Cellulose acetate membranes for osmosedimentation: Performance and morphological dependence on preparation conditions

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Osmosedimentation is a new membrane-assisted separation technique, based on the rapid approach to sedimentation equilibrium when macromolecular solutions are contained within dialysis cells, in contact with solvent via a permselective membrane. Cellulose acetate membranes, cast from ternary solvent (acetone, acetic acid, water) solutions are suitable for osmosedimentation of proteins at low (2000 rpm) centrifugation speeds. Solute retention is improved when acetone-rich casting solutions are used. These membranes were examined by electron and optical microscopy, showing considerable morphological changes in the membrane support layer as the casting solution composition is changed.

(Keywords: osmosedimentation; membrane morphology)

INTRODUCTION

Osmosedimentation is a membrane-assisted method with analytical and preparative capabilities in the study of macromolecular solutions and colloidal dispersions[1-4].

Earlier, theoretical work from this laboratory showed that mass transfer (and consequently approach to sedimentation equilibrium) in a system under constant temperature and external pressure, in the absence of chemical reactions, is speeded up by increasing the number of phase boundaries within the system[2].

In this laboratory, it was observed experimentally that sedimentation of macromolecules in solution is accelerated by bringing the solution into contact with its solvent through a semipermeable membrane. This new phase boundary leads to coupled osmotic and sedimentation mass currents, the net result of which is the enhancement of solute sedimentation as depicted in *Figure 1*. The magnitude of this effect is a function of the membrane permeability to the solvent used. Therefore it is an essential condition that semipermeable membranes with high permeability are available. Current commercial ultrafiltration flat membranes and hollow fibres should be adequate for osmosedimentation experiments. However, these experiments require large amounts of membranes, with a range of sizes and shapes. For this reason, we were prompted to develop our own membranes, made of cellulose acetate.

Cellulose acetate provides membranes with a wide range of permeability and retention characteristics; some binary cellulose acetate solutions[5-13] have been investigated for the casting of reverse osmosis membranes. Much work has been done with acetone as the solvent[13-16], providing highly selective membranes but with low permeability, suitable for use only under very high pressures. In this laboratory, membranes cast from cellulose acetate solutions in acetic acid and water showed high permeability and good performance in osmosedimentation of high molecular weight solutes ($MW > 500\,000$). We are not aware of any detailed report about the performance of this kind of membrane in ultrafiltration. In this paper we report on the morphological characteristics and performance of asymmetric cellulose acetate membranes cast from ternary solvent solutions (acetone–acetic acid–water), which are found to have both solute retention and solvent permeability characteristics making them suitable for osmosedimentation experiments.

EXPERIMENTAL

Membrane preparation

Reagents used in membrane preparation were: cellulose acetate (Carlo Erba, 53% acetyl content, $\bar{M}_{visc} = 3 \times 10^4$), acetic acid and acetone (reagent grade), and water. Solutions were prepared and centrifuged, to remove bubbles. They were then poured onto glass panes of suitable area (typically, 35×20 cm) and spread by moving a glass rod horizontally over the glass panes. To achieve uniform membrane thickness two pieces of nichrome wire were fastened to the glass panes; the glass rod was supported by the nichrome wire, which can be changed to give films of various thicknesses[17].

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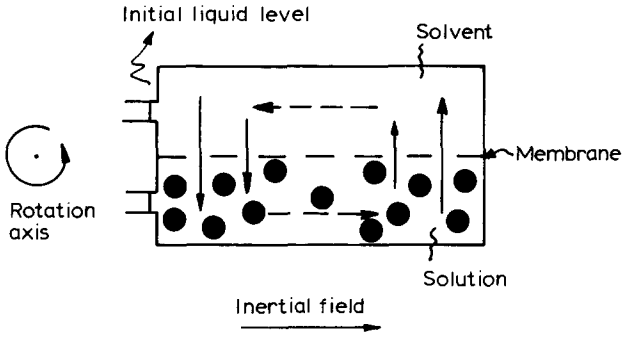


Figure 1 Diagram of mass currents in an osmosedimentation experiment, at short centrifugation times; (——→) refers to solvent osmotic and reverse osmotic mass currents; (– – →) refers to liquid bulk flow

The spread solution films were allowed to evaporate for 2 min in the laboratory atmosphere (except where the effect of evaporation time was investigated). The film-covered glass was then immersed in distilled water, at room temperature, and kept under water until it was completely coagulated and free of acetic acid and acetone.

The amount of water in the cellulose acetate solution was adjusted so as to obtain a uniform, coagulated film; an insufficient water concentration in the casting solution yields a curdy, useless precipitate. Thus, for a given cellulose acetate concentration the concentrations of acetone and acetic acid cannot be varied independently.

Membrane characterization

The osmosedimentation experiments were performed in 7.5 cm-high dialysis cells, at 4°C and 2000 rpm in a Sorvall RC-38 centrifuge. One compartment was filled with 1% protein solution (globulin, bovine albumin or ovalbumin) in 0.1 M NaCl and the other with 0.1 M NaCl aqueous solution. After 24 h samples at different heights were withdrawn and solution absorbances were read.

