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## Magnetic Orientational Effects during the Aggregation Kinetics of a Steroid/Cyclohexane Gelling System

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Magnetic fields are used to study orientation effects on the aggregation of a small amphiphilic steroid derivative in cyclohexane. When a magnetic field is applied to the first steps of steroid aggregation, it can give an unstable gel phase which separates into collapsed oriented filaments. The degree of alignment of filaments is evaluated from both optical microscopy and X-ray diffraction experiments. It is shown that only the initial steroid aggregates can act as oriented growth precursors for the filamentary steroid gel network growth.

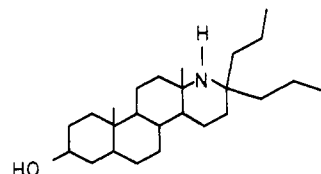
### Introduction

Gels are heterogeneous systems composed of a solute-rich solidlike three-dimensional network bicontinuous with a solvent-rich fluid phase. The dilute system shows a viscoelastic behavior which sets up via a critical transition. Properties below and just above the gelation threshold are respectively that of large clusters and infinite cluster in solution. In a percolation model the related quantities such as viscosity and modulus of elasticity are known to follow power laws with universal exponents.<sup>1,2</sup> This universality characteristic originates from a geometrical invariance property valid within a given length range. As a consequence, the gel network in conventional experimental preparation procedures is random and fractal. Numerous gelling systems are known to show such a regular random network. Among large molecules gelatin,<sup>3</sup> poly(benzyl glutamate),<sup>4</sup> and polyacrylamide<sup>5</sup> gels reveal these typical networks. However, the same is also true for some small amphiphilic molecules in apolar media. This is the case for gels concerned with the present study and made with a steroid derivative in cyclohexane.<sup>6</sup> The random gel network is obtained when the constitutive fibers are allowed to grow in an isotropic medium in the absence of any external orientational force (mechanical, electrical, or magnetic). Strong orientation effects can be obtained by use of shear stresses either with micellar systems<sup>7</sup> or with polystyrene coils in dilute solution.<sup>8</sup> Evidence of orientation in concentrated solutions of some biological polymers has also been reported when strong magnetic fields are used. For instance, oriented gel phases have been obtained with collagen fibrils<sup>9</sup> and polymerized fibrinogen proteins.<sup>10</sup>

Here we are concerned with gels formed with the above-mentioned small amphiphilic steroid derivative in cyclohexane. Aggregation of the steroid molecules gives very long filaments, the structure and the network of which have been studied by small-angle neutron scattering<sup>11</sup> and freeze-etching electron microscopy<sup>6</sup> experiments, respectively. We report in this paper the orientation effects obtained with a gelling steroid solution in a strong magnetic field (4.2 T) as observed by optical microscopy and X-ray diffraction experiments.

### Experimental Section

Gelling solutions are prepared by dissolving the steroid in hot



cyclohexane by vigorous shaking as previously described.<sup>12,13</sup> The aggregation kinetics of the steroid derivatives in cyclohexane are highly dependent upon the steroid concentration.<sup>14</sup> The steroid amine derivative concentration is adjusted to get long gelation times at 20 °C ( $C \sim 2.1 \times 10^{-2}$  M;  $t_G \geq 60$  min). A 4.2-T magnetic field of a Bruker spectrometer is used for the experiment. An Olympus BH-2 microscope equipped with crossed polarizers is used for optical characterization.

X-ray studies have been performed using an Elliot rotating anode generator GX20. A double focusing system consisting of two Frenk's mirrors gives a point-focused beam. With such a beam, a perfectly oriented sample results in sharp reflections while

