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A Microosmometer and Its Use for the Determination of the Molecular Weight of Hyaluronic Acid

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A technique for construction, filling and operation of small osmometers is described. Using this technique, the molecular weight of hyaluronic acid prepared from human umbilical cord is estimated at 4.7 $\times$ 10^5.

On the basis of measurements of double refraction of flow and viscosity Meyer and Palmer [1] and Blix and Snellman [2, 3] have arrived at estimates of the molecular weight of hyaluronic acid ranging from 2×10^5 to 5×10^5. The purpose of the present investigation is to estimate the molecular weight of the acid from osmotic pressure measurements.

The material used was prepared from human umbilical cord [4].

As the acid is only sparingly soluble, and its viscosity is high when dissolved in water, concentrations of more than 2 weight per cent could not be used for the present purpose. An estimation shows that the expected osmotic pressure of such a solution corresponds to a water column of about 10 mm.

An outer solution of aqueous 0.2 molar sodium chloride and an inner solution consisting of a 2 per cent solution of hyaluronic acid in the outer solution, were used for the measurements.

Because of the preciousness of the acid it was necessary to work with small quantities of the material.

For the purpose of correction for capillary rise of the solution the technique of measuring osmotic pressure by means of toluene described by Güntelberg and Linderstrøm-Lang [5] was adopted. Using this method, measurements are made of the difference between the heights of the toluene columns in osmometer and correction capillary, the latter immersed in toluene.

The osmometer is constructed as follows:

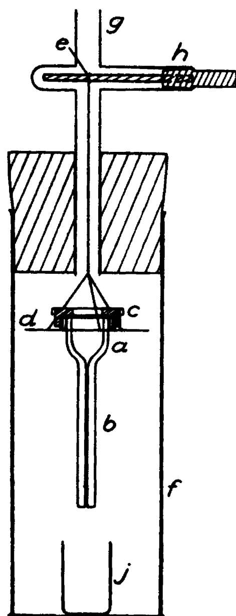

Fig. 1. Microosmometer.

a: bell-shaped cell, b: capillary, c: nut, d: ring, e: metal pin, f: glass cylinder, g: tube, h: ground glass joint, j: sampling tube.

A capillary of 0.88 mm bore, outside diameter approximately 7 mm and length approximately 100 mm is provided at one end with a bell-shaped cell *a*, height about 15 mm, inside diameter about 10.5 mm, thickness of wall about 1 mm. The open end of the cell is ground plane, and a male thread brass ring is pressed down and cemented onto the cell with a zinc oxide-zinc phosphate cement (dental cement) permitting the plane edge of the cell to protrude from the threaded ring by a few tenths of a millimetre. A milled nut *c* with a circular hole of a diameter of about 9.5 mm screws onto the threaded ring. A 50 mm piece of the capillary is cut off, and the cuts are ground plane, the spare capillary being used later for correction for capillarity.

The membrane used is a collodion membrane prepared according to the method described by N. Bjerrum and E. Manegold [6], a collodion solution obtained from British Drug House (BDH) being diluted in the ratio 16 to 100 with equal parts of ethanol and ether, all constituents by volume. After drying for 1—1/4 hours at room temperature (without a fan) the prepared membranes were stored in distilled water covered with toluene. Disks of a diameter of about 13.5 mm are punched out from the central part of the membrane. Immediately before use the disk is fastened in the dried osmometer with a washer of celluloid inserted between nut and membrane.

The osmometer is filled by means of the apparatus shown in Fig. 1.

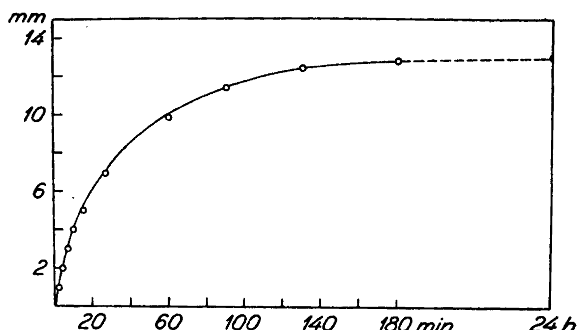

Fig. 2. Difference between heights of menisci in osmometer and correction capillary as a function of time.

The osmometer is turned upside down, and the membrane is covered with a few drops of the outer solution. Still upside down it is suspended in the glass cylinder *f* from a ring *d* hung by a thread to be wound round a metal pin *e* which is inserted in the ground glass joint *h*. By this device the osmometer can be raised and lowered from outside. In the sampling tube *j* about 2 millilitres of hyaluronic acid solution prepared by weight is placed and covered with a layer of about 2 millilitres of toluene. By means of a T-tube with two stopcocks the tube *g* is connected on one side to a glass filter pump and on the other side to a flask of about 1 litre. A manometer with scale division is inserted between the two stopcocks.

The evacuation is begun with both stopcocks open. When the pressure has been reduced to about 5 mm above the pressure of water vapour, the stopcock to the flask is closed, and evacuation is continued for a few minutes. In this way the air is driven out of the osmometer, being swept off with water vapour from the liquid layer on the membrane.

Then the stopcock to the pump is closed, and the osmometer is lowered to the bottom of the aqueous solution of hyaluronic acid. Now the stopcock to the flask is opened, the solution thereby being pressed up into the osmometer. When the osmometer is about 3/4 full, it is raised so much that the opening of the capillary is in the toluene layer where it is held till the osmometer is completely filled. Now the stopcock to the flask is closed, and the pump is stopped, whereafter the stopcock to the pump is opened carefully, allowing the cylinder to be filled with air of atmospheric pressure. Any entrapped bubble of air will then be so small that it can escape through the capillary when the osmometer is turned back into position.

For measurement the osmometer is placed in an optical cell filled with the outer solution covered by a layer of toluene. The optical cell again is placed in a glass walled rectangular container through which water from a thermostat is circulated, thus keeping the temperature of the container constant at 20° C (variations about 0.003° C).

The whole system of container and optical cell can be moved vertically by means of a screw to adjust the boundary line between aqueous phase and toluene layer in the optical cell to the level of that in the osmometer.

Osmometer and correction capillary are clamped close to each other in the same fittings to permit the correction capillary to dip into the outer layer of toluene.

The difference between the liquid levels of osmometer and correction capillary is measured by means of a vertical reading microscope with micrometer eyepiece (100 scale divisions = 5.0 mm). The microscope rotates on a vertical axis, and its vertical displacement is read on a millimetre scale with an accuracy of 0.1 mm by vernier. The heights of menisci in the capillaries are always read on a level with the center line of the eyepiece scale.

It proved to be the most convenient procedure in taking readings of the difference in heights to begin with arbitrary levels of the solution columns near the expected difference, and await establishment of equilibrium.

Check runs of the apparatus were made with a known albumin solution kindly supplied by the Carlsberg Laboratory.

The result of a characteristic experiment: No. 2, Table 1, is illustrated in Fig. 2 showing that osmotic equilibrium was reached in approximately three hours.

The results of five different experiments all made at 20° C are given in Table 1. It should be mentioned that the solutions are prepared by weight, and that the specific gravity values of toluene and sodium chloride solution used are 0.866 and 1.008, respectively.

Table 1.

Experiment	no. 1:	454 000
»	» 2:	470 000
»	» 3:	461 000
»	» 4:	489 000
»	» 5:	467 000
	Mean:	468 000

It will be seen that the result is in good agreement with the highest values found by Blix and Snellman.

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