

High pressure cell for small- and wide-angle x-ray scattering

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A compact high pressure cell and its control environment designed for small- and wide-angle x-ray scattering experiments under hydrostatic pressure up to 3000 bar and temperatures between -20°C and $+80^{\circ}\text{C}$ are described. With this system x-ray scattering experiments can be performed at constant pressure and temperature with a conventional laboratory x-ray source and it can be easily modified to carry out time resolved pressure jump studies at synchrotron radiation sources. The computer-aided pressure adjustment has a calibrated accuracy of $\pm 0.5\%$, and temperature is computer-controlled to a precision of $\pm 0.05^{\circ}\text{C}$. The instrument has been designed to investigate systems of biological interest, especially lipid-water dispersions, but it is equally possible to measure highly viscous or solid samples. The performance is illustrated by a pressure dependent small- and wide-angle x-ray scattering study of a phospholipid-water dispersion at constant temperature. © 1997 American Institute of Physics. [S0034-6748(97)03312-1]

I. INTRODUCTION

The investigation of macromolecular and supramolecular structure as a function of pressure¹ has attracted growing interest over the recent years. Conformational properties, aggregation and crystallization of polymers are well recognized as being affected by pressure.² Particular interest, however, arises from the specific effects of pressure on biological systems. Pressure causes proteins to dissociate and to unfold,³ it can inhibit or enhance the crystallization of proteins,^{4,5} kills microorganism,⁶ inactivates enzymes,⁷ reverses the physiological effect of anesthetics⁸ and induces phase transition in model membranes.⁹⁻¹¹ The industrial applications of high pressure in food processing^{12,13} further illustrate the importance of understanding the effect of pressure on structure.

While for spectroscopical methods, such as fluorescence,¹⁴ vibrational spectroscopy,^{15,16} or nuclear magnetic resonance¹⁷ techniques, suitable high pressure instrumentation for laboratory use has already been developed, such is not the case for x-ray scattering and diffraction. For x-ray diffraction, which has a particular high potential for obtaining relevant structural information, the approaches so far have been limited to large installations such as synchrotron radiation sources¹⁸⁻²⁰ (or for neutron diffraction, to high flux reactors²¹).

In the present work, a compact high pressure equipment for the use on conventional laboratory-based x-ray generators has been developed. The task was to design a high pressure x-ray cell (HPXC) for small angle x-ray scattering (SAXS) and wide-angle x-ray scattering (WAXS) experiments to investigate biological and related systems. Since

these systems are affected by relatively modest pressures^{22,23} and temperatures, as compared to solid-state applications, desired pressures should reach a few kbar and temperatures should stay below the boiling point of water. Temperature control and pressure adjustment should be highly precise, as observations of phase transitions in phospholipid-water dispersions have shown to take place in very small temperature intervals.²⁴ Additionally, high pressure measurements should be possible either with fluid, viscous, or solid samples.

When using conventional laboratory-based x-ray generators attention must be paid to highly transparent x-ray windows to avoid high absorption of the scattered x rays and perfect separation of the investigated material from the pressure transmitting fluid to prevent mixing during the more extended exposure times as compared with measurements done with synchrotron radiation.

Design, construction and performance of a high pressure system for liquid, viscous and solid samples, capable of producing pressures up to 3000 bar within temperatures between -20° and $+80^{\circ}$, are described. In addition to being useful for laboratory-based SAXS and WAXS experiments, the system can be easily modified for use in fast time-resolved pressure jump studies at synchrotron light sources.

II. DESCRIPTION OF THE APPARATUS

A. General remarks

Two main types of high pressure cell designs presently exist. One is the window-type cell, which consists of a pressure vessel made from high tensile-strength x-ray opaque material, and hence needs an input and output x-ray window for the incident and scattered x-ray beam.^{19,20} The cells of the second type are completely made from one piece of material penetrable for x rays. Generally these are beryllium rods because they are simple to produce.^{18,25,26} The difficul-

