$Zn^{II}-OH^-$ species coexists and remains reactive, as we saw above.

The more acidic $Zn^{II}$ in $L_4$ ($pK_a(H_2O) = 7.3$) has ca. 25 times stronger affinity to the dianions than less acidic $Zn^{II}_{aq}$ ion ($pK_a(H_2O) = 9.0$) (see Table IV). The $K(A^-)$ valuesu of the phosphodiester monoanions $BPP^-$ and $BNP^-$ with our model are much smaller than those with the phosphomonoester dianions $PP^{2-}$ and $NP^{2-}$. This trend holds for the affinities with more basic $PP^{2-}$ ($pK_a = 5.8$) vs less basic $NP^{2-}$ ($pK_a = 5.1$). Previously, Sigel et al. reported a similar relationship between the phosphomonoester dianion affinity with $Zn^{II}_{aq}$ ion and phosphate ligand basicity.[22] In addition, the present results in phosphate anion complexation using our model have first disclosed that $Zn^{II}$ acidity is also a significant factor determining the binding stability between $Zn^{II}$ and phosphate anion.

## Conclusion

Zinc(II) complex **4** of a 12-membered macrocyclic triamine ([12]ane$N_3$, $L_4$) has been shown to be the most suitable (structurally and kinetically) model for the zinc enzyme that catalyzes phosphate ester hydrolysis. We have found that the nucleophilic power of the $Zn^{II}-OH^-$ species from **4** effects phosphotriester and phosphodiester hydrolysis. The kinetic studies on the hydrolysis of phosphoesters and carboxyester by our model complex **4** have clearly disclosed that the $Zn^{II}$ in the rigid triamine ligand acts as a hybrid-type catalyst. As shown in phosphate ester hydrolysis promoted by other metal (e.g., $Co^{III}$, $Ir^{III}$, $Mg^{II}$) complexes, the $Zn^{II}$ bound to the phosphorus oxygen atoms may also assist P–O bond rupture as a leaving group effect. The strongest affinity of **4** to $OH^-$ ion surpasses any other anions including the phosphodiester substrate or the hydrolysis products phosphomonoester dianion and phosphodiester monoanion. The former fact allows the hybrid mechanism, and the latter fact works favorably in the catalytic cycles. For the alkaline phosphatase **5** which has two $Zn^{II}$ ions around different environments, it is conceivable that one $Zn^{II}$ ion captures the phosphate ester or the phosphorylated (to serine) equivalent, and another one being $Zn^{II}-OH^-$ species to assist their hydrolysis to make a more efficient hybrid catalyst. Finally, further structural modification of the basic structure of **4** (e.g., attachment of an intramolecular hydroxy group for the phosphate binding site or a guanidyl group for substrate recognition) is likely to yield even closer alkaline phosphatase and other $Zn^{II}$-containing hydrolytic enzyme models, which is currently under investigation in our laboratory.

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# *Communications to the Editor*

## Structured Self-Aggregates of 4-Methoxy-(*E*)-cinnamic Acid at the Air/Solution Interface As Detected by 2π + 2π Photodimerization and Their Role in the Control of Crystal Polymorphism

I. Weissbuch, L. Leiserowitz, and M. Lahav*

*Department of Materials and Interfaces*
*The Weizmann Institute of Science, Rehovot 76100, Israel*

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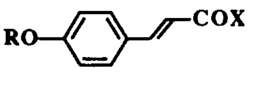
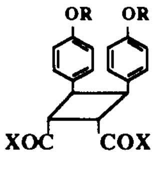
Self-organization of molecules into structured clusters plays an important role in the early stages of crystal nucleation and growth. The different molecular arrangements within such clusters may lead, at the onset of crystallization, to precipitation of crystal polymorphs.[1-3] In the absence of analytical tools to probe the structure of such clusters in solution or in the melt, we have still to rely, for a while yet, on indirect methods. Here we describe the detection of structured self-aggregates of 4-methoxy-(*E*)-cinnamic acid (**1**) at the air/solution interface by topochemical photodimerization and the possible function of such aggregates for inducing the precipitation of a new crystalline polymorph.





