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# Calibration of ultrafiltration membranes against size exclusion chromatography columns

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## A B S T R A C T

Using the extension of the concept of universal calibration parameter, yielding a relation between the hydrodynamic volume of molecules and the elution volume in size exclusion chromatography (SEC), to retention coefficients in ultrafiltration (UF), we propose a direct calibration of UF membranes against chromatography columns. Plotting the retention coefficient by one given UF membrane of a series of probe molecules versus their elution volume in SEC chromatography provides a calibration curve for this membrane. For a wide range of retentions, such calibration can be directly used to predict the retention of any molecule: one only needs to measure its exclusion volume by the SEC column, and read the retention by the calibrated membrane on the calibration curves.

The method has been tested with dextran and PEG for the calibration, and milk proteins as test molecules, for three different membranes. The predicted values of the retention are in rather good agreement with those experimentally measured in a UF cell.

## 1. Introduction

Despite a widespread use of ultrafiltration (UF) in various industrial sectors and biological or pharmaceutical laboratories, the characterisation of UF membranes has not been standardised yet, and the characteristic parameters most often used, namely the molecular weight cut off (MWCO) and the permeability to water ( $L_p$ ) provide not more than a vague idea of the selectivity and flux when in operation. This is easily explained by several aspects of membrane filtration. Fouling is one, which depends not only on the membrane material properties and structures, but also on the fluid to be processed and of its interactions with the material, which are many. Another source of unreliability of MWCO values lies in the various ways of measuring it. Operating conditions (concentration, pressure, hydrodynamics, temperature, etc.) modulate the concentration polarization that is a major factor affecting the observed value of the rejection coefficients, from which the MWCO is derived. The variables chosen to represent the selectivity might not be totally appropriate: The molecular weight very well characterises a molecule within a class (e.g. the degree of polymerization),

but it does not represent its absolute size when in solution and it is quite well known that whether a membrane has been characterised using dextran, polyethylene glycols (PEG) or proteins, its MWCO will appear different.

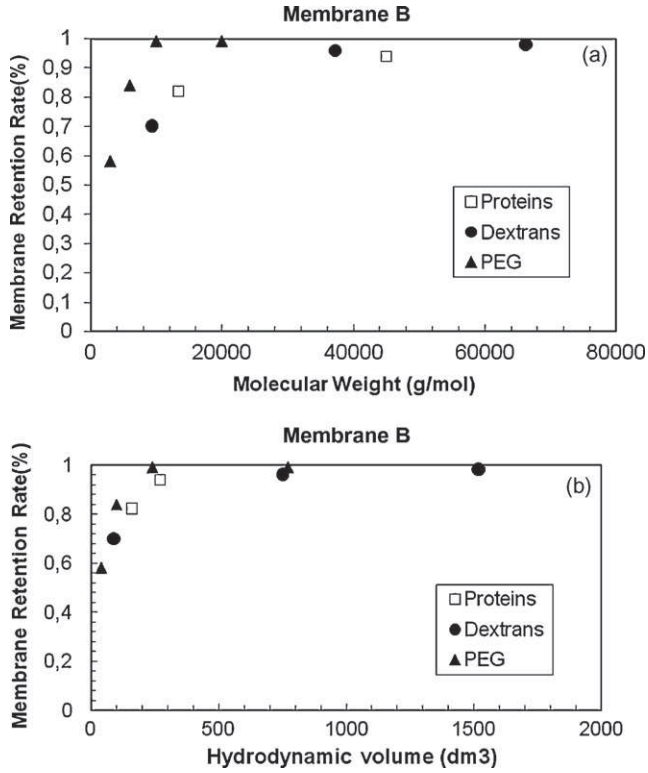
This later issue is particularly important whenever an end-user would like to select which membrane (often characterised by its "MWCO") is needed to perform the separation of macromolecules within a mixture. Field or lab tests remain today the safer way to choose the most appropriate membrane from a short list.

In 1995 [1], we proposed to address this question by analogy with the so-called "universal calibration" method proposed by Grubisic et al. [2] for the calibration of size exclusion chromatography (SEC) columns. These authors demonstrate by a series of experiments using a given SEC column, that plotting the elution volume of solutes of a wide range of molecular architectures (e.g. linear "comb" and "star polystyrene, linear metacrylates and various copolymers) versus their "hydrodynamic volume" instead of their molecular weight or their Stoke radius, allowed all the data to fall on the same calibration line. More recently, Hamaliec and Meyer [3] and Jackson et al. [4] have generalized the SEC universal calibration curve for complex polymers. Via a similar approach based on the analogy between SEC chromatography and ultrafiltration operated in conditions where retention only relies on a size exclusion mechanism we showed that for four different membranes (40, 100, 200 kDa and 0.1  $\mu\text{m}$ ), made of different materials (sulfonated polysulfone (SPS) and PVDF), plotting the solute retention versus the hydrodynamic volume of the molecules drove to the same type of result: i.e. a single calibration curve, independent of

*Abbreviations:* BSA, bovine serum albumin; TDS, total dissolved solutes; SEC, size exclusion chromatography; UF, ultrafiltration.

