The permselectivity of poly(glyceryl methacrylate) membranes: hydrogel analogues of glomerular basement membrane

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The ultrafiltration behaviour of highly hydrated thin films of poly(glyceryl methacrylate) (PGMA) hydrogel has been studied and compared with the behaviour of the glomerular basement membrane (GBM). PGMA hydrogel films paralleled the behaviour of GBM as compressible ultrafilters, showing selectivity for proteins of different sizes. The pore theory and fibre-matrix hypothesis were used to model permeation behaviour. Pore radii predicted by the pore theory were 2.3–4.0 nm for PGMA and 3.5–6.0 nm for GBM. Fibre radii predicted using the fibre-matrix hypothesis generally were 0.8 ± 0.05 nm for both materials, consistent with the view that these materials are networks of randomly arranged flexing fibres. The fibre-matrix hypothesis was judged to provide a more realistic correlation between structural characteristics and permeation behaviour than the pore theory. The results indicate that GBM possesses hydogel-like properties.

(Keywords: hydrogel; basement membrane; fibre-matrix hypothesis; pore theory; permeation; size-dependent rejection)

INTRODUCTION

Polymers are commonly used to replace biological materials for many purposes including biomedical uses[1–3]. Here the use of polymers is explored in modelling renal glomerular basement membrane (GBM), the natural material which serves as an ultrafilter in the walls of glomerular capillaries[4]. Blood is ultrafiltered by this membrane barrier in the initial step of urine formation.

Studies of the permeation properties of basement membrane show that it behaves as a highly hydrated matrix of fibres[5] primarily composed of type IV collagen. Creating analogues of this structure using polymers offers several advantages. Basement membrane is a low-fouling ultrafilter and analogues might therefore be expected to share this property. Studies of basement membrane *per se* are constrained by the nature of the material; it is difficult to change its properties to explore structure–function relationships and it can only be isolated as tiny fragments which cannot be studied readily by the conventional methods of materials science. In contrast, polymer analogues can be made with deliberately varied structures so providing a means for studying structure–function relationships which may then yield insights in understanding basement membrane.

In earlier studies[6,7] it was reported that thin films of highly hydrated poly(glyceryl methacrylate) (PGMA) showed ultrafiltration properties resembling those of basement membrane when myoglobin was used as the permeant. Here these studies are extended to explore more fully the ultrafiltration properties of PGMA using cytochrome c, lysozyme, α-lactalbumin and bovine serum albumin (BSA), allowing a more detailed comparison between the synthetic and natural materials.

EXPERIMENTAL

Preparation of PGMA filters

The monomer 2,3-dihydroxypropyl methacrylate (glyceryl methacrylate) was prepared by the mild acid hydrolysis[8] of 2,3-epoxypropyl methacrylate (glycidyl methacrylate, 97% technical, Aldrich, UK).

Thin hydrogel ultrafilters were prepared according to previous procedures as described elsewhere[9]. Typically, the polymer formulation was 10 volumes of glyceryl methacrylate in 30 volumes of water to which was added one volume each of analar grade 6% (w/v) ammonium persulphate and 12% (w/v) sodium metabisulphite (BDH, Poole, Dorset, UK) as initiators. This solution mixture was allowed to polymerize at ambient temperature between siliconized glass plates held together by clips. After 30–40 min the plates were separated and a thin layer of hydrogel membrane was retained on one of the glass plates.

Owing to its friable nature, the hydrogel membrane needed to be supported before it could be used as an ultrafilter. For this purpose, porous cellulose acetate/nitrate filtration membranes (type MF HAWP, 0.45 μm exclusion, Millipore UK Ltd, Watford) were laid over the slightly wet film which was left for a further 90 min. The resultant polymer composite membrane was then peeled off the glass plate using a razor blade and soaked at room temperature in sodium chloride (0.15 mol dm^{-3}) Tris–hydrochloric acid (0.01 mol dm^{-3}) buffer pH 7.4 containing sodium azide (0.002% w/v) as preservative (buffered saline) to remove all traces of initiators and unpolymerized monomers, and to allow the membrane to swell to equilibrium. Films stored in this way

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maintained normal filtration characteristics for several months.

