Conformations of a Tethered Membrane: Crumpling in Graphitic Oxide?

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We have used freeze-fracture electron microscopy and static light scattering to study the conformations of two-dimensional sheets of graphitic oxide suspended in aqueous solution. Electron micrographs show the graphite sheets are flat with some curling at the edges. Scattering experiments yield a fractal dimension of 2.15 (±0.06). Lowering the solvent polarity with acetone enhances the intramembrane affinity and leads to a compact structure. Under no conditions did we find a crumpled phase, which had been hypothesized as possible for 2D tethered membranes.

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A recent area of considerable interest in theoretical physics has been the statistical mechanics of fluctuating membranes and surfaces [1]. Much of this work has centered around tethered or solid membranes. In contrast to flexible membranes composed of lipid bilayers, a tethered membrane contains a permanently cross-linked network of constituent molecules forming the two-dimensional analog of conventional polymers. However, theoretical results indicate these sheetlike macromolecules should have dramatically different properties than linear polymers [2]. In solution, the membrane undergoes random motion which is constrained by its fixed connectivity. Unlike a fluid membrane, such as the L_α phase in surfactant systems, or a hexatic membrane (L_β), a solid membrane has a shear elasticity so it resists in-plane strains caused by out-of-plane fluctuations (undulations), giving it a large entropic bending rigidity. There has been much theoretical speculation that these membranes undergo a transition from a low-temperature flat phase to a high-temperature crumpled phase [1,3]. Some experimental evidence of a crumpled phase in the system of graphitic oxide in aqueous solution has been reported [4]. However, due to experimental limitations the results were not conclusive. In this work, we use a combination of freeze-fracture transmission electron microscopy and static light scattering to show that there is no crumpled phase of graphitic oxide.

In the absence of self-avoidance, a polymerized membrane is expected to adopt a crumpled random structure somewhat like a coiled polymer [3]. Such an object is self-similar and is characterized by fractal behavior, with its radius of gyration scaling as $R \sim L^\nu$, where L is the characteristic size of the membrane. The fractal dimension, defined via $M \sim R^{d_f}$, is given by $d_f = 2/\nu$. For a flat surface, $R = L$ and $d_f = 2$, while for a completely compact object, $R \sim L^{2/3}$, yielding a fractal dimension of 3. A crumpled surface has a fractal dimension between these two limits.

Theoretical predictions of the fractal dimension of a self-avoiding tethered membrane were first obtained by extending the Edwards model for self-avoiding polymers [5] to obtain a continuum free energy for a two-dimensional polymer sheet suspended in d-dimensional space [3]. In the Flory mean-field approximation, $\nu = 4/(d+2)$ or 0.8 in $d = 3$, indicating a crumpled phase with $d_f = 2.5$ [3]. This result was supported by Monte Carlo simulations [3] and renormalization group calculations [6]. Monte Carlo results that included an explicit bending rigidity suggested the possibility of a finite temperature crumpling transition [7]. However, the upper critical dimension for such a system is infinite; hence, long-range interactions due to self-avoidance are always important and may lead to a flattening of the membrane. More extensive computer simulations [8] and molecular dynamics studies [2,9] found no crumpling of self-avoiding tethered membranes in a good solvent. A poor solvent leads to an attractive interaction and the formation of a collapsed or compact phase [10].

While much work has been done on the theoretical side, finding appropriate systems to test these theories has remained an experimental challenge. A major difficulty is finding solid membranes with a width to thickness aspect ratio of around 1000:1 and an experimentally appropriate size (microns). Possible systems for investigating tethered membranes include metal dichalcogenide layers [11], polymerized adsorbed monolayers [12], spectrin protein skeletons of red blood cells [13], two-dimensional polymers of chiral precursors [14], and suspended layers of graphitic oxide in aqueous solution [4]. Light scattering experiments on suspended membranes of graphitic oxide were consistent with a fractal dimension of 2.5, suggesting a crumpled phase [4]. Graphitic oxide (GO) membranes are micron size sheets of solid carbon with thicknesses on the order of 10 Å formed by exfoliating carbon with a strong oxidizing agent [15]. Microscopy results in Ref. [4] were limited to samples dried on a copper grid, which are not representative of the configurations in solution [16]. Our original intent in this work was to examine the confirmations of crumpled GO membranes using freeze-fracture electron microscopy. However, all micrographs of GO suspensions indicated a flat

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structure. To confirm the microscopy results, we performed static light scattering experiments which yielded a smaller fractal dimension than the one obtained in Ref. [4]. However, the limited range of wave vectors over which fractal behavior was observed made interpreting the light scattering difficult.