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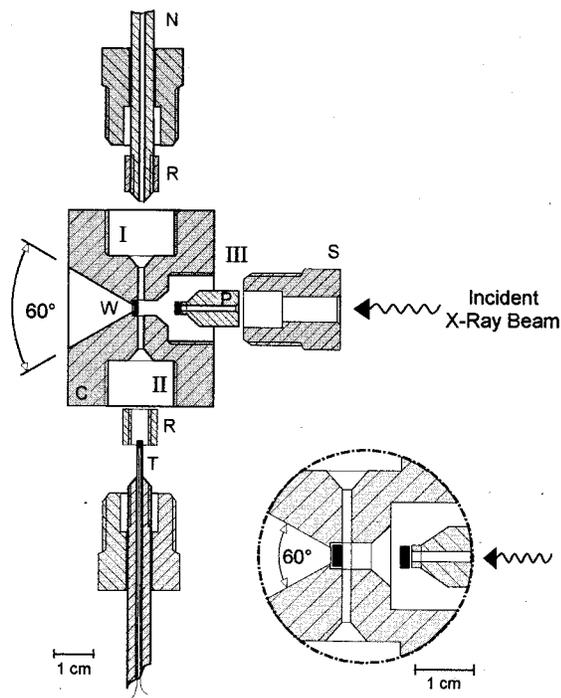


FIG. 1. Schematic view of the high pressure x-ray cell (HPXC). (C) high pressure cell body (made of Cr–Ni–Mo–Ti steel) with an upper pressure limit of 3000 bar; (P) sealing part of connection III and support of the x-ray input window; (W) x-ray window (beryllium-discs with a diameter of 3.5 mm and a thickness of 1.5 mm); (T) iron-constantan thermocouple seated in a stainless steel casing; (N) standard high pressure network (outer diameter of 6.35 mm, inner diameter of 1.6 mm); (S) standard support plug; (R) standard sealing collar; (I) connection to the high pressure network N, (II) connection to the thermocouple T, (III) x-ray beam input.

ties in heating these beryllium rods and in sealing the junction between the beryllium rod and the high pressure network, the hazards of breakage (beryllium oxide dust is extremely toxic), and the high manufacturing price led us to choose the window-type cell.

We used beryllium as it is the material of choice for high pressure x-ray windows if a conventional laboratory based x-ray generator is used. The x-ray absorption of beryllium is very low because of its low atomic number and additionally it possesses relatively high tensile strength due to traces of beryllium oxide.²⁵ Diamond, owing to its higher x-ray absorption, is more suitable if synchrotron radiation sources are used.²⁰

B. High pressure x-ray cell

The details of the design of the HPXC are outlined in Fig. 1. The body of the cell, made of high-strength Cr–Ni–Mo–Ti steel and outside dimensions of $(50 \times 36 \times 22)$ mm³ is a modified standard high pressure T-element from Nova Swiss (Nova Werke AG, Effretikon, Switzerland). The required high pressure connections already exist, and no changes in these standard connections were needed, hence the manufacturing time and consequently the cost for the HPXC could be kept low. The three connections have the screw dimension M16×1.6.

Connections I and II are used to join the HPXC to the high pressure network N and the thermocouple T. Connection III is used as an x-ray beam input. The sealing part of connection III is the plug P with its conical middle section, instead of the tube in conjunction with the screwed collar R, as it is in the case of connection I and II. Plug P additionally serves as a support for the x-ray input window, which is placed on the cylindrical top of the plug, possessing on its top a wall thickness of 1 mm and an inner bore of 1.5 mm in diameter as x-ray beam throughput. It is a nonstandard part and like the body of the cell made of Cr–Ni–Mo–Ti steel.