Measurement of hydrogel void volume

Gels were polymerized between glass plates at ambient temperature for 2 h using microscope slides as spacers ($\sim 150\ \mu m$). The gel sheets were cut into buttons at about 1.5 cm diameter which were then equilibrated in buffered saline at room temperature. For each button, the wet weight was determined by blotting twice with filter paper to remove excess water before weighing, and the dry weight was measured after drying to constant weight. The void volumes (fractional water volumes) of samples of gel swollen to equilibrium in buffered saline was 0.88 ± 0.02; except for the sample used for the filtration of α-lactalbumin which was conducted across a film with a void volume of 0.91 ± 0.02.

Film thickness measurements

Hydrogels were cast on Millipore membranes which had been previously marked with fine lines of water-proof ink. Following filtration studies, 0.5 mg of *Micrococcus lysodeikticus* capsules suspended in 5 cm^3 buffered saline were filtered down onto the film surface at a pressure of 200 kPa. After being kept in buffered saline for 1 h, the film thickness was measured using a phase contrast microscope by focusing first on the *Micrococcus* capsules and then on the fine ink lines, while noting the vertical movement of the microscope from the fine adjustment. Measurements of at least 20 randomly chosen points were averaged; film thicknesses were 9.6–13.3 μm ($\pm$ 0.79–0.82 μm).

Ultrafiltration procedure

The composite hydrogel film/Millipore membranes were mounted in type 52 Amicon filtration cells and washed with buffered saline pressurized with oxygen-free nitrogen at 200 kPa. The proteins used were horse cytochrome c, horse myoglobin, hens egg lysozyme, bovine α-lactalbumin and bovine serum albumin (Sigma Chemical Co., Poole, Dorset, UK). These were dissolved in buffered saline (0.5 mg cm^{-3}) and were microfiltered (0.45 μm exclusion) before use. Protein solution was placed in each filtration cell (60 cm^3 capacity with filtration area of 13.2 cm^2) and each filtration was conducted at each of several known pressures; cells were stirred at 1000 rev min^{-1} to minimize the effects from concentration-polarization. Filtrate samples were collected over timed periods and A_{280} of the filtrates was measured. This permitted measurement of solvent flux, J_v, and solute flux, J_s. Protein rejections were calculated as:

$$R = 1 - C_f/C_b$$

where C_f is the filtrate solute concentration and C_b is the concentration of the overstanding bulk solution corrected for losses during filtration. Three filtration cells were run in parallel and at least three consecutive readings were taken from each; the flux values were normalized to a film thickness of 10 μm and were averaged. Flux values showed a standard deviation of $\pm 10\%$ from the consecutive readings from each of three cells; rejection values were $\pm 5\%$.

Measurement of permeability coefficient

Measurements were performed at 20°C using a horizontal diffusion cell with a vertically mounted composite membrane film. The cell had a large (80 cm^3) and a small (5 cm^3) reservoir, designed so that both compartments could be continuously stirred at 1000 rev min^{-1} to reduce boundary layer effects. Solute concentrations were monitored by radioisotope measurement of tracer proteins labelled using *N*-succinimidyl-[2,3-^3H]-propionate[10]. Radioactivity was measured using scintillation counting with internal standardization. Flux values were determined by frequently exchanging the solution in the small chamber for fresh buffer so that the concentration difference across the membrane was that of the concentration of protein in the large chamber; this remained constant, within measurement error, during experiments. The permeability coefficient was obtained from plots of steady-state J_s against concentration difference (ΔC) according to:

$$P_m = J_s/\Delta C$$

Counting errors were $\pm 2\%$ standard deviation and >99% of isotope was coupled to the protein.

RESULTS

The solvent and solute flux values for filtrations with individual proteins across PGMA films are shown in *Table 1*. *Table 2* includes parallel values for basement membrane taken from reference 11. The results are corrected to a film thickness of 10 μm. Filtrations using basement membrane were conducted at pH 5.7, the pI for basement membrane, to minimize charge effects. The behaviour of both sets of membranes was closely comparable except that fluxes were somewhat higher for GBM than for PGMA and basement membrane was

