Self-assembly of chlorophenols in water

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ABSTRACT In saturated solutions of some di- and trichlorophenols, structures with complex morphologies, consisting of thin, transparent sheets often coiling into helices and ultimately twisting into filaments, were observed under the optical microscope. Freeze-fracture electron microscopy, x-ray diffraction, phase diagrams, and molecular modeling were performed to elucidate the observed phenomena. Here, we present evidence that the chlorophenols studied, when interacting with water, self-assemble into bilayers. The fact that some chlorophenols form the same supramolecular structures as those described previously for structurally nonrelated surfactants sheds light on the mechanisms of self-assembly.

Chlorophenols are ubiquitous molecules of both practical and fundamental interest (1). Their widespread formation and their use in the forest industry and as disinfectants make them important from an environmental point of view. By investigating the water phase diagrams of various chlorophenols, we observed that in saturated solutions, depending on the number and the position of the chlorine atoms in the molecule, they formed either rigid, brittle crystals (e.g., 2,6-dichlorophenol) or a voluminous, cotton-like matter (e.g., 2,4-di- and 2,4,6-trichlorophenol). Transmission optical microscopy revealed that the structures obtained in the latter case consisted of thin (from 1 μm to several μm), long (hundreds of μm to several cm) filaments, which were remarkably flexible (Fig. 1*A*). These structures remain perfectly stable for at least 1 year at room temperature if kept in the initial aqueous solution, but form crystals when dried. To elucidate the mechanism of formation of the filaments, a hot, saturated solution of 2,4,6-trichlorophenol was examined on cooling, by using an optical microscope with polarized light. In the initially black, isotropic solution, the individual, bright spots of an anisotropic phase appeared first. On the edges of the latter structures, thin, transparent sheets formed rapidly, propagating outwardly and twisting into filaments (Fig. 1*B*). Those among the sheets that formed long ribbons (Fig. 1*C*) writhed into helices (Fig. 1 *D* and *E*). Some helices formed closed tubules, depending on their pitch. In several cases, a rapid transformation of helices into filaments was observed, after the ribbon had twisted into a tight rope at the end of the helix. This dramatic movement propagated along the helix, leading to the disappearance of its turns. The nascent filaments (Fig. 1*F*) spontaneously adopted various irregular as well as regular, coiled spatial arrangements. The low-pitch coils formed tubules (Fig. 1 *G* and *H*), as occurred previously in the case of helices. The sequence of these events is summarized schematically in Fig. 2.

The above structures are surprising, given the nature of the molecular building blocks. Twisted strips, helical ropes, helical ribbons, and tubules have been observed in a variety of systems composed of synthetic and natural, usually chiral, surfactants

self-assembling into bilayers (2–19). Chlorophenols, however, have no molecular features in common with any of these surfactants, so questions arise concerning the structure of the aggregates they form and the nature of the interactions leading to aggregation (20).

Freeze-fracture electron microscopy of the structures formed by 2,4,6-trichlorophenol in bulk water confirmed the presence of long, mainly longitudinally fractured filaments like those shown in Fig. 3*A*. These filaments consist of periodically stacked, smooth sheets (Fig. 3*B*), bearing a strong resemblance with those currently observed in the fracture of lamellar lipid-water phases (21) and suggesting a bilayer arrangement of 2,4,6-trichlorophenol molecules. The repeating distance between the sheets, as measured by optical diffraction on the filaments fractured in the direction perpendicular to the stacked sheets (Fig. 3*C*), is of the order of 50 Å. This relatively large value, compared with the calculated 18-Å thickness of a 2,4,6-trichlorophenol bilayer suggests that if the structure of filaments involves lamellar stacking of bilayers, the latter are separated by fairly thick water layers. The fact that the fracture planes are smooth indicates that they propagate through these water layers. The possibility cannot be ruled out, however, that two-dimensionally ordered 2,4,6-trichlorophenol bilayers with rather small parameters could be involved, which might have escaped detection after platinum shadowing.