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**Fig. 1.** Comparison of the same retention data obtained for a SPS membrane 100 kDa, when plotted against the molecular weight (a) or the hydrodynamic volume (b) of the tracers molecules. The latter offers a single characteristic which does not depend on the kind of molecule used as a tracer.

the nature of the tracer used. Fig. 1 offers an illustration of the kind of improvement one can obtain when plotting the retention versus the hydrodynamic volume (1b) instead of the molecular weight (1a).

Obtaining a true sieving characteristic curve for UF membranes was certainly a progress in their characterisation, however many aspects of the problem of using these characteristics for the prediction of the membrane performance remained obscure.

As for an example, using such characteristic curves to predict the selectivity that should be obtained for any given molecule is still a problem since the hydrodynamic volume is seldom known *a priori*. Probably for this reason, this approach has not been often used. However size exclusion chromatography appeared as an obvious additional tool which can help complete the membrane users' toolbox, and its use has been further discussed and improved as shown in [5,6]. In 2006, Molek and Zydney [7] have however evidenced differences in the apparent hydrodynamic radii in SEC and ultrafiltration due to the influence of shear stress on the shape of flexible polymer, which does not exist in chromatography.

This is the topic of this paper to discuss on the combination of UF, SEC and various kinds of tracers in order to obtain a fast first approximation of the membrane selectivity for any given macromolecule, prior to any filtration run.

In UF membranes and SEC columns, a simplified model for the selectivity can be obtained by assuming that the selective porous medium is made of ideal capillaries which are straight, cylinders of radius  $r$ , and that molecules to be separated can be characterised by a radius  $a$  of an equivalent sphere.

In SEC, mass transfer is limited by diffusion of the molecules through the pores of the beads. The volume of elution which is the experimental characteristic of one particular molecule of radius  $a$ , depends on the pore volume,  $V_p$ , on the exclusion volume,  $V_o$ , and

on the partition coefficient  $\Phi$  [8]:

$$V_e = V_o + \Phi V_p \quad (1)$$

The partition coefficient has the form:

$$\Phi_c = \left(1 - \frac{a}{r_c}\right)^2 \quad (2)$$

where  $a$  is the solute radius and  $r_c$  is the average pore radius of the chromatography beads.

The relative resistance of a membrane on the transfer of a molecule A is characterised by the observed retention coefficient  $R_{obs,A}$ , defined as a function of the permeate concentration  $C_p$ , and the bulk concentration  $C_b$ :

$$R_{obs,A} = 1 - \frac{C_p}{C_b} \quad (3)$$

The selectivity of a membrane for a molecule A with regards to a molecule B can be expressed as  $S_{A,B} = [1 - R_{obs,A}] / [1 - R_{obs,B}]$ .

Because of concentration polarization effects, the concentration of the solution in contact with the high pressure side of the membrane,  $C_m$ , is larger than the bulk concentration. The retention calculated between both sides of the membrane  $R_m (= 1 - C_m/C_b)$ , and the observed retention are tight together via the Peclet number in the boundary layer adjacent to the membrane [9]:

$$R_{obs} = \frac{1}{1 + ((1 - R_m)/R_m)\exp(Pe_{bl})} \quad (4)$$

where  $Pe_{bl}$  is the Peclet number in the boundary layer, defined as:

$$Pe_{bl} = \frac{J\delta}{D} \quad (5)$$

where  $J$  is the convective flux density,  $\delta$  is the thickness of the boundary layer and  $D$  is the diffusion coefficient. In particular, one notes that the limit of  $R_{obs}$  when  $Pe_{bl}$  tends towards zero is  $R_m$ .

$R_m$  is known as the membrane retention coefficient, and depends on both diffusion and convection through the pores, which means that  $R_m$  is not an intrinsic membrane property, since it may change with operating conditions, as follows:

$$R_m = \frac{1 - \exp(-Pe_{pore}) - K_h \Phi_m (1 - \exp(-Pe_{pore}))}{1 - \exp(-Pe_{pore}) - K_h \Phi_m} \quad (6)$$

$\Phi_m$  is the partition coefficient,  $K_h$  is a hindrance factor depending on the ratio of the molecule to pore radii and

$$Pe_{pore} = \frac{Jl}{\varepsilon D} \quad (7)$$

$l$  is the pore equivalent length and  $\varepsilon$  is the membrane porosity.

