

Neutron sample cell suitable for the diffraction of aligned biomaterials and capable of exerting up to 370 MPa of hydrostatic pressure

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(Received 12 February 2002; accepted 3 February 2003)

We describe a temperature controlled sample cell suitable for the study of biomimetic materials (e.g., lipid bilayers) using neutron diffraction, and capable of exerting hydrostatic pressures of up to 370 MPa. The advantage of this sample cell, compared to previous high-pressure cells of its type, is that it allows for the use of samples aligned on a solid support which, compared to “powder” or so-called liposomal preparations, requires only small amounts of sample and allows for the clear differentiation between in-plane and out-of-plane structure. [DOI: 10.1063/1.1568555]

I. INTRODUCTION

Neutrons, discovered by Chadwick in 1932, are neutral, elementary subatomic particles that are found in all atomic nuclei, except hydrogen. They have a mass similar to that of a proton, a nuclear spin of 1/2 and a magnetic moment. Neutrons suitable for scattering experiments are presently produced either in nuclear reactors, by the fission of uranium nuclei resulting in neutrons of energies between 0.5 and 3 MeV, or by spallation sources where accelerated subatomic particles (e.g., protons) strike a target material (e.g., tungsten, lead) releasing neutrons from the nucleus. In nuclear reactors, similar to the National Reactor Universal (NRU) at Chalk River, the fission neutrons are thermalized in a moderator of light or heavy water with the resulting thermal neutrons having an average energy of ~ 0.025 eV. It is these neutrons, which have wavelengths similar to those of x rays, which are used to study condensed matter.

Although neutrons and x rays can have similar wavelengths, there are real differences between the two that make them, in many cases, complementary probes. Unlike x rays, the scattering ability of a neutron does not increase monotonically with increasing atomic number but is randomly distributed throughout the Periodic Table. For example, the coherent scattering lengths of two very different atomic mass elements (such as, nitrogen and platinum) can be very similar.¹ Moreover, this differential scattering ability extends to the element's isotopes; the classic example being hydrogen (H) and deuterium (D) where the scattering length of H is negative. These qualities make neutrons an ideal probe for the study of hydrogenous materials.^{2,3} Of central relevance to the present article is the fact that neutrons are neutral particles and interact weakly with matter.

The study of materials under difficult environments (e.g., high magnetic fields, temperatures and pressure) is not trivial under any circumstance but made simpler by the fact that neutrons interact weakly (thus nondestructively) with many common materials and in particular, aluminum (Al). The incoherent and absorption neutron cross sections for aluminum

are such that Al, for the most part, is considered to be practically transparent to neutrons.¹ Al is an excellent conductor of both electricity (64.94% of the international annealed copper standard) and heat ($0.5 \text{ cal. s}^{-1} \text{ cm}^{-1} \text{ K}^{-1}$), and some of its alloys are easily machined and are resistant to most forms of corrosion. The relatively inexpensive cost of Al and its alloys together with its useful physical characteristics make Al the material of choice when it comes to constructing sample environments at neutron scattering facilities worldwide.

In biology, pressure is as an important thermodynamic variable as temperature, especially in consideration of living organisms that live in harsh environments, such as in the depths of the oceans. For biologically relevant systems such as lipid bilayers⁴ in water, pressure provides a means of separating the effects of volume and temperature on their phase behavior. Over the past two decades or so, a variety of techniques have been employed to study the influence of pressure on the structural parameters of lipid bilayers.⁵⁻⁹ By far, most of the samples studied were “powders” or liposomal dispersions, which give rise to an isotropic signal. In the case of x-ray or neutron diffraction the signal is spread over scattering angles of 2π . On the other hand, the signal from an aligned system is nonisotropic allowing for the clear differentiation between the intra-bilayer (hydrocarbon chain correlations) and the inter-bilayer (lamellar repeat spacing) organization.¹⁰ Moreover, due to the fact that the signal is anisotropic, much less sample material is required. This is an important consideration when studying samples whose availability is of the order of a few milligrams or are simply too costly.