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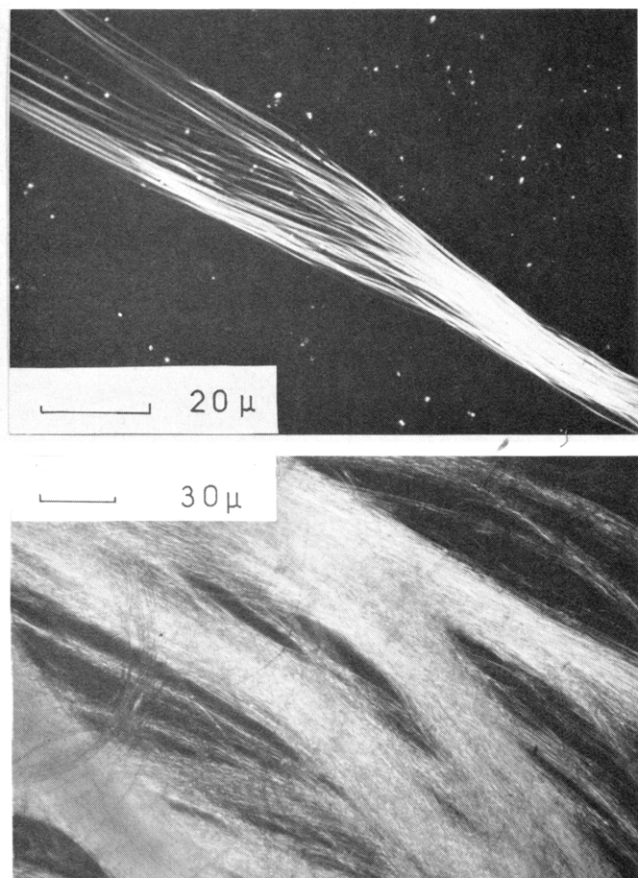
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**Figure 1.** Polarizing optical microscopy of the phase-separated gel network showing (top) highly birefringent oriented steroid fibers; (bottom) defects in the fiber alignment as nonparallel overlapping of fiber bundles.

extended diffraction rings are obtained with slightly disoriented samples. The specimen-film distance is 98 mm.

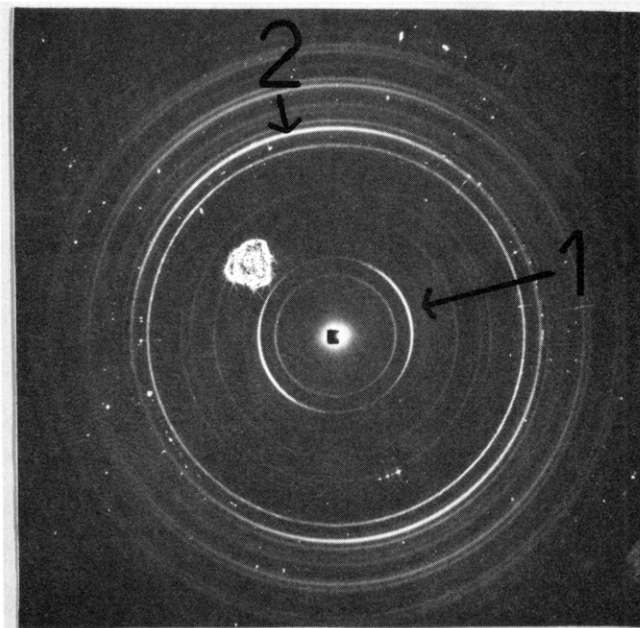
### Results

The large majority of samples gelled in the magnetic field are macroscopically identical (visual examination) with those obtained without the magnetic field. However, some samples gelled within the magnetic fields are mechanically softer and can give, after a very variable time delay of several days, a solid collapsed bundle of fibers in a liquid cyclohexane phase. This behavior is statistically very surprising in the steroid system for which several hundred gel samples were prepared without the use of magnetic fields and have always been very stable even over several years.

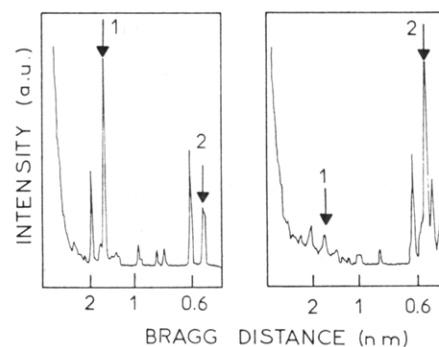
When observed under crossed polars, the fibers appear highly birefringent and oriented (see Figure 1). The birefringent core of oriented fibers shows some domains with various colors from purple-red to blue when the microscope stage is rotated.

