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An alternative method to the osmotic stressing polymers: the osmomanometer

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Abstract Although stressing polymers have been widely and successfully used to determine the osmotic properties of solutes in aqueous media, the osmotic stress method presents some limitations. To overcome these drawbacks, an alternative and more direct method, which has been named the osmomanometer, is described in this letter. The osmotic pressure accessible by this method ranges typically from 1 to 30 kPa using a simple hydrostatic effect and can be extended to higher pressures by using pressurized gas. This method needs neither a pressure sensor nor calibration.

Keywords Osmometer · Osmotic pressure · Stressing polymers

Discussion

During the last few decades, the "osmotic stress" technique has been extensively and successfully used in order to determine the thermodynamic properties of solutes like DNA, polysaccharides or proteins (see the general review by Parsegian et al. 1986). As recalled in part by Cohen and Highsmith (1997), this method has provided information on compacting forces (LeNeveu et al. 1976; Podgornik et al. 1995), electrostatic interactions (Essafi 1996; Raspaud et al. 2000; Hansen et al. 2001), hydration changes (Reid and Rand 1997) and protein binding properties (Garner and Rau 1995; Highsmith et al. 1996). More recently, the compaction of the *Escherichia coli* nucleoids was also analysed (Cunha et al. 2001). An "osmotic stress" experiment consists in changing the osmotic pressure of a solution (containing the solute of interest) in thermodynamic equilibrium with a reservoir (the stressing solution). Both solutions may be separated by a semi-permeable membrane. The "stressing solution" is usually composed of a "stressing" agent, a hydrophilic polymer [poly(ethylene glycol), PEG; poly(vinylpyrrolidone), PVP; or dextran], diluted in water at a given salt concentration. The osmotic stress or the osmotic pressure magnitude is reported on different websites (http://www.mgsl.dcrt.nih.gov/docs/osmdata/osmdata.html; http://aqueous.labs.brocku.ca/osfile.html) as a function of the polymer concentration. The typical applied pressure ranges from 1 kPa to 10 MPa. It should be also noted that these polymers have been astutely used in an inverse osmotic pressure experiment by Dubois et al. (1998) in which the sample becomes the reservoir. As mentioned by Podgornik et al. (1995), the stress exerted on the solute via the membrane is similar to the application of pressure by a semi-permeable piston, where permeating species (water and other small molecules) may move freely. The thermodynamic equilibrium is reached when the chemical potentials of the permeating species are equal on both sides of the membrane.

Even if this kind of experiment is simple and allows us to access a wide pressure range, there are some experimental and theoretical limitations:

1. As mentioned by T. Odijk (personal communication) and as also observed in a previous experiment (Raspaud et al. 2000), there is a tendency of clogging the membrane, which is usually a simple dialysis bag. Polymers may obstruct the pores, thus limiting the exchange between the two compartments.
2. Depending on the pore size of the membrane but also on the quality or more exactly on the polydispersity of the polymers, a fraction of the polymers may pass through the membrane and contaminate the samples. This means that the solute may not be re-used for experiments that are very sensitive to the contamination.
3. The pressure tables on the websites are generally given for experiments performed at room temperature and in water. As a consequence, if one needs to perform

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the experiments in a different solvent or at a lower temperature in order to diminish the action of contaminant enzymes, pre-calibration is required.

4. The last limitation reported here, being more of a theoretical concern, is due to the inert part of the polymers, which are only considered to repel the solute via their strong affinity for water and via depletion forces. One may not exclude the possibility of specific interactions between the solute and the hydrophilic polymers (see Timasheff 1998).