**1** $R=CH_3$ $X=OH$
**3** $R=CH_3$ $X=NH_2$
**4** $R=C_{18}H_{37}$ $X=OH$
**5** $R=C_{18}H_{37}$ $X=NH_2$
**2** $R=CH_3$ $X=OH$
**6** $R=C_{18}H_{37}$ $X=OH$

Surface tension and optical second harmonic generation measurements of aqueous solutions of **1** reveal that the solute molecules are sufficiently hydrophobic to accumulate at the solution surface,[4] with an estimated coverage of ~30%. These studies, however, do not provide information on the structure of the surface aggregates. Structural considerations, based on packing modes of molecules in crystals,[5] suggest that molecules of **1** should form, at the air/solution interface, close-packed stacks of "translationally" related molecules, separated by 4 Å, stabilized by aromatic ring interactions and Coulombic forces between the carboxyl groups. This organization should influence the photochemical behavior of these molecules as was established from photoreactivity in the crystalline state.[6] Thus while **1** undergoes trans/cis photoisomerization in bulk solution, as do all (*E*)-cinnamic acids, the self-organized molecules at the interface are expected to undergo a 2π + 2π photodimerization leading to the mirror-symmetric photodimer, 4,4′-dimethoxy-β-truxinic acid (**2**).

In order to differentiate between products formed in the bulk of the solution and at the interface, aqueous solutions of **1** ($4.0-5.6 \times 10^{-4}$ M) were irradiated ($\lambda > 320$ nm) in two different experimental setups: closed glass vessels completely filled with solution and open glass vessels with a large solution/air interface (Figure 1B,A). Irradiation of the solution in the closed vessel resulted[7] in a trans:cis ratio of about 1:3 (Figure 1b), whereas the open vessel yielded, in addition, formation of 5–20% of the photodimer **2**, depending upon the exposure time (Figure 1a). The molecular organization at the surface could be gradually removed by addition of EtOH (35%) to the solution, as made manifest by the formation of only traces of the photodimer **2**.

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[7] The photoproducts were assigned by reversed-phase HPLC analysis against synthetic standards.

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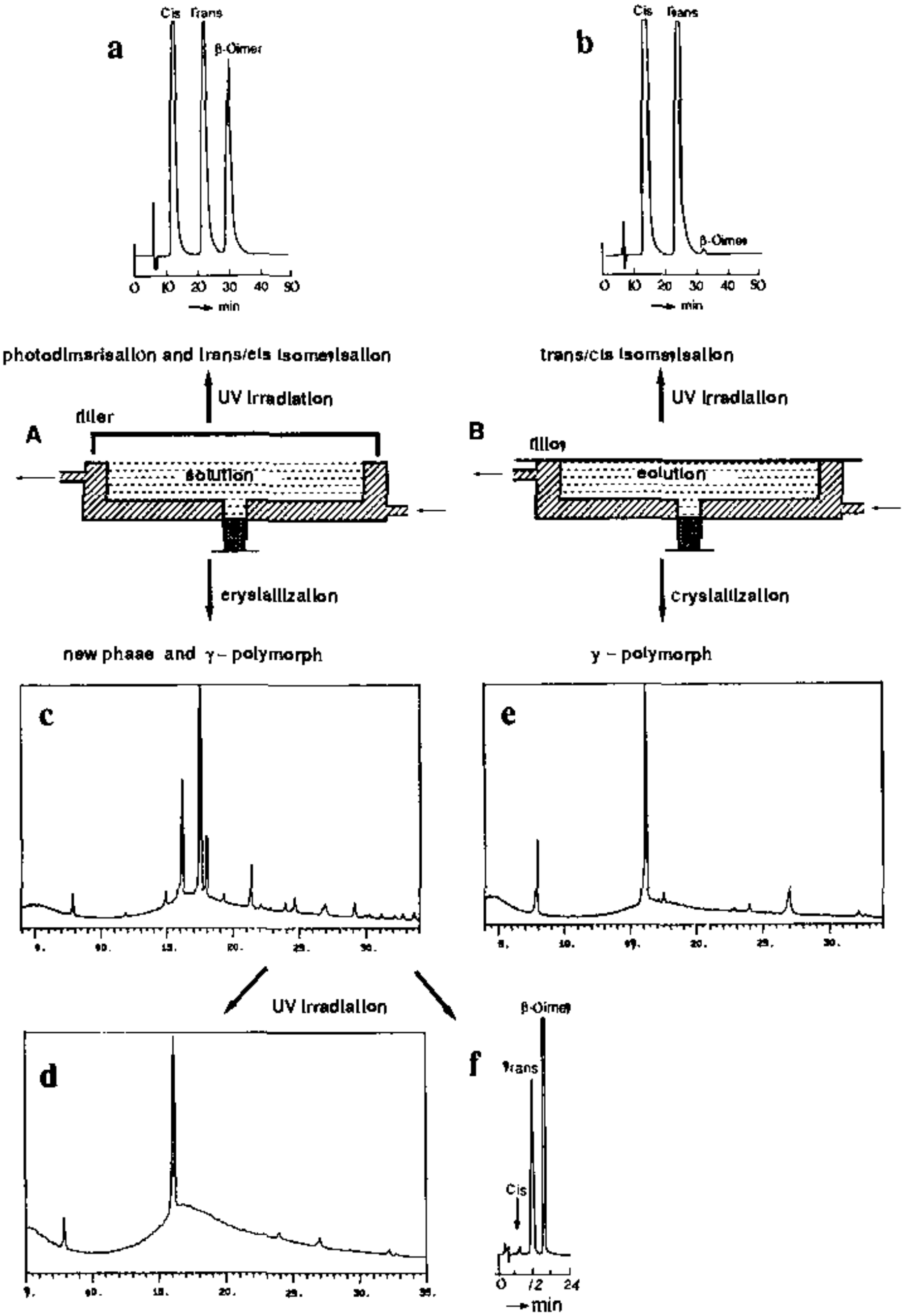

