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Characteristics of a Protein Concentrating Anisotropic Cellulose Acetate Membrane

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Summary

A high flux anisotropic ("skinned") protein concentrating membrane made of cellulose acetate is described. Its method of preparation is given and the influence of the thickness of the membrane on its flux determined. The membrane's characteristics under different pressures and when ultrafiltering protein solutions of varying concentration are studied. A slightly denser variant of the same membrane, useful for the concentration of some of the smaller pathological serum proteins, is also described. The characteristics of both new membranes are compared with those of some of the commercially available membranes.

INTRODUCTION

Membranes have been used for the concentration of proteins for some time (*1*), although this use has never become widespread. Apparently the main obstacle to wider use has been the low flow rates attainable with the available membranes.

The older membranes were either not provided with a skin, or when they were, this was not suspected (for a more complete review of the early literature and discussion of "cryptoanisotropy," see *2*). Since the discovery that some membranes were provided with a skin (*3*), purposely skinned membranes suitable for the rapid desalinization of water have been developed, but comparable applications to macromolecular solutions have only recently received attention (*4–9*).

Skinned cellulose acetate membranes suitable for the concentration

of dilute protein solutions have been recently described (*10*). This membrane allows the rapid concentration of 400 ml protein solution to 1 ml in about 3hr. Its manufacture is simple and its properties are reproducible.

MATERIALS AND METHODS

Membranes

CA 50 membranes (so called because of their composition) are prepared as follows: 25 g of cellulose acetate 39.8% acetate, ASTM viscosity 3, (Eastman #4644) is slowly added under constant stirring with a pestle to 75 ml of acetone and 50 ml of formamide in a mortar of about 130 mm opening. Stirring is continued until all lumps are dissolved. Alternatively the mixing process utilizes a Servall Omnimixer (Ivan Sorvall, Inc., Norwalk, Conn.). Because the use of a closed chamber cuts down the solvent loss (primarily of acetone), the initial acetone level is then reduced from 75 to 65 ml so that the proportions in the final mixture are the same as in the conventionally prepared mixture.

The mixture is then poured into a 500-ml filtering flask which is placed in a 55°C waterbath. About 40 cm Hg vacuum is applied to the flask until all bubbles disappear. Approximately 15 ml of the mixture is then poured in a ribbon across the short side of a 17 × 25 cm glass plate (along its two long edges metal runners are clamped, the membrane thickness is controlled by the runners' thickness). The mixture is then quickly and evenly spread over the glass plate with the help of a 15-cm long glass tube by drawing the tube in one long smooth horizontal motion along the runners. An excess of about two thirds of the applied mixture is swept off the plate and discarded. The glass plate with the spread-out mixture is immerged as quickly as possible in an ice-water bath and kept there for at least 1 hr before the membrane is lifted from the plate.

The almost instantaneous coagulation of the upper surface of the mixture upon its immersion in cold water causes the membrane to be provided with a very thin skin which is denser than the rest of the membrane structure. This is the actual protein-stopping skin and care must always be taken to use the membrane with the side up that was away from the glass plate when it was formed. As it is well nigh impossible to distinguish the top of a wet membrane from its bottom, it is advisable to mark one edge of every newly made membrane with

an asymmetric perforation pattern, so as to record the direction of its anisotropy.

With the help of 4 different sets of metal runners of 0.10, 0.15, 0.25 and 0.30 mm thickness, a series of membranes of varying thicknesses were made. The ultimate thickness of the membranes after coagulation is about ⅔ of that of the runners used in their manufacture. But as the thickness of the membranes themselves is not easy to measure with great precision, we shall allude to them by the thickness of the runners.

CA-35. The CA-50 membrane stops all proteins with a molecular weight larger than 35 000, which is sufficient for the majority of protein concentration requirements. But for stopping microglobulins (such as the monomer of Bence-Jones protein which has a molecular weight of 23 000), a somewhat tighter membrane may be needed. Such a membrane is easily made by using 35 ml of formamide instead of 50 ml in the recipe described above. The membrane thus obtained is called the CA-35 membrane.