Further, X-ray diffraction photographs (Figure 2) show on the one hand a set of uniform rings and on the other hand a set of nonuniform rings typical of orientational effects. Among the nonuniform rings we can distinguish a first class of reflections with a 3.1-nm periodicity over 4 orders of diffraction, the first one being weak, and a second class of anisotropic diffractions in an orthogonal direction with an intense reflection at 0.55 nm. Figure 3 shows the corresponding densitometric diffraction patterns in two orthogonal directions where the above-mentioned second diffraction order (1.52 nm) and 0.55-nm reflections are clearly seen.

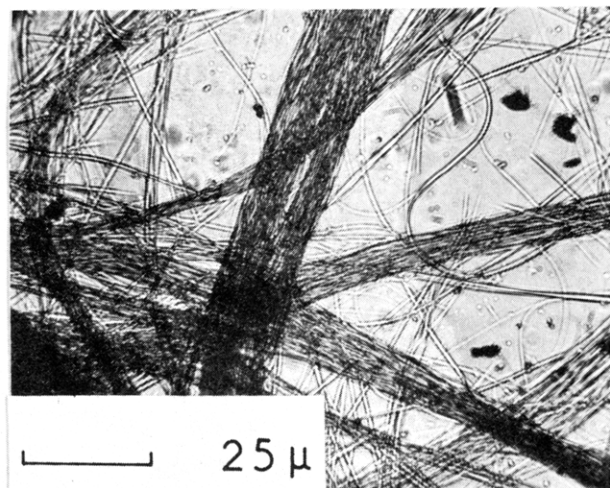
Finally, when a small amount of cyclohexane is added to the oriented filaments between two slide glasses, the disorientation process can be observed by optical microscopy under crossed polars. The birefringent rigid filaments detach from each other to curve gradually over a several micron length scale (see Figure 4) and reconstitute a bidimensional gelling membrane. The X-ray diffraction pattern of such a membrane is made up entirely of



**Figure 2.** X-ray diffraction photograph of the dried magnetically oriented steroid gel network. Copper  $K\alpha$  radiation. The arrows point to the two main nonuniform rings. The corresponding spacings are respectively 1.52 and 0.55 nm. The diffraction pattern is that of a poor alignment of filaments within the fiber bundles which indicate an important random angular orientation about the fiber axis in the individual diffracting units.



**Figure 3.** X-ray diffraction patterns obtained by densitometry measurements along two orthogonal directions. Orientational effects are noticed on the 1.52- and 0.55-nm Bragg reflections indicated by arrows, respectively numbered 1 and 2.



**Figure 4.** Polarizing optical microscopy of a bidimensional gelling membrane reconstituted from a cyclohexane swelling experiment of the dried oriented steroid gel network. The oriented fiber network is progressively randomized by dispersion in cyclohexane.

uniform rings. Once fiber concentration in solution is high enough, typical Schlieren textures of nematiclike domains are observable.

Finally, a volumic reconstituted gel is again quite stable.

## Discussion

Usually ordering of organic compounds in magnetic fields is only possible for anisometric species with a strong anisotropy of the diamagnetic susceptibility tensor ( $\Delta\chi$ ). If it is unreasonable to expect any orientation effect in the entangled gel network once formed or of the "monomeric" steroid species, the situation may occasionally be favorable during the kinetics of steroid aggregation. Previous electron microscopy experiments<sup>6,15</sup> suggest that the growing filaments in the sol phase could reasonably be approximated to rigid rodlike particles.

In a static experiment with rodlike particle orientation in a magnetic field, the degree of orientation  $\Phi$  depends on a balance between the stabilization orientational energy  $W_{OR}$  and thermal agitation. The energy  $W_{OR}$  is proportional to the resultant diamagnetic anisotropy of the rodlike aggregate susceptibility tensor which possesses an axis of rotational symmetry. This anisotropy is an additive property of the molecular quantity  $\Delta\chi_{mol}$  ( $\Delta\chi = \chi_{||} - \chi_{\perp}$ ). The energy balance expression is thus defined by eq 1, where  $H$  is the magnetic field,  $K$  is a form factor of the rodlike

$$\Phi \propto K\Delta\chi_{mol}(\cos^2 \theta)H^2/2k_B T \quad (1)$$

aggregate ( $K = 1$  for a linear aggregate, for instance),  $k_B$  is Boltzmann's constant,  $T$  is the absolute temperature, and  $\theta$  is the angle between the particle symmetry axis and the field direction.