Figure 1. Schematic drawing of the two experimental setups: (A) open vessel; (B) closed vessel; diameter 10–14 cm, depth 2 cm, Pyrex filter, and water circulation at 5 °C from a thermostat. HPLC analyses of the solutions irradiated in the two types of vessels: separation conditions, column I RP-18, 5μ, 25 cm; mobile phase, 43% methanol and 57% 0.05 M $KH_2PO_4$; flow, 0.5 mL/min; UV detection at 235 nm. (a) Sample irradiated in the open vessel. (b) Sample from the closed vessel. X-ray powder patterns of the crystalline materials: (c) sample precipitated in the open vessel; (d) sample precipitated in the open vessel after solid-state irradiation; (e) sample precipitated in the closed vessel. (f) HPLC analysis of the sample as in part d: separation conditions, column II RP-18, 5μ, 10 cm; mobile phase, 30% acetonitrile and 70% 0.05 M $KH_2PO_4$; flow, 0.5 mL/min; UV detection at 235 nm.

A similar structural analysis of the 4-methoxy-(*E*)-cinnamamide (**3**) implies that it should organize, at the interface, into two-dimensional clusters within which the molecules form close-packed stacks interlinked by N—H⋯O=C hydrogen bonds.[8] Because the hydrogen-bonding repeat distance is about 5 Å, the stack repeat cannot be less than about 5.5 Å. In this arrangement the C=C double bonds will be separated by too large a distance to allow photodimerization.[6] Indeed, irradiation of aqueous solutions of **3** in the two experimental setups yielded only trans/cis isomerization, in a ratio of about 1:3.5.

Similar photochemical behavior was observed with the use of the insoluble amphiphilic analogues[9] **4** and **5** spread on a water subphase in a Langmuir trough, either in the compressed (10 mN/m) or in the uncompressed state with a surface coverage of 50–80%. Here again, while the acid yielded about 10% of the mirror dimer **6** and ~16% of the cis isomer, the amide monolayer underwent only a trans/cis isomerization in both the compressed and uncompressed states.

In order to probe the role played, if any, by the surface-ordered self-aggregates of **1** at the interface in promoting the formation

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of new crystalline forms, this material was precipitated from aqueous solutions, by cooling, in the two experimental setups described above. X-ray powder patterns of the crystalline materials are shown in Figure 1c–e. The crystallization experiments in the open vessel yielded a new crystalline phase, which precipitated together with the known phase[10] previously defined as the $\gamma$-form[6] (Figure 1c). Such samples display four new and distinct diffraction peaks with *d* spacings of 6.0, 5.1, 5.0, and 4.2 Å.

Solid-state UV irradiation of the mixture of the crystalline phases yielded the mirror photodimer **2** (Figure 1f). This dimer must arise from the new crystalline phase because the $\gamma$-form is light stable and the diffraction peaks associated with the new phase disappear upon irradiation[11] (Figure 1d).

From the experiments performed in the closed vessels, the $\gamma$-phase precipitates, either in pure form (50% of experiments) or with only small amounts of the new phase (Figure 1e).

The formation of the mirror-symmetric $\beta$-dimer **2** from irradiation of the solutions in the open vessel and from the solid precipitated in the same vessel strongly suggests the formation of ordered two-dimensional aggregates at the air/solution interface. Furthermore, the common feature of the mirror symmetry of the dimer **2** implies that the molecules are stacked by "translation symmetry" within the clusters at the interface and in the new polymorph. Thus it is reasonable to deduce that the clusters are nuclei en route to crystal formation. Observation of these clusters by more direct methods, such as grazing incidence X-ray diffraction,[12] might provide a deeper insight into crystalline nuclei of precritical size.

**Acknowledgment.** We thank the U.S.–Israel Binational Science Foundation, Jerusalem, and the donors of the Petroleum Research Fund, administered by the American Chemical Society, for financial support.