Commercial Membranes

UM 1 and UM 10 membranes were obtained from the Amicon Corporation (Lexington, Mass.). PEM membranes were obtained from the Gelman Instrument Company (Ann Arbor, Mich.). Pellicon membranes were obtained from the Millipore Corporation (Bedford, Mass.) and Sartorius LSG 60 T membranes were obtained from Brinkman Instruments (Westbury, N.Y.).

Ultrafiltration

Ultrafiltration was carried out in cells provided with magnetic stirrers for the prevention of concentration polarization, or clogging of the membrane's pores by a build-up of very high protein concentrations localized on top of the membrane. Pressure was provided by compressed nitrogen from a high pressure tank provided with a 1–100 psi pressure regulating valve and 1–100 psi and 1–3000 psi gauges.

Test Solutions

The three test solutions used consisted of saline (0.15 *M* NaCl in water); a diluted protein solution, prepared by diluting 1 part of normal human serum with 400 parts saline (about 0.02% protein);

and 10% serum prepared by diluting 1 part normal human serum with 9 parts saline (about 0.8% protein).

Protein concentration was measured either with the help of an Abbe refractometer or of a Beckman DU spectrophotometer (at 280 mμ). Ultrafiltrates were evaluated for the presence of protein by their ability to precipitate or lack of ability to precipitate with 30% trichloracetic acid (TCA) or tannic acid. In the former, levels as low as 0.2% protein will give a precipitate with TCA. Ultrafiltrates that failed to give TCA reactions were checked with tannic acid (*11*), which is sensitive to levels as low as 0.002%.

RESULTS

Flux through a skinned membrane of this type depends on several parameters. Among these are membrane thickness, operating pressure, the protein concentration of the solution being filtered, and concentration polarization at the membrane surface. Results from experiments in which these parameters were systematically varied one by one (with the exception of concentration polarization) will be described.

Membrane Thickness

Substantial increases in flux may be obtained by using thinner membranes (Fig. 1), although the increased danger of leakage through the very thinnest membranes obviates their usefulness for the concentration of protein solutions. For that reason the thinnest membrane used was one made with runners of 0.10 mm thickness, which sometimes showed signs of beginning leakage. On the plot of concentration of proteins vs time it becomes obvious that the very thinnest membrane we made was not the most advantageous but rather the next thicker one, which was made with runners of 0.15 mm thickness. As the main purpose of these membranes is the fast concentration of protein solutions, these data are given in terms of protein concentration attained vs time (Fig. 1).

Scanning electron micrography of the CA-50 membrane have shown the skin to be approximately 1 μ in thickness. The skinned side is extremely smooth (see top of Fig. 2), and the pores of the skin are too small to be discerned with the scanning electron microscope. The bottom of the same membrane has very deep and large pores, varying in diameter from 0.1 to 0.5 μ (see bottom of Fig. 2).

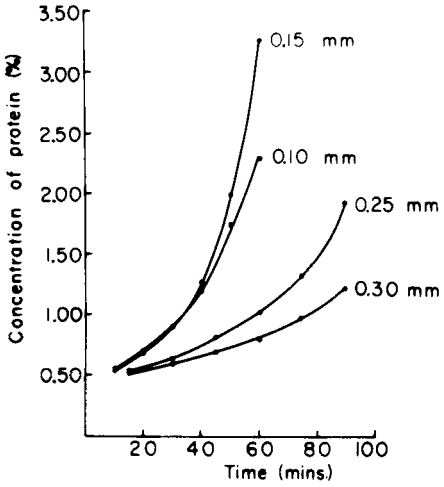

FIG. 1. Graph of the protein concentration attained vs time with 4 CA-50 membranes of different thicknesses. The optimal thickness appears to be 0.15 mm.