X-ray scattering experiments were carried out on filaments, as well as on dry 2,4,6-trichlorophenol, by using a focusing Guinier camera (Beaudoin, Paris) with monochromatic CuK$_\alpha$ radiation and linear collimation. Although the scattered intensity was relatively weak, possibly because of the high absorption of the chlorine atoms, numerous reflections in the scattering patterns of the initial, dry compound and the filaments were observed in the wide-angle region from about 1/8 Å, indicating a crystalline molecular order in both cases. With the filaments, however, three reflections, absent in the dry 2,4,6-trichlorophenol, were detected at about 1/18, 1/13, and 1/11 Å. These reflections can be assigned to the 3, 4, 5 order of a lamellar repeat distance of 53.5 Å, a value close to that observed after freeze fracturing. At the present time, there is no obvious explanation accounting for the absence of the low-angle first- and second-order reflections.

The above findings called for some thermodynamic clarifications concerning the behavior of 2,4,6-trichlorophenol in water. This goal was achieved by performing phase-equilibrium experiments. Liquid-liquid equilibrium is observed at high temperatures, down to the triple monotectic point, i.e., T = 334.2 K. At this temperature solid chlorophenol appears and remains in equilibrium with the liquid phase up to the triple eutectic point, i.e., T = 272.8 K. The phenomena described in this paper occurred, however, between the two triple point temperatures in a metastable, supersaturated solution. The first anisotropic phase (i.e., the bright spots
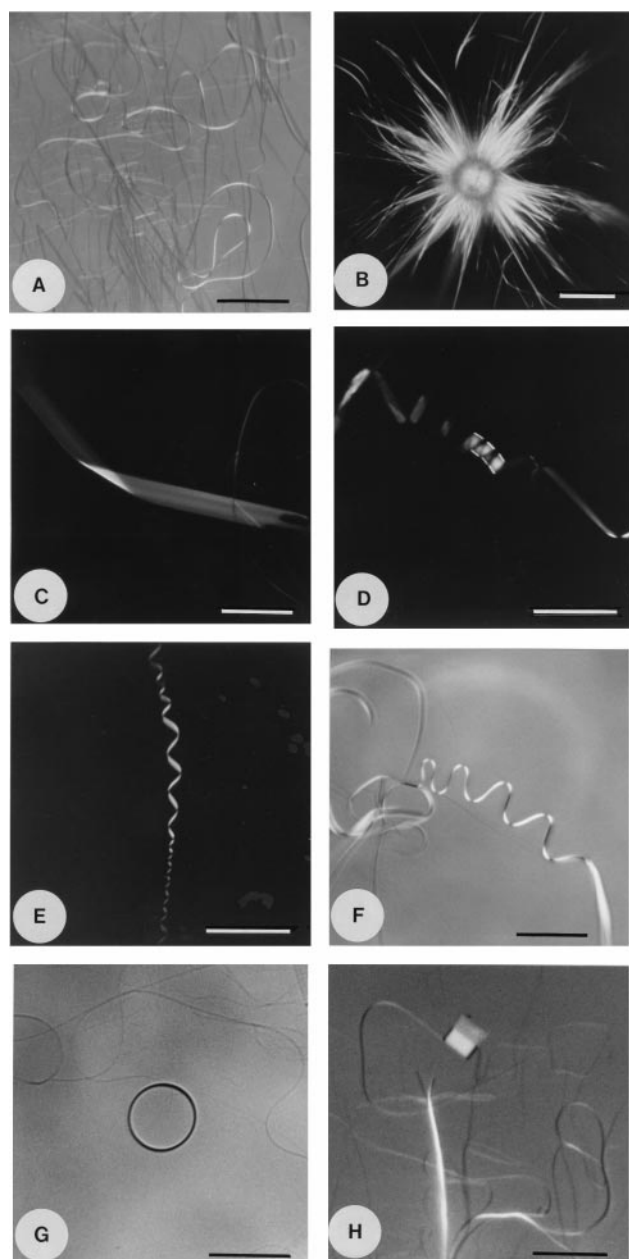