Permeability and solute retention were determined by fitting the membranes in Millipore Swinnex 47 filter holders, measuring solution flux under 2 atm and reading effluent solution absorbance in a PMQ-II Zeiss spectrophotometer. The retention in ultrafiltration experiments was taken as $R=(1-A_F)/A_{init}$, where A_{init} is the initial solution absorbance and A_F is the filtrate absorbance.

Wet membranes were observed in a Nikon Apophot optical microscope. After freeze drying the membranes were coated with a layer of gold and observed in a JEOL JSM-P15 electron microscope.

RESULTS

Membranes cast from various solvent mixtures were used in osmosedimentation experiments. Three different solutes were used: γ-globulin, bovine serum albumin and ovalbumin. Aqueous solutions of these proteins do not develop appreciable concentration gradients, when they are spun at 2000 rpm for some hours. However, when the solutions were centrifuged within one of the compartments of a dialysis cell, fitted with a membrane prepared as described above, while the other cell compartment was filled with solvent, large concentration gradients were obtained. This is described in *Figures 2* and *3*, where the following conclusions can be drawn: (i) bovine and ovalbumin retention is the better in membranes cast from acetone-richer solutions, γ-globulin is well retained in all the membranes used, which suggests that their average pore diameters are smaller than the γ-globulin molecule diameter; (ii) membranes showing poor retention do not form the largest concentration gradients,

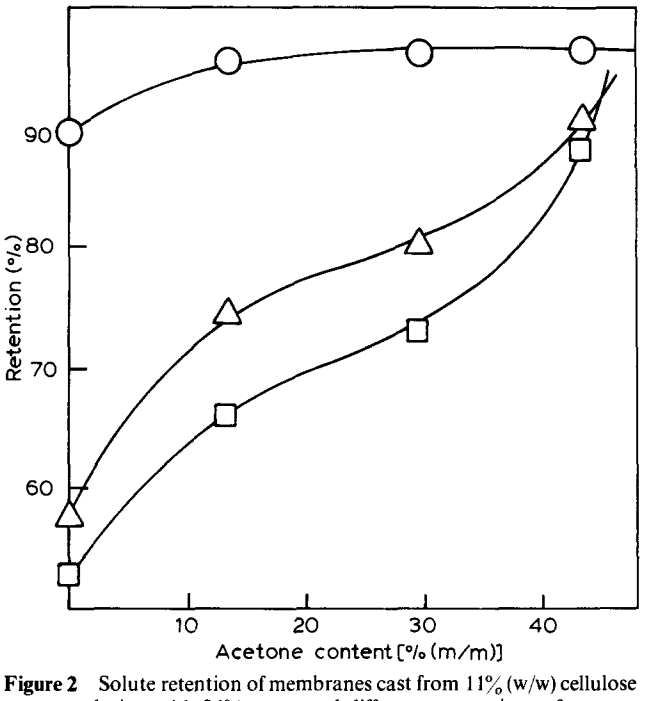


Figure 2 Solute retention of membranes cast from 11% (w/w) cellulose acetate solution with 24% water and different proportions of acetone (and acetic acid). 24 h osmosedimentation at 2000 rpm. Test-solutions: (○) 1% γ-globulin, (△) 1% bovine albumin and (□) 1% ovalbumin

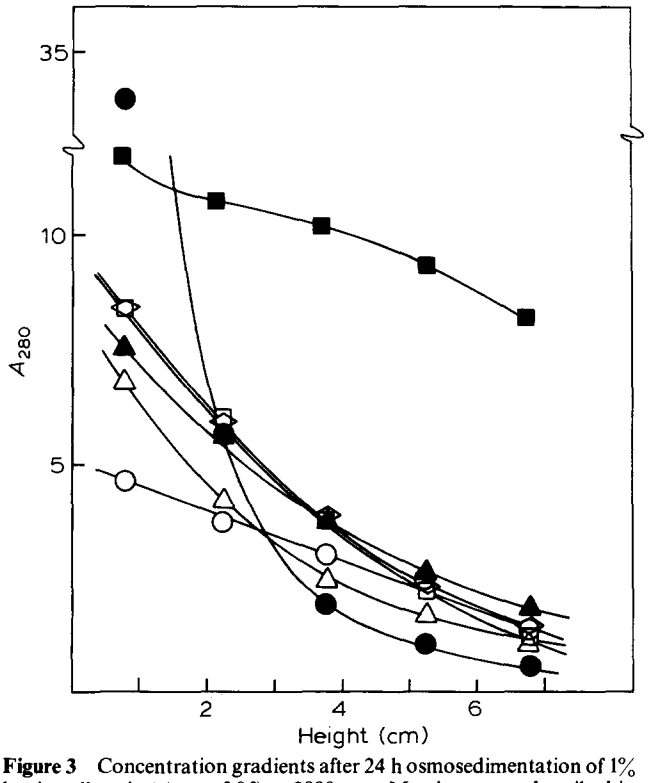


Figure 3 Concentration gradients after 24 h osmosedimentation of 1% bovine albumin ($A_{280}=5.85$) at 2000 rpm. Membranes as described in *Figure 2*, cast from (○) 0%, (△) 13.4%, (□) 29.5% and (◇) 43.2% acetone solutions; (●) 1% γ-globulin solution ($A_{280}=11.9$) using membrane cast from 13.4% acetone solution; (▲) 1% ovalbumin solution ($A_{280}=5.73$) using membrane cast from 43.2% acetone solution. (○) is a control run, performed with 1% γ-globulin and using a polythene film instead of the cellulose acetate membrane

