

Sample cell capable of 100% relative humidity suitable for x-ray diffraction of aligned lipid multibilayers

J. Katsaras

National Research Council, Bldg. 459, Sm. 18, Chalk River Laboratories, Chalk River, Ontario K0J 1P0, Canada

M. J. Watson

Atomic Energy of Canada Limited, Bldg. 459, Sm. 18, Chalk River Laboratories, Chalk River, Ontario K0J 1P0, Canada

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We describe a sample cell capable of attaining 100% relative humidity that is suitable for the study of aligned lipid multibilayers using x-ray diffraction. The basic principles of this cell are applicable to sample environments used in a variety of physical techniques (e.g., nuclear magnetic resonance, light scattering, infrared spectroscopies, etc.) thus enabling these techniques, for the first time, to study aligned samples having the same physical characteristics (i.e., repeat spacing, transition temperature, etc.) as their liposomal counterparts immersed in water. © 2000 American Institute of Physics. [S0034-6748(00)04304-5]

I. INTRODUCTION

Compared to powder samples or, as they are commonly known, liposomal preparations, highly aligned biomimetic membrane systems have enabled a variety of techniques to extract unambiguous structural information.¹⁻⁵ In certain cases, structural solutions were the direct result of an aligned sample becoming available.^{3,4,6,7} In the case of diffraction, aligned samples allow for the clear differentiation between in-plane (hydrocarbon chain organization) and out-of-plane (lamellar repeat spacing) correlations.^{4,6} As such, the use of aligned samples is highly desirable, however, their major drawback has been that they could not be hydrated from water vapor at 100% relative humidity (RH) to the same extent as their liposomal counterparts in contact with liquid water.^{3,8,9} Since the chemical potential of water vapor in equilibrium with bulk water is the same as that of liquid water, the discrepancy came to be known as the “vapor pressure paradox.”¹⁰

The widely accepted vapor pressure paradox persisted until recently, when neutron diffraction experiments employing a newly developed sample cell¹¹ proved that the paradox was the result of cells containing large temperature gradients and not enough “evaporative capacity”¹² to overcome condensation occurring as a result of the presence of these gradients. Thus, the inherent deficiencies present in previous cells constructed over the last three decades, humidities of better than 99% were never achieved. As such, all of the studies using aligned, L_α phase biomimetic samples were carried out thus far using “dehydrated” samples,¹³ which when compared to fully hydrated samples may have very different structural characteristics.

In this article, we report on a variable temperature sample cell capable of fully hydrating aligned samples and suitable for high-resolution x-ray diffraction studies. This sample cell has now opened up possibilities to revolutionize

the approaches taken to determine the bilayer structure under physiologically relevant conditions.¹⁴

II. SAMPLE CELL

The sample cell shown in Fig. 1 was predominantly constructed from aluminum on a design based on two principles.¹¹ The first being the minimization of temperature gradients. This was achieved by the standard practice of employing a massive outer jacket to thermally isolate the inner sample chamber. The temperatures of the jacket and the sample chamber were individually controlled *via* two refrigerated water circulators (Fig. 2). We took advantage of this design feature to set the jacket temperature slightly higher ($\sim 2^\circ\text{C}$) than the temperature of the sample chamber in order to eliminate condensation on the low mass sample chamber windows. The second principle (P. Rand, private communication) was to increase the water's effective evaporative area using a porous, not highly absorbent sponge saturated with water placed in close proximity (~ 3 mm) to the curved sample as shown in Fig. 2. This feature allows for the presence of small temperature gradients to exist while achieving 100% RH.

III. DIFFRACTION DATA

Synchrotron data were obtained using the D-1 station at the Cornell High Energy Synchrotron Source (CHESS). Monochromatic x rays from a dipole source (10 keV photons) were monochromatized by a W/B4C synthetic multilayer having a periodicity of 22.5 Å. The charge coupled device (CCD) detector, built by Tate *et al.*,¹⁵ had a 1024^2 array of pixels each of active size $50\ \mu\text{m}^2$.

The specimen consisted of a dipalmitoylphosphatidylcholine (DPPC) multibilayer stack attached to a curved glass substrate. The incident x-ray beam was oriented perpendicular to the axis of the cylindrical sample (tangential to the

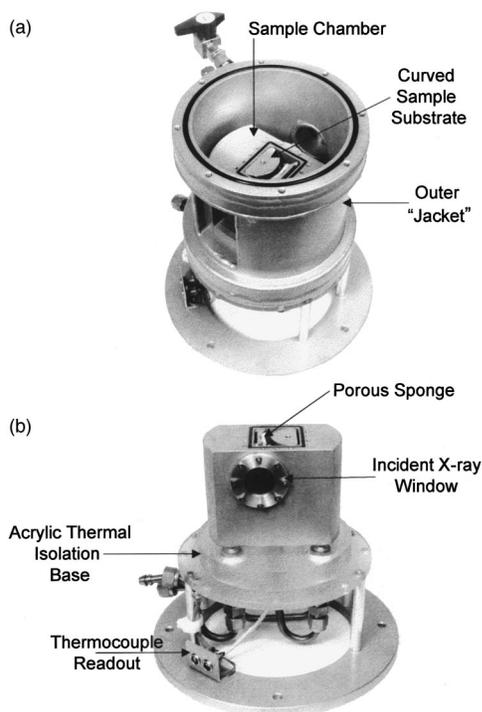


FIG. 1. Perspective photographs of the sample cell (a) with and (b) without the temperature controlled "jacket." With the exception of the windows (either aluminized mylar or kapton), acrylic thermal isolation base and brass cooling/heating lines, the sample cell was constructed from aluminum. The inner sample chamber is a sealed unit (shown with sealing panel removed) and contains the water-saturated evaporative sponge used in hydrating the sample and the cylindrical substrate enabling the collection of multiple Bragg reflections. The sample chamber is thermally isolated from the outer jacket *via* the use of an acrylic base. Moreover, the space between the sample chamber and the outer jacket can either be evacuated or purged with a thermally conductive gas. Operating temperature range of the sample cell estimated between 5 and 80 °C.

curved substrate, Fig. 3). As a result of the "texture" created by the curved substrate, the various Bragg maxima were reflected simultaneously. It should be noted that the sample is well aligned only in the direction normal to the plane of the multibilayer stack.