Here one notes that at high flux,  $R_m$  tends towards  $(1 - K_h \Phi_m)$ , this asymptotic value of  $R_m$ , noted  $R_\infty$ , then characterises the ratio of the molecules apparent size to the pore apparent or equivalent average radius.

This is on such considerations that we have tried to correlate the experimental characteristics of membranes ( $R$ ) and of columns ( $V$ ) for a series of molecules.

## 2. Material and method

As the experimental part of this paper is the same as the one published in our former paper, we provide here a summary of it.

### 2.1. Membranes

Ultrafiltration Tech-Sep membranes (Novasep, Miribel, France) were made of sulfonated polysulfone, of 100 kDa (Membrane B – permeability after protein adsorption:  $3.1 \times 10^{-10}$  m/Pa/s) and 200 kDa (Membrane C – permeability after protein adsorption:

**Table 1**

Main characteristics of the macromolecular probes used in the present study. Unless mentioned,  $[\eta]$  was measured by capillary viscosimetry in our laboratory. Dextran and PEG molecular weights are as given by the supplier.

Probe	Molecular weight (kg/mol) $M$	Stokes radius	Hydrodynamic volume $[\eta] M$ (m <sup>3</sup> /mol)
PEG3	3	1.5 [11]	0.04
PEG6	6	2.2 [11]	0.10
PEG10	10	2.6 [11]	0.24
PEG20	20	3.5 [11]	0.77
PEG35	35	4.5 <sup>a</sup>	1.94
Dextrans T10 <sup>b</sup>	9.3	2.3 [12]	0.09
Dextrans T40 <sup>b</sup>	34.2	4.3 [12]	0.75
Dextrans T70 <sup>b</sup>	66.3	5.6 [12]	1.52
$\alpha$ -Lactalbumin	13.3	2.1 [13]	0.04
Ovalbumin	45	2.8 [13]	0.16
Bovine serum albumin (BSA)	69	3.6 [13]	0.27
$\gamma$ -Globulin	156		0.94

<sup>a</sup> Extrapolated from [11].

<sup>b</sup>  $[\eta]$  as by the supplier.

$3.6 \times 10^{-10}$  m/Pa/s) nominal molecular weight cut off, and a micro-filtration membrane (Membrane D – permeability after protein adsorption:  $4.6 \times 10^{-10}$  m/Pa/s) made of PVDF, rated 0.1  $\mu$ m. The filtration cell was a stirred dead-end one, and the membrane area was 13.4 cm<sup>2</sup>.

Prior to any use, the membranes were rinsed in distilled water, then packed during 1 h, by filtrating distilled water at a pressure difference of 100 kPa.

In order to reduce the role of membrane fouling during the characterisation steps, and because proteins are more foulant than dextran or PEG, we used the same pre-fouling procedure for all membrane samples used in this study. For this, we contacted the skin side of the membrane with a BSA solution (1 g/L) during 12 h at 10 °C. By this pre-treatment, we made sure that adsorption could reach equilibrium, and therefore that the characterised membrane would have a surface structure that would resemble (although not being exactly the same as) the one of the membrane in operation.

## 2.2. Solutes

In order to avoid any effect of the type of buffer on the molecular conformation of polymers or proteins, we used the same buffer for proteins and polymer solutions, whether for chromatography or UF experiments. This buffer was made of Tris–HCl buffer at pH 8.6 prepared in RO water, completed with 0.5 mol/L of NaCl. All solutes well dissolved in the buffer at a concentration of 1 g/L.

Solutes are polyethylene glycols (PEG) (Fluka Chemie), dextran (Pharmacia Chemicals) and Proteins (Sigma Chemicals), whose main characteristics have been reported in Table 1.

## 2.3. Ultrafiltration

Experiments were conducted using a stirred dead-end cell, employing a fresh 13.4 cm<sup>2</sup> disk for each experiment. The observed retention coefficients were determined from the permeate,  $C_p$  and bulk,  $C_b$ , concentrations as experimentally measured:

$$R_{obs} = 1 - \frac{C_p}{C_b} \quad (8)$$

In order to account for the effect of concentration polarization on the retention coefficient, we used the film model equation which links the observed retention  $R_{obs}$ , to the membrane retention ( $R_m$ ) via the Peclet number ( $J/k$ ):

$$\ln \left[ \frac{1 - R_{obs}}{R_{obs}} \right] = \ln \left[ \frac{1 - R_m}{R_m} \right] + \frac{J\delta}{D} \quad (9)$$

We then measured  $R_{obs}$  for different permeate fluxes obtained at different applied pressures between 10 and 100 kPa, and derived the value of  $R_m$ , by plotting Eq. (9) for each membrane and each probe. In this work, we considered that in ultrafiltration regime, the changes in  $R_m$  with flux are minimal (diffusion of solute is small as compared to convection), and therefore the extrapolation at zero flux of Eq. (9) provides a characteristic of the membrane, although not an absolute characteristic parameter.