GO was prepared using the same procedure reported by Hwa and co-workers [4,17] based on the earlier work of Ref. [18]. Bulk graphite consists of sheets of covalently bonded carbon held together by van der Waals forces. Graphite powder dissolved in sulfuric acid was oxidized using potassium permanganate, breaking the interlayer carbon bonds and replacing them with hydroxyl groups [15]. The rate of this reaction was carefully controlled to avoid explosion. The resulting yellowish liquid was diluted in 3% hydrogen peroxide, filtered, and treated in ion-exchange resin. After drying, the GO formed a brown sooty solid that was redispersed in alkaline solution to neutralize the acidic hydroxyl groups. In accordance with previous work indicating the maximum solubility at a pH of 11–12, we dispersed 0.5 mg of GO per ml of $0.01N$ NaOH ($p\mathrm{H} = 12$) solution. The suspension was left standing for at least one week, allowing the unreacted graphite to precipitate. The yellowish supernatant was then used in further experiments. Optical microscopy revealed micron size particles moving randomly in the solution.

Samples for transmission electron microscopy (TEM) were prepared using the standard freeze-fracture method [19]. Freeze fracture has been used successfully to image microstructured fluids such as tobacco mosaic virus nematics [20], ternary microemulsions [21], bilayer vesicles [22], and various liquid crystalline phases [23]. A small droplet (0.5 μl) of GO suspension was sandwiched between two thin copper planchettes, equilibrated in a chamber at 25°C and >95% relative humidity, and then quickly plunged into liquid propane which provides a cooling rate of $\sim 10^4$ K/sec, causing vitrification of the solution [19]. As the time scales for membrane fluctuations are much slower than for solvent crystallization, the membranes must retain their room temperature configurations. The samples were then transferred under liquid nitrogen to freeze etch device, where they were fractured under vacuum at $-110\,^\circ$C and etched for 5 sec to remove ~ 1 nm of solvent. The exposed sample surface was then shadowed with a 1.5 nm layer of platinum at a 45° angle with respect to the surface, followed by a 15 nm layer of carbon deposited normal to the surface. The copper

