A high pressure cell for small angle neutron scattering up to 500 MPa in combination with light scattering to investigate liquid samples

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We report on a high pressure cell to use with small angle neutron scattering (SANS) in a pressure range up to 500 MPa. The cell offers the new possibility to investigate liquid samples by a specially designed sample chamber, which allows changing of samples relatively easily. Since the cell construction uses sapphire as window material, also light scattering investigations can be performed simultaneously to the SANS measurements. In this article we describe the construction of a high pressure cell and we demonstrate the applicability of the construction for SANS in combination with dynamic light scattering showing data on the biological molecule lysozyme. © 2007 American Institute of Physics. [DOI: 10.1063/1.2817632]

I. INTRODUCTION

The use of high pressure in natural sciences has gained more and more attention since the pioneering work of Bridgeman.¹ The solid-solid phase transitions, phase equilibria in general, or the use of high pressure to elucidate reaction kinetics via the concept of activation volumes are examples to name only a few. The use of small angle neutron scattering (SANS) together with high pressure is a rapidly growing field of research because neutron scattering yields the proper q range for studying conformational changes of matter. It further offers the possibility of using contrast variation techniques which have just recently gained much attention through a variety of biological/physical problems that have been tackled by high pressure techniques and SANS.^{2–6} Recent developments in this area report on time resolved measurements using pressure jump techniques to explore the kinetics of phase behavior and structure formation in complex matter. However, this has so far only been reported with X-rays and laser light scattering.^{7,8} We present in this article a setup which combines SANS and dynamic light scattering (DLS) up to 500 MPa at the same time. An option for performing pressure jumps is also implemented. Technically, one of the most prominent problems is the so called media separation, which is needed to separate the pressure transmitting liquid, mostly hydraulic oil, from the liquid samples. Early attempts on a high pressure cell for liquid, viscous polymeric samples have been reported⁹ in a pressure range up to 250 MPa. There, an O-ring separates the sample from the pressure transmitting oil. However, no container was used and, therefore, sample change was difficult and time consuming. Other high pressure cells have been described to use with SANS.^{10–13} However, so far, no device has been reported to allow the simultaneous light scattering measurement. The cell by Bonetti and Calmettes¹² has, in principle,

this option for a 90° scattering angle, but no such data are published. Another technical drawback is the usually relatively large beam diameter in neutron scattering compared to X-rays or light. Therefore, a rather big sample size is needed and, thus, the high pressure device has to take care about that constrain. We will present in this article a method by which a relatively easy change of liquid samples can be achieved. This method uses a small container which carries the liquid sample (the so-called inner container method). It is sealed against the pressure transmitting oil by an O-ring, which at the same time allows the pressure to be transmitted and performs separation of media. A further advantage of our construction is its defined sample thickness which does not change during the experiment. The thickness is controlled by the dimensions of the inner container and can be varied according to transmission requirements by changing the container window thickness. In this article we will introduce our new SANS high pressure cell design with relatively easy sample change and the online possibility to do dynamic light scattering. In order to prove its functioning, we will present light scattering data on egg-white lysozyme up to 500 MPa and SANS data up to 300 MPa.

II. THE HIGH PRESSURE CELL FOR SANS

The basic ideas behind the SANS high pressure cell are the following: easy change of sample, reliable media separation which means that the pressure transmitting hydraulic oil does not mix with the sample, use of window material which is also optically transparent, and last, but not the least, a relatively large pressure range accessible with the possibility to change temperature and perform pressure jump experiments. We have constructed a pressure jump generator on the basis of a moving piston that allows changing pressure in a range of ΔP about maximally 100 MPa in about 1 s. Also, a repetitive mode is possible as the whole setup is computer controlled via self-written software. First results from that

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FIG. 1. Middle cut through the high pressure cell.