In this letter, an alternative and complementary method is proposed. Instead of measuring the osmotic pressure exerted by a solute diluted at different known concentrations, an osmotic pressure is imposed manometrically by a column of solvent and the concentration of solute in thermodynamic equilibrium is measured. This method does not need stressing polymers but uses only the gravitational effect of the solvent. The general idea is quite simple and is illustrated in Fig. 1. The upper compartment, which contains the solute, is separated from the rest by a semi-permeable membrane. On the other side of the membrane, the lower compartment is connected to the reservoir by a column. The two free surfaces of the liquid located in the upper compartment and in the reservoir are open to the external atmosphere. Both surfaces are not at the same height and one may note the height difference, Δh. The differential pressure which is exerted on the two free surfaces is then simply given by the hydrostatic formula $\pi = \rho g \Delta h$, with ρ being the liquid density and g the acceleration of free fall, 9.81 m s^{-2}. In water at a temperature of 20 °C, $\rho = 0.997$ g $cm^{-3} \approx 10^3$ kg m^{-3}, the pressure becomes equal to $\pi = 9.78 \times \Delta h$ kPa. Therefore the pressure is only proportional to the height difference; in order to vary the exerted pressure, a change in Δh is sufficient. In a standard laboratory, one may typically move Δh from 0.1 to 3 m, leading to the accessible pressure range 1–30 kPa.

This range is quite limited when compared to the pressure range explored using the stressing polymers (1 kPa to 10 MPa). However, it could be extended using the same accessories and just by changing the method as follows. The reservoir may be moved so that there is no height difference and then the upper compartment may be connected to a pressurized gas (like nitrogen) source with a classical manometer. In this more classical configuration, the pressure exerted on the solute compartment corresponds to the gas pressure which is regulated by the manometer. In this case, the accessible pressure ranges from 10 kPa to some MPa, thus covering the upper limit of the osmotic stress method.

To test the validity of the method illustrated in Fig. 1 and its application, the curve of pressure versus concentration has been determined using the stressing polymer dextran (Fluka, MW 110,000) diluted in water, and has been compared to the data reported on the website http://aqueous.labs.brocku.ca/osfile.html (data provided by C. Bonnet-Gonnet). The cell, which is composed of the two compartments separated by the

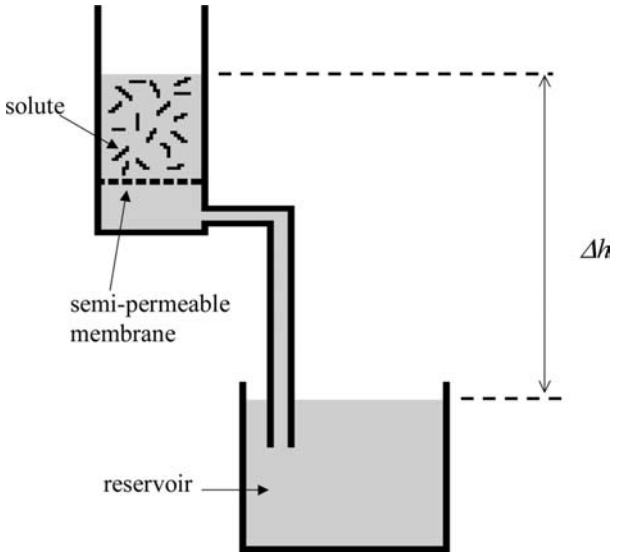

Fig. 1 Schematic illustration of the osmomanometer. A semi-permeable membrane separates the upper compartment which contains the solute from the lower compartment which is connected by a solvent column to a solvent reservoir. The membrane is not permeable to the solute but permeable to the solvent (water, etc.). The two free surfaces (of the upper compartment and of the reservoir) are open to the external atmosphere and are at a height difference Δh. Without a membrane, all the solvent and solute would fall into the reservoir, but in the presence of the membrane a fraction of the permeating species is retained in the upper compartment. This is due to the solute affinity for the solvent or, more precisely, this effect is related to the mixing free energy G. This fraction may be monitored by changing the height difference Δh or equivalently by changing the osmotic pressure $\pi = \rho g \Delta h$. Thermodynamically, the osmotic pressure is defined by $\pi = -\partial G / \partial V$, V being the retained volume