FIG. 1. Optical microscopy images (*A–F* and *H*: polarized light) of structures observed in a saturated 2,4,6-trichlorophenol-water solution. The solutions were prepared by heating an excess quantity of 2,4,6-trichlorophenol in water to about 80°C, followed by vigorous shaking and cooling to room temperature. The snapshots were taken at room temperature. (*A*) Filaments obtained in a vial and transferred onto the microscope slide (scale bar, 40 μm). (*B–H*) Structures obtained on the microscope slide in a drop of the initially isotropic solution. (*B*) Sheets and filaments forming on the edges of a disk-shaped, anisotropic phase (scale bar, 20 μm). (*C*) Winding of a ribbon-shaped sheet (scale bar, 40 μm). (*D*) A ribbon writhed into a three-turn, low-pitch helix (scale bar, 30 μm). (*E*) A multiturn, high-pitch helix (scale bar, 30 μm). (*F*) A ribbon twisting into a filament (scale bar, 40 μm). (*G*) A filament coiling into a tubule, viewed along its long axis (scale bar, 30 μm). (*H*) A side view of a nascent tubule (scale bar, 20 μm).
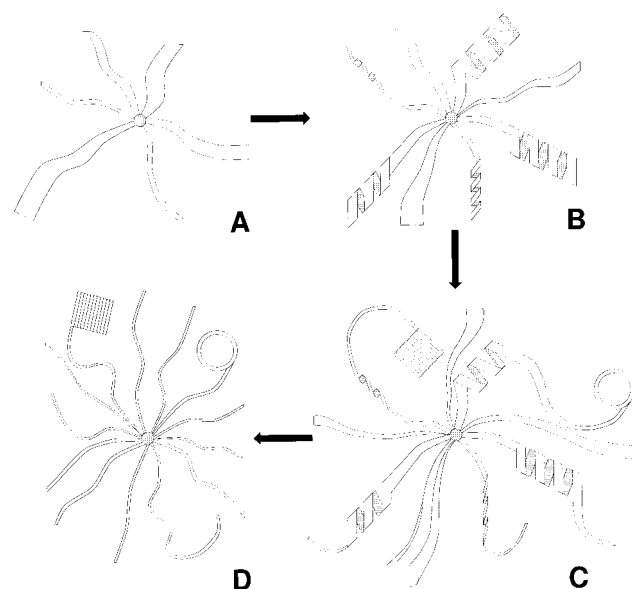

FIG. 2. Schematic representation of the formation of filaments. (*A*) The first nucleus of an anisotropic phase and the thin sheets growing outwardly. (*B*) The narrow sheets (ribbons) writhing into helices. (*C*) Subsequent, tighter twisting of the ribbons into filaments, which coil eventually into tubules; wider sheets rolling up into filaments. (*D*) The final filaments.

observed with the optical microscope), which developed in the undercooled solution probably as the result of the nucleation of a chlorophenol-rich phase (82 mole % of chlorophenol at the monotectic temperature), triggered the formation of the liquid-crystalline structures described above. An explanation for this phenomenon should be, nevertheless, proposed, be-

cause in this temperature range, crystals can be expected to form under equilibrium conditions. Our explanation is based on the entropy/enthalpy balance. The entropy of the formation of the orientational hydrogen bonding between chlorophenol and water is large and negative, because of the bulky chlorine atoms directly adjacent to the hydroxyl group. The formation of such hydrogen bonds, which is one of the key factors in the self-assembly of water/chlorophenol bilayers, nevertheless may be achieved by overcoming the entropy penalty. The latter becomes possible on cooling, thanks to the increase of the enthalpy of the hydrogen bond formation.