## 2.4. Size exclusion chromatography

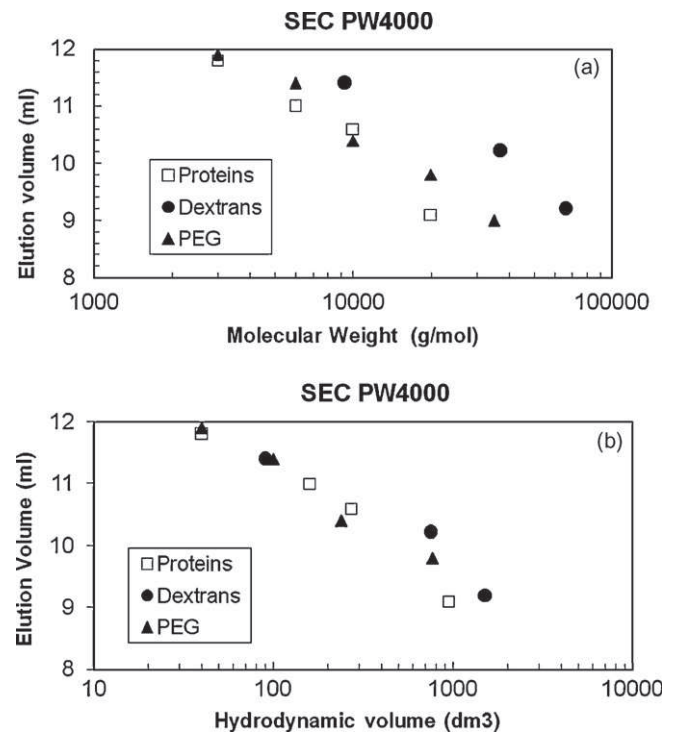
Two types of HPLC columns have been used: Micropak TSK-gel PW3000 and PW4000 (Interchim, Montluçon, France). Such columns are 300 mm long and 7.5 mm in diameter. They are packed with polymer gel beads, of 10 and 13  $\mu$ m in diameter respectively, their average pore size being 25 and 50 nm respectively. The buffer flow was set at 1 mL/min and the volume of the injection loop was 100  $\mu$ L. Detectors in series were used for the analysis of polymers (refractive index) or proteins (UV – 280 nm).  $C_p/C_b$  was calculated as the ratio of the heights of the peaks measured on the chromatograms, and  $R$  was then obtained from Eq. (3).

## 3. Results

As explained earlier, the plot of the elution volume as a function of the hydrodynamic volume of the eluted solutes gives a characteristic of a given column, as shown in Fig. 2.

We note that the elution volume varies as the log of the hydrodynamic volume, and that a characteristic equation can therefore be derived from these plots. These equations are given in Table 2, for the two columns which were used in this study.

The  $R_m$  data collected for the B, C and D membranes are shown in Figs. 3–5.



**Fig. 2.** Comparison of the same elution volumes obtained on a Micropak TSK-gel PW 4000 Column – 1 mL/min – buffer Tris pH 8.6 (same as for UF experiments) when plotted against the molecular weight (a) or the hydrodynamic volume (b) of the tracers molecules. Note that in this system of coordinates, the characteristic is a straight line with a reasonable correlation coefficient.