Pressure

Because the 0.15-mm membrane gave the best concentration performance without leakage, a series of runs was made in which pressure was varied in increments of 10 psi. Prior to each run, cells were fitted with new 0.15 mm membranes and charged with 50 ml of saline, 0.25 or 10% serum. Flow rate was estimated in each case by measuring the volume, at regular intervals, of ultrafiltrate collected in a graduated cylinder. Since the flow rate remained fairly constant over the first hour of ultrafiltration, a comparison of the average fluxes during the first half hour of ultrafiltration was made at different pressures with the three different solutions, see Fig. 3.

As can be seen, optimal flow rate when the membrane was stopping appreciable amounts of protein was obtained at a pressure of 30 psi, and this rate was about 45% less than when solvent alone was passed. At higher pressures the decrease in flux must be attributed to clogging of the membrane. This may be deduced from the observation that the membranes then also began to pass traces of protein, as evidenced by slightly positive TCA reactions obtained with the ultrafiltrates from the 40, 60, and 80 psi runs.

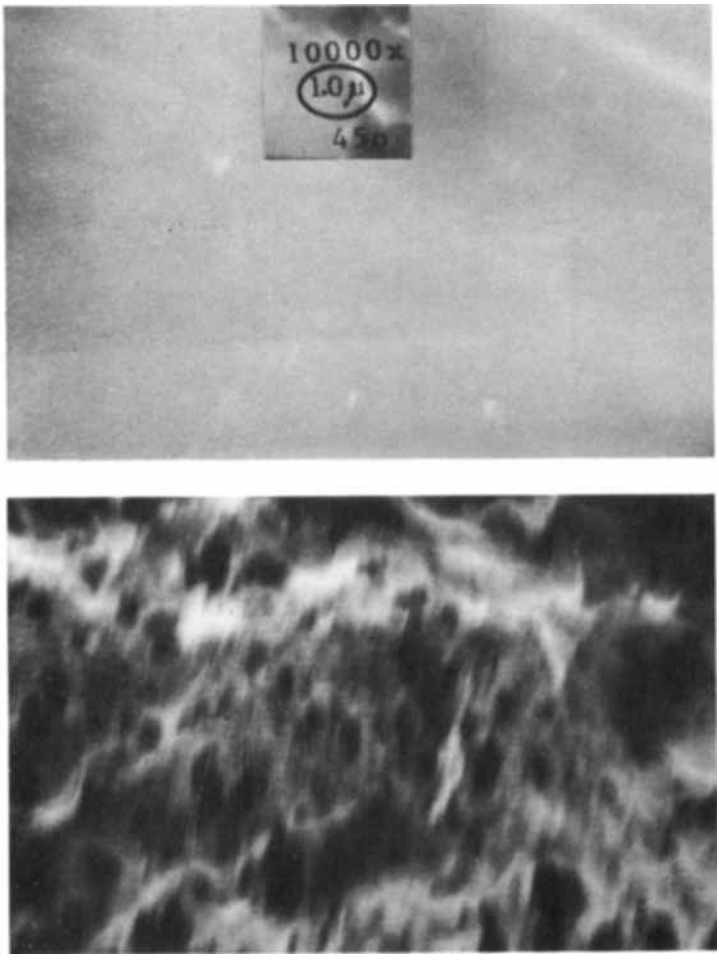

FIG. 2. Scanning electron micrograph of the CA-50 membrane. Top: "Skinned" top of the membrane (approximately 1 μ thick). Bottom: Coarse-pored bottom of the same membrane. 10 000×.