for a given solute; (iii) better retentions and larger concentration gradients are obtained as the solute protein molecular weight increases, as expected; (iv) concentration gradients in the control runs (see *Figure 3*), in which the cellulose acetate membrane is replaced by a polythene film (impermeable to solvent) are much smaller than these obtained using cellulose acetate. Using γ-globulin as the solute, a greater than 60-fold concentration difference is observed, from cell top to bottom; using polythene film this difference decreases to less than 50%.

Visking membranes are unsuitable for osmosedimentation under gravity. They were also found inadequate for centrifugation experiments such as those described above; when they were used instad of the cellulose acetate membranes, no solute concentration gradients were detected. The membrane itself underwent considerable bulging, due to the pressure difference between the two cell compartments. Commercial ultrafiltration membranes (Amicon XM 100) were also tested. Their performance was comparable with that of the membranes used in this work. However, the high cost of commercial ultrafiltration membranes and the ready availability of the membranes described in this work make them very attractive. Attempts to make osmosedimentation experiments using hollow fibres (Amicon 3P10) were unsuccessful. Indeed, the fibres themselves did not stand centrifugation and were ruptured.

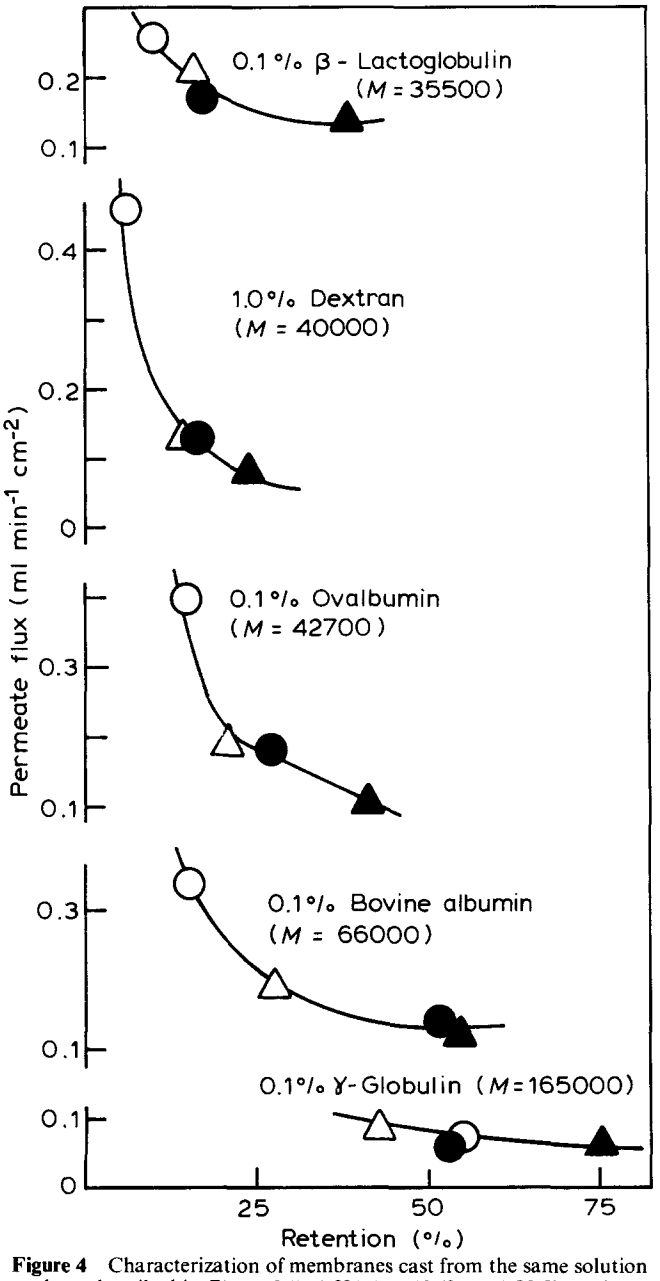


Figure 4 Characterization of membranes cast from the same solution as those described in *Figure 2* ((○) 0%, (△) 13.4%, (●) 29.5% and (▲) 43.2% acetone) in ultrafiltration experiments at 2 atm

Ultrafiltration experiments were performed to compare protein solute retention in the membranes, both in osmosedimentation and ultrafiltration modes. *Figure 4* shows that solutions richer in acetone yield membranes having a lower water permeability but a greater ability to retain solutes. Comparison of the results in *Figures 2* and *4* shows that retention is higher in the osmosedimentation experiments, for a given membrane and solute. This is understandable considering that in an ultrafiltration experiment a stagnant, concentrated solute layer builds up quickly, which is pressed over the membrane. As a result, what is actually being filtered is a progressively (locally) concentrated solution, from which solute leaks in a higher proportion. In the osmosedimentation experiments, concentrated solute layers contiguous to the membrane are displaced to the cell bottom, by convection. As a result, the area of concentrated solution contacting the membrane is very small, as compared with an ultrafiltration experiment.

