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SHORT COMMUNICATION

MEASUREMENT OF COLLOID OSMOTIC PRESSURE OF MICROLITRE AND SUB-MICROLITRE VOLUMES

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This paper describes two devices for the measurement of colloid osmotic pressure (COP) in volumes of fluid of 0·5 μl or larger. Both devices are based on a design introduced by Hansen (1961) in which COP is measured directly. An ultrafiltration membrane is clamped over a fluid-filled reference chamber which communicates with the sensitive surface of a blood pressure transducer. When colloid-containing fluid is placed on the upper surface of the membrane, fluid is drawn out of the reference chamber, lowering the hydrostatic pressure there. If the membrane is impermeable to the colloid, the pressure drop in the reference chamber is directly proportional to the COP of the colloid-containing fluid.

As shown in Fig. 1, a miniature pressure transducer (model XCQ-080-10G, Kulite Semiconductors Ltd, Basingstoke, England) is held by a PTFE washer and stainless-steel cap in the lower half of a threaded and bored out length of acrylic rod. Glued into the upper half of the rod is a glass cylinder the bore of which provides a pressure reference chamber of approx. 0·5 μl. The cylinder is made from thick-walled capillary which has been pulled in a flame and the ends broken square and ground flat on fine alumina. The top portion of the device is made of stainless steel. It consists of a disc of 1 mm thickness which has a 0·5 mm hole in its centre and a thicker piece whose centre has been bored to a Luer taper. The Luer taper permits connection to a calibration manometer.

The osmometer is assembled as follows: the space to contain the pressure transducer is filled with saline, and the pressure transducer is inserted until its upper edge contacts the bottom of the glass capillary. The stainless-steel cap is then screwed down and tightened whilst cap and acrylic portion are gripped with pliers to maximize the force exerted. Correct insertion of the pressure transducer forces saline to fill the reference chamber. Whilst viewing with a stereomicroscope, the top of the pressure transducer and bore of the reference chamber are inspected for air bubbles. A small disc (approx. 1·5 mm diameter) of ultrafiltration membrane which has been soaked in saline beforehand is placed over the opening of the reference chamber. The top part of the device is then screwed down on the membrane by tightening the four screws evenly. This process is best done whilst monitoring output from the pressure

Key words: colloid osmotic pressure, electro-osmometer, pressure changes.

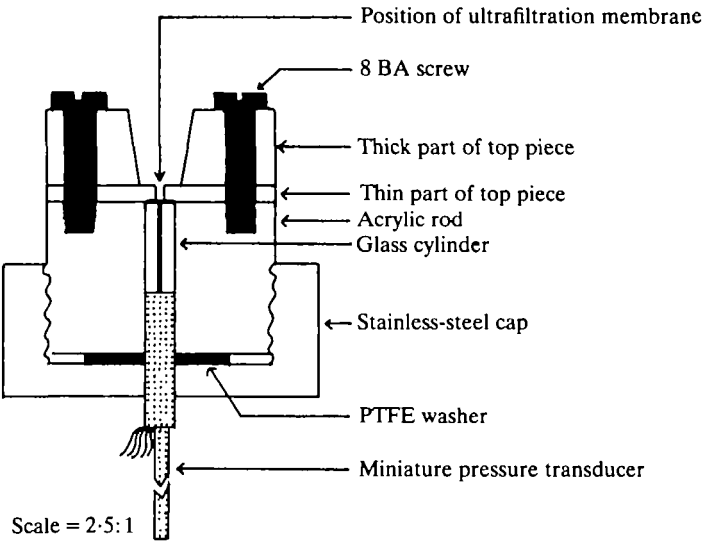

Fig. 1. Detail of a membrane electro-osmometer (designated 'miniature device') for the measurement of the colloid osmotic pressure of fluid volumes of approx. 0·5 μl or larger.

transducer; quite large transient pressures are generated which could damage the transducer.