Protein Concentration

As is already clear from Fig. 3, the evaluation of membranes when retaining protein in appreciable concentrations (that is to say, in concentrations over 0.5%) is more complicated than just evaluating the fluxes of membranes when passing saline solutions or very dilute protein solutions. Figure 4 shows that at the higher protein concen-

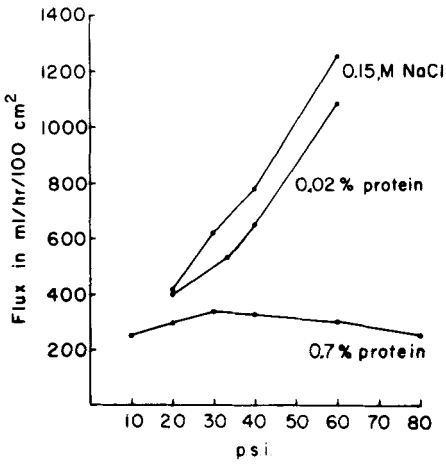

FIG. 3. Variation of flux of a CA-50 membrane with pressure. The optimal pressure appears to be 30 psi when concentrating appreciable amounts of protein.

trations the flow rate gradually decreases as the concentration of the retained protein increases. This roughly parallels the resultant increase in viscosity (*1*). At higher protein concentrations than 3% however, a rather stronger decrease in flow rate is observed which most likely is due to an increase of surface polarization of the membrane. Nevertheless, protein concentrations of 5% or even higher can be attained

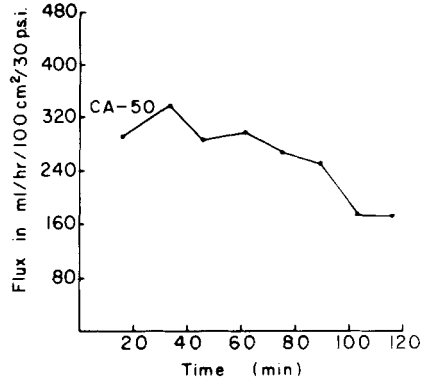

FIG. 4. Graph of flux vs time of a CA-50 membrane when concentrating a protein solution of an initial concentration of 0.7%. After 1½ hr the flux decreases somewhat more than would be expected from the increased viscosity due to the higher protein concentration alone.

with these membranes at still quite adequate rates of flow. It has been shown (*10*) that a pint of dilute proteins solution can be concentrated about 400 times within not more than 3 hr. The average starting flow rates during the first half hour of ultrafiltration are shown in Table 1 for a number of membranes for the three test solutions used in this project.

TABLE 1

Flow Rates (averaged over the first half hour of ultrafiltrations) in ml/hr/100 cm² Membranes Area/30 psi

Membrane	Solutions		
	0.15 *M* NaCl	0.02% protein	0.7% protein
CA-50	672	550	340
CA-50, glycerol treated and dried	585	427	363
CA-50, SDS treated and dried	600	490	276
PEM	735	589	275
UM-1	900[a]	ND	190
UM-10	427	308	175
CA-35	236	297	173
CA-35, glycerol treated and dried	201	148	143
CA-35, SDS treated and dried	269	198	119

[a] According to Amicon's data for the flow rate with distilled water; *Amicon Product Bulletin*, No. 201, May 1967.

CA-50 vs CA-35 Membranes

Table 1 also allows the comparison of flow rates between the CA-50 and the CA-35 membrane mentioned above. The latter has a slightly smaller pore size, which is sometimes desirable for the retention of somewhat smaller proteins than is possible with the CA-50 membrane. For instance, for a protein with a molecular weight of 23 000 (L-chains of human immunoglobulins), about 5% passes through the CA-50, while the CA-35 membrane is completely impervious to it. The CA-35 membrane, though slower than the CA-50, still has a perfectly appreciable flow rate (see Table 1).

Drying of the Membranes

Although the very highest fluxes can only be obtained when the membranes are continuously kept in water from the moment of their

manufacture, if a certain loss of flux is acceptable, the membrane can be dried if certain precautions are taken. The simplest method is to soak the membrane for approximately 1 hr in 50% glycerol water solution. After soaking the membranes can be dried at room temperature. Prior to use the dried membranes must, of course, be thoroughly rinsed in water or buffer. This process does not change the pore size of the membranes but it does tend to reduce their flow rates, see Table 1. It is also possible to dry the membranes after pretreatment in a 0.1% sodium dodecyl sulfate solution (SDS). Even greater care must be taken to wash off the excess of dodecyl sulfate prior to the use of the membranes as the presence of even small amounts of detergents can materially change the effective pore size of the membrane to certain proteins (*1, 2, 12*). With the dried membranes it is no longer necessary to mark their edges in an asymmetrical manner, because in the dried state the skinned side of the membrane is quite visibly much shinier than the other side.