The throughput for the scattered radiation is a cone with an aperture of 2 mm and an opening angle of 60°. The cone enables scattering experiments up to the wide-angle region. A simple cylindrical throughput would only allow one to carry out experiments in the lower part of the small-angle region [see Fig. 2(A)]. However, the cone does not prevent

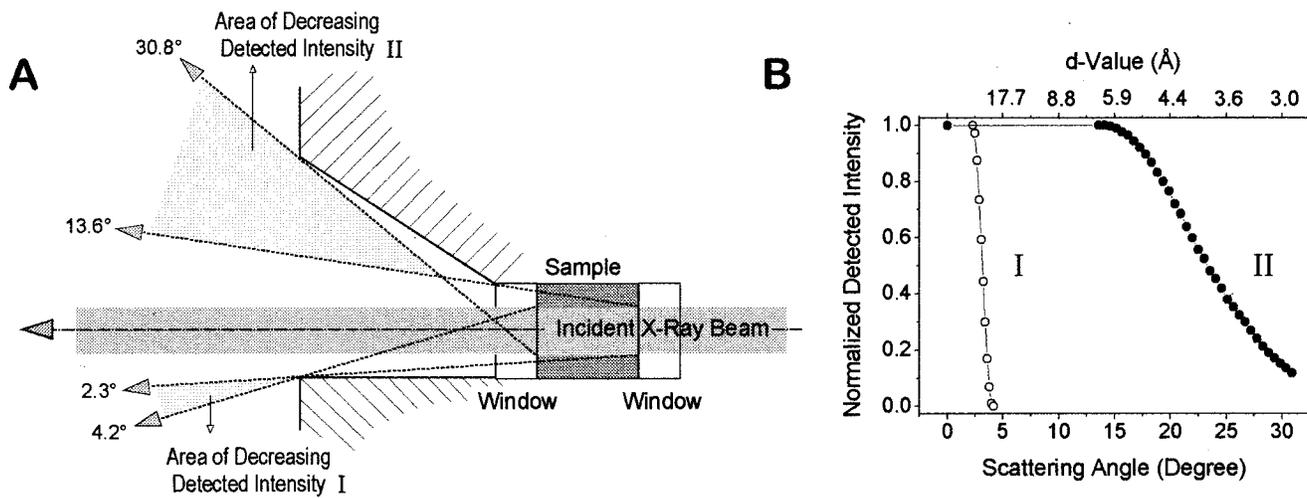


FIG. 2. (A) Schematic representation of the partial obstruction of the scattered radiation caused by the output window support and the cylindrical I or conical (opening angle=60°) II opening. (B) Corresponding intensity decrease calculated with Monte Carlo simulation for a primary beam diameter of 0.5 mm and the dimensions of the HPXC.

partial obstruction by the window support of the scattered radiation at larger angles, which leads to a decrease of the detected intensity with increasing scattering angle. Figure 2(B) shows this intensity decrease at higher scattering angles for a cone with an opening angle of 60° and a simple cylindrical x-ray throughput (opening angle = 0°), calculated with Monte Carlo simulation for a primary beam diameter of 0.5 mm. With conical opening the detected intensity decreases between the scattering angle of 13.6° and 23.7° by 50% and this shows that WAXS measurements of lipid-water systems can be performed in the wide-angle region of 4 \AA taking into account the nonlinear intensity decrease with increasing scattering angle.

The two x-ray windows are of the Poulter type²⁷ (see Fig. 1). Consequently, special care has to be taken to provide clean and polished surfaces of high quality at the windows and their support to guarantee perfect sealing. Since Poulter-type windows can be troublesome in the case of ambient or initially low pressure, an additional gel seal (viscous security seal Cat.No. 63814 from Loctite, Munich, Germany) in combination with a two-component glue (UHU Plus Endfest 300) is used to fix the windows on their support.

The disc-shaped x-ray windows (thickness = 1.5 mm, diameter = 3.5 mm) are made of beryllium (purity $\geq 98.5\%$). They are coated with a $5 \mu\text{l}$ polyimide film (Heraeus, Hanau, Germany) at the surface facing the cell interior, to protect it from contact with the pressure transmitting medium. The theoretical x-ray transmittance of the x-ray windows is 55% for $\text{Cu } K_\alpha$ radiation.

The bore in the direction of the incident x-ray beam possesses a diameter and a length of 3.6 mm and 4.6 mm, respectively. Taking into account the volume of the two x-ray windows a total sample volume of $\sim 16.3 \text{ mm}^3$ is available and assuming an x-ray beam with diameter of 0.5 mm the irradiated sample volume is $\sim 0.3 \mu\text{l}$. Viscous and solid samples are introduced in opening inlet III where the plug P can be easily removed. In case capillaries are used for sample containment the irradiated volume of the sample is roughly $0.15 \mu\text{l}$ and the connection of the high pressure network allows for capillary changing.