To rotate the rodlike particles, the driving magnetic torque has to compete with a viscous torque characterized by the rotational diffusion coefficient  $D_R$ . The Brownian motion of the rods follows various models for different rod concentration ranges. Here we assume that the rod concentration  $C$  (g/cm<sup>3</sup>) is such that  $C^* \ll C \ll C^{**}$ , where  $C^* = M_R/L^3$  and  $C^{**} = M_R/dL^2$ , where  $L$  is the rod length,  $d$  is the rod diameter, and  $M_R$  is the molecular mass of the rod. These are the conditions of the "semi-dilute entangled regime" where the rotational diffusion coefficient dependence on the rod length is given by

$$D_R \propto D_{RO}C^{-2}L^{-7} \quad (2)$$

where  $D_{RO}$  is the rotational diffusion coefficient at zero concentration and  $C$  is the mass rod concentration.

In the steroid system, the orientation experiments are carried out under these conditions ( $C_0 \sim 8 \times 10^{-3}$  g/cm<sup>3</sup> ( $\sim 2.0 \times 10^{-2}$  M)). Indeed, an indicative value of  $C^{**} = 14 \times 10^{-3}$  g/cm<sup>3</sup> ( $\sim 3.5 \times 10^{-2}$  M) can be calculated following the specific expressions

$$C^{**}_{ster} = C_0 + C^{**} \\ M_R = 70LM_0/N \quad (3)$$

where  $C_0 \sim 4 \times 10^{-3}$  g/cm<sup>3</sup> ( $\sim 10^{-2}$  M) at 20 °C is a limit concentration below which no long filaments can exist,<sup>11,13,14</sup>  $M_0$  is the steroid molecular mass, 70 is the number of steroid molecules per rod nanometer,<sup>11</sup> and  $N$  is Avogadro's number.

By contrast, in a dynamic experiment of steroid aggregation, the rod length is a kinetic function of the initial steroid concentration. This is the actual situation of the magnetic orientation experiments for which the initial state is always the unstable sol phase. The kinetic function is deduced from Avrami plots of small-angle neutron scattering (SANS) kinetic data<sup>13</sup> during the first stages of the unidirectional aggregation reaction and can be approximated by

$$L(t)/L_0 = 1 - \exp(-Kt) \quad (4)$$

where  $L_0$  is the mean rod length equilibrium value and  $K$  is a kinetic constant (s<sup>-1</sup>). Electron microscopy experiments<sup>6</sup> frequently show a mean  $L_0$  value of 10<sup>4</sup> nm. Various aggregation kinetic data<sup>14</sup> indicate  $K \sim 0.1 \text{ mn}^{-1} = 2 \times 10^{-3} \text{ s}^{-1}$  in this range of low initial steroid supersaturation. The number of steroid rods  $n$  per cubic centimeter of solution is found to be equal to  $C/M_R = 10^{-13}/\text{cm}^3$  for a 10<sup>-2</sup> M ( $\sim 4 \times 10^{-3}$  g/cm<sup>3</sup>) steroid concentration involved in the rod structure (eq 3).

TABLE I: Reduced Rotational Diffusion Coefficient  $D_{RO}/D_R$  for Three Different Distributions of  $n$  Growing Rods (Equilibrium Length  $L_0 = 10^4$  nm) at Various Kinetic Steroid Aggregation Times<sup>a</sup>

$t, s$	$L(t), \text{nm}$	$D_{RO}/D_R$		
		$n = 10^{13};$ $d = 100 \text{ nm}$	$n = 10^{10};$ $d = 3163 \text{ nm}$	$n = 10^9;$ $d = 10000 \text{ nm}$
2	40	65 <sup>b</sup>	0	0
5	99	$2 \times 10^{15}$	0.2	0.002
10	198	$\infty$	117 <sup>b</sup>	1.1
20	392	$\infty$	$5 \times 10^4$	546 <sup>b</sup>
50	952	$\infty$	$\infty$	$2 \times 10^6$
1000	8647	$\infty$	$\infty$	$\infty$

<sup>a</sup>  $d$  is the corresponding gel network mesh size at equilibrium.