system will be published elsewhere. Our cell design allows a maximum pressure of 500 MPa which has been tested and approved by a finite element calculation. Irreversible damage of the cell occurs at pressures exceeding 800 MPa. The cell can be operated in a temperature range between 4 and 80 °C by means of a surrounding jacket in which a thermostating liquid circulates. The temperature accuracy is within 1 K. In our experiments, we have used pressures up to 500 MPa which were achieved by a screw-type positive displacement pressure generator (Sitec) and were measured by a pressure transducer (Burstner) with an accuracy of 0.5 MPa. The cell is shown in Figs. 1 and 2. It is equipped with two sapphire windows (optical grade heat exchange method sapphires of 62 mm diameter and 35 mm thickness, Kyburz Sapphire AG, Switzerland) which are mounted vis-à-vis on a highly polished inner cell holder. A Teflon foil between sapphire windows and inner cell holder guarantees good sealing and helps avoiding scratches in the windows during the assembly of the cell. The sample container, called “pill,” is placed in an

FIG. 2. (Color online) Details of high pressure cell after one half of the steel body was removed. What is seen is the other half of the steel body on which the inner cell holder lies. Mounted on that is one big sapphire which in the completely mounted state is hidden within the other half of the steel body. The sample pill is located on the bottom of the sapphire in the middle of the inner cell holder. The silver ring which seals the inner volume filled with oil (and pill) against the sapphire lies around the sample pill. Also, a Teflon sealing foil is seen between sapphire and inner cell holder.

FIG. 3. Shown is an inner circular section of the cell as in Fig. 1 with details of the sample container (pill). The cut is along the holes of the brass ring to illustrate where the hydraulic oil can transmit the pressure via the Viton O-ring.

empty inner space which is formed between both sapphire windows in the inner cell holder. This inner space is sealed against the windows by silver rings. For pressures up to 250 MPa also a viton O-ring is good enough. The pressure transmitting oil can access the inner space via holes normal to the polished surfaces. This detail is shown more explicitly in Figs. 3 and 4. The inner space is filled with the pill. It consists of a brass ring which has on its periphery a number of small holes. Prior to the high pressure experiment, this pill has to be filled with the sample. To do so, a sapphire plate is pressed into one side of the brass ring by means of a special tool. Then a Viton O-ring is placed into the brass ring, and the sample is filled in very carefully in order to avoid air bubbles. This assembly is then closed by another sapphire plate which is pressed in the other side of the brass ring using the same tool as before. The dimensions are chosen such that the plates now squeeze the O-ring which in turn

FIG. 4. (Color online) Components of the pill: the brass ring with holes on the periphery, the two sapphire plates, and the O-ring. The sample in the pill is sealed against the hydraulic oil when the two sapphire plates press the O-ring against the brass ring holes from inside.

will seal the holes on the periphery of the brass ring. The plates have typically a thickness of 3 mm which leaves an open space of about 1.3 ml volume and about 2 mm thickness for the sample. In principle, also other thinner plates can be used. By that, a larger volume and hence larger path lengths for the neutrons can be achieved. That might be useful for highly deuterated samples, where usually the transmission is high. Placing this pill now in the inner space of the high pressure cell will allow the hydraulic oil to transmit the pressure on to the sample via the holes on the periphery of the brass ring. The thickness of the pill is chosen such that the sapphire windows are in contact with the sapphire plates when the cell is ready to use. Due to this contact against the sapphire windows, the sample thickness is fixed and does not change with pressure (less than 10 μm , which corresponds to a 1% variation in path length and, thus, in transmission). A thin film of hydraulic oil might form between sapphire plates and sapphire window and, therefore, add incoherent scattering to the SANS spectra, but the effect is negligible due to the very thin film thickness and can furthermore be reduced by the proper choice of hydraulic liquid. The inner part of the cell which contains the pill is held in place via two rather massive stainless steel parts which are connected by eight steel bolts. The mounted pressure cell is 220 mm wide, 150 mm high, and 160 mm deep with a sapphire window aperture of 42 mm which allows for a maximum forward scattering angle of 20°.