The HPXC withstands pressures up to 3000 bar and raising the pressure limit up to 3500 bar is quite conceivable.

1. Sample capsule

Liquid samples can be capsuled by a quartz-glass capillary (Mark-capillary Hilgenberg, Malsfeld, Germany) with an outer diameter of 1 mm, which is sealed by a plug of mercury to avoid contamination of the sample with the pressurizing fluid. This practically friction-free plug enables pressure equality inside and outside of the capillary.

Viscous samples are separated from the pressurizing fluid by a Teflon plug fitted in the bore between the sample volume and the high pressure network connection.

C. Pressure adjustment

The pressure is generated by a standard piston-type pressure generator (High Pressure Equipment Co. HIP, Erie, Pennsylvania, USA) capable of producing a hydrostatic pressure up to 4500 bar. The generator is operated by a geared

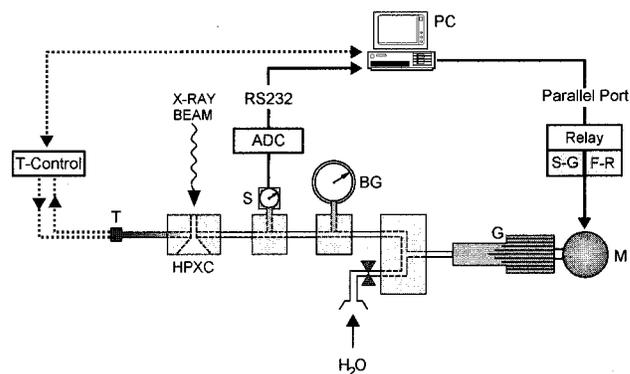


FIG. 3. Schematic diagram of the high pressure network, the pressure adjustment and the temperature control. The pressure measured by the sensor (S) is read into the computer (PC) through an analog-to-digital converter (ADC). Through a relay switching unit the PC commands the motor (M) to operate the piston of the high pressure generator (G). Manual adjustment of the desired pressure is possible by operating the stop-and-go button (S-G) and the forward-reverse button (F-R). The actual pressure can also be monitored by the Bourdon tube gauge (BG). Pressure and temperature are constantly on display. The circuit of temperature control with the thermocouple (T) and the reference junction is depicted in a very rudimentary way just for completeness.

three-phase ac motor. The pressure transmitting fluid is deionized water, which has the advantage of clean handling and low cost. Alternatively, oil as a pressure transmitting fluid can be used, if contact between the solid sample and water is undesirable.

We have taken advantage of the recent availability of commercial high pressure components²⁸ for building the high pressure network. The complete network, displayed in Fig. 3, is mounted on a portable Dural work bench. The network tubing has an outer diameter of 6.35 mm and an inner diameter of 1.6 mm, and is specified for pressures up to 7000 bar. Pressure can be adjusted either by manually switching the motor controls or automatically via personal computer (PC). The piston of the pressure generator, moving with a speed of about 0.5 mm/s, displaces approximately 30 mm^3 of the pressure transmitting fluid per second. Consequently the pressure rises from 1 bar to 3000 bar within roughly 60 s, supposing $dp/dt = -(1/\kappa V)(dV/dt)$ (κ = compressibility of water at 20° , V = total volume of the pressure transmitting fluid = $1.4 \times 10^4 \text{ mm}^3$).

With automatic control the compression proceeding at constant piston speed stops when the time, necessary for increasing the pressure by the difference between the set pressure and the actual pressure, lies within the periodic pressure readout interval of 80 ms. This leads to a maximal difference between the set and the adjusted pressure of $\pm 0.5\%$, taking into account the time to stop the movement of the piston, the intrinsic dead time of the pressure sensor and the delay caused by trapped air in the high pressure network which might occur especially at low pressures.