membrane and which has been used, is of type "Stirred Ultrafiltration Cell" (Amicon) with a semi-permeable membrane ("Ultrafiltration Membrane" in regenerated cellulose, NMWL = 10,000, Millipore). This cell is placed on a magnetic stirring table. For the rest of the accessories, the column is a flexible tube of different lengths and the reservoir a simple flask of water. Before operating on the dextran solution, the cell and the column are previously filled with water using a nitrogen pressure source. A solution of diluted dextran is then added to the upper compartment and equilibrated during at least two days for a given Δh value. At the equilibrium, the value Δh is re-measured and the final dextran concentration W (%) is determined by weighting. For DNA or proteins, this concentration could be also measured by UV absorption. To obtain a series of pressures, Δh is changed. The measured pressure versus concentration curve (filled symbols) is shown in Fig. 2 and compared to the known data (open symbols). The two curves are in very good agreement, thus proving the validity of the method.

One may note some limitations of this experiment due to the types of membrane and cells that have been used here and which are not perfectly adequate. The major drawback comes from the membrane, which is

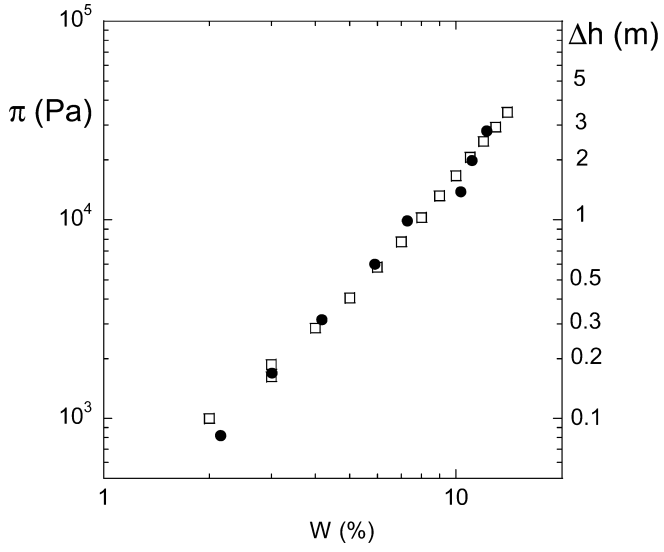

Fig. 2 Simple test of the osmomanometer application with the polymer dextran diluted in water. The *open* and *filled* symbols correspond to the data given on the website and to the measurements performed with the proposed method, respectively. The *right vertical axis* indicates the imposed height difference Δh and the corresponding osmotic pressure π is given on the *left axis*. The *horizontal axis* is the dextran weight fraction in the upper compartment

not symmetric on both sides. This means that the permeating species may only pass in one direction. In the present case, the dextran solution in the upper compartment may only be concentrated and not diluted by the height difference due to this membrane asymmetry. Another problem is the presence of air in the liquid column, which may disrupt the correct exchange between the reservoir and the dextran compartment. It probably comes from the insufficient air tightness of the cell. The last limitation corresponds to the fact that a quite large final volume (ca. 0.5–1 mL) is required to obtain the correct exchange and to avoid contact between the air and the membrane. Finally, all these limitations could be improved by using appropriate cells and membranes. On the other hand, compared to the osmotic stress method, this osmotic manometer has the advantage of dealing with the absolute osmotic pressure without calibration. This means, for instance, that the experiments may be performed either at room temperature of 20 °C or in a cold store at 2–7 °C.

To conclude, because of its simplicity, this method seems very attractive and educative in the sense that the user imposes the height difference on the human scale (meters). For heights of the order of 2–3 meters, the experimenter just needs a step ladder to perform the experiment! To approach the pressure condition of the internal bacterial medium [see reference (32) in Kindt et al. 2001], a few tens of meters are required.

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