It was also observed that acetone-richer casting solutions yielded thinner membranes, the other preparation conditions being constant. On the other hand, it is possible to prepare membranes of various thicknesses and retentions by varying the thickness of the nichrome wires used on the casting glass panes (*Figure 5*). The thickness of osmosedimentation membranes can be varied without serious problems related to mechanical weakness. This is because hydrostatic pressure gradient across a membrane, in a typical osmosedimentation experiment, is very small. For instance, the density difference between solutions on both sides of the membranes is rarely greater than 10^{-2} kg dm^{-3}; the inertial fields are usually below 3000 × gravity and cell heights are less than 0.1 m. The resulting pressure gradients are thus less than 0.3 atm, much less than what is supported by an ultrafiltration membrane.

Membrane morphology

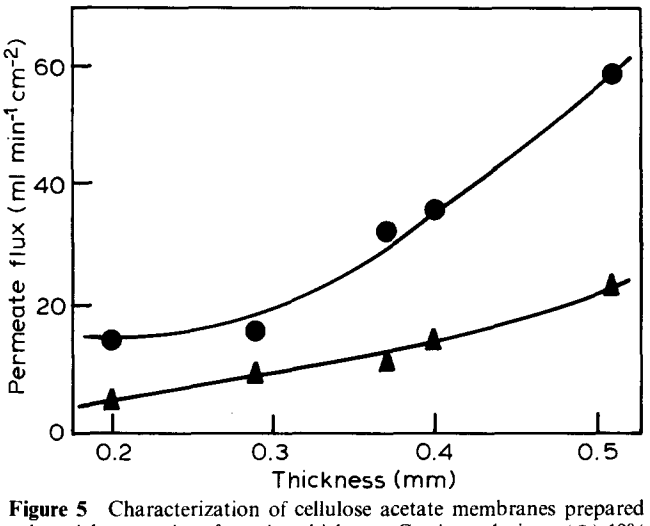
The most relevant morphological information which could be obtained on an ultrafiltration or osmosedimentation membrane is the shape and size of the filtering pores. Unfortunately, to obtain this data it would be necessary to achieve a resolution of 1–2 Å in the micrographs, which is seldom obtained.

However, membrane morphological examination at lower resolution can still be informative: the asymetric nature of the filtering membrane is revealed, the structure of the support layer can be assessed, and flaws and defects can be detected.

The support layer of asymmetric membranes cast from different solutions show different morphologies, revealed by optical and electron microscopy (*Figures 6*, *7* and *8*). In the scanning microscope the examination of the filtering (selective) membrane side shows just a blank,

unstructured field without pore resolution; the opposite side shows holes of irregular contour, usually less than 1 μm in diameter. Membrane views from the top in the optical microscope and the cut-off views both in optical and electron microscopes show finger-like cavities in the support layer, perpendicular to the membrane plane. The number of cavities increases with the acetic acid proportion and they can be even superimposed in membranes cast from solutions richer in acetic acid. Membranes cast from solutions richer in acetone show isolated cone-like cavities which are less frequent, the higher is the acetone concentration. It seems that the cavities allow easier liquid flow, but they are a characteristic of the support layer and should not affect any further the permeability and retention of the selective layer.

Membranes were allowed to dry under the microscope. One then sees that the liquid does not evaporate evenly.



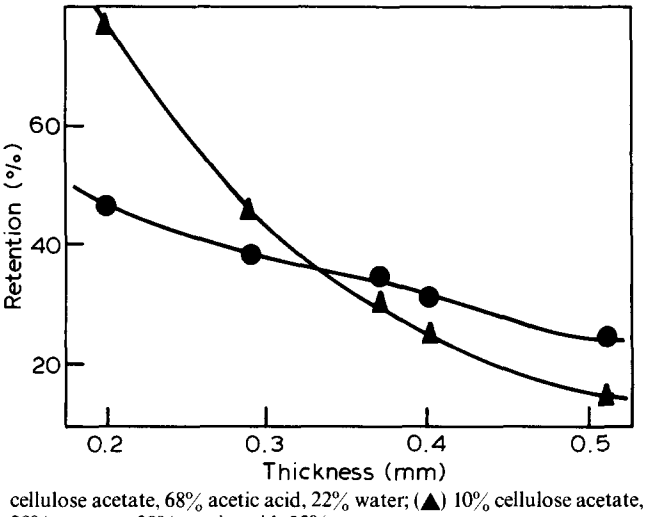


Figure 5 Characterization of cellulose acetate membranes prepared using nichrome wire of varying thickness. Casting solutions: (●) 10% cellulose acetate, 68% acetic acid, 22% water; (▲) 10% cellulose acetate, 29% acetone, 38% acetic acid, 23% water