Fig. 2 illustrates a colloid electro-osmometer which is useful for volumes of about 5 μl and upwards. The entire device is constructed in stainless steel. It consists of a top piece which is threaded to facilitate connection to a calibration manometer. The bottom piece has been bored with a flat-end mill to a diameter appropriate to the pressure transducer whose acrylic dome the device replaces. The bottom piece is threaded over part of its outer diameter with a size appropriate to the clamping ring of the pressure transducer. A 0·5 mm hole is drilled in the centre of the bottom piece and the central 4 mm is circumscribed by a groove of approx. 0·1 mm depth. The device shown in Fig. 2 was made to fit Elcomatic blood-pressure transducers (Elcomatic Ltd, Glasgow, Scotland).

The second device is assembled as follows: the bottom piece is immersed open end up in a dish of saline. The silicon seal (which must be flat in cross section) is placed in position, and the pressure transducer is inserted avoiding the entrapment of air bubbles. The clamping ring is tightened, and the pressure transducer and bottom piece are removed from the saline. Whilst viewing with a stereomicroscope, an approx. 6 mm diameter disc of ultrafiltration membrane which has been soaked beforehand in saline is centred over the hole in the bottom piece. The top piece is then screwed down evenly onto the filtration membrane, observing the same precaution mentioned in the description of the first device.

The second device requires about the same sample volume as a device described by Aukland & Johnsen (1974). However, the ultrafiltration membrane is clamped over a flat surface, rather than the curved surface of the Aukland & Johnsen design. Therefore, the risk of distorting the ultrafiltration membrane is avoided. This feature, taken together with the greater simplicity of its construction, suggests that

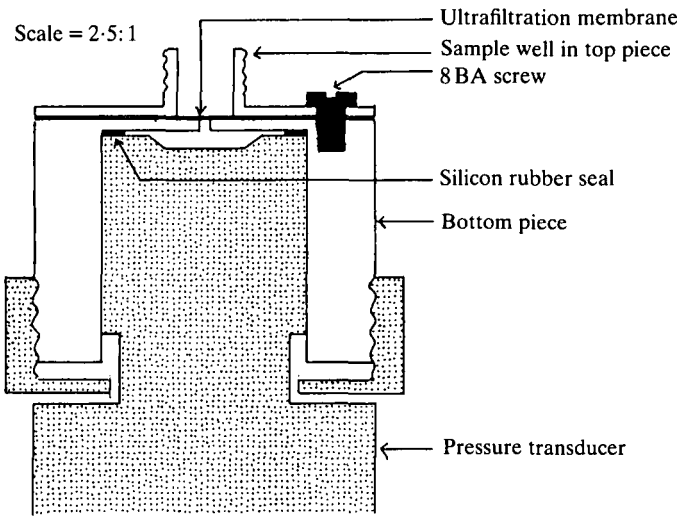

Fig. 2. Detail of a membrane electro-osmometer (designated 'larger device') for the measurement of the colloid osmotic pressure of volumes of approx. 5 μl or larger. Only the upper portion of the pressure transducer body is shown.

the present design is superior to that of Aukland & Johnsen, especially in applications where thin ultrafiltration membranes are used.

The two devices described above were used in the following identical procedure. A device was fixed in the viewing field of a stereomicroscope illuminated with a glass-fibre light guide. Ringer solution was delivered into the sample well between colloidal solutions. The sample well was flushed with an aliquot of each sample prior

