

# RELATION BETWEEN END USE SEPARATION PROPERTIES AND SUBSTRATE CHARACTERISTICS FOR NEW PROTEINIC MEMBRANES

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**Scientific topic:** Advanced processing for high performance materials

**Abstract:** A new nanofiltration/low ultrafiltration membrane was prepared by tangential filtration of protein solutions on macroporous ceramic supports. A unique feature of these membranes is their ability to work under mild conditions ( $\Delta P$  and tangential velocity). Moreover they are biocompatible and may be easily regenerated. All these characteristics make them very good for concentration and purification of food stuff, biological and pharmaceutical solutions.

**Keywords:** Membrane, processing, formed-in-place, protein, ceramic.

## INTRODUCTION

A new membrane type for nanofiltration or low ultrafiltration is prepared by cross-flow filtration of protein solutions on an  $\alpha$ -alumina cylindrical support. The deposited protein layer is then modified and stabilised by a tanning reaction involving a formaldehyde solution, before a final oven drying is carried out [1].

Since their typical working conditions are particularly mild, e.g., 0.2 MPa transmembrane pressure drop and 1 m/s tangential velocity, such protein membranes are attractive potential candidates for the concentration and purification of food stuff, biological and pharmaceutical solutions that are sensitive to high temperature and shear, and require compatible filter material.

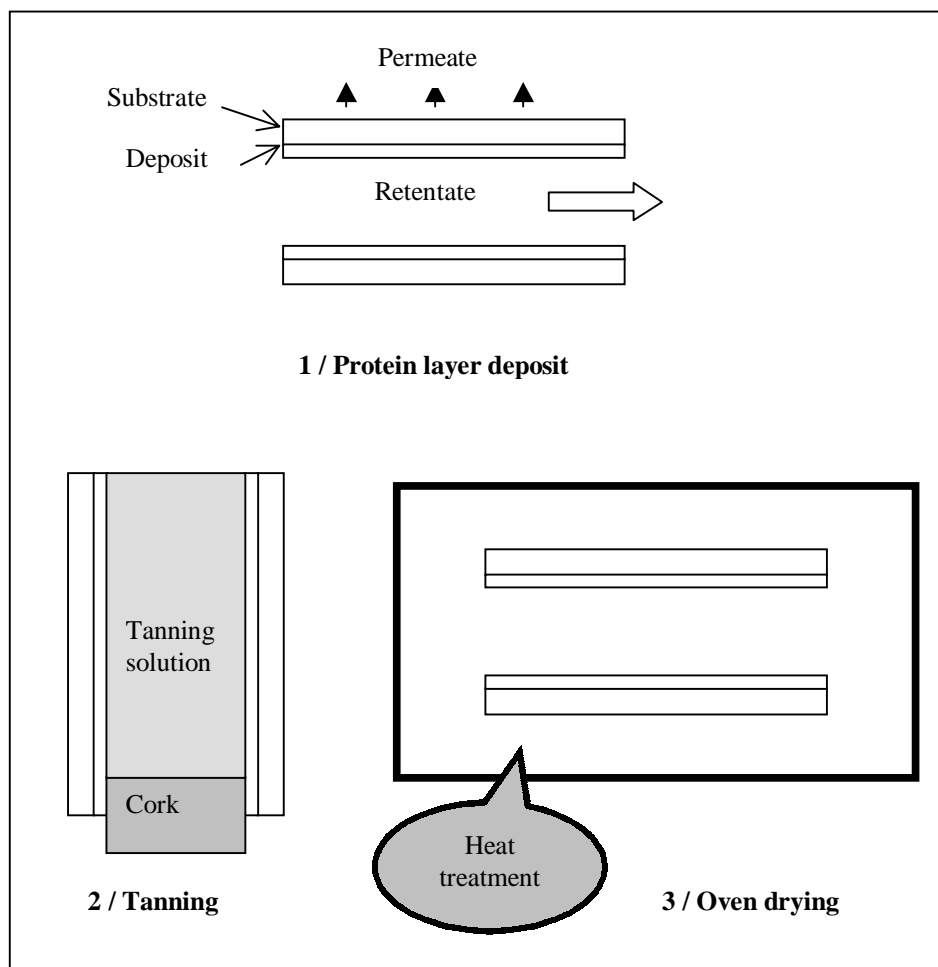
## MATERIAL PROCESSING AND END-USE PROPERTIES CONTROL