Previously, there have been high pressure cells designed that were suitable for neutron scattering^{7,11} and in one case, the neutron powder cell¹¹ was modified to study aligned systems.⁶ The pressure cell designed by Neilson *et al.* was made out of a titanium-zirconium (Ti-Zr) alloy (34% Ti and 66% Zr) and was capable of 250 MPa of isotropic pressure.¹¹ However, its main drawbacks were the nature of the pressurizing medium (light oil) and, compared to Al, the elevated values for both the incoherent and absorption neutron cross sections of the Ti-Zr alloy. Also, since lipids are primarily

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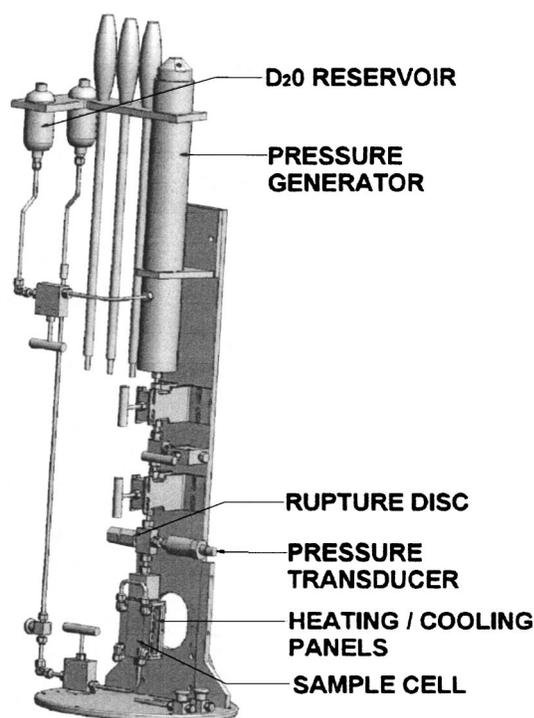


FIG. 1. Pressurized sample cell assembly with the three pressure generator handles stored on the support frame. The system is rated for hydrostatic pressures up to 370 MPa and is suitable for neutron diffraction of aligned biomimetic systems. The hand-operated pressure generator contains only 7.5 ml of fluid and is capable of a maximum hydrostatic pressure of 400 MPa. The water heating/cooling panels or jackets are modular and are bolted onto the sample cell block; 0–400 MPa hydrostatic pressure (accuracy $\pm 1\%$) was measured using a wire strain gauge pressure transducer (Sitec model 770–6171).

hydrogenous materials, the use of oil as a pressurizing medium poses a real problem in contaminating the sample.

The other sample cell suitable for neutron scattering was described by Winter and Pilgrim.⁷ The cell was made out of pure molybdenum (Mo) and was capable of withstanding up to 300 MPa of hydrostatic pressure. However, it was not designed to interrogate aligned samples and both the incoherent and absorption cross sections of Mo are almost a factor of 5 and 10, respectively, greater than those of Al.

In this article, we report on the construction of a temperature controlled (-10 to 100 °C) pressure cell capable of exerting up to 370 MPa of hydrostatic pressure on biomimetic samples aligned on solid substrates, usually silicon single crystals. The pressure cell allows for the study of samples where quantities of the sample are limited and in conjunction with a two-dimensional detector allows for the study of both in-plane and out-of-plane correlations as a function of temperature and pressure.

II. PRESSURIZED SAMPLE CELL ASSEMBLY

The custom designed pressurized sample cell assembly is shown in Fig. 1. Fittings, tubing, valves, and the hand-operated pressure generator were purchased from Sitec-Sieber Engineering AG (Zürich, Switzerland) while all of the other components, including the sample cell block, were machined at Chalk River Laboratories (Chalk River, ON,

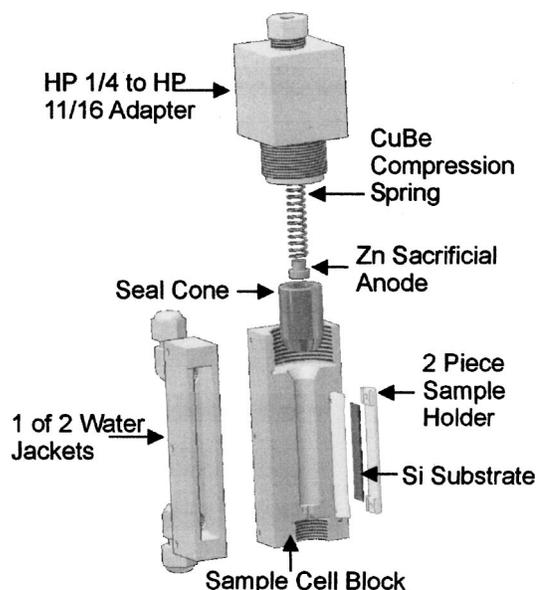


FIG. 2. Neutron sample cell assembly constructed out of 7075-T6 Al alloy with an inner bore diameter of 1.02 and 6.00 cm bore height. The cell block was anodized and was fitted with helicoils on both ends. The heating/cooling water jackets or panels are modular and bolt on to the cell block. The sample holder was constructed out of 6061 Al alloy and displaced 1.90 cm³ of water.