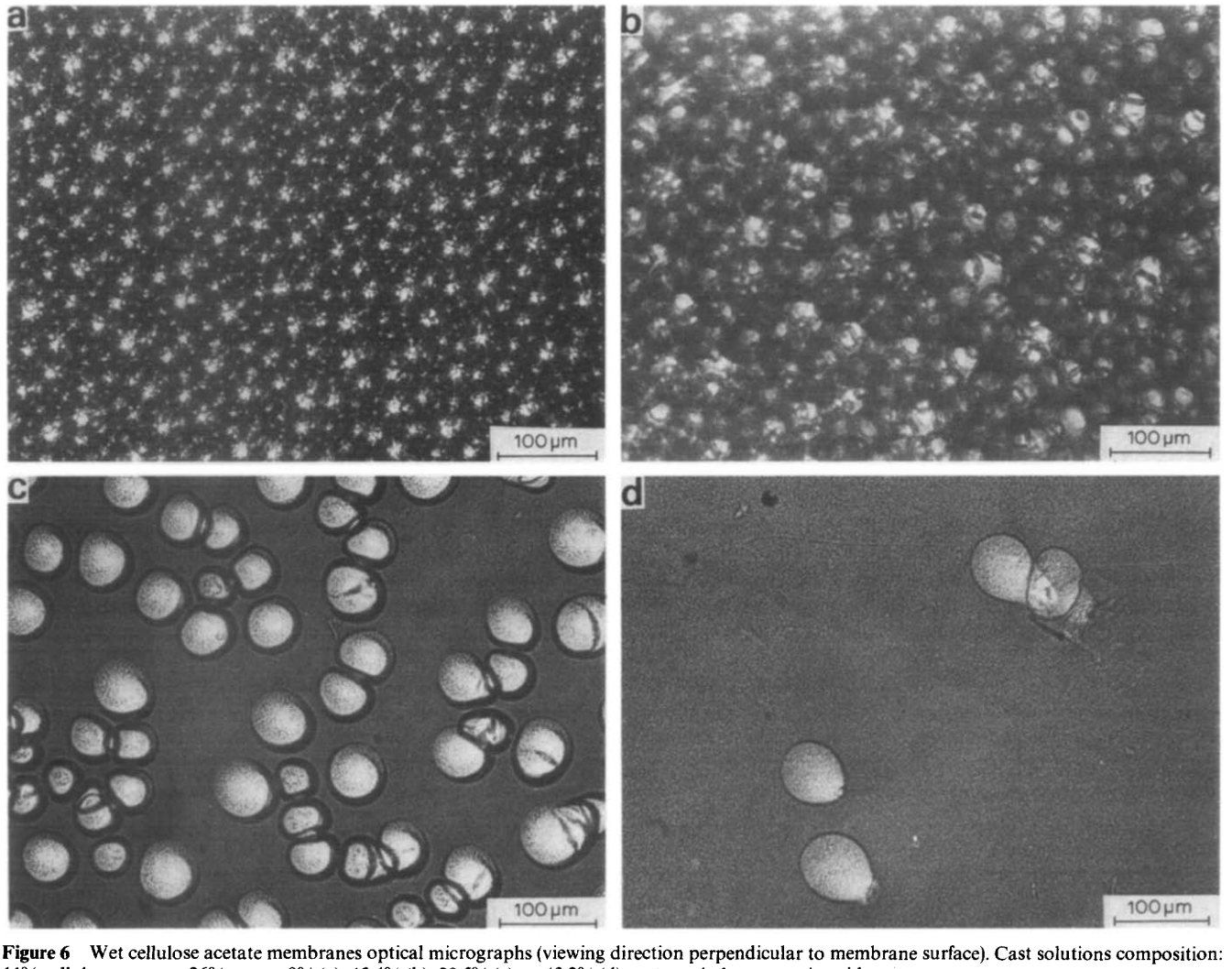


Figure 6 Wet cellulose acetate membranes optical micrographs (viewing direction perpendicular to membrane surface). Cast solutions composition: 11% cellulose acetate, 26% water, 0% (a), 13.4% (b), 29.5% (c) or 43.2% (d) acetone; balance, acetic acid