III. LIGHT SCATTERING AT HIGH PRESSURE

It is possible to perform DLS on a sample in the pressure cell. As the pressure cell was designed for SANS, several drawbacks for DLS had to be overcome. The thick sapphire windows induce a parallel offset of nonperpendicular beams and slightly scramble the polarization of the incoming and outgoing photons. The latter can be overcome by placing an accordingly oriented polarizer in front of the detector system, filtering out the scrambled part of the scattered light. The parallel offset in combination with the comparably small illuminated part of the relatively thin sample simply causes a more delicate alignment procedure. Another optical problem arises from the reflections from all the surfaces in this stack of parallel flat windows. Multiple reflections are scattered back and forth and superpose with the scattered light from the sample. By orienting the incident beam nonperpendicularly to the window surfaces and by tilting the whole scattering plane against them, the scattering volume can be accessed by the detection system. Due to the rather complicated beam path through the pressure cell windows, an additional calibration measurement has to be performed to properly determine the precise scattering angle θ . To do so, we have measured the diffusion coefficient of our sample in a standard commercial ALV light scattering goniometer. From the so obtained exact relation between relaxation rate and scattering angle for our sample, it is then possible to calculate the scattering angle of the DLS setup at the SANS instrument. Thus, the scattering angle in the high pressure experiment presented in this article could be determined to be $\theta=138^\circ$. The definition of the scattering vector q is

FIG. 5. Schematic sketch of the setup which allows simultaneous SANS and DLS measurements.

$q=|\mathbf{q}|=(4\pi n/\lambda)\sin(\theta/2)$, where n is the refractive index, λ is the excitation wavelength, and θ is the scattering angle. Performing high pressure light scattering experiments, one has to take into account that, due to measurements at a fixed scattering angle, the value of q is pressure dependent because the refractive index n is pressure dependent. In a recent publication, we have demonstrated how data have to be treated in order to account for this effect.¹⁴ In our DLS setup in the SANS sample environment, a vertically polarized beam from a 17 mW He-Ne laser (Melles-Griot) with a wavelength of 632.8 nm illuminates the sample. The scattered light passes a vertical polarizer, is collimated into a monomode fiber beam splitter, and is then fed into two avalanche photodiodes (APD, Perkin Elmer). The output signal of both APDs is then processed with an external two channel correlator (correlator.com Flex99OEM-12) in order to perform a pseudo-cross-correlation which eliminates undesired detector side effects (after pulsing). The used setup is sketched schematically in Fig. 5.

From the experimental setup, it is rather difficult to determine the exact scattering angle. Please note that with a few modifications, the DLS setup described beforehand can also be used for diffusing wave spectroscopy (DWS) to characterize the dynamics of turbid samples. We have already successfully measured turbid samples by using an enhanced DWS setup which allows for the distinction between ergodic and nonergodic samples (the so-called double-cell DWS setup).

IV. PERFORMANCE TEST OF THE HIGH PRESSURE CELL USING A LYSOZYME STANDARD SAMPLE

To test the performance of the high pressure cell, we have prepared a solution of egg-white lysozyme at a concentration of 50 mg/ml in 50 mM acetate buffer in D_2O (that corresponds to about 1.3% added salt to the D_2O). The experiments have been performed at the temperature $T=24^\circ\text{C}$.

A. Dynamic light scattering (DLS) under high pressure

For the homodyne case, the experimentally measured intensity autocorrelation function $G^{(2)}(q, t) = \langle I(q, t)I(q, 0) \rangle$ is related to the normalized autocorrelation function $g^{(1)}(q, t)$ via the Siegert relation,¹⁵

$$G^{(2)}(q, t) = \langle I \rangle^2 [1 + f |g^{(1)}(q, t)|^2], \quad (1)$$

where f is an experimental factor and $\langle I \rangle$ is the mean intensity. The field autocorrelation function $g^{(1)}(q, t)$ is related to an apparent diffusion coefficient D_{app} of the system with equilibrium concentration fluctuations via $g^{(1)}(q, t) = \exp(-D_{\text{app}}q^2t)$. This is usually written as $g^{(1)}(q, t) = \exp(-\Gamma t) = \exp(-t/\tau)$, where Γ is the relaxation rate and τ is the relaxation time. To model the field autocorrelation function $g^{(1)}(q, t)$ for a size distribution of diffusing particles, we used the cumulant expansion for $g^{(1)}(q, t)$,

