen, and B represents its dimer, and we introduce a parallel set of reactions for the other antimer and its dimer, denoting those species as A′ and B′, respectively. We also introduce the enol-mediated enantiomerization

reaction (4) with first order rate constant k_e.

$$A \leftrightarrow A' \tag{4}$$

Here, Eq. (1) might represent transfer of monomers across the interface between the layers, and Eq. (3) might describe adsorption of dimers at either the interface or the glass surface. The model is clearly oversimplified and omits, for example, the possibility of mixed dimers, AA′, as well as many other aspects of the system. We can hope, at best, for some qualitative insight into the observed behavior.

If we introduce diffusion and assume that monomers diffuse more rapidly than dimers, we can derive a set of partial differential equations from Eqs. (1)–(4) and the counterparts of Eqs. (1)–(3) for the reactions of A′ and B′. These equations characterize the behavior of A, B, A′, and B′ in one layer of our model system. The fact that A and A′, B, and B′ are enantiomers requires that all primed rate constants be identical to their unprimed counterparts. However, a_0 and a_0' may differ, because the two layers have different enantiomeric compositions. The resulting equations are

$$\frac{\partial a}{\partial t} = k_0 a_0 - 2k_d a^2 b - k_e(a - a') + D\nabla^2 a \tag{5}$$

$$\frac{\partial b}{\partial t} = a^2 b + \frac{k_2 b}{b + K} + d\nabla^2 b \tag{6}$$

$$\frac{\partial a'}{\partial t} = k_0 a_0' - 2k_d a'^2 b' - k_e(a' - a) + D\nabla^2 a' \tag{7}$$

$$\frac{\partial b'}{\partial t} = k_d a'^2 b' + \frac{k_2 b'}{b' + K} + d\nabla^2 b' \tag{8}$$

where

$$\nabla^2 a = \frac{\partial^2 a}{\partial x^2} + \frac{\partial^2 a}{\partial y^2}$$

We choose zero flux boundary conditions and impose a small random fluctuation in A with the initial uniform concentrations of all varaibles given by $a(0) = 2$, $b(0) = 0.1$, $a'(0) = 0.01$, $b'(0) = 0.01$. Numerically integrating the equations with the parameters $k_0 a_0 = 1$, $k_0 a'_0 = 0.2$, $k_d = 1$, $k_2 = 1$, $K = 0.1$, $k_e = 0.1$, $d = 0.1$, $D = 1$, on a grid of size $L_x = L_y = 60$ yields the results shown in Figure 6. All lengths, times, and concentrations have been made dimensionless by appropriate normalization of the parameters.

A linear stability analysis of Eqs. (5)–(8) shows that, for the chosen ratio of diffusion constants $d/D = 0.1$, homogeneous oscillation is the stable state of the system, but if the intial perturbation is sufficiently

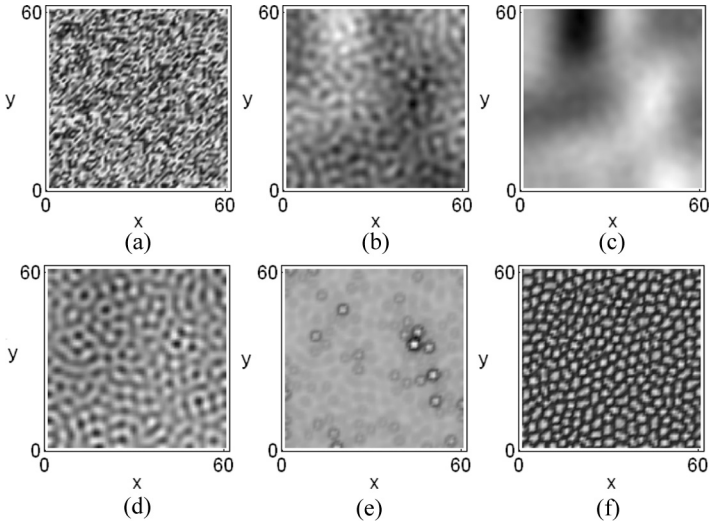


Figure 6. Spatial distribution of enantiomeric excess, $e.e. = (a+2a'-b-2b')/(a+2a'+b+2b')$, for t = 0 (a), 17.5 (b), 24.5 (c), 46 (d), 64 (e) and 100 (f). White corresponds to $e.e. = -1$, and black to 1, respectively.

large, as in Fig. 6a, a long spatially inhomogeneous transient arises (Fig. 6b–f), which shows a remarkable resemblance to some of the patterns seen in our experiments. While our model clearly has many limitations and is only a caricature of the actual system, the appearance of such complex patterns in even this simple model may provide some insight into the origin of the surprising behavior observed in profen solutions.

ACKNOWLEDGMENT

The authors thank Merck KGaA (Darmstadt, Germany) for supplying the TLC plates used in our experiments. I.R.E. and M.L were supported by U.S. National Science Foundation grant CHE-0615507.

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Received September 22, 2008
Accepted October 20, 2008
Manuscript 6469F