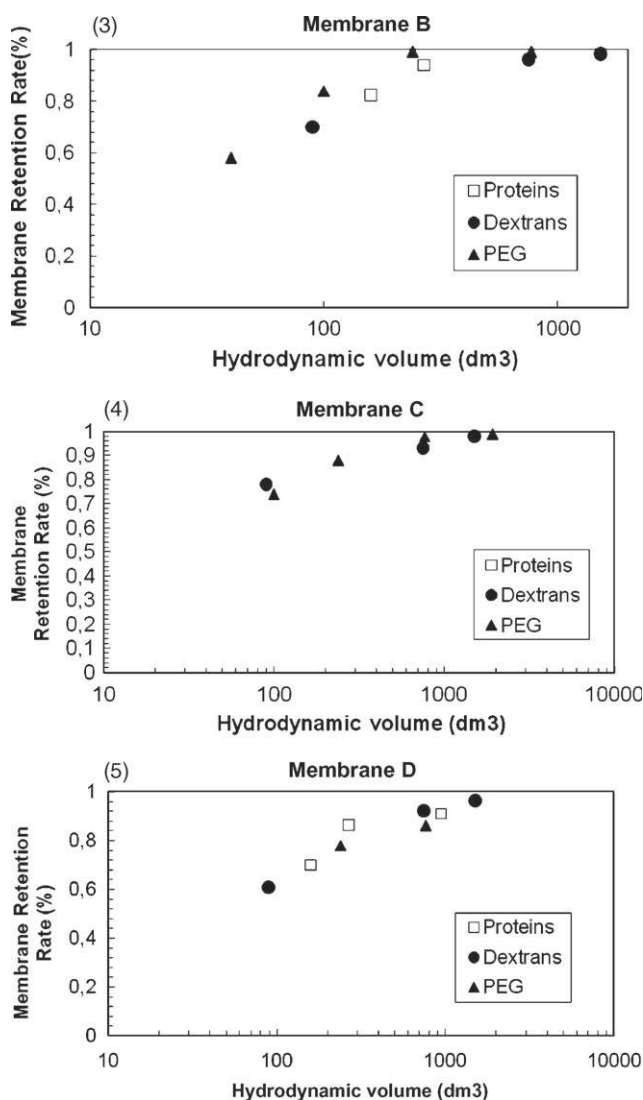
**Table 2**

Relationships derived from experimental values, between the hydrodynamic volume ( $V_h$ ) of macromolecules and their elution volume ( $V_e$ ) in two different size exclusion chromatography columns. The range of hydrodynamic volumes for which the calibration curves apply is [0.04–1.90 m<sup>3</sup>/mol].

Column	Equation ( $V_e$ in mL; $V_h$ in m <sup>3</sup> /mol)		$r^2$
TSK PW3000	$V_e = 6.70 - 0.83 \log V_h$	$\log(V_h) = 8.07 - 1.20V_e$	0.976
TSK PW4000	$V_e = 7.60 - 1.00 \log V_h$	$\log(V_h) = 7.60 - V_e$	0.956

Our previous study [1 – Fig. 4] also showed similar linear relationships between retention and hydrodynamic volumes in semi-log coordinates. However, we observed that the straight lines broke near  $R_m = 0.95$ . We also commented that in this range of retention, the permeate concentration is in general rather low and subject to larger experimental errors than for the rest of the data range. For this reason, our calibrations are based on retention data ranging between 0.2 and 0.95. The range of hydrodynamic volumes for which the calibration curves apply is [0.04–1.90 m<sup>3</sup>/mol].

Equations found in Table 3 have been obtained by fitting the data obtained with PEG and dextran only, since we wanted to use the data obtained with proteins to cross check the method.



**Figs. 3–5.** Membrane retention coefficients measures on three different membranes, for three different types of macromolecular probes and plotted here versus their hydrodynamic volumes, in semi-log coordinates.

**Table 3**

Characteristic retention equations for three different UF membranes, obtained by fitting experimental retention ( $R_m$ ) versus elution volumes ( $V_e$ ) plots for dextran and PEG molecules. Proteins data were not included in the calculation.

Membrane	Equation	$r^2$
B (SPS – 100 kDa)	$R_m = 0.40 + 1.04 \log(V_h)$	0.92
C (SPS – 200 kDa)	$R_m = 0.21 + 0.99 \log(V_h)$	0.92
D (PVDF – 0.1 $\mu$ m)	$R_m = 0.31 + 0.95 \log(V_h)$	0.93

**Table 4**

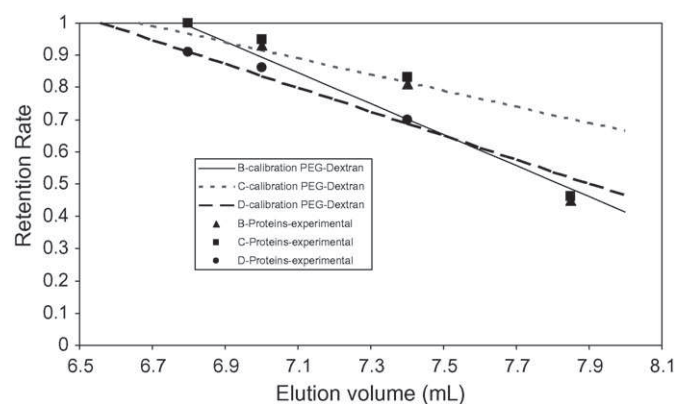
Calibration equation of each membrane against a SEC column, obtained by combinations of equations presented in Tables 2 and 3.