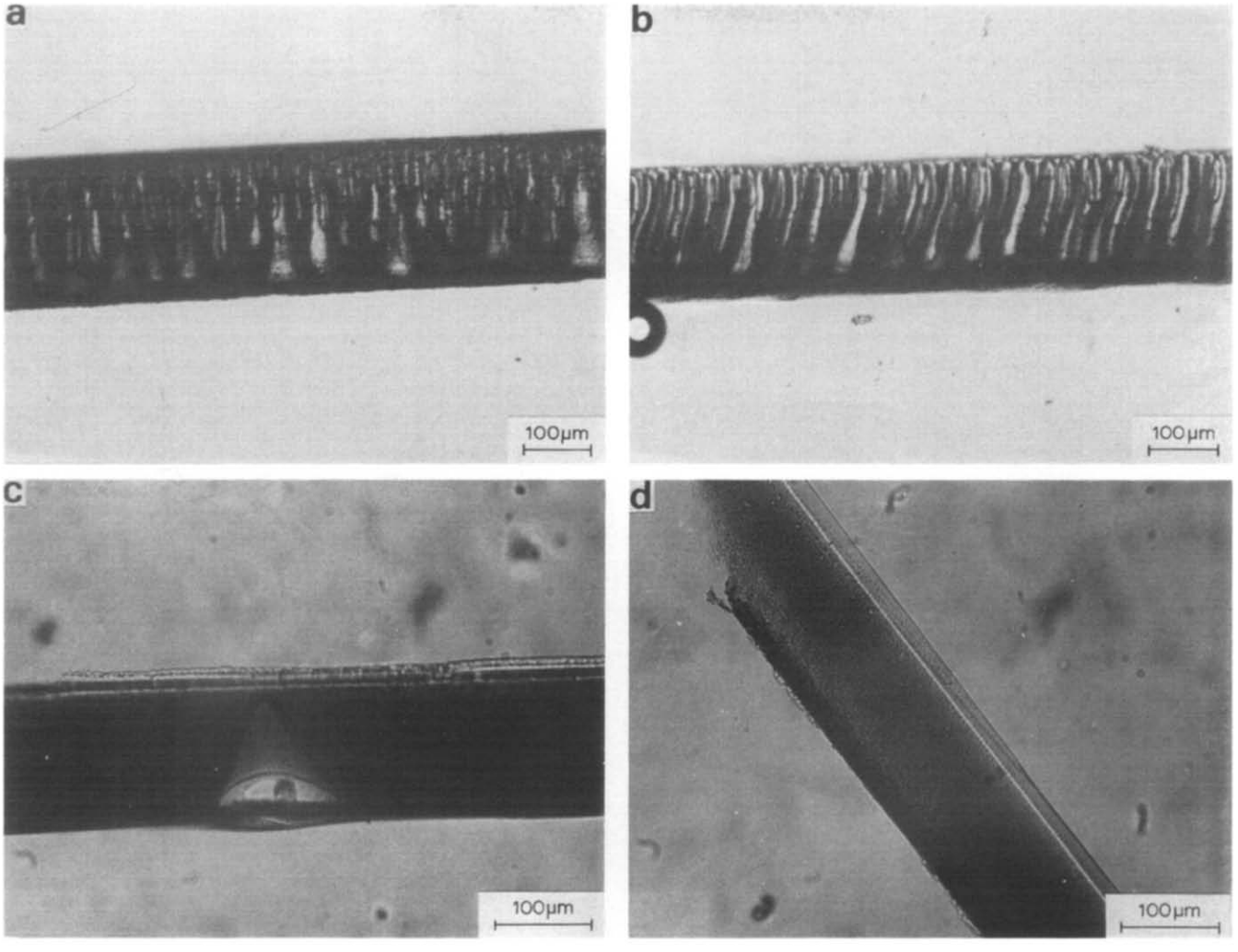


Figure 7 Optical micrographs of cross-sections of the membranes in *Figure 6*. Finger-like cavities are seen in (a) and (b); large, cone-shaped openings in (c) and fine pores in (d)

Instead, a succession of bursts is observed, as if a large number of droplets was undergoing evaporation in an unconnected fashion. *Figure 9* gives successive pictures of a same, drying membrane. It is then clear that water in the membrane is divided among unconnected compartments, from which evaporation is more or less difficult. It should be noted in these pictures that the cone-like structure remains unaltered when nearly all of the water in its surroundings has evaporated. This behaviour can be understood following two different arguments: first, the internal walls in the cone-like structure may be more hydrophilic than those in smaller holes; second, it is possible that both are equally wettable by water. In this case, these walls should be hydrophobic, in which case evaporation from a wider pore is more difficult than from a narrower pore. There is still a third possibility, that is, the cone-like structure may be closed by denser, less permeable skins than the thinner cavities.

DISCUSSION

The casting solvent plays a very important role in membrane characteristics, for many reasons. Rates of water and polymer solvent transfer, between the casting solution and the coagulating medium, may differ considerably from one system to another, thus affecting the polymer concentration with the onset of precipitation[5-7]. Following this argument, the membranes obtained using solutions richer in acetone are more retentive because acetone migrates outside the polymer film more quickly than this absorbs water. Relative rates of water migration to the interior of the casting films should be proportional to the relative free-energies of mixing of their solvents with water[9,18]. Assuming that the entropic contribution is the same, the dominating factor should be the mixing enthalpy, and this is much more positive for acetone than for acetic acid 10.5 and 1.7 kJ mol^{-1} at infinite dilution and $t = 10°C$ and 7°C, respectively[19]. That means that water should penetrate films richer in acetic acid faster, giving less opportunity for polymer chain rearrangement in denser, less porous aggregates. In this regard, the contribution of solvent–cellulose acetate interaction to the rate of film coagulation (and thus to film density) should be of the same order of magnitude for acetone and acetic acid, because this will depend on the closeness of polymer and solvent solubility parameters[9,10] which, for the two solvents considered, acetone and acetic acid, differ by less than 2%.