$$\ln g^{(1)}(q, t) = -\Gamma t + \mu_2 t^2, \quad (2)$$

with μ_2 being the second cumulant. The ratio μ_2/Γ^2 is a measure of the size polydispersity of the system. If the system is monodisperse, then μ_2 is zero and the cumulant expansion recovers a simple single exponential fit to $g^{(1)}(q, t)$. From the concentration dependence of the obtained diffusion coefficient D_{app} , we can calculate the translational diffusion coefficient at zero concentration D_0 according to¹⁶

$$D_{\text{app}}(c) = D_0(1 + k_d c), \quad (3)$$

where k_d is a parameter describing the particle interactions and c is the concentration. The so obtained D_0 is related to the particle size by the Stokes-Einstein relation,

$$D_0 = \frac{kT}{6\pi\eta r}. \quad (4)$$

Here, kT has the usual meaning, η is the solution viscosity, and r is the hydrodynamic radius of the diffusing particle. The pressure dependence of k_d has been reported by Banachowicz and was found to be a monotonically decreasing function with pressure.¹⁷ It seems as if k_d approaches a constant value at pressures beyond 100 MPa of about 7×10^{-4} ml/g. We have performed DLS measurements in the high pressure cell configuration, as shown schematically in Fig. 5. The obtained normalized intensity autocorrelation functions from the sample in our SANS cell are shown as a function of pressure in Fig. 6. From the cumulant analysis of the data at $P=0.1$ MPa at a q value of $q=1.85 \times 10^5$ cm⁻¹, we find, according to Eq. (2), an apparent diffusion coefficient of $D_{\text{app}}=1.16 \times 10^{-6}$ cm²/s in good agreement with the data of Banachowicz.¹⁷ Since the product of $k_d c$ is in the order of 10^{-5} , we can safely approximate, following Eq. (3), the pressure dependence of D_0 by the one of D_{app} . The latter pressure dependence is plotted as $\Gamma = D_{\text{app}}q^2$ in the inset of Fig. 6. Obviously, there is no or at least a very small variation of the apparent relaxation rate with P . Intuitively, one would expect that the rate is slowed down with increasing P . However, there are two effects which tend to cancel each other and we rationalize it in the following way: Eq. (4) relates the rate with viscosity and, hence, we find $\Gamma_{\text{corr}} \approx q^2(P)/\eta(P)$, where the P dependence of q is given by the

FIG. 6. Normalized intensity autocorrelation functions for lysozyme (50 mg/ml) in 50 mM acetate buffer as a function of pressure as indicated. In the inset, the values for the pressure dependence of the bare relaxation rates Γ and Γ_{corr} are shown (the latter is corrected for the pressure dependencies of η and n).

P dependence of n . $\eta(P)$ can be found in Ref. 18, and to calculate the respective changes in n according to the recipe given in Ref. 14, we used literature values²⁷ for the density of light and heavy water. Contrary to η and n , the pressure dependence of radius r is negligible as is later rationalized by SANS from the shape invariant scattering curves with varying pressure. So, for the pressure dependence of the viscosity and index of refraction, corrected relaxation rates, also shown in the inset of Fig. 6, show no dramatic P dependence and are up to 140 MPa in agreement with Banachowicz.¹⁷ This finding supports the assumption that pressure is usually not able to change the conformation of lysozyme unless pressure exceeds a certain critical pressure threshold value. Then, typically, an irreversible rearrangement of structure occurs. The structural stability of lysozyme against pressure is probably buried in the dense structure stabilized by interplay between hydration and electrostatic interaction. For pressures up to 200 MPa, for example, volume changes in the order of 2.4% have been reported.¹⁹ This small variation is in agreement with our findings. We find by dynamic light scattering lysozyme to be structurally stable up to 450 MPa and this is also supported by SANS monitoring the structure directly as described in the following section. We should note here that, at $P=500$ MPa, we find a different behavior by performing light scattering experiments, namely, exactly this beforehand mentioned structural changes. This is indicated by a loss of the apparent P independence of the correlation function (in Fig. 6 the curve for 500 MPa does not superimpose with all other pressures) and the unusual time dependent long-time tail of the correlation function. Moreover, this effect is irreversible upon release of pressure. Hence, we conclude that structure has changed. Unfortunately, we have no SANS data for this case since we ran short with beam time, but since the effect has been established, we will study it in future work.