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## Ice Nucleation: A Test To Probe the Packing of Amphiphilic Alcohols at the Oil–Water Interface

R. Popovitz-Biro,* M. Lahav,* and L. Leiserowitz*

*Department of Materials and Interfaces*
*The Weizmann Institute of Science, Rehovot 76100, Israel*

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*Revised Manuscript Received August 20, 1991*

Amphiphilic molecules tend to accumulate in the form of monolayers at the interface between water and an immiscible liquid. Such monolayers have been extensively studied by classical techniques, such as interfacial tension and surface potential measurements.[1-4] However, experimental work probing structural details on the molecular level is very limited[5,6] although some theoretical simulations of the liquid–liquid interface have been reported.[7-9]

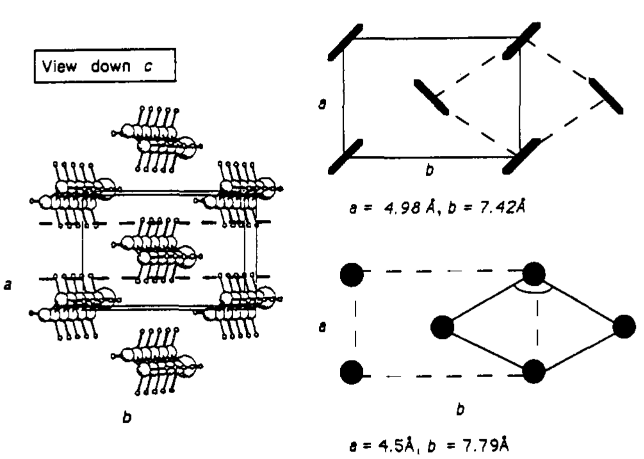

**Figure 1.** Packing arrangement viewed perpendicularly to the water surface of models of alcohol monolayers in crystallites formed at 5 °C over pure water (left). Schematic representation of the orthogonal packing of the hydrocarbon chains (right top). Schematic representation of the *ab* lattice of hexagonal ice (right bottom).

We have demonstrated previously that monolayers of insoluble alcohols, when deposited on water drops at the air–water interface, act as efficient ice nucleators.[10] Grazing incidence X-ray diffraction measurements have shown that at temperatures below 9 °C these alcohols self-aggregate to form two-dimensional crystalline domains whose *ab* net is close in dimension to the *ab* lattice of hexagonal ice (Figure 1). Moreover, the analysis of the grazing incidence diffraction data indicates not only a good lattice match but also a reasonable structural fit of the alcohol OH moieties to that of the ice layer.[11] Such domains may induce the formation of ice-like embryos at the interface, leading to efficient nucleation. Freezing temperatures were strikingly sensitive to minor structural changes in the amphiphilic alcohols, such as chain length, carbon parity, and surface area per molecule.

In the present study we exploit the structural match between monolayer and ice to probe the oil–water interface on a molecular level.[12] Experiments were carried out by measuring the freezing points of water drops placed in organic solutions of long-chain aliphatic alcohols. As references, the solutions of the corresponding carboxylic acids or the pure solvents have been used,

Figure 2a shows that aliphatic alcohols $C_nH_{2n+1}OH$ with $n > 22$ nucleate ice at higher temperatures than the corresponding carboxylic acids, as was found at the air–water interface. Moreover, temperatures of freezing were observed to depend on alcohol chain length and carbon parity. The largest difference, of about 6 °C, was obtained between the freezing points induced by alcohols with $n = 30$ and $n = 31$. This experiment strongly suggests that the amphiphilic alcohols aggregate at the oil–water interface into crystalline clusters. The results also indicate that surfaces or templates created by aggregates of the odd-numbered alcohols have a better structural fit with ice than the even-numbered homologues, possibly due to somewhat different orientations of their OH groups. The fact that ice nucleation at the oil–water interface was observed with alcohols having a chain length of $n > 22$, whereas at the air–water interface it required only $n > 16$,

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(12) In contrast to our structural approach, J. Rosinski (*J. Phys. Chem.* **1980**, *84*, 1829) proposed that heterogeneous ice nucleation can take place at the liquid–liquid interface as a result of ordering by dipole moments of the organic liquid.

(13) All materials, except alcohols with $n = 23$, 29, and 31, were purchased from Sigma and were specified >98% pure. Alcohols with $n = 23$, 29, and 31 were synthesized by reducing the corresponding methyl ester with $LiAlH_4$ and purified by column chromatography on silica gel, using methylene chloride as eluent. A Mili-Q water purification system was used to get high-resistivity water.

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