Effect of the Membrane Concentration Method on Proteins

The remarkable lack of denaturation of proteins concentrated with the membrane ultrafiltration method has already been described (*10*). The two major physical criteria used for studying the denaturation of proteins are electrophoresis (to study possible changes in the electrophoretic mobility of proteins) and analytical ultracentrifugation (to study possible polymerization or depolymerization of proteins). Figure 5 shows the electropherogram of normal human serum on the one hand, and 400 times diluted normal human serum reconcentrated 400 times by the membrane ultrafiltration method on the other hand. There are no distinguishable differences between the two electropherograms, which indicates that the ultrafiltration method does not change the electrophoretic mobility of blood serum proteins.

Analytical ultracentrifugation, which permits the analysis of the molecular weight distribution of proteins in a mixture, has shown clearly that the membrane ultrafiltration method has no measurable influence on the molecular weight distribution of blood serum proteins. Figure 6 shows the molecular weight distribution of normal human serum proteins before and after 400 times dilution and reconcentration by membrane ultrafiltration.

Extensive routine work with concentration by membrane ultrafiltration of diluted antibody-containing solutions has shown that this

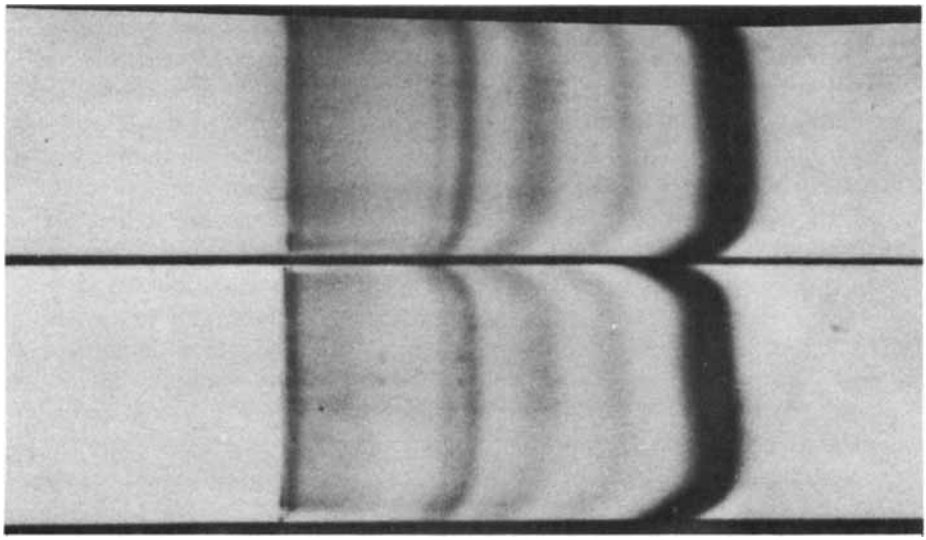

FIG. 5. Cellulose acetate electropherogram of normal human serum (top) and of the same serum 400 × diluted with saline and concentrated back to its initial protein concentration by ultrafiltration with a CA-50 membrane (bottom).

method of concentration in no way impairs the biological activity of the proteins.

Comparison of the CA-50 and CA-35 Membranes with Some Commercial Membranes

Table 1 not only allows the comparison between our CA-50 and CA-35 membranes and between their properties in the wet and previously dried state, but also between the properties of these membranes and a few commercially available membranes.

It will be noticed that the membranes are classified in Table 1, from top to bottom, according to their fluxes when stopping appreciable amounts of protein. This, after all, is not only their most important property when they are used for the rapid concentration of protein solutions, but these fluxes are also their most stable characteristic. Fluxes obtained with water, saline, or very dilute protein solutions fluctuate much more widely, even with membranes of the same batch, and are somewhat less meaningful.