To get an insight into the atomic-level details of a water/2,4,6-trichlorophenol bilayer arrangement, molecular modeling involving large-scale statistical simulations was carried out. The model system consisted of 110 molecules of trichlorophenol arranged in two leaflets, between two lamellae of 1,147 TIP4P (22) water molecules. Each lamella was in equilibrium with its low-density vapor phase. A hybrid Monte Carlo (HMC) approach (23, 24), implemented in the COSMOS (25) program, was used to simulate the system in the (N, P$_\perp$, γ, T) ensemble. Our simulations showed that, after thorough equilibration, and within the time-scale explored of 7,000 HMC passes, the system remained lamellar (Fig. 4), which can be partly explained by the large molecular mechanics association energy of -4.7 kcal/mol for the stacked arrangement of two chlorophenol molecules, compared with the basis set superposition error-corrected MP2/6–31+G* association energy of -3.7 kcal/mol. The free energy profile for the transfer of one 2,4,6-trichlorophenol from the gas phase to the bulk water, derived from a separate 20.8 ns (N, E, V) molecular dynamics simulation, gave a free energy of hydration of -2.7 kcal/mol, compared with our experimental estimate of -4.5 kcal/mol, and a free energy of adsorption of -3.2 kcal/mol, which is roughly that of phenol (26). Probability distribution functions computed over the ensemble of configurations indicated that, on average, all the rings remain parallel to each other, exposing their hydroxyl moiety toward the aqueous phase. Our results further show that the rings are slightly tilted with respect to the normal to the interface, which suggests that a parallel-displaced arrangement rather than a stacked one is involved,
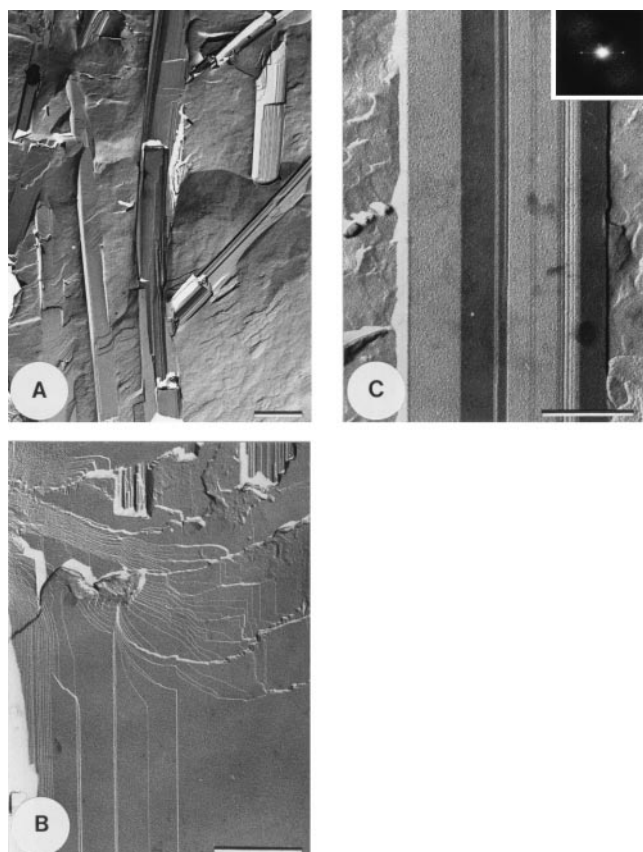
FIG. 3. Freeze-fracture electron microscopy of the filaments. A drop of the sample, about 20–50 μm thick, was deposited onto a thin copper holder, and the holder was either directly quenched in liquid propane, or the sample was first squeezed between the holder and a thin copper plate and then quenched in liquid propane. Both types of preparations were fractured *in vacuo* (about 10^{-7} torr) with the liquid nitrogen-cooled knife inside a Balzers 301 freeze-etching unit. The squeezed preparations were fractured by removing the upper plate with the cold knife. The replication was carried on by using the unidirectional shadowing with platinum carbon at an angle of 35°. The mean thickness of the metal deposit was 1–1.5 nm. The replicas were washed with ethanol and distilled water and then observed in a Philips EM 410 electron microscope. The contrast of the images depended on the fluctuations in the depth of the metal deposit. (*A*) Low magnification freeze-fracture electron micrograph of 2,4,6-trichlorophenol/water assemblies. Note the presence of very long filaments displaying well-defined longitudinal fracture planes (scale bar, 1 μm). (*B*) Higher magnification image of freeze-fractured 2,4,6-trichlorophenol/water filament showing periodically ordered, smooth lamellae (scale bar, 0.5 μm). (*C*) Part of a long filament fractured perpendicularly to the stacked lamellae. The optical diffraction inset shows the interlamellar spacing of 50 Å (scale bar, 0.25 μm).

as previously described in the case of simple aromatic molecules (27). The fact that the reorientational correlation times of one 2,4,6-trichlorophenol in bulk water were *ca.* 50 ps indicates that this molecule reorients itself very slowly, i.e., approximately 17 times more slowly than benzene. These reorientation times are expected to be even longer in the bilayer.