Canada). The most critical component in the system is the sample cell which has to withstand hydrostatic pressures of up to 370 MPa while at the same time, being “transparent” to thermal neutrons.

The sample cell (Fig. 2) was machined from a single block of 7075-T6 Al alloy. As mentioned earlier, Al was chosen because it is practically transparent to thermal neutrons while the 7075-T6 Al alloy was chosen because of its high yield strength (525 MPa). The elemental composition of 7075 Al alloy and the sample cell dimensions chosen to withstand the requisite pressures resulted in a cell that was transparent to $>70\%$ of the incident neutrons.

The wall thickness of the sample cell was determined by using Lame's equation, $t = D/2 [(S + P/S - P)^{1/2} - 1]$ where t is the wall thickness, S is the yield strength of the material, P is the applied pressure, and D is the bore diameter. Based on this relationship, the minimum wall thickness for the requisite 1 cm bore diameter turned out to be ~ 1.4 cm. From the total sample cell volume (4.9 cm³) we calculated the amount of stored energy in the system to be ~ 150 J. However, when the sample mount is inserted into the sample cell it displaces 1.9 cm³ of water consequently reducing the stored energy of the system to ~ 93 J.

Despite its high yield strength, 7075-T6 Al alloy is not suitably corrosion resistant, especially at elevated temperatures. To overcome this inherent deficiency, the sample cell was hard anodized to a total Al_2O_3 plating of 50 μm . Moreover, a “sacrificial” zinc anode (cathodic protection) was used to further minimize the damage to the sample cell. Finally, the cell block was fitted, on either end, with helicoils as there was a measurable stretching of the threads with repeated use.

The temperature of the cell block was controlled by at-

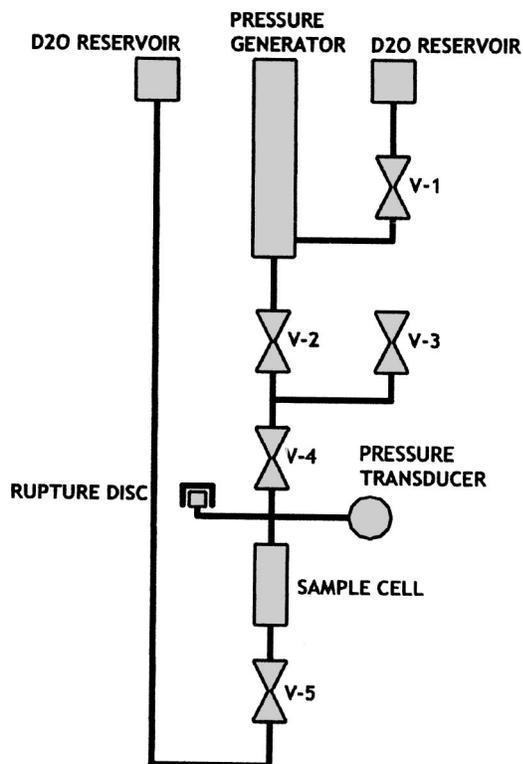


FIG. 3. Flow diagram of pressurized sample cell assembly shown in Fig. 1.

taching water jackets on the outside of it. A circulating water bath maintained the temperature to ± 0.05 °C. The pressure during the experiment was measured using a strain gauge pressure transducer (Sitec model 770-6171).

III. PRESSURIZING THE SYSTEM

Figure 3 shows a flow diagram of the pressurized sample cell assembly (Fig. 1). There are two water reservoirs filled, in our case, with heavy water (D₂O). One reservoir is used to prime the manually operated pressure generator while the other water reservoir was used to prime the sample cell. Once the system was fully assembled it was purged of all trapped gases and then primed.

Starting from its maximum stop, the pressure piston draws water from the first D₂O reservoir through valve V1. The water was then forced through valve V2 and expelled through V3, with V4 closed, purging the connecting region of air. The pressure generator was then again primed with D₂O from the reservoir.

With V2 in the closed position the sample cell was primed by allowing water to flow slowly under gravity through the open valve V5, the sample cell, V4, and finally leaving the system at V3. The backflow rate must be very slow or the sample can be stripped off of the silicon substrate as the water rushes into the cell. Unseating the substrate mount assembly was not a problem because the CuBe compression spring overcomes the head of water that is present when the priming reservoir is valved into the system (Fig. 2). Once bubble free water exited V3 all valves were closed and the system was ready to be pressurized. To pressurize the