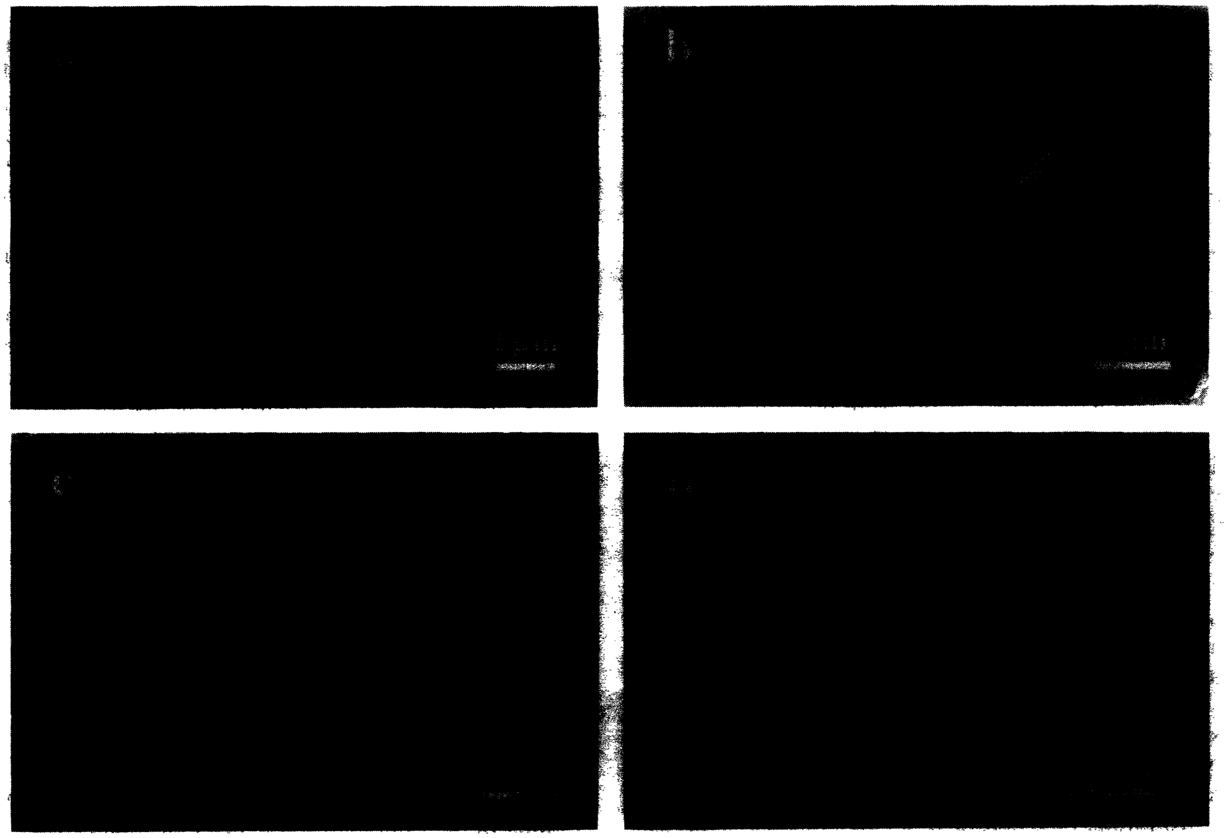

FIG. 1. Electron micrograph of freeze-fracture replicas of graphitic oxide membranes dispersed in $2N$ NaOH solution. Large flat membranes are seen in (a)–(c). Membranes protruding from the fracture plane are seen in (d). No evidence of crumpling is seen in any of these pictures.

planchettes were then dissolved in chromic acid, leaving the platinum-carbon replicas behind. The replicas were cleaned in doubly distilled water and collected on a formvar-coated electron microscope grid. The replicas were imaged using a JEOL 100 CX II operating in conventional transmission mode at 100 kV.

Typical structures appearing in these images are shown in Fig. 1. The lighter areas in the micrographs indicate shadowing (absence of platinum). These pictures show flat, randomly shaped objects which are typical of the structures seen in replicas of GO suspensions. These objects have sizes of 0.5 to 5 μm and appear to have a smooth texture. Figures 1(a) and 1(b) show a large section of replica containing many membranes. Two large membranes are seen in Fig. 1(a) as well as a number of smaller ones. The surfaces of the GO membranes appear smoother than the surrounding fluid. The roughness of the background is due to small variations in surface evaporation during the etching process. In Fig. 1(c) we see an enlarged view of a representative membrane. This membrane appears very flat in the middle with some curling near the edges. This is consistent with molecular dynamics simulations, where large fluctuations are seen near the edge of the membrane [2]. Figure 1(d) shows GO membranes at orientations approximately normal to the photograph. From the shadowing we can calculate that these membranes protruded a few tenths of a micron from the fracture plane at the time of replication. We cannot determine the amount of edge curling from this micrograph since we do not know the angle of the membrane with respect to the layer normal. However, this picture is clearly inconsistent with a crumpled conformation. In addition, the edge views show the membranes to be very thin and probably monodisperse.

As the micrographs in Fig. 1 are inconsistent with previous light scattering experiments on the same material [4], we performed static light scattering on these samples. A fractal object is scale invariant when probed at wavelengths smaller than its size R and larger than the atomic spacing. In this regime, the orientationally averaged structure factor $S(\mathbf{q})$ has the asymptotic form $S(\mathbf{q}) \sim q^{-d_f}$ for $qR \gg 1$, where $\mathbf{q}$ is the scattered wave vector [24,25]. To ensure we are in this regime, membranes smaller than the shortest lengths probed, $1/q_{\min} \sim 0.5$ μm, must be removed. We therefore filtered the GO suspension through a 1 μm polycarbonate filter and resuspended the trapped particles. It was necessary to redisperse the membranes in a larger volume of solution to eliminate multiple scattering. We estimate the final concentration of graphite to be about 20 μg/ml. This suspension was further filtered through an 8 μm polycarbonate filter to remove any remaining large particles and placed into a large diameter (0.98 in.) scattering cell to minimize small angle reflections. The results of our light scattering are shown in Fig. 2. The linear behavior of the structure factor when plotted on a log-log graph versus q indicates a power law decay. The solid line in this figure shows a fit of the data which yield a value of $d_f = 2.15 \pm 0.06$. The two dashed lines indicate the best fits fixing d_f at 2.0 and 2.5. Although the data are clearly closer to the expected behavior for a flat phase, it is difficult to distinguish between flat and crumpled over this narrow range of wave vectors. Unfortunately, our experimental setup limits us to only one decade in q, making it difficult to conclusively distinguish between flat and crumpled on the basis of scattering data alone. In addition, it has been predicted that for a rough surface, characterized by a roughness exponent ζ, there should be a crossover to $1/q^{3-\zeta}$ behavior at some length scale $l \sim R^{\zeta}$ characteristic of the size of the flat domains [25]. As these membranes appear very flat in our micrographs, we expect $l \ll R$, so that the regime $q > 1/l$ is not experimentally accessible using light scattering. Further experiments using x-ray scattering may provide evidence of this behavior.