Table 1 Data obtained from filtration of proteins through PGMA films

Filtration pressure (kPa)	J_v (cm min^{-1} × 10^2) and [J_s (mg cm^{-2} min^{-1} × 10^3)]				
	Myoglobin	Cytochrome c	Lysozyme	BSA	α-Lactalbumin
200	3.09 [2.30]	3.33 [4.23]	2.85 [8.72]	3.28 [n.d.][a]	4.13 [1.63]
150	2.70 [2.43]	3.02 [4.51]	2.62 [8.90]	3.00 [n.d.]	3.63 [2.88]
100	2.12 [2.35]	2.39 [4.18]	2.12 [7.40]	2.49 [n.d.]	2.95 [3.35]
50	1.40 [2.21]	1.59 [3.65]	1.43 [5.41]	1.58 [n.d.]	1.97 [3.17]
30	1.01 [1.98]	1.13 [3.07]	1.03 [4.05]	1.13 [n.d.]	1.38 [2.88]
10	0.49 [1.43]	0.51 [1.75]	0.47 [1.96]	0.50 [n.d.]	0.60 [1.92]

[a][n.d.] = not detectable

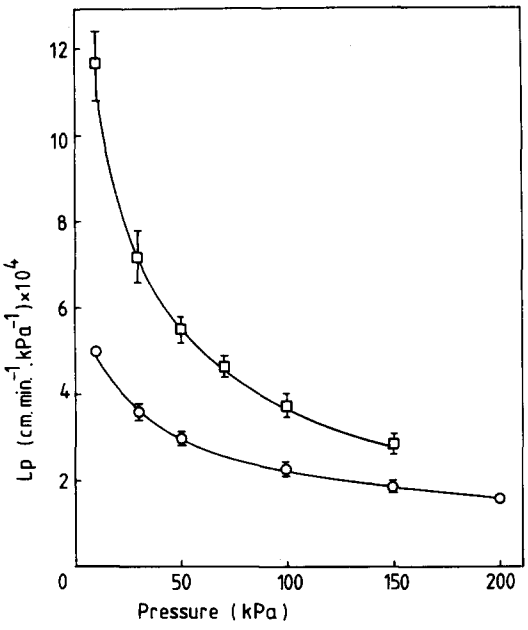

Figure 1 Comparison of the hydraulic permeability coefficients for PGMA (○) and GBM (□)

Table 2 Data obtained from filtration of proteins through GBM films (ref. 11)

Filtration pressure (kPa)	J_v (cm min^{-1} × 10^2) and [J_s (mg cm^{-2} min^{-1} × 10^3)]			
	Myoglobin	Cytochrome c	Lysozyme	BSA
150	4.54 [4.60]	4.41 [6.16]	4.35 [8.20]	4.62 [1.93]
100	3.93 [4.72]	3.92 [6.16]	3.85 [8.20]	3.93 [1.77]
70	3.39 [4.82]	3.36 [6.16]	3.26 [8.94]	3.47 [1.62]
50	2.77 [4.72]	2.94 [5.88]	2.85 [8.94]	3.05 [1.62]
30	2.08 [4.62]	2.03 [6.02]	2.17 [7.95]	2.54 [1.77]
10	– [–]	1.26 [4.62]	1.21 [5.25]	1.54 [1.39]

permeable to BSA. The hydraulic permeability coefficient, L_p, for the membranes at different pressures are compared in *Figure 1*. The decline in L_p with pressure results from progressive compression of the membranes[5–7]. The values for the two types of membrane tend to converge at high pressure but the hydrogel exhibits appreciably lower L_p values at low pressures. The protein rejection values are compared in *Figures 2a* and *2b*. Rejection increases with protein size for both materials and increases with applied pressure reflecting compression of the barriers. The difference in permeability to BSA is clearly illustrated; myoglobin shows consistently higher rejections with PGMA while cycochrome c shows very similar behaviour with both materials. However, lysozyme shows striking behaviour. For both materials this protein exhibits lower rejections than cytochrome c, although it is a larger molecule (M_w = 14 500 compared to 12 000), and this difference in rejection is most marked for PGMA films. The P_m values (normalized to 10 μm thickness) were found to be 2.04 × 10^{-3}, 2.53 × 10^{-3}, 2.92 × 10^{-3} and 2.72 × 10^{-3} cm min^{-1}, respectively for myoglobin, cytochrome c, lysozyme and BSA; the corresponding values for basement membrane were 1.12 × 10^{-3}, 2.21 × 10^{-3}, 2.09 × 10^{-3} and 5.44 × 10^{-4} cm min^{-1}, respectively. These values generally reflect the behaviour of the proteins during ultrafiltration, diminishing with increasing size of protein and again showing anomalous results for lysozyme.