According to Negrel's [2] standard procedure, membrane preparation basically involves three main operations (figure 1). At first, a protein layer is deposited by tangentially filtrating a gelatin solution (Merck art. 4078, 10 g/l, pH 2) over a macroporous  $\alpha$ -alumina support (pore diameter 0.2  $\mu\text{m}$ ). The filtration takes place according to the following operating conditions: tangential velocity  $U = 1$  m/s, transmembrane pressure drop  $\Delta P = 0.2$  MPa, temperature 20°C, duration 45 min. Then the protein layer is stabilised and modified by tanning and drying. The tanning reaction is carried out in a static way, at 20°C during 120 min, using formaldehyde (18 g/l solution). The tubular support is placed vertically and plugged with a cork of its bottom, so that it may be filled with the tanning solution. This operation results in a covalent bond reticulation between protein amino groups. Finally drying is carried out in an oven at 110°C for 45 minutes. This standard protocol also includes two rinsing (filtration with distilled water) steps, the first one between membrane deposition and tanning, and the second one between tanning and drying.

In a more recent work Bullon [3] proposed to yet optimise the Negrel's procedure by bringing down to 25 minutes the time for protein deposit, and by changing reaction conditions (50 g/l solution during 30 minutes). Furthermore rinsing steps are eliminated. In what follows this procedure was adopted unless otherwise specified.

The required end-use properties of the filtering element, i.e. an active layer with pore diameters ranging between 1 and a few nanometers which correspond to classical nanofiltration or low ultrafiltration characteristics, are checked through standardised filtration runs ( $U = 1$  m/s,  $\Delta P = 0.2$  MPa,  $T = 20^\circ\text{C}$ ). Dynamic characterisation is conducted with 15 g/l model aqueous solutions of polyethylene glycol (PEG) from Merck with molecular weights, MW, equal to 600 or 1500 Daltons. As shown elsewhere [4] these molecules present apparent radii of about 1.2 and 2.0 nanometers. The results are expressed as volume flux ( $\text{l/h.m}^2$ ) and retention yield  $R$  (%):

$$R (\%) = \left(1 - \frac{C_P}{C_F}\right) 100$$



**Figure 1 :** Standard procedure for membrane preparation

were  $C_F$  and  $C_p$  are respectively the feed and permeate concentrations. PEG concentrations are determined by using a differential refractometer (Shimadzu RID-6A model).

In the approach thus described, optimisation of material performance is obtained owing to continuous "coming and goings" between membrane processing and control steps. New results concerning the effect of some substrate characteristics on membrane performance are reported.

### **EFFECT OF SUBSTRATE PORE DIAMETER AND MATERIAL**

Five different proteinic membranes were prepared using two kind of materials: titania ( $TiO_2$ ) and  $\alpha$ -alumina ( $Al_2O_3$ ) cylindrical supports provided by SCT Tarbes (France). Various pore diameters were investigated. Each membrane, elaborated as described above, were then characterised. Results are presented in Table 1.

**Table 1:** Flux and retention versus substrate pore diameter and material

Support	pore diameter ( $\mu\text{m}$ )	membrane	$J_{\text{water}}$ ( $\text{l.h}^{-1}.\text{m}^{-2}$ )	$J_{\text{PEG}}$ ( $\text{l.h}^{-1}.\text{m}^{-2}$ )	$R_{\text{PEG600}\%}$	$R_{\text{PEG1500}\%}$
$\alpha$ -alumina	0.2	$M_1$	20	15	55	85
	0.1	$M_2$	30	20	24	50
	0.06	$M_3$	12	10	56	87
Titania	0.13	$M_4$	150	50	10	65
	0.04	$M_5$	35	15	45	85

### 1 Pore diameter

Considering membranes  $M_1$  and  $M_3$  prepared using  $\alpha$ -alumina supports with 0.2 et 0.06  $\mu\text{m}$  pore diameters, characterisation experiments show that permeation fluxes are all the higher as pore diameter is larger. But retention rates are roughly the same for these two elements.

Membrane  $M_2$  elaborated on 0.1  $\mu\text{m}$  pore diameter support is very different. Its permeability is higher and the retentions of PEG 1500 and PEG 600 are very low. These results should be questionable if the evolution of flux and retention observed when passing from  $M_4$  to  $M_5$  (titania substrate) did not present a similar tendency to the one recorded with  $M_2$  and  $M_3$  (alumina substrate with roughly the same pore diameters).

No decisive explanation has yet been found even if one can hint at particular arrangements of the deposited layer either on the internal surface of the ceramic tube or inside pores depending on pore size. Further experiments and investigations are thus needed.

### 2 Material

For the same substrate pore diameter, membranes built on titania support exhibit fluxes significantly higher than the ones prepared on alumina support. Retention rates are quite similar with PEG1500, but slightly higher on alumina supports with PEG600. Obviously interactions between inorganic substrate and macromolecules play a main part as regards performance. More specially it may be underlined that the crossing of species through the deposited layer is all the easier with titania as compared to alumina as the size of molecules is smaller (specially solvent and to a lesser extent PEG600). Some attempts have been made to relate these findings to a better structuration of gelatin chains on alumina due to stronger interactions with proteins. It is worth recalling that the isoelectric point of  $\alpha$ -alumina is around 7-9 against 5-6 for titania.