The fastest nonleaking commercially available membrane is the

PEM membrane which, though not exactly the same as our CA-50 membrane, has the greatest resemblance to it in composition because it is also made of cellulose acetate. As the PEM membrane, like most modern commercial membranes, is marketed in the dried state, it is not surprising that it is somewhat slower than the wet CA-50. Its properties are fairly close to those of the dried CA-50 membranes.

No fluxes are given for the new Millipore membranes (Pellicon PSAC and PSED), because the ones we purchased and tested consistently leaked serum protein at concentrations of 0.02% as well as at 0.7%. The same holds for the Sartorius LSG 60 T membranes, although these only leaked serum protein at a concentration of 0.7% and over, and not at 0.02%. A peculiarity of this last membrane is that the fluxes obtained with it are the same, irrespective of which side of the membrane is used. As these fluxes are relatively high, we suspect that the membrane may be skinned on both sides.

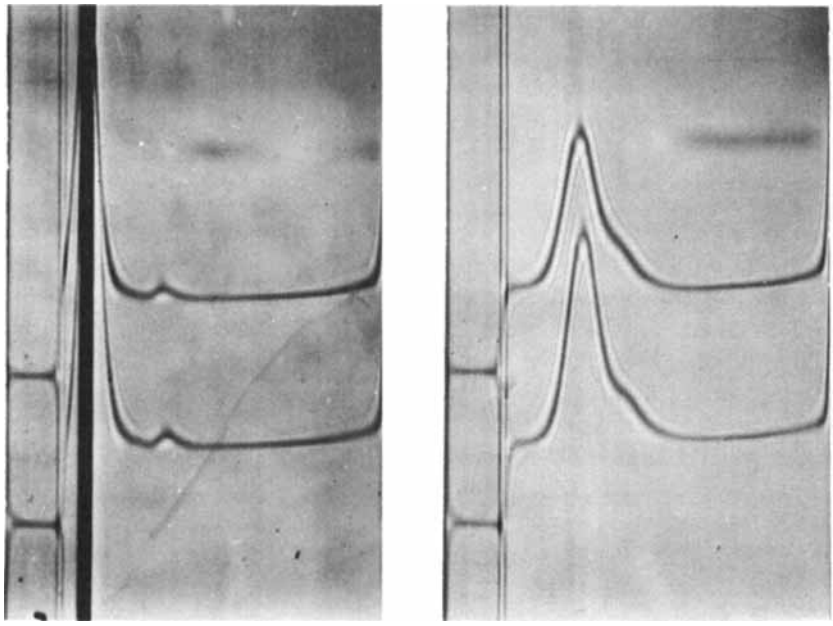

FIG. 6. Analytical ultracentrifugation patterns of normal human serum (top curves) and of the same serum 400 × diluted with saline and concentrated back to its initial protein concentration by ultrafiltration with a CA-50 membrane (bottom curve). The protein concentration was adjusted to 1.0%. The left picture was taken 12 min and the right picture 44 min after attaining 59 786 rpm. The Schlieren angle was 60°.

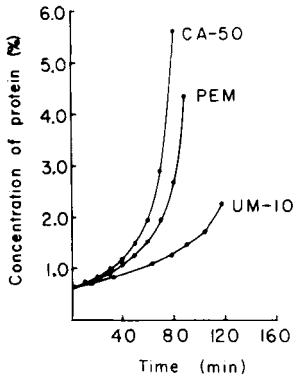

FIG. 7. Graph of the protein concentration attained vs time with the CA-50 membrane, a PEM membrane (Gelman Instruments Co.), and a UM-10 membrane (Amicon Co.).

As the most important use of the membranes here described is the rapid concentration of protein solutions, a comparative graph of the speeds of protein concentration of the CA-50, the PEM, and the UM-10 membranes is given in Fig. 7.

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