DISCUSSION

Previous studies of permeation through basement membrane *in vitro* led to the conclusion that the behaviour of the material was best explained using the

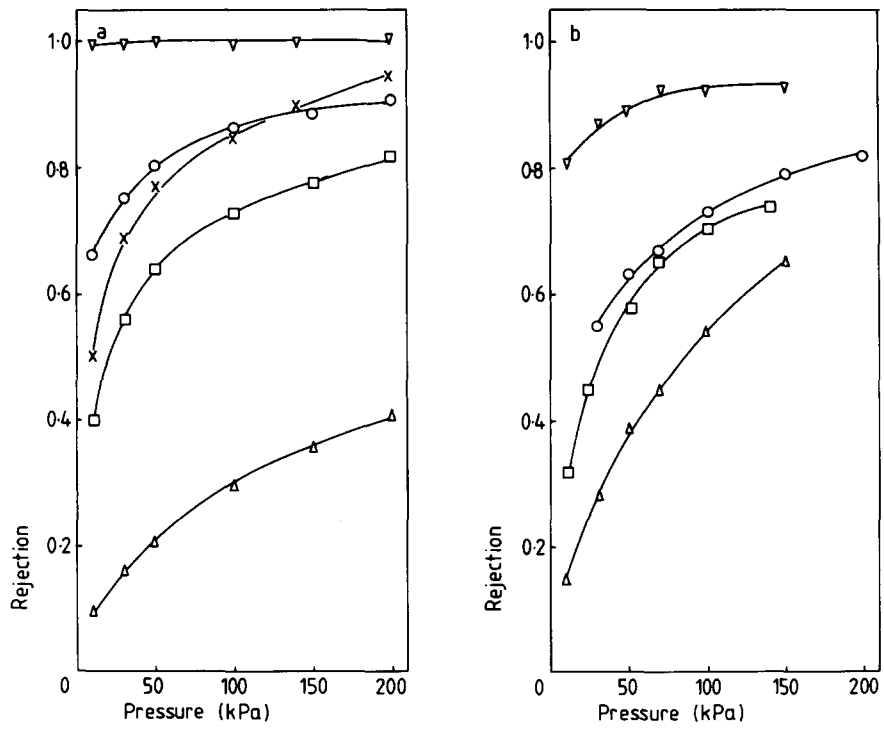

Figure 2 Filtration of proteins through films of (a) PGMA and (b) GBM. Rejection of myoglobin (○), cytochrome c (□), lysozyme (△), BSA (▽) and α-lactalbumin (×)

fibre-matrix hypothesis[12-14]. The membrane behaved as a matrix of randomly arranged fibres, radius ~0.8 nm, with a void volume of 0.9. Studies of myoglobin permeation through PGMA films also led to the conclusion that the fibre-matrix hypothesis offered a reasonable explanation of the properties of this material[6,7]. It is therefore of interest to judge whether this view holds for the extended range of proteins studied here.

Previously we have discussed in detail[6] the difficulties in applying theoretical analyses to ultrafiltration measurement such as reported here. Briefly, the filtration of water through a membrane barrier is described by:

$$J_v = L_p(\Delta P - \sigma \Delta \pi) \quad (1)$$

where J_v is the solvent flux (cm min^{-1}); L_p is the hydraulic permeability coefficient (cm min^{-1} kPa^{-1}); ΔP is the applied hydraulic pressure difference; σ is the Staverman reflection coefficient of the macromolecular species; $\Delta\pi$ is the osmotic pressure of the solution.

At high J_v, solute is swept to the membrane face forming a zone of local concentration which tends to hinder solvent flow and to reduce the measured rejection, since permeation flux is determined by solute concentration at the filter surface rather than the concentration in bulk solution. A polarization parameter, Ω, can be estimated[15], and for the conditions used in this study $\Omega < 1$, indicating that polarization effects are unlikely to have influenced the results[6]. A second difficulty lies in judging whether the measured rejection, the fraction of solute rejected by the barrier, is close to σ, given by:

$$R = \sigma(e^{Pe} - 1)/(e^{Pe} - \sigma) \quad (2)$$

$$Pe = J_v(1 - \sigma)/P_m \quad (3)$$

$$J_s = J_v(1 - \sigma)C_b + P_m(C_b - C_f)Pe/(e^{Pe} - 1) \quad (4)$$

where Pe is the membrane Peclet number; J_s is the solute flux (mg cm^{-2} min^{-1}); P_m is the membrane permeability coefficient (cm min^{-1}).

