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Water uptake and protein release characteristics of a new methacrylate-based polymer system

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A novel polymer system comprising poly(ethyl methacrylate) (PEMA) powder gelled with hydroxyethyl-methacrylate (HEMA), n-butylmethacrylate (nBM) monomer mixtures has been produced. The monomers were combined in different ratios to vary the relative hydrophobicity of the system. Surface and bulk properties of this copolymer system were investigated. Surfaces were relatively featureless with little variation due to composition. Contact angles ranged from 76 to 83°. Equilibrium water content of the polymers was directly related to the mole fraction of HEMA content. The uptake of water in the earlier stages was proportional to $t_{1/2}$, consistent with a diffusion process; the slope of this plot enabled diffusion coefficients to be measured. The maximum equilibrium water content was 16%. Water uptake was reduced in phosphate-buffered saline, but addition of bovine serum albumin did not affect water uptake. Desorption was also linear on a $t_{1/2}$ plot in the early stages. There was a direct relationship between water uptake and loss. The polymer system was capable of releasing albumin; the amount of albumin released was inversely related to the HEMA content of the system. The relationship of the properties of the polymer system to biological interactions and potential applications are discussed. © 1997 Elsevier Science Ltd.

(Keywords: poly(ethyl methacrylate); hydroxyethyl methacrylate; n-butyl methacrylate; equilibrium water content; protein release)

INTRODUCTION

Poly(methyl methacrylate) (PMMA), a homopolymer comprising PMMA polymer and methacrylate monomer, is widely used as a bone cement, and yet it exhibits a number of disadvantageous physicochemical properties, such as shrinkage, a high exothermic polymerization reaction, release of a cytotoxic monomer and a porous particulate surface[1]. Whilst considering alternatives to this it would be prudent to evaluate polymer properties which favour polymer biocompatibility for this and other applications.

The success of polymers used as biomaterials depends upon their bulk and interfacial properties with biological fluids. It is widely considered that initial cell attachment and proliferation is a useful indication of the biocompatibility of a material. A number of factors including surface topography, roughness, chemistry and energy have been shown to influence cell attachment to material surfaces. Hydrophilicity and hydrophobicity, functions of surface energy, have been cited as influencing water uptake and cellular interactions at polymer surfaces[2–4], but these terms are broad and insufficiently definitive[5]. Previous studies have shown it is not simply the wettability of surfaces that influences cell adhesion, associated factors are also important such as the number of hydroxyl groups at the surface, equilibrium water content (EWC) and the organization of water within the polymer[5,6]. Although hydrophilicity and water uptake are related properties, hydrophilic polymers do not necessarily show high water uptake. In addition, the degree and nature of water uptake varies, for example poly(hydroxyethyl methacrylate) swells uniformly whereas poly(tetrahydrofurfuryl methacrylate) takes up water more slowly and water appears in clusters[7]. In theory, materials with high diffusion coefficients should release water-soluble additives more rapidly, because the transfer of water into the material allows the additives to be solubilized and released. This is of interest, as there is a wide range of water-soluble proteins, including growth factors which can stimulate tissue repair around medical devices. Polymers can be loaded with growth factors prior to incorporation in the body or absorb growth factors and cytokines during natural tissue repair. Previously we have used calcium phosphate ceramics, methyl methacrylate polymers, bioartificial materials and gelatin microspheres to deliver growth hormone both *in vivo* and *in vitro*[8–11].

Poly(ethyl methacrylate) (PEMA) powder polymerized with n-butyl methacrylate (nBM) monomer was developed as an alternative bone cement[12–14], and has been used in clinical trials as the London Hospital

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Medical Centre Bone Cement (LHMCBC). The advantages of this system over PMMA are that it has a lower exotherm and shrinkage than PMMA, it is ductile with superior fatigue properties and it forms an interpenetrating network where discrete beads are less obvious. PEMA has also been used with tetrahydrofurfuryl methacrylate (THFMA), originally developed as a low-shrinkage material for dental applications[15,16], and has also been shown to possess good biological properties in dental mucosa[17], bone and cartilage[18]. Low shrinkage of this material has been associated with the high molar volume of THFMA[19]. It also exhibits high water uptake up to 34% and rising after 3 years[20]. Recently it has been found that the uptake is critically dependent on the osmolality of the external solution[20].

The incorporation of a hydrophilic component such as hydroxyethyl methacrylate (HEMA) into the PEM/nBM system may modify the polymer system to control water uptake and swelling associated with HEMA hydrogels. Such a controlled, variable system may also prove amenable as a model to evaluate the relative importance of properties such as hydrophilicity and EWC on cell adhesion and spreading. In an attempt to address these objectives, we have produced a series of polymers based on PEMA polymerized with a monomer mixture of HEMA and nBM in various ratios. The basic bulk and surface properties of wettability, surface morphology, water absorption/desorption and protein release have been investigated with incremental changes in the monomer ratios.