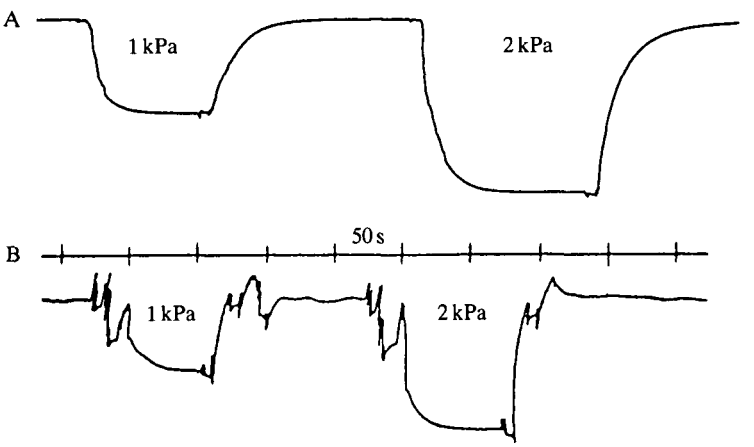

Fig. 3. (A) Oscillograph trace showing the response of the larger device to approx. 5–10 μl samples of Ficoll 70 solutions whose calculated colloid osmotic pressure was 1 kPa and 2 kPa. (B) Oscillograph trace showing the response of the miniature device to 0·5 μl samples of Ficoll 70 solutions whose calculated colloid osmotic pressure was 1 kPa and 2 kPa.

to each determination. This procedure considerably reduced the variability between readings, but it required larger samples. Fig. 3 illustrates the oscillograph traces obtained in the estimations of the COP of standard solutions of Ficoll 70 (Pharmacia Ltd, Milton Keynes, England), which has an average molecular weight of approx. 70 000 Da. The miniature device (Fig. 3B) responds to any form of pressure so that each time a sample was added or removed rapid recorder-pen oscillation occurred due to contact between the sampling pipette and the sample well. When the flushing volume was removed as completely as possible, evaporation from the surface of the membrane caused a relatively large pen deflection. When the definitive sample was added, the pen deflection attained a value which was characteristic for the solution under study. The trace shown was taken from a study in which the pen deflection was measured of approx. 0·5 μl samples of each of two Ficoll 70 solutions whose COP was calculated using a formula devised by Gamble (1983). The reproducibility of which the device is capable can be seen by the following: ten samples of Ficoll 70 having a calculated COP of 1 kPa gave an average pen deflection equivalent to 1·0 ± 0·10 kPa (range = 0·92–1·2 kPa). Ten samples of Ficoll 70 having a calculated COP of 2 kPa gave an average pen deflection equivalent to 2·0 ± 0·19 kPa (range = 1·8–2·3 kPa).

Attempts were made to estimate the COP of standard samples whose volumes were only a few tenths of a microlitre. The pen deflections from such samples did not come to a steady value. Therefore, evaporation from samples that are much smaller than about 0·5 μl would appear to limit the usefulness of the device for precise COP determinations of such volumes.

A typical trace from the larger of the two devices described here is shown in Fig. 3A. This device was much less responsive than the smaller device to pressure changes brought about by mechanical disturbance or evaporation from the ultrafiltration-membrane surface; possibly this is due to the greater volume of the pressure reference chamber. The average pen deflection of ten samples of Ficoll 70 of 5–10 μl volume with a calculated COP of 1 kPa was equivalent to 0·99 ± 0·07 kPa (range = 0·92–1·1 kPa). The average pen deflection of ten samples of Ficoll 70 of 5–10 μl volume with a calculated COP of 2 kPa was equivalent to 2·0 ± 0·17 kPa (range = 1·8–2·3 kPa).

Both devices enable relatively accurate measurements of pressure changes brought about by fluid movement through ultrafiltration membranes in response to a COP gradient. However, it is important to emphasize that the interpretation of those pressure changes depends upon less precise information, namely the number of colloidal molecules and the permeability of the ultrafiltration membrane. The colloid used in the present study has been studied by Gamble (1983) who has derived a formula relating concentration to COP using Amicon UM-10 ultrafiltration membranes. In the present study, Millipore PTGC ultrafiltration membranes (Millipore Ltd, Harrow, Middx, England) were used. The permeability characteristics of PTGC membranes appear to be similar to the UM-10: solutions of Ficoll 70 generated hydrostatic pressure changes across PTGC membranes of a magnitude predicted by Gamble's formula. The membranes were calibrated independently using a water manometer.

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