The pressure is measured by a pressure-to-voltage converter (HP/9991-01, it possesses an internal voltage amplifier and a nonstandard male pressure port with bore diameter of 1.6 mm and screw dimension M16 \times 1.6, Sensotec, Columbus, Ohio). The sensor is capable of measuring pressures up to 3450 bar with a calibrated full scale accuracy of 0.5%. For

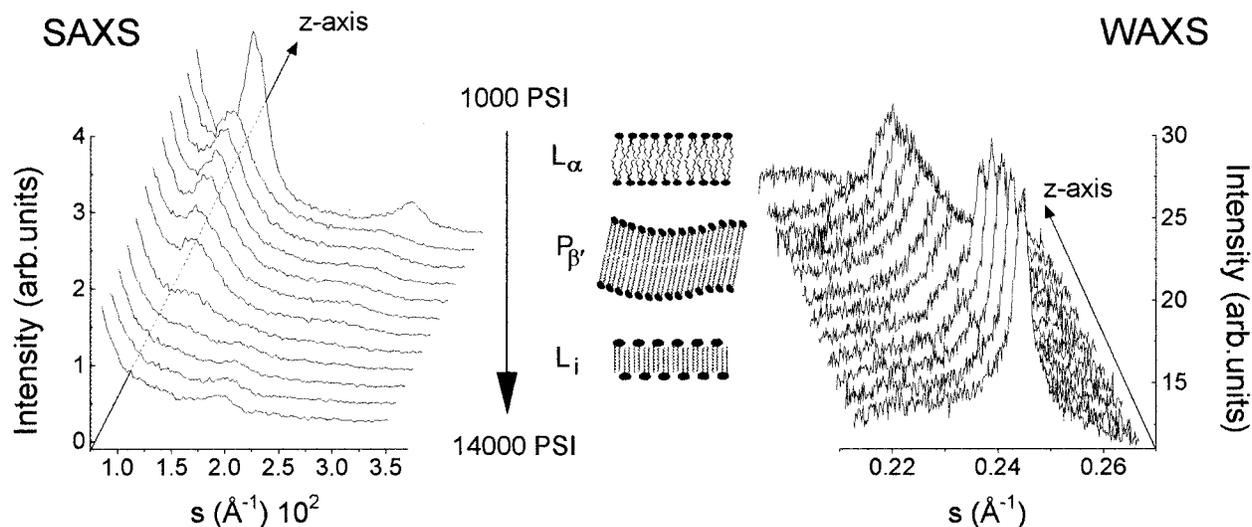


FIG. 4. Simultaneously measured SAXS and WAXS curves of distearoyl phaspatidylcholine (DSPC) in excess water between pressures of ~ 69 bar (1000 psi) and ~ 965 bar (14 000 psi) at a temperature of 56°C are shown. The sequence of the different lamellar phases of DSPC as a function of pressure is graphically depicted in the corresponding pressure region: the fluid phase L_α , the ripple phase $P_{\beta'}$, and the interdigitated phase L_i of the investigated phospholipid DSPC. Measuring protocol: from ~ 69 bar to ~ 552 bar (1000–8000 psi) and from ~ 758 bar to ~ 965 bar (11 000–14 000 psi) in steps of ~ 69 bar (1000 psi) with an equilibration time of 1800 s and an exposure time of 1200 s.

safety and convenience the pressure can also be monitored by a Bourdon tube gauge.

D. Temperature control

The present system is designed with a particular view on investigations of supramolecular structures and phase transitions of aqueous lipid dispersions. For pressure studies on systems undergoing phase transitions it is essential to warrant isothermal conditions. Lipid-water dispersions for example can exhibit a Clausius–Clapeyron coefficient dp/dT up to $50 \text{ bar}/^\circ\text{C}$.²⁹ Hence special care has been taken providing precise temperature control. The temperature control presented below allows temperature variation between -20°C and $+80^\circ\text{C}$ with a precision of $\pm 0.05^\circ\text{C}$.