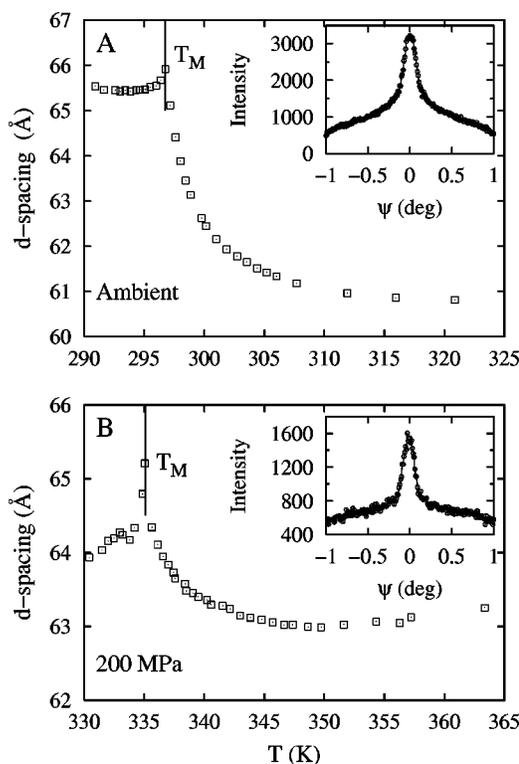


FIG. 4. The *d* spacings of aligned DMPC multilayer stacks as a function of temperature at ambient [(A)] and ~ 200 MPa [(B)] pressure. The “rocking” curves (insets) show that the samples are highly aligned, with respect to the Si surface, with a mosaic spread of $< 0.2^\circ$ (full width at half maximum). The rocking curves, for samples above T_M , were obtained by fixing the detector at a Bragg maximum and recording the intensity as a function of sample angle, Ψ . At ~ 200 MPa the pressure changed $\sim 10\%$ over the experimental temperature range (35 °C) and was not adjusted as the system contains no active feedback loop.

system, V2 and V4 were opened and the pressure adjusted. Once the desired pressure was attained, V2 and V4 were closed.

IV. DIFFRACTION DATA

Neutron diffraction experiments were carried out at the NRU reactor (Chalk River Laboratories), using the N5 and E3 triple-axis spectrometers. Monochromatic neutrons of suitable wavelengths ($\lambda \sim 1 - 3.5$ Å) were obtained using either a pyrolytic graphite or germanium single crystal. The samples were oriented from a concentrated lipid solution, typically 20 mg lipid in methanol, on a clean Si substrate of dimensions 1 cm \times 4.5 cm. After initial evaporation of the solvent, the samples were kept under vacuum for ~ 24 h to remove any traces of methanol.

Figure 4(A) shows how, at constant atmospheric pressure (101.3 Kpa), the lamellar repeat spacing (*d* spacing) of highly aligned dimyristoyl phosphatidylcholine (DMPC) bilayers as a function of temperature, changes. The interesting phenomenon is that, in the vicinity of the main transition temperature (T_M) the *d* spacing is increasing in a nonlinear fashion and is not typical of a first-order phase transition. This so-called anomalous swelling¹² is commonly believed to be evidence of a critical point being preempted by the first-order gel-to-liquid-crystalline main transition.¹³ The

“rocking” curve [Fig. 4(A), inset] is evidence that the sample under study was highly aligned. Increasing the pressure to 200 MPa shows a decrease in the nonlinear portion of the d spacing versus temperature curve [Fig. 4(B)] while the bilayers remained highly aligned [Fig. 4(B), inset] and their d spacing increased. Moreover, we observed an increase in T_M of ~ 40 K, consistent with previous DMPC studies of T_M increasing ~ 20 K per 100 MPa of applied pressure.¹⁴

V. DISCUSSION

We have constructed a sample cell uniquely suitable for the study of aligned biomimetic materials and capable of up to 370 MPa of hydrostatic pressure and a broad range of operating temperatures (-10 and 100 °C). The sample cell has been successfully operated at 350 MPa of hydrostatic pressure and temperatures of <60 °C for extended periods of time (more than three days). However, at temperatures approaching 100 °C, the maximum working pressure attained was 250 MPa. At elevated temperatures, corrosion becomes a problem with sample cells capable of withstanding only a few cycles (less than five experiments) at which point, they either cannot make a seal or succumb to mechanical failure. At 60 °C, or less, both the performance and life span of the cell increase dramatically. The addition of helicoils, anodization, and the zinc sacrificial anode contribute significantly in increasing the duty cycle of the cell for all temperatures.

This sample cell will allow the study of both the in-plane and out-of-plane structure of biologically relevant materials at a range of hydrostatic pressures up to 370 MPa. Moreover, the amounts of sample required, compared to pressure cells designed for liposomal suspensions, are much reduced. This is an important feature especially when one is dealing with samples which are expensive or whose availability is limited.

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