In the light of the results presented here, it can be inferred that small, flat, perfectly rigid, aromatic molecules of 2,4,6-trichlorophenol can assemble spontaneously into flexible bilayers on contact with water. Whereas aromatic π-π interactions stabilize each leaflet, the interplay of hydrophobic and hydrophilic interactions, analogous to those present in lipid bilayers, is envisioned to be responsible for the orientation of hydroxyl groups toward the water layer and for the segregation of the hydrophobic region of the molecule from the solvent,
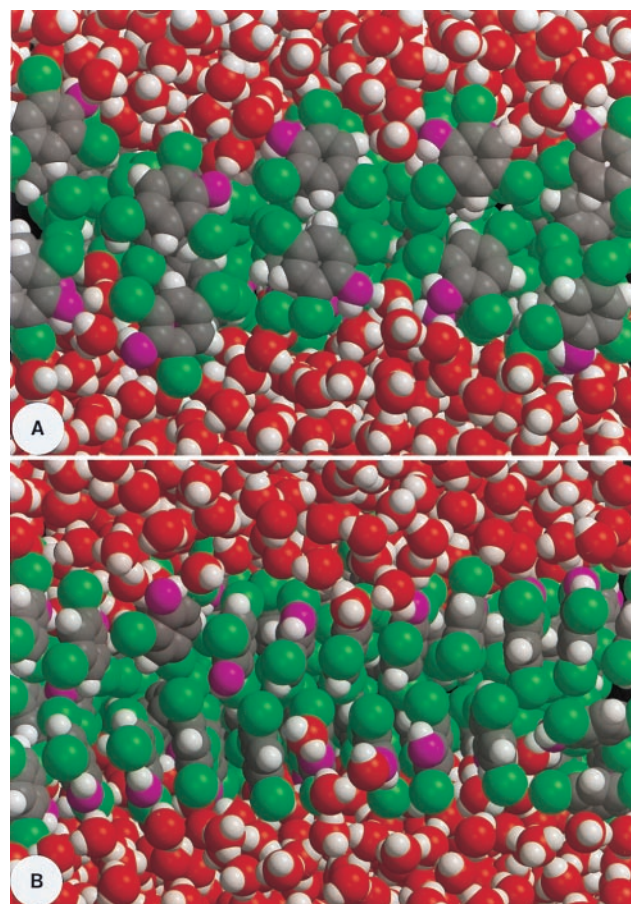

FIG. 4. Front (*A*) and side (*B*) views of a 2,4,6-trichlorophenol/water bilayer system, in the directions Oy and Ox parallel to the interface, respectively, obtained from an HMC simulation in the (N, P$_\perp$, γ, T) ensemble. The normal pressure to the interface, P$_\perp$, the surface tension, γ, and the temperature, T, were set at 1 atm, experimental value of 48.4 mN/m, and 297.1 K, respectively. At thermodynamic equilibrium, the molecular area of 2,4,6-trichlorophenol in the x, y-plane was *ca.* 36.5 Å^2. Each HMC pass corresponded to 250 molecular dynamics steps, by using a 1.5-fs time-step. The system was equilibrated for 10,500 HMC steps. The parameters of the effective potential energy function were taken from the AMBER all-atom force field (31), except for the chlorine atoms, the van der Waals parameters of which were recalibrated from state-of-the-art quantum chemical dimerization energies and experimental free energies of hydration—R$^*_{Cl}$ = 1.953 Å and ε_{Cl} = 0.350 kcal/mol. Non-bonded interactions between small, electrically neutral groups of the chlorophenols and water were smoothly truncated beyond 9.0 Å. Color code: carbon, gray; 2,4,6-trichlorophenol oxygen, magenta; water oxygen, red; chlorine, green; and hydrogen, white.

hence for the bilayer formation. Because not all isomers of the di- and trichlorophenols form flexible filaments, the question still remains what structural features of chlorophenols are critical for self-assembly. Concerning the morphology of the aggregates observed under microscope, our results showed that molecular chirality is not a prerequisite for the coiling of macroscopic structures (28–30). The driving force involved in this phenomenon can be ascribed to the anisotropy of chlorophenol organization in the Ox and Oy directions (Fig. 3), together with the tilting (27) of the monomers. In all, this work demonstrates that the choice of the structural parameters conditioning self-assembly is probably more complex than hitherto believed.

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