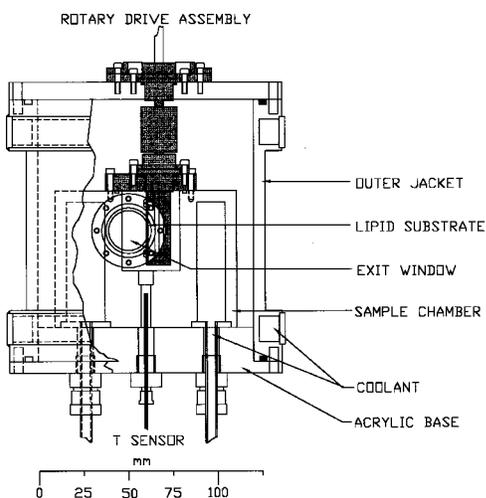


FIG. 2. Elevation assembly of the specimen holder with the optional rotary drive assembly used for flat samples. From this perspective, it can easily be seen that the coolant is only present at either end of the outer jacket.

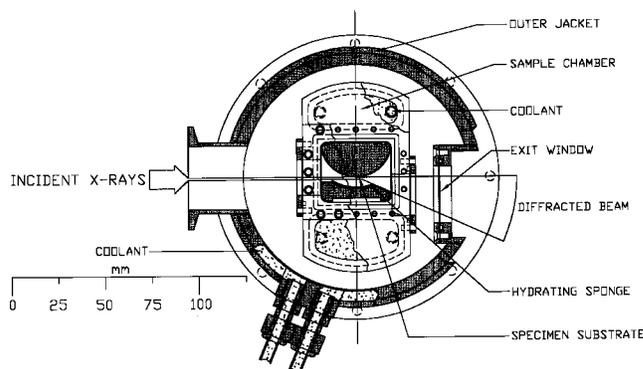


FIG. 3. Top view cross section of sample cell indicating the relationship of the incident x rays to the sample and the hydrating sponge to the specimen.

Figure 4 shows a two-dimensional (2D) x-ray diffraction pattern obtained from fully hydrated rippled ($P_{\beta'}$) bilayers at 39 °C using the sample cell in Fig. 1. From this diffraction pattern one can easily extract the repeat spacing (d) of the bilayers, the wavelength of the ripple (λ) and the angle of the 2D unit cell. The results of this study resolved issues concerning the $P_{\beta'}$ phase that were ambiguous from previous powder studies. These issues could not be resolved until the advent of fully hydrated, aligned samples as the various lattice parameters of the $P_{\beta'}$ phase vary substantially as a function of water concentration.^{16,17} As such, only fully hydrated aligned samples could be compared to the previous studies using fully hydrated liposomal preparations.^{18–20}

IV. DISCUSSION

Dispelling the existence of the vapor pressure paradox has opened up the possibility for the wide spread use of

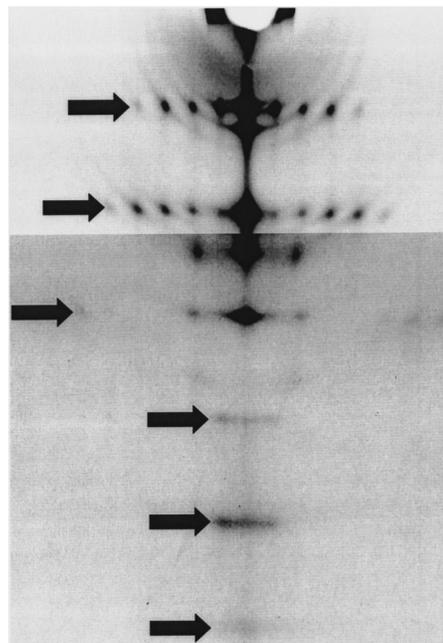


FIG. 4. 2D diffraction pattern of rippled racemic DPPC bilayers at 39 °C formed on cooling from the disordered L_{α} phase. From the diffraction pattern it is clear that this phase is made up of two populations of ripples, a long ripple ($d=82.2$ Å, $\lambda=255$ Å, and $\gamma=90^{\circ}$) whose family of Bragg maxima are identified by the bold arrows and a short ripple ($d=68.3$ Å, $\lambda=144$ Å, and $\gamma=92^{\circ}$). The diffraction pattern is a single 10 s exposure with different contrasts to emphasize the different Bragg maxima.

aligned samples. We foresee the construction of sample cells appropriate for the various techniques presently used to study biological structure and function as the ideas described in the present report are not only applicable to diffraction sample cells suitable for diffraction alone.

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¹²By "evaporative capacity" we mean the rate of producing water vapor which in the present case, is related to the surface area of the sponge. However, increasing the evaporative capacity of the system is not sufficient. Placing the porous sponge in close proximity to the aligned sample is imperative to achieving fully hydrated samples.

¹³In the case of L_α egg phosphatidylcholine bilayers, a decrease of only 1% in RH (100% to 99%) resulted in a 12 Å decrease in interbilayer (water portion) separation (see Ref. 10).

¹⁴Physiologically relevant conditions state that the membrane bilayers are fully hydrated and in the disordered L_α phase.

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