At low values of J_v, especially for smaller molecules with higher values of P_m, diffusional movement will contribute significantly to J_s and $R < \sigma$. Increasing J_v diminishes this discrepancy, so that R approaches σ. Rigorous evaluation of Pe is impossible for compressible membranes since P_m can only be measured under conditions where there is no compression, i.e. no convective flow. However, at higher pressures, where J_v is high, R is expected to be close to σ.

Bearing in mind these uncertainties, analyses of permeation were attempted using the fibre-matrix model[16]:

$$\sigma = \{1 - \exp[\pi l_f(2ar_f + a^2)]\}^2 \quad (5)$$

$$L_p = \frac{r_f^2 \varepsilon^3}{4\kappa\eta\,\Delta x(1 - \varepsilon)^2} \quad (6)$$

where ε $(= 1 - \pi r_f^2 l_f)$ represents the void volume; a is the solute radius (nm); r_f is the fibre radius (nm); l_f is the length of fibre per unit volume (nm cm^{-3}); κ is the Kozeny-Carman coefficient[17] (taken to be 5 for a random fibre array); η is the solution viscosity; Δx is the membrane thickness.

From R, measured for a particular protein at a given pressure, r_f can be estimated using equations (5) and (6) by choosing a value for r_f which predicts the measured value for L_p. The validity of r_f can then be judged by using this assumed value to calculate L_p at each different filtration pressure using R measured separately at each pressure. R (measured) and r_f (assumed) allows calculation of l_f, which then leads to the value for the void volume at each pressure. It is then possible to calculate Δx for the films while under pressure since the equilibrium thicknesses and void volumes are known; compression reduces thickness by squeezing water from the films and increasing l_f. These results are shown in *Table 3* and the agreement between measured and calculated values is shown in *Figure 3a*. For myoglobin, cytochrome c and α-lactalbumin, reasonable predictions for L_p were obtained by assuming a fibre radius of 0.80 ± 0.05 nm. A difference in r_f of ± 0.05 nm did not affect results appreciably[7]. Thus the three different proteins showed consistent results. BSA was completely rejected so that this mode of analysis could not be employed. However, if r_f is taken as 0.8 nm with l_f as 1.5×10^{13} nm cm^{-3}, σ is calculated to be 0.999, as expected from observation. The results for similar calculations with GBM are shown in *Table 3* and *Figure 4a*; it can be seen that the model yields similar results except that fibre packing is less intense, as might be expected since basement membrane appears to be a more permeable material than PGMA. As noted earlier, at low pressures R might not be close to σ; however, such behaviour is not obvious from *Figure 3a* where the results at low pressure (high L_p) fall close to the line of identity.

The results may also be analysed using the pore theory[15,18,19] where:

$$\sigma = [1 - (1 - \alpha)^2]^2 \quad (7)$$

$$L_p = \frac{n\pi r_p^4}{8\eta\Delta x} \quad (8)$$

where $\alpha = a/r_p$, r_p is the pore radius; n is the number of pores per unit area; Δx is the membrane thickness. For pores at right angles to the plane of the membrane ε $(= n\pi r_p^2)$ represents the void volume.

The pore density can be calculated from the known

Table 3 Filtration parameters predicted from analysis using the fibre-matrix hypothesis (ref. 11)

Proteins	Membranes	r_f (nm)	Δx (μm)	ε	l_f (nm $cm^{-3} \times 10^{-13}$)
Myoglobin	PGMA	0.80	5.3–10.1	0.65–0.82	1.38–0.72
	GBM	0.83	5.7–9.9	0.76–0.86	1.16–0.64
Cytochrome c	PGMA	0.80	5.4–9.7	0.68–0.86	1.41–0.61
	GBM	0.80	4.2–10.0	0.75–0.89	1.21–0.52
Lactalbumin	PGMA	0.75	4.5–9.8	0.65–0.88	1.55–0.53
BSA	GBM	1.15	6.4–10.9	0.74–0.84	0.64–0.38