Finger-like cavities in the support layer structure have been observed by many authors[8,9,11,18,20-23] and there is general agreement in assigning this feature to polymer

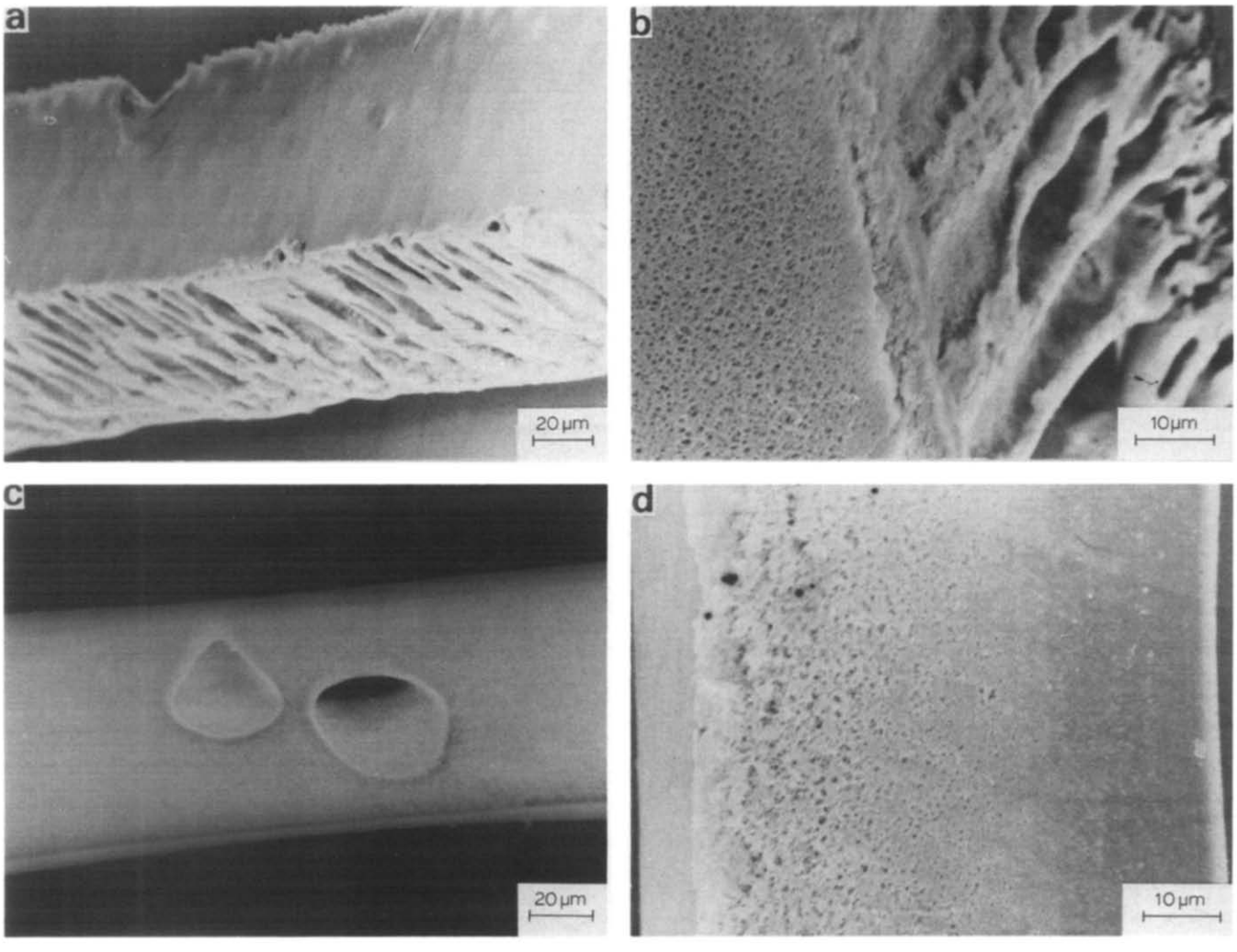


Figure 8 Scanning electron micrographs of cross-sections of the membranes in *Figure 6*. Lower part of (a) shows finger-like cavities, the plane around the corner is the filtering surface. Right side in (b) shows finger-like cavities, the plane around the corner shows a membrane bottom

precipitation associated with irregular access of the non-solvent (water). Mass transfer leading to cavity formation may be due to: (i) surface tension gradients in the coagulating system[8]; (ii) rupture of a thin skin formed in the early stages of coagulation[11]; (iii) water transport initiated in localized, polymer-poor regions[18], perhaps associated with heterogeneity in the polymer aggregation pattern, in the coagulating film surface[20–23]. Generally speaking, finger-like cavity formation seems to occur when affinity between the polymer solvent and the non-solvent is considerable, i.e. when heats of liquid–liquid mixing are the least positive, in accordance with the experimental results from this work.