Membrane	Column	Equation
B (SPS – 100 kDa)	TSK PW 3000	$R_m = 8.79 - 1.25V_e$
C (SPS – 200 kDa)	TSK PW 3000	$R_m = 8.20 - 1.19V_e$
D (PVDF – 0.1 $\mu$ m)	TSK PW 3000	$R_m = 7.97 - 1.14V_e$
B (SPS – 100 kDa)	TSK PW4000	$R_m = 8.30 - 1.04V_e$
C (SPS – 200 kDa)	TSK PW4000	$R_m = 7.73 - 0.99V_e$
D (PVDF – 0.1 $\mu$ m)	TSK PW4000	$R_m = 7.53 - 0.95V_e$

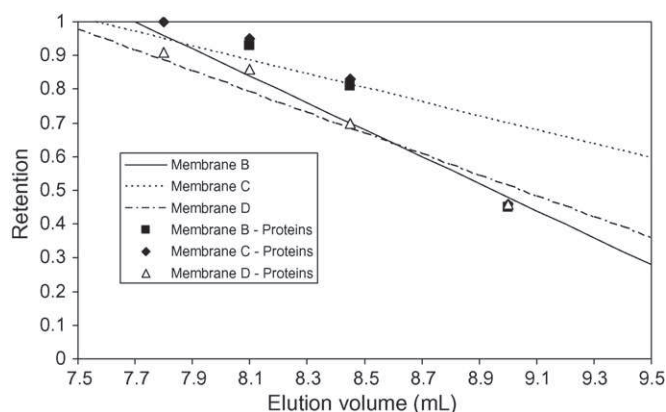
The next step was the substitution for  $\log(V_h)$  in equations of Tables 1 and 2, so as to obtain direct relationships between the retention of a molecule by a given membrane, and its elution time through a SEC column. The resulting equations are reported in Table 4.

The calibration equations reported in Table 3 then allow a direct calculation of the retention of a given molecule, provided that its elution volume through the calibration column has been measured. We then assume from here on that once a membrane and a column have been calibrated against each other using some tracer macromolecules, it will be possible to estimate a retention coefficient by this membrane of any target molecule, provided its elution volume can be measured by a simple SEC chromatography analysis. This membrane retention coefficient ( $R_m$ ), can be compared from one membrane to another one, or from one molecule to another one. However, to get an estimation of the observed coefficient at a given flux, Eq. (4) should be used. Another option would be to apply the calibration discussed in the present paper to observed retention coefficients obtained at a given polarization modulus  $C_m/C_b$  i.e. for a given filtration flux. The calibration would be closer to field conditions, but would depend on the level of concentration polarization or the Peclet number.

This is what is exemplified in Figs. 6 and 7, in which the straight lines plotted in the ( $R_m, V_e$ ) plane have been obtained from PEGs and dextran experimental data, for three different membranes and



**Fig. 6.** Plot of the retention versus elution volume for three tested membranes and one column PW4000. The lines correspond to the values predicted by equations shown in Table 4 (then obtained by combination of those in Tables 2 and 3), which were established from retention and elution measured with PEG or dextran. The symbols correspond to retention and elution volumes for proteins. The agreement is quite good except for membrane B.



**Fig. 7.** Plot of the retention versus elution volume for three tested membranes and a GPC column PW3000. The lines correspond to the values predicted by equations shown in Table 4 (then obtained by combination of those in Tables 2 and 3), which were established from retention and elution measured with PEG or dextran. The symbols correspond to retention and elution volumes for proteins. The agreement is quite good except for Membrane B.

one column (Table 4). On the same figure, the measured elution volumes and retention coefficients for proteins are also reported (symbols). Therefore the lines and the symbols have been obtained from totally independent experiments. The agreement between the calibration curve and the actually measured data is not excellent, especially for membrane B, for which the calibration underpredicts the retention for the largest proteins (BSA and  $\gamma$ -globulin). However, the data obtained with Membranes C and D are rather good, and these method would have been predictive if applied with one of the test proteins and these membranes. The standard deviation calculated for the data reported in Figs. 6 and 7 was found at 0.095. The retention coefficient of a molecule from its elution volume in SEC would then be estimated within reasonable confidence.

Despite the experimental uncertainty thus introduced, this method provides a comparison of various membranes, and/or of various protein retentions prior to making actual membrane filtration experiments, and is based on now rather standard types of SEC equipments. The concept of hydrodynamic volume, which was very useful to understand the question of the non-universal calibration based on MW, can now be dropped, as shown in equations of Table 4, since simple empirical relationships exist between  $V_h$  and  $V_e$  and  $R_m$ .