<sup>b</sup> Critical time and length values for which the high  $D_{RO}/D_R$  values prevent any rod orientation.

We assume  $n$  is constant during the growth step. For various time values of the kinetic aggregation reaction, Table I indicates the length  $L(t)$  (eq 4) and the ratio  $D_{RO}/D_R = L^9(t)/(5 \times 10^{-20}n)^2$  for different numbers of rods  $n$  or germination sites. The first case ( $n = 10^{13}$ ) corresponds to the statistical case where all the germination sites are growing at the same time. The second case ( $n = 10^{10}$ ) concerns a situation where the germination sites are growing by domains, which is more realistic in this range of low steroid supersaturation as demonstrated by previous kinetic experiments.<sup>14</sup> The third case ( $n = 10^9$ ) corresponds to the limit case of the minimum number of rods ( $C^*$ ) of length  $L_0$  to gelify the system. The corresponding mesh-size values of the porous network are calculated from a simple model in which rods of a finite range  $L$  are placed in cubes of size  $d^3$ . We get the mean distance between the rods of radius  $R$  from

$$d = R\pi^{1/2}\phi^{-1/2} \quad (5)$$

where  $\phi$  is the volume fraction of the rods in solution. The first case ( $n = 10^{13}$ ) which implies a mass conservation relation at equilibrium gives a mean size of 100 nm which is experimentally observed by electron microscopy experiments.<sup>6</sup>

It is clearly seen that whatever the effective situation, any rotation becomes impossible in a few seconds (or minutes), the system being completely "frozen" by excluded volume effects. This is why we can only expect to build oriented rods of finite lengths (198 nm in the third case) which act as oriented precursors for the consequential growth steps. The oriented xerogel-phase separation confirms that the first rodlike aggregates of the sol phase have been oriented and have guided the subsequently growing fibers. Similar behavior has been reported with collagen in which small assembly of molecules are precursors for fibrils.<sup>9</sup> A very comparable situation is found in the polymerization reaction of fibrinogen<sup>10</sup> where the reaction has to take place slowly to give highly oriented fibrin gels. Additionally, collagen above a certain denaturation pH assumes a sufficient rigidity of the triple-helical structure for a spontaneous formation of an isotropic dilute phase and concentrated anisotropic ordered phase. The observed phase separation in the steroid case is in agreement with the Flory lattice model for rigid rods in solution which predicts an extension of the heterogeneous biphasic domain when the ordered phase is aligned.<sup>17,18</sup>

The orientation effect becomes inefficient as the length and concentration increase during the steroid aggregation kinetics and specially when entanglements occur in the filament dispersion. This explains the relative poor local ordering observed on X-ray photographs. Nevertheless, orientation effects are clearly seen on the two above-mentioned sets of reflections where it may be noticed that the corresponding Bragg distances of 1.52 and 0.55 nm could be correlated with the steroid molecular length in a bilayer structure and a lateral molecular spacing in such mesomorphic states,<sup>19</sup> respectively.

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By contrast, in the optical-wavelength range, birefringence observed with the oriented bundle of fibers indicates an effective microordering. The color variation is symptomatic of a compatibility of the visible light wavelength range with a spatial periodicity in the structure of the oriented solid sample. Previous structural studies<sup>6,11,15</sup> have emphasized that chirality which is an intrinsic property of the steroid "monomers" is also present in both the filament structure of the native gel (pitch  $\sim 5$  nm (ref 6)) and the xerogel fibers (pitch  $\sim 60$  nm (ref 15)). In this situation, the transmitted light beam has crossed nematic layers of oriented chiral filaments which behave like a cholesteric-nematic system.

The swelling experiment described using a dried oriented bundle of fibers shows that, in the absence of any orientational external

perturbation, a random gel network sets up where only occasional long-range orientation effects are observed in domains with typical optical textures.