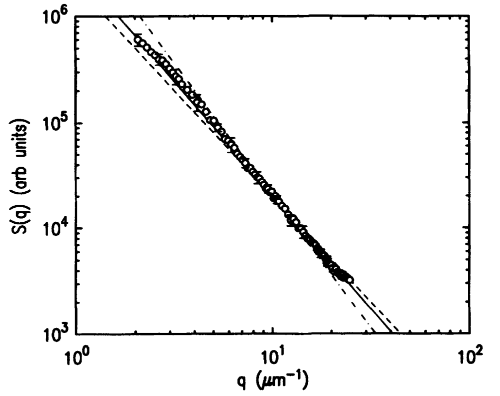

FIG. 2. Static structure factor of GO membranes in alkaline solution as a function of scattered wave vector q determined by light scattering. The solution contains membranes between 1 and 8 μm at a concentration of about 20 μg/ml. The solid line indicates a fit of the data yielding a fractal dimension of $d_f = 2.15 \pm 0.06$, while the two dashed lines are the best fits using $d_f = 2.0$ (— — —) and 2.5 (— · —).

Upon addition of 10 vol % acetone, we find that the GO membranes collapse into small compact objects. Electron micrographs of replicas from such structures are shown in Fig. 3. Figure 3(a) shows a small structure typical of what is seen in these replicas. We observe that this object appears much more three dimensional than those seen in Fig. 1. However, the membrane surface is still smooth. It shows no evidence of crinkling or crumpling, but rather appears folded. This is even more evident in the larger membrane seen in Fig. 3(b). This structure appears to be a micron size sheet folded down the middle. These results may be consistent with molecular dynamics simulations of tethered membranes with an attractive potential, which indicate a collapsed structure is formed via a series of folding transitions [10]. We were unable to obtain consistent data from this suspension using static light scattering. This is understandable since we are no longer in the limit $qR > 1$ and we expect a wide

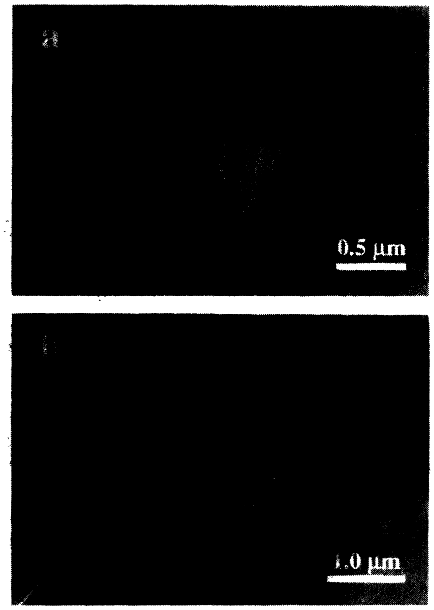

FIG. 3. Electron micrographs of replicas from GO membranes suspended in solution with 10 vol% acetone. These membranes appear more three dimensional and folded that those seen in Fig. 1. However, they do not appear crumpled.

distribution of sizes and shapes. Dynamic light scattering was consistent with extremely polydisperse objects with an average hydrodynamic radius of ~260 nm.

These experimental results indicate that graphitic oxide membranes adopt a flat configuration when suspended in an alkaline solution. Examination of dozens of freeze-fracture replicas using TEM failed to find any membranes showing the crumpled conformation previously reported in this system [4]. Static light scattering over a limited range of wave vectors also indicates a flat structure. Although these experiments were all carried out at room temperature, we do not expect to observe a crumpled phase in GO membranes as the temperature is increased. Since one expects a covalently bonded, solid membrane to have a bending rigidity several orders of magnitude larger than that for conventional surfactant bilayers, our results are not surprising. The possibility still remains that a crumpled phase could exist in much looser bound tethered membranes, such as polymerized phospholipid bilayers. It still remains an experimental challenge to synthesize appropriately sized membranes in these systems.