### 3.1. Calibration-summary

The calibration step consists in challenging a membrane and a SEC column against a series of macromolecular probes. The requirements on these probes are that they are soluble, easy to assay and hopefully commercially available in a wide range of molecular dimensions. The knowledge of their molecular dimensions helps when choosing the right SEC column. Dextrans, PEG's or globular proteins meet these requirements. Once the characteristic curves have been experimentally determined for the column ( $V_e = f(\log(V_h))$ ) and for the membrane ( $R_m = g(\log V_h)$ ), a direct calibration curve is readily derived between  $R_m$  and  $V_e$ .

To use this calibration curve for predicting the retention of a given macromolecule or of a mixture of macromolecules by this membrane without running a filtration test, one has to inject a solution of this (or these) macromolecule(s) in the SEC column, get its elution volume and read the predicted retention on the calibration curve. Modern high pressure SEC systems are capable of yielding such information within 10 min, which is incomparable to the time required for lab tests commonly performed to select the most suitable membrane.

## 4. Discussion

This calibration, as presented here, offers an interesting perspective to the characterisation based on the hydrodynamic volume we had proposed some years ago. The correspondence between the elution volume and the hydrodynamic volume is the way we used to by-pass the difficult question of knowing the exact value of the hydrodynamic volume or radius, *ab initio*, either of the tracers or of some unknown macromolecules one needs to process.

The calibration curve between the elution volume in SEC and the retention coefficient in the ultrafiltration system can be directly related if plotted against one another without the use of hydrodynamic volume, molecular weight, or other intermediary. It might happen that the relationships between  $V_e$  and  $\log(V_h)$  and  $R_m$  and  $\log(V_h)$  on the other hand are not linear, for some particular case. If so, such a calibration remains possible provided that the relationships are monotonous, although the final form of the calibration curve might not be as simple, neither in an analytical form.

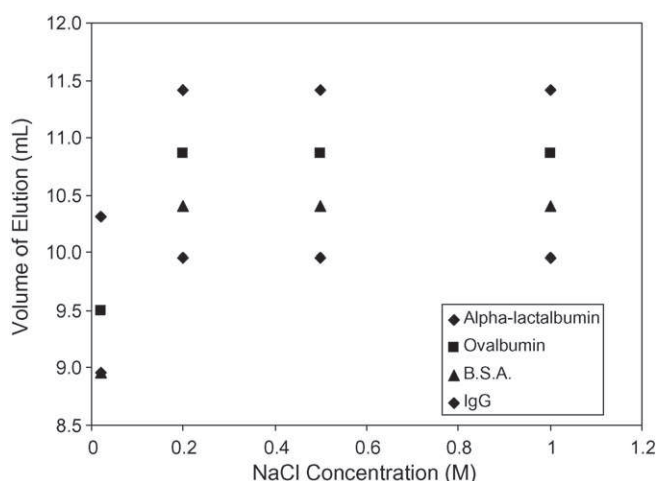
As mentioned earlier, in this work the calibration is proposed between the membrane retention  $R_m$  and the Elution volume  $V_e$ . In the case of membranes of similar geometries, which could be used in same modules, or at least in modules in which the hydrodynamics would be comparable, then a calibration involving the observed retention  $R$  can be used.

Within the range of conditions (temperature, total concentration, pH, ionic strength and composition, etc.) a SEC column can stand, one can run the column calibration in conditions close to those met in the filtration process, and this avoids tedious and uncertain corrections to account for operating away from ideal conditions (very dilute solutions, pH, room temperature, etc.). If the SEC analysis and the process conditions cannot be matched, then only relative information will be accessible and pilot or field experiments become the only way to get a more precise answer to the major questions regarding selectivity. As in our study, the accuracy of the calibration will be higher if the UF and SEC buffer are the same and if not, if their ionic strength and pH values are close to each other.

Protein concentration maybe an issue if so low that the corresponding peak cannot be distinguished from the chromatogram baseline. However, the advantage of this method over one that would require true membrane filtration of the sample, is that because the elution volume, and not the concentration of the test molecule in the sample is searched for, the method can cope with much lower concentrations than if a quantitative analysis were required. On the other hand, industrial fluids with high total dissolved solutes loading will probably have to be diluted, so as to avoid plugging the SEC columns. Such dilutions often need attention as the buffer used might change the structure in solution of the macromolecules of interest.