The temperature is measured by an iron-constantan thermocouple with the sensor placed at about 1 mm distance from the irradiated sample volume. The interference by galvanic potentials which occur upon contact of the measuring and reference junctions with water is prevented by protecting these junctions in stainless steel and glass tubes, respectively.^{18,30}

The desired temperature is adjusted by two Peltier modules (Melcor, Trenton, New Jersey) sandwiched between the broadsides of the HPXC and two water-cooled copper blocks. This assembly is held together by a stainless steel cage which allows one to change the sample without dismounting the Peltier modules and the copper blocks.

The electronic temperature control mainly consists of a 16-bit microprocessor (6303 MCU) with a programmable erasable programmable read-only memory (EPROM), a 16-bit analog-to-digital converter, and a 16-bit digital-to-analog converter. The temperature is controlled such that in periods of 100 ms the required thermoelectric current is adjusted according to the actually measured temperature by a digital proportional plus integral (PI) controller. The performance

characteristics of the PI controller are adapted to the thermoelectric power of the Peltier elements, to the mass of the HPXC and to the required control limits by control parameters.

A serial RS-232 port enables communication with a personal computer, which allows convenient input of the desired temperature, the control parameters and a calibration table. The calibration, necessary owing to the nonlinear temperature-voltage behavior of the thermocouple, is done with a calibrated Pt-1000 resistance thermometer and can be stored in the random access memory (RAM) of the microprocessor.

III. SYSTEM TEST AND PERFORMANCE

A. X-ray camera setup

The above described HPXC and its environment is designed for easy insertion into standard x-ray optical systems. The experiments are performed using a camera system based on a pin-hole collimator $200 \mu\text{m}$ in diameter attached to a rotating-anode generator (RU-200B, Rigaku, Japan) with a Ni filter and Cu K_α radiation ($\lambda = 1.54 \text{ \AA}$). The generator is operated at a power of 7.5 kW. The scattered radiation is collected using a two-dimensional image processing detector system (Photek IPDX40, Instrument Technology Limited, St. Leonards-On-The-Sea, UK) in the small-angle region and a one-dimensional position-sensitive detector [(PSD-50), MBraun, Garching, Germany] in the wide-angle region. The distance of the evacuated path of the scattered radiation between the sample and the detector is 290 mm and 200 mm for small- and wide-angle measurements, respectively.

The exposure time of each scattering pattern was 1200 s. The SAXS curves, obtained by the two-dimensional detector, are integrated over an angle of 10° and radially normalized.

B. Test measurements

Test measurements were done in order to show the performance of the HPXC by simultaneous SAXS and WAXS experiments with several phospholipid-water dispersions. Aqueous dispersions of phospholipids show a variety of different phases, depending on hydration, temperature and pressure.^{31,32} The properties of these lipid phases and their thermodynamics regarding hydration and temperature are intensively studied.^{33–36} But without investigations concerning the thermodynamic variable pressure^{9,37} knowledge of the conditions governing phase formations and phase transitions would be incomplete.

Our experiments under elevated pressure show that with an exposure time of 1200 s diffraction patterns with reasonable counting statistics can be performed using a conventional laboratory x-ray source. In Fig. 4 the uncorrected diffraction patterns of phospholipid-water dispersion (DSPC) at 56 °C and pressures between ~69 bar (1000 psi) and ~965 bar (14 000 psi) are displayed. At 56 °C and ~69 bar (1000 psi) the DSPC is in the lamellar-fluid L_{α} phase. Increasing the pressure by ~69 bar (1000 psi) pushes the system in the corrugated $P_{\beta'}$ phase. Further increase of the pressure lets the system gradually immerse into the interdigitated L_i phase.

Beside studying lipid phases under near equilibrium conditions, investigations under nonequilibrium conditions are indispensable to understand the mechanism and rates of the transition between various phases. In view of this necessity the high pressure apparatus described in this paper can be easily upgraded for pressure jump relaxation experiments, with a time resolution in the millisecond range, using high flux synchrotron x-ray radiation. The modifications necessary to enable these experiments will be described in detail in a subsequent article.

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¹The following units of pressure are provided for the convenience of the reader: 1 atm = 1.013×10^6 dyn cm⁻² = 1.013 bar = 1.013×10^5 Pa (= N m⁻²) = 14.696 psi = 76 cm Hg (at 0 °C) = 760 Torr (at 0 °C).

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