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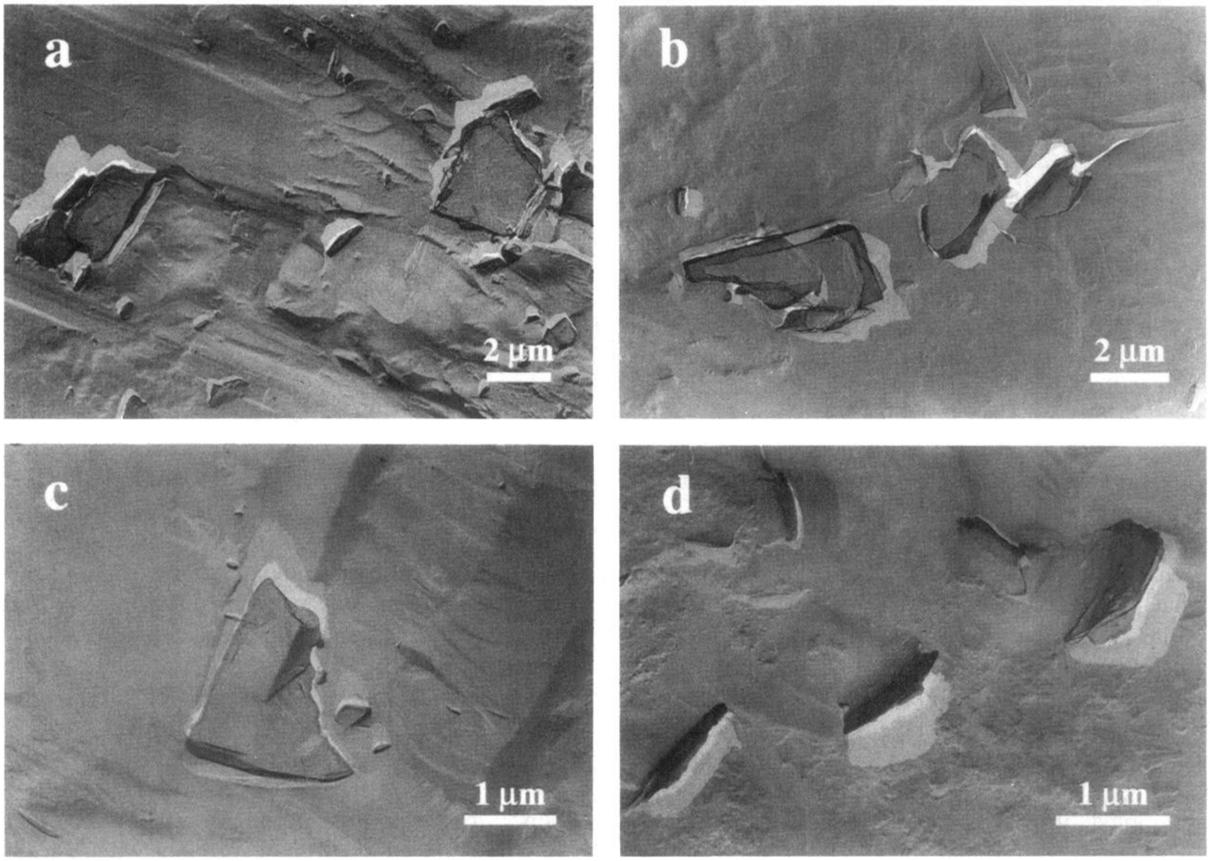

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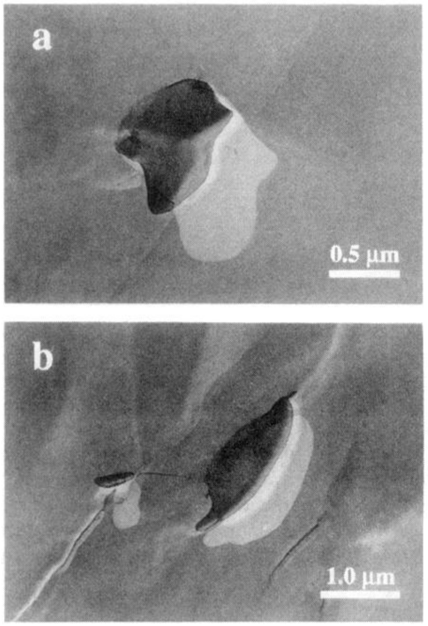

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