Magnetic field orientation experiments have underlined some analogies with polymeric systems. Complementary structural studies of orientation in these low molecular weight gelling compounds are now in progress using stronger magnetic fields but also mechanical shear stresses.

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## Temperature-Independent Electron Transfer: Rhodamine B/Oxide Semiconductor Dye-Sensitization System

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The fluorescence spectrum and decay of rhodamine B (Rh B) adsorbed on insulator ( $\text{SiO}_2$ ) and oxide semiconductors ( $\text{ZrO}_2$ ,  $\text{TiO}_2$  (anatase)) were measured in vacuo at temperatures in the range 4–300 K. The effect of temperature on both the intensity and decay rates of the fluorescence is very weak, indicating that the electron transfer (ET) from Rh B in the excited state to those semiconductors (SC) is almost an activationless process. The result leads to the conclusion that continuous levels in the conduction band of the SC serve as the electron acceptor state.

### Introduction

Photoinduced electron-transfer (ET) process from an adsorbed molecule in the excited state to substrate semiconductors (SC) in vacuo has been studied.<sup>1–4</sup> The characteristics of the ET between adsorbate and solid may be quite different from that of the ET between molecules. When the solid serves as the electron acceptor, there are two possible candidates for the electron acceptor level. One is the conduction band composed of continuous energy levels, and the other is surface states with localized discrete levels. The ET rate dependence on substrate oxide SC for Ru(II) complexes<sup>3</sup> and Rh B has recently been reported.<sup>4</sup> Based on the results, it was suggested that continuous levels in the conduction band serve as the electron acceptor levels.

In this paper studies are presented of the intensity and decay rate of the fluorescence from rhodamine B (Rh B) adsorbed on SC surfaces in vacuo over a wide temperature range, 4–300 K. The fluorescence measurement of such an adsorbate–substrate system in vacuo has advantages for studying the temperature dependence of the ET process because it is a solvent-free system and the distance between donor and acceptor is fixed. The fluorescence of Rh B adsorbed on  $\text{SiO}_2$  (Rh B/ $\text{SiO}_2$ ) as a reference in the absence of an ET process was also measured. The ET rate was found to be almost temperature independent.

### Experimental Section

Commercial Rh B was recrystallized three times from water. The oxide powders were purchased from Kojundo Kagaku Corp.

(purity, 99.999%; particle diameter, ca. 1  $\mu\text{m}$ ; surface area, 5–10  $\text{m}^2/\text{g}$ ) and used without further treatment. Rh B was adsorbed on the powders by the following procedure: 1 g of the powder was suspended in 10 mL of  $10^{-7}$  M aqueous solution of Rh B for about 20 min; then it was filtered. The powder was dried by evacuation ( $10^{-3}$  Torr) at 50  $^\circ\text{C}$  for at least 24 h. The surface coverage estimated was less than ca. 1/10000. Here the radius of the Rh B molecule was assumed to be 8 Å and all the molecules in solution were adsorbed uniformly on the surface. The samples sat in an optical cryostat (Oxford Instruments Ltd, CF 1104) and were evacuated to ca.  $10^{-6}$  Torr. Fluorescence spectra were recorded on a Spex Fluorolog 2 spectrometer. Decay curves were measured with several time scales (5, 25, and 50 ps/channel; 1024 channels) by using picosecond photon-counting apparatus in the instrument center of IMS (excitation wavelength, 548 nm; observation, 600 nm time resolution, ca. 10 ps).<sup>5</sup> We analyzed the decay curves by the least-squares autofitting method reported previously.<sup>3</sup>

### Results and Discussion

**Fluorescence Spectra.** The fluorescence spectra of Rh B adsorbed are almost the same regardless of the substrates studied here. It is reported that the peak wavelength shifts to longer wavelength as the surface coverage increases.<sup>6,7</sup> This is explained by a mixing of a dimer emission<sup>6</sup> and/or by a fluorescence spectral change induced by an interaction between an excited Rh B monomer and neighboring monomers.<sup>7</sup> The peak wavelength observed in the present study was 570 ( $\pm 2$ ) nm, which is coincident with the literature value of the peak of monomer Rh B fluorescence

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