B. SANS under high pressure

We have performed the SANS experiments at the SANS I machine at the SINQ spallation source at the PSI.²⁰ We

FIG. 7. SANS results for lysozyme in D_2O as a function of pressure as indicated. Also shown for comparison is the background of the high pressure cell. It shows no q dependence and amounts to about 0.12 cm^{-1} .

used thermal neutrons of wavelength $\lambda=0.5 \text{ nm}$ featuring a wavelength spread $\Delta\lambda/\lambda$ of about 0.1. The data analysis was performed using the BERSANS software package,²¹ which accounts for all necessary corrections due to background, transmission, count rate/dead time ratio, possible multiple scattering, masking, and radial averaging of the raw data. A standard water sample was used for calibration to absolute scattering intensities and also to account for nonuniform detector efficiency.²² We have measured at 2 m detector distance, which gives access to a q range of $0.5 < q(\text{nm}^{-1}) < 3.5$, where q is the scattering vector, defined as $q=|\mathbf{q}|=(4\pi/\lambda)\sin(\theta/2)$, with λ being the incident neutron wavelength and θ the scattering angle. Note that light scattering q 's are at least one order of magnitude smaller than those from SANS; however, its variation is smaller.

The results of the SANS measurements are shown in Fig. 7 as a function of pressure up to 300 MPa. Basically, one notices an increase of scattering intensity with pressure which results in a change of contrast due to the compression of the solvent, similar as in the case of glucose isomerase. As in that case, lysozyme seems to possess a stable conformation with regard to the exertion of pressure since the curves can be scaled on top of each other without any noticeable change of shape. Without doing any analysis on the q dependence of $I(q)$ of the lysozyme, we process only the values of the forward scattering for $q=0$.

For the forward scattering $I(q=0)$, we can write²³

$$I(q=0)K = c \frac{N_A}{M_w} \left(\frac{b}{V} - \rho_0 \right)^2 V^2 S(q=0)$$

$$= c \frac{N_A}{M_w} (b - \rho_0 V)^2 S(q=0), \quad (5)$$

where K is a calibration constant, $\Delta\rho=b/V-\rho_0$ is the scattering contrast of the lysozyme, $b=4.50 \times 10^{-10} \text{ cm}$ is the scattering length, $V=17.8 \text{ nm}^3$ the volume,²⁴ b/V the scattering length density of the lysozyme, and ρ_0 is the scattering length density of the solvent (heavy water with a scattering length density of $\rho_0=6.36 \times 10^{10} \text{ cm}^{-2}$). Clearly, b does not depend on P since it is entirely given by the atomic compo-

FIG. 8. Comparison between the pressure variation of the forward scattering and the density change for heavy water. Density data from Ref. 18.

sition; also, V does not change much with P since the scattering curves show no change in shape. This finding is supported by recent SANS results on glucose isomerase, where the shape of the scattering curves did not also change with P .²⁵ Therefore, b/V is considered as being not pressure dependent, and, likewise, the structure factor at $q=0$ which was calculated using a rescaled mean spherical approximation calculation is $S(q=0)=0.68$.²⁶ What differs is mainly the scattering length density ρ_0 of the D_2O , because the number density of scatterers depends on P . Following Eq. (5), a plot of $I(q=0)$ as a function of pressure should follow the pressure dependence of the density for heavy water.¹⁸ This relation is shown in Fig. 8 and shows an excellent agreement between experiment and literature values.

V. CONCLUSIONS

We report on a SANS high pressure cell which uses fused sapphire as window material and, thus, allows simultaneously to perform light scattering investigations beneath the SANS measurements. The cell works for pressures up to 500 MPa in a temperature range between 4 and 80 °C and uses a special container technique that allows separating sample and pressure transmitting oil easily. SANS and simultaneous DLS data on an egg-white lysozyme solution under high pressure are presented and show the functioning of the setup.

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