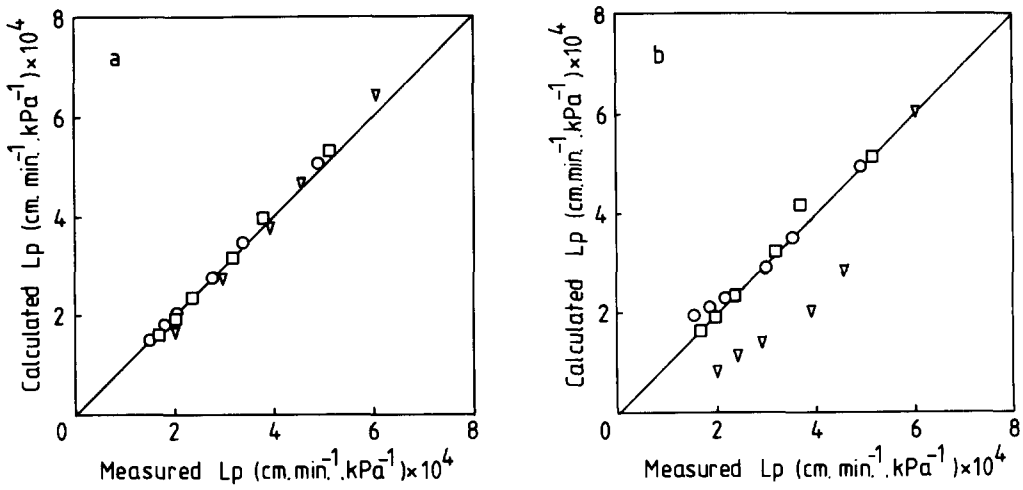

Figure 3 Comparison of measured L_p and values predicted by (a) the fibre-matrix hypothesis and (b) the pore theory for films of PGMA. Proteins are myoglobin (○), cytochrome c (□) and α-lactalbumin (▽)

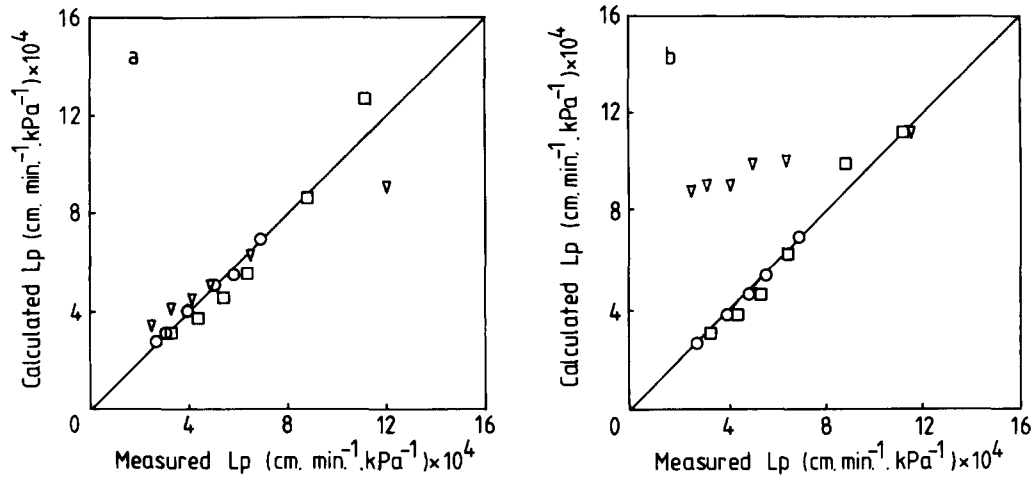

Figure 4 Comparison of measured L_p and values predicted by (a) the fibre-matrix hypothesis and (b) the pore theory for films of GBM. Proteins are myoglobin (○), cytochrome c (□) and BSA (▽)

Table 4 Filtration parameters predicted from analysis using the pore theory (ref 11)

Proteins	Membranes	r_p (nm)	Δx (μm)	ε	n (cm^{-2} × 10^{12})	R
Myoglobin	PGMA	2.43–3.47	6.2–10.0	0.26–0.54	1.40	0.63
	GBM	2.74–3.97	5.7–10.0	0.28–0.59	1.20	0.55
Cytochrome c	PGMA	2.32–3.54	5.9–10.0	0.24–0.55	1.40	0.49
	GBM	2.56–4.60	3.4–10.0	0.23–0.74	1.10	0.32
Lactalbumin	PGMA	2.40–4.36	6.6–10.0	0.13–0.43	0.71	0.50
BSA	GBM	4.35–5.02	7.3–10.0	0.45–0.60	0.67	0.83