## EFFECT OF PROTEIN TYPE AND TREATMENT

As membrane properties are very closed up with proteinic deposit structure, different membranes prepared with different types of protein were compared. Moreover tanning conditions were varied.

### 1 Protein

Six kinds of proteins were tested: bovine milk  $\beta$ -lactoglobulin (MW # 18 kDa), bovine milk casein (MW # 25 kDa for the monomers), chicken egg albumin (MW # 45 kDa), and three kinds of gelatin (Merck gelatin -mean MW # 63 kDa-, Sanofi 150 bloom gelatin -mean MW # 87 kDa-, Sanofi 250 bloom gelatin -mean MW # 110 kDa-). In this part of the study, membranes were prepared as previously described [3] except for the formaldehyde concentration which was fixed at 0.185%. Results of characterisation experiments conducted with the two previously mentioned PEG and sucrose, a disaccharide with a molecular weight of 342 Daltons, are shown in Table 2.

**Table 2 :** Flux and retention for various proteins

	Merck gelatin	Sanofi 150 gelatin	Sanofi 250 gelatin	Egg albumin	Milk casein	Milk $\beta$ -lactoglobulin
<b>J (l.h<sup>-1</sup>.m<sup>2</sup>)</b>						
J <sub>water</sub>	107	20	48	0	43	21
J <sub>disaccharide</sub>	43	15	13	0	36	23
J <sub>PEG 600</sub>	47	15	12	0	33	21
J <sub>PEG 1500</sub>	43	12	9	0	31	19
<b>Retention (%)</b>						
R <sub>disaccharide</sub>	7	13	7	-	0	0
R <sub>PEG600</sub>	18	47	17	-	10	12
R <sub>PEG1500</sub>	23	74	41	-	28	36

Egg albumin is unsuitable for membrane building. Owing to its known heat-coagulation property, no flux can be observed with this type of membrane. The best performance in regard of retention rate is obtained with gelatin membranes and more

especially with Sanofi 150 bloom gelatin. It is thought that the molecular weight distribution of macromolecules plays a main part in proteinic deposit.

## 2 Tanning conditions

Cross linking of protein layer is also important for membrane properties. Actually Table 3 shows that membrane performance is very dependent on the concentration of formaldehyde during tanning. Built with higher formaldehyde concentration (5%), membrane prepared with  $\beta$ -lactoglobulin becomes impermeable. Tanning is a chemical reaction by which the reactive side chains of proteins (amino groups contained in residues lysine and arginine) may be linked together through covalent bounds. The bound numbers are very dependent of the protein type (number of reactive amino acids) and the cross-linking concentration. If formaldehyde concentration is too low, reaction could not be correctly achieved. For a high concentration, the formation of formaldehyde bridges which brings closer protein chains could create a so dense proteinic layer that no more liquide permeation can be observed. But as the gel layer is an easy-to-distort structure, such a high concentration could sometimes also be responsible for local spaces in the proteinic membrane thus leading to decrease in retention.

**Table 3:** Fluxes and retention versus formaldehyde concentration

Formol conc.	Merck gelatin		Sanofi 150 gelatin		Milk $\beta$ -lactoglobulin	
	0.185%	5%	0.185%	5%	0.185%	5%
<b>J (l.h<sup>-1</sup>.m<sup>2</sup>)</b>						
J <sub>water</sub>	107	20	20	22.5	21	0
J <sub>dissacharide</sub>	43	17	15	9.5	23	0
J <sub>PEG 600</sub>	47	15	15	9.5	21	0
J <sub>PEG 1500</sub>	43	15	12	9.5	19	0
<b>Retention (%)</b>						
R <sub>disaccharide</sub>	7	10	13	0	0	-
R <sub>PEG600</sub>	18	55	47	23	12	-
R <sub>PEG1500</sub>	23	85	74	39	36	-

## CONCLUSION

If the properties of proteinic membrane seems to be mainly dependent on the deposit structure, this study has shown the effect of substrate pore diameter and material. Obviously  $\alpha$ -alumina appears to be a better candidate than titania to get higher retention with smaller species. The structure of deposit is strongly related to the protein as well as to the applied chemical treatment. Proteins with a large molecular weight distribution seem to be more suitable to prepare nanofiltration/low ultrafiltration membranes. But for each choice of protein, the concentration of formaldehyde solution must be optimised.

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