Anomalous behaviour in the diffusion of polyethylene oxide through dialysis membrane

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Introduction

Dialysis is a common technique adopted in biochemistry to purify biopharmaceutical drugs. This methodology is also of interest in macromolecular chemistry and pharmaceutical nanotechnology in order to purify synthetic macromolecules and nanodrug carriers designed for drug delivery purposes. However, based on their original applications, the diffusion characteristic of the dialysis membrane is given in respect to the diffusion rate of globular proteins. So the diffusion capacity is function of molecular weight cut-off, i.e. corresponding to the maximum molecular weight of a globular macromolecule to be able to cross the membrane.

The diffusion kinetics of synthetic macromolecules is expected to differ significantly from globular proteins due to at least the following differences :

- ×Specific relationship between hydrodynamic diameter and molecular weight,
- ×Flexibility
- ×Ionic density
- ×Solubility/miscibility/adsorption behaviour with the dialysis membrane
- ×Polymer chain entanglement above a critical concentration.

In view to validate the application of this technique to purify synthetic macromolecules, we have compared the diffusion ability of neutral polyethyetylene oxide (PEO) standards or poly(dimethyl-aminoethyl-methacrylate) (PMADAM) to protein standards (human insulin and ovalbumin).

Materials and methods

Tthe diffusion ability of the macromolecules has been analyzed in terms of:

- × Concentration (0.1, 1, 10% (W/V))
- × Molecular weight (PEO of Mw of 4, 10, 20 kDa)
- × Macromolecules conformation (neutral PEO vs charged PMADAM)

The tightness and cut-off of dialysis membrane of 1 KDa (Spectrum Laboratories, Inc) has been assessed adopting either Human Insulin (HI ; Mw : 5,807) and Ovalbumin (Ov ; Mw :43,000) as reference proteins (concentrations 0.01 and 0.1 %). The diffusibility of PEO (4, 10 and 20 KDa) has been compared with the one of PMADAM (10 kDa). These synthetic polymers have been dissolved at 3 different concentrations: 0.1 ; 1 and 10 % (W/V)).
24h after dialysis to achieve thermodynamic equilibrium, the amount of macromolecules diffusing out of the membrane has been quantified by :

Polymers
Lyophilisation - Gravimetry
SEC

Proteins
BCA

The dialysis has been performed against water at room temperature under lateral agitation.

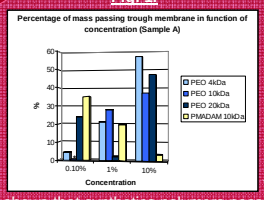
Results and discussion

Surprisingly enough all the synthetic polymers evaluated have crossed significantly throughout the 1 KDa membrane, in spite to have mean Mw well beyond this cut-off (see figure 1). By comparison no diffusion has been observed for the two reference proteins. (Fig. 2)

Our results have also highlighted that the diffusion of PEO's is facilitated when considering higher concentrations of polymer solutions. The opposite observation has been done in the case of the polyelectrolyte. (Fig. 1)

The SEC profiles of the polymers recovered out of membrane are identical to the original polymers placed inside the membrane. (Fig. 3)

Fig. 1.

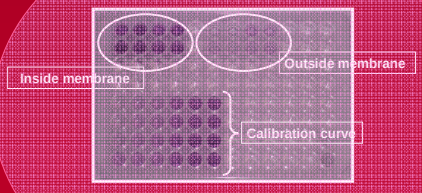


* No result.

This graph shows that synthetics polymers pass trough the dialysis membrane.
For PEO, this diffusion is increasing with concentration.
In contrast, the efficiency of diffusion of PMADAM is inversely proportional to his concentration.

Fig. 2.

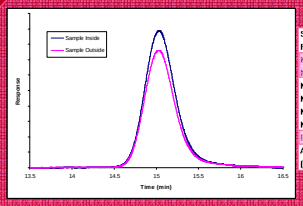
BCA multiplates



As expected, the concentration outside membrane is near zero in opposition to the inside.

Fig. 3.

PEO 4 kDa (10%)



Sample		
FRCF	1.0000	1.0000
Mp	5729	5774
Mn	5354	5371
Mw	5584	5605
Mz	5775	5798
Mz+1	5944	5968
Mv	5553	5574
Disp	1.043	1.044
Area [Response Minutes]	195217	167182

Conclusion and perspectives

We can conclude that synthetic polymers of molecular weight well beyond the cut-off of dialysis membrane diffuse substantially across this barrier. It can be anticipated that this diffusion should occur through a reptation mechanism within the cellulose membrane. The difference in diffusion rate in function of the polymer concentration and nature could be explained on the basis of their difference in respective hydrodynamic diameters and chain entanglement.

Independently of their interest for purification purposes, the results of our study could also find applications in the in vitro analysis of kidney clearance of hydrosoluble synthetic polymers.

Acknowledgements

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