L_p, and from the calculated L_p, at low pressure. It is assumed that compression reduces L_p by deforming the membrane material into the space occupied by pores so that r_p narrows as Δx is decreased. L_p can then be calculated from r_p (compressed) and compared with measured values. Filtration parameters calculated according to the pore theory are shown in *Table 4*. For different proteins, predictions for the pore radii are generally consistent and were found to be in the range of $r_p = 2.32–4.36$ nm, with the smaller pore sizes found at higher pressures. However, the calculated void volume, defined as fractional pore volume, is grossly underestimated. The calculated and measured values of L_p are compared in *Figure 3b*. Predictions of L_p are not consistently good for all proteins. A problem encountered using this model is that n, the pore number, is calculated from R and L_p measured at the lowest filtration pressure when membrane compression is least. As noted earlier, R may differ from σ at low pressures when J_v is low and diffusion may contribute significantly to J_s. Modelling using the fibre-matrix hypothesis, however, did not indicate major inconsistencies attributable to this error. On these grounds it seems that the pore theory is a less satisfactory model. Attempts to allow for diffusional flow

using P_m values did not provide analyses that were any more consistent.

Similar results for the basement membrane are shown in *Table 4* and *Figure 4b*. Pore radii were predicted in the range of $r_p = 2.38-5.02$ nm, but again the model predicts void volumes which are too low. A further inconsistency is that when BSA is studied, a larger pore size is predicted, which seems unrealistic. Previously we concluded that the fibre-matrix hypothesis provided a more consistent and a more realistic view of hydrogels than the pore theory[6,7] and this conclusion seems to be supported by these studies.

Lysozyme is not included in the calculations as it shows much lower rejections than expected. Hence, α-lactalbumin, a homologous protein with similar molecular size, was filtered, and shows rejection values as expected from its dimensions. The two proteins possess opposite charges at pH 7.4, but since the PGMA film is uncharged, charge difference is unlikely to explain the marked differences in rejection. One possibility is that lysozyme may be more flexible, unfolding and refolding as it penetrates the matrix structure[20,21].

CONCLUSION

The results demonstrate that thin hydrogel films of PGMA behave as size-selective ultrafilters, though their behaviour is affected by compression, unlike the materials used commercially for ultrafilters. Homogeneous hydrogels are highly hydrated matrices of randomly arranged polymer filaments and as such their permeation behaviour can be modelled using the fibre-matrix hypothesis, as demonstrated in this study. Modelling predicts that the polymer fibre radius is 0.8 nm; there is no independent confirmation for this value though it seems reasonable from molecular modelling studies. The model illustrates that the density of fibre packing (l_f) is an important determinant of permeation behaviour and l_f depends upon both the intrinsic structure of the hydrogel and upon the degree of compression of the material under operating conditions.

The pore theory also allows modelling of the behaviour of the material but is inconsistent in predicting void volumes and pore radii; intuitively it seems unlikely that a random structure such as a hydrogel should possess defined pores, as required by this theory.

PGMA films show a behaviour which closely resembles that of basement membrane. The hydrogel formulation was chosen to provide a material with a void volume similar to that of basement membrane so as to be closely analogous. Their very similar behaviour and the consistent predictions of the behaviour of both materials by the fibre-matrix hypothesis confirm an earlier view that basement membranes are best regarded as hydrogel-like rather than as materials possessing a porous structure. It is of interest that basement membrane seems to possess a somewhat more permeable structure than is provided by PGMA. This suggests that there may be some ordering of the protein framework of this material, maintaining a slightly more open structure than is found in the polymer hydrogel. Basement membranes appear to be low-fouling ultrafilters and this property seems to be shared by PGMA. In the present study, the same PGMA films were used to filter myoglobin, cytochrome c, lysozyme and BSA over a period of several weeks. At the termination of these measurements the performance of the films remained unaltered and L_p values for buffer flow was unchanged. The open structure of the hydrogel and the absence of any extensive solid surface presumably minimizes protein adsorption; in this respect the hydrogels again mimic basement membrane.

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