The volume of sample required for tests are low with this method as the amounts injected in SEC systems are counted in fractions of millilitres, and the largest amounts of sample required are those needed to pre-condition the membrane by adsorption, which is never more than 5 cm<sup>3</sup>/cm<sup>2</sup> of membrane.

Membrane fouling is another important issue, which may scatter data far beyond what has been reported in this paper. Obviously a clean and a fouled membrane would not have the same calibration line against a SEC column, neither would it show the same retention coefficient for a given molecule. If fouling occurs during the calibration tests, then this probably bias the calibration lines a lot. All along our experimental study, we did prepare the membrane in the same way: contact it to a protein solution for 12 h at 10 °C, so as to reach adsorption equilibrium. In an operational mode we would of course recommend to contact the membrane to the fluid to be



**Fig. 8.** Changes in elution volumes as a function of the concentration in sodium chloride added to a Tris-HCl buffer. Beyond 0.3 mol/L in NaCl added, one cannot detect any change in the elution volumes, which suggests that both the probes and the column have reached a steady structure.

processed better than to a BSA solution, as it would create surface conditions much closer to the field ones than the model solution. However, in the absence of availability of this fluid, better adsorb a standard BSA solution, than use a bare membrane which would certainly not respond as a pre fouled one.

Charge effects have been reported in ultrafiltration especially for proteins, and smart separations have been described and industrialised, based on a fine tuning of the charge effects, under proper conditions [10]. This type of selectivity, which combines size exclusion and other (mainly electrostatic) effects is not accounted for in the calibration as presented here. We report in Fig. 8 (After [14]) the changes in the elution volumes of four different molecules, as a function of the buffer ionic strength. These curves suggest to operate at moderate buffer ionic strength (around 0.2 molar with the columns used in these experiments), although one observes a relatively low change in  $V_e$  with the ionic strength. Considering the nature of the columns packing, this is an expected independence.

However, the positive effect of electrostatic repulsive forces on protein retention in UF has often been interpreted as if the protein were larger at low ionic strength. The size difference is influenced by the Debye length (Munch et al. [15]). Therefore, the calibration as proposed here might be run with different buffers in SEC and in UF. Would this be done, the results should be considered very carefully and their application restricted to the specific case simulated by the chosen experimental conditions.

A final reference to the calibration of membrane in non-aqueous media is relevant here as well. Recent improvements in membrane fabrication allow their use in non-aqueous solvents, and this is particularly interesting in nanofiltration. Now the question of the characterising these membranes in solvent media has become a crucial one. The calibration of these membranes versus SEC column, provided they can stand the relevant solvents, might offer an interesting approach to this problem.

## 5. Conclusion

The calibration of UF membranes against SEC columns is possible by using the same macromolecules as tracers, such as PEGs or dextran or even proteins, to first characterise these two systems.

The calibration then consists of an experimental measurement of each of these tracers' retention rate by the UF membrane and

elution volume through a given SEC column. The observed retentions are converted into intrinsic membrane retention, as defined in the text. Finally a membrane retention-elution volume relationship can be derived.

In our conditions, we showed that these calibrations allowed us to estimate the retention of proteins by membranes of various structures and materials with a standard deviation of 0.095, from elution volumes measurements, thus allowing to by-pass some tedious UF experiments.

We have discussed how this calibration can account for the effects of fouling, ionic strength and pH to some extend.

## Nomenclature

$a$	solute radius of gyration (m or nm)
$C_b$	solute concentration in the bulk (g/L)
$C_m$	solute concentration near the membrane high pressure side (g/L)
$C_p$	solute concentration in the permeate (g/L)
$D$	diffusion coefficient (in bulk) ( $\text{m}^2/\text{s}$ )
$K_h$	hindrance factor
$Pe_{bl}$	Peclet number defined with regard to the boundary layer thickness
$Pe_{pore}$	Peclet number defined with respect to the length of a membrane pore
$r_c$	average pore radius for the SEC column (m or nm)
$R$	observed retention coefficient
$R_m$	membrane retention coefficient
$R_\infty$	asymptotic membrane retention coefficient
$r_m$	average pore radius for the membrane (m or nm)
$V_e$	elution volume in SEC ( $\text{m}^3$ or mL)
$V_o$	exclusion volume of a particular SEC column ( $\text{m}^3$ or mL)
$V_p$	pore volume of a SEC column ( $\text{m}^3$ or mL)
$V_h$	hydrodynamic volume of a molecule ( $\text{m}^3/\text{mol}$ or mL/mol)

## Greek letters

$\delta$	thickness of the mass transfer boundary layer (m)
$\varepsilon$	membrane surface porosity
$\Phi$	partition coefficient between the porous phase and the liquid phase

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