Improvements in membrane characteristics, achieved by changing solvent composition, evaporation time and film thickness, have made possible their application to osmosedimentation of proteins, such as serum albumin, ovalbumin and γ-globulin. The results given in this article show that these can be concentrated at low centrifugation speeds and with small losses, using the appropriate membranes.

Recent work has shown that molecular weight determinations are feasible by osmosedimentation under gravity[3]. Using these newly developed membranes it should be possible to do molecular weight determinations by low-speed centrifugation, bringing to within reach of this technique a number of interesting systems, with molecular weights in the range 10^4–10^5.

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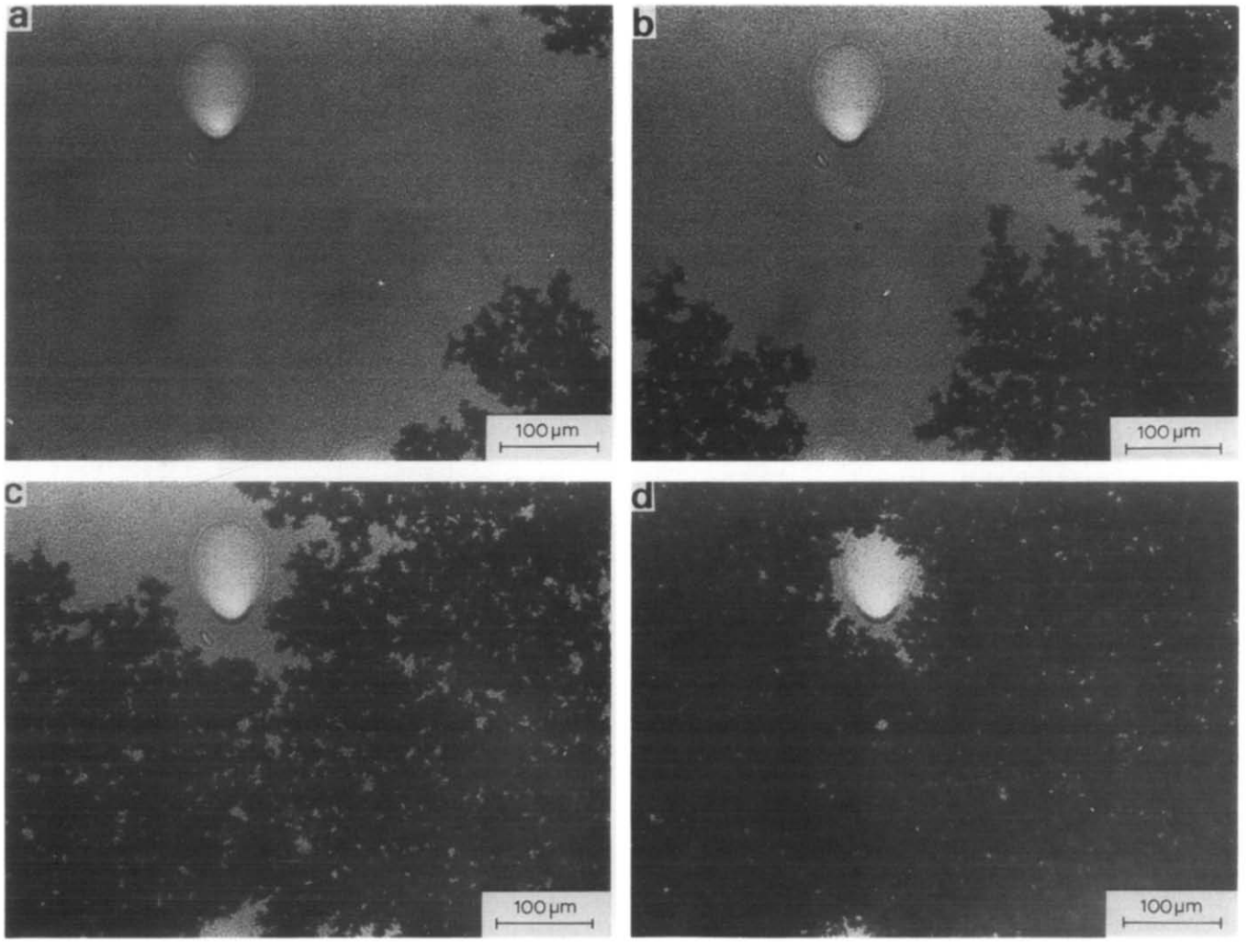

Figure 9 Optical micrographs of cellulose acetate membrane (cast solution with 29.5% acetone) during drying. Pictures from (a) to (d) were taken at successive times. They show that the dry (darker) areas move stepwise, as expected assuming that the membrane-swelling water is compartmentalized

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