MATERIALS AND METHODS

Polymer preparation

Polymer discs were prepared using a 1 : 1 ratio of PEMA powder (Bonar Polymers Ltd, Newton Aycliffe, C. Durham, UK) to HEMA (Aldrich, Dorset, UK) and nBM (Bonar Polymers Ltd, Newton Aycliffe, C. Durham, UK) monomer containing 2.5% v/v *N*, *N*-dimethylparatoluidine (Aldrich, Dorset, UK). The 1 : 1 ratio was used as higher ratios of PEMA-to-monomer mixtures polymerized too quickly to be cast in the desired form. The components were thoroughly mixed, then cast into 15 mm diameter polyethylene moulds and left overnight to polymerize. Discs were cast in an incremental series of ratios of HEMA:nBM from 10/90 to 90/10.

Contact angle measurement

Advancing water contact angles were measured using a Rame Hart 100 contact angle goniometer. Polymer discs were cast, in the ratios described above, against acetate sheets in Teflon moulds to produce flat surfaces. Measurements were taken at ambient temperature using a 2 μl drop of 18Ω de-ionized water. Reported values are the mean of four measurements taken at different points on the surface of each polymer formulation $\pm$ SD.

Water absorption and desorption

Polymer discs were autoclaved at 120°C for 1 h to remove any residual monomer. Each specimen was weighed, then placed into either sterile distilled water or phosphate-buffered saline (PBS) and maintained at 37°C. At regular intervals, the discs were removed, blotted dry on filter paper and weighed until the specimens reached equilibrium. The ratio of M_t (mass water uptake at time t) to M_{inf} (mass water uptake at equilibrium) was calculated and plotted against the square root of time. The diffusion coefficient for each polymer ratio was calculated by a method described previously[7].

To measure water desorption, the discs were removed from their solutions, blotted dry on filter paper, weighed and then transferred to dry 12 well plates and maintained at 37°C. The discs were weighed initially and then at regular intervals until equilibrium was reached.

Protein incorporation and release

Polymer discs containing 5% bovine serum albumin (BSA, Sigma, Dorset, UK) were produced by adding 0.1 g of BSA to 2 g of PEM with 2 ml of monomer and casting as described above. Each disc was placed into a 'universal' (Sterilin, UK) containing 5 ml of distilled water with 0.03% sodium azide (Sigma, Dorset, UK) and maintained at 37°C. BSA release was monitored *in vitro* by the following procedure. At regular time points the elution fluid was removed and replaced with fresh distilled water containing sodium azide. The eluant was assayed for protein using the Bio-Rad protein method (Bio-Rad Laboratories, Hertfordshire, UK).

Scanning electron microscopy

Scanning electron microscopy was used to examine the surfaces of the polymer discs. The discs were washed in double-distilled water, air dried in a dessicator under vacuum for 48 h, gold coated, then examined using a Jeol 6200 scanning electron microscope operating at 10 kV.

RESULTS

All the polymer combinations, except the one containing HEMA/nBM in the ratio 10 : 90, polymerized at room temperature to produce stable polymer discs. The polymerization process involved an exothermic reaction with peak temperatures ranging from 44 to 60°C. The temperature of polymerization and the opacity of the polymer discs were directly related to the HEMA content of the polymer system. This is consistent with the higher reactivity of HEMA over nBM. Scanning electron microscopical examination of the surfaces revealed a smooth, undulating surface with occasional convex protrusions (*Figure 1*), probably PEMA beads similar to those observed at the surface of PEM/nBM by Revell *et al.*[13]. The apparent topography was consistent for all polymer combinations. Contact angles measured for the polymer series fitted into a narrow range, ranging from 76 to 83° with increasing nBM content (*Table 1*).

Water uptake

The water uptake was linear on a $t^{1/2}$ plot in the early stages (*Figure 2*). With the exception of the 10/90 (HEMA/nBM) polymer sample, there was an incremental rise in the amount of water uptake with increasing HEMA content (*Figure 2a*). Water uptake in PBS followed a similar pattern with time and HEMA content; however, the amount of water taken up for a specific polymer combination was consistently less than for water (*Figure 2b*). This divergence was reduced with increasing HEMA content. The addition of BSA to either distilled water or PBS did not significantly affect water uptake (*Figure 3*). All the $t^{1/2}$ graphs were linear for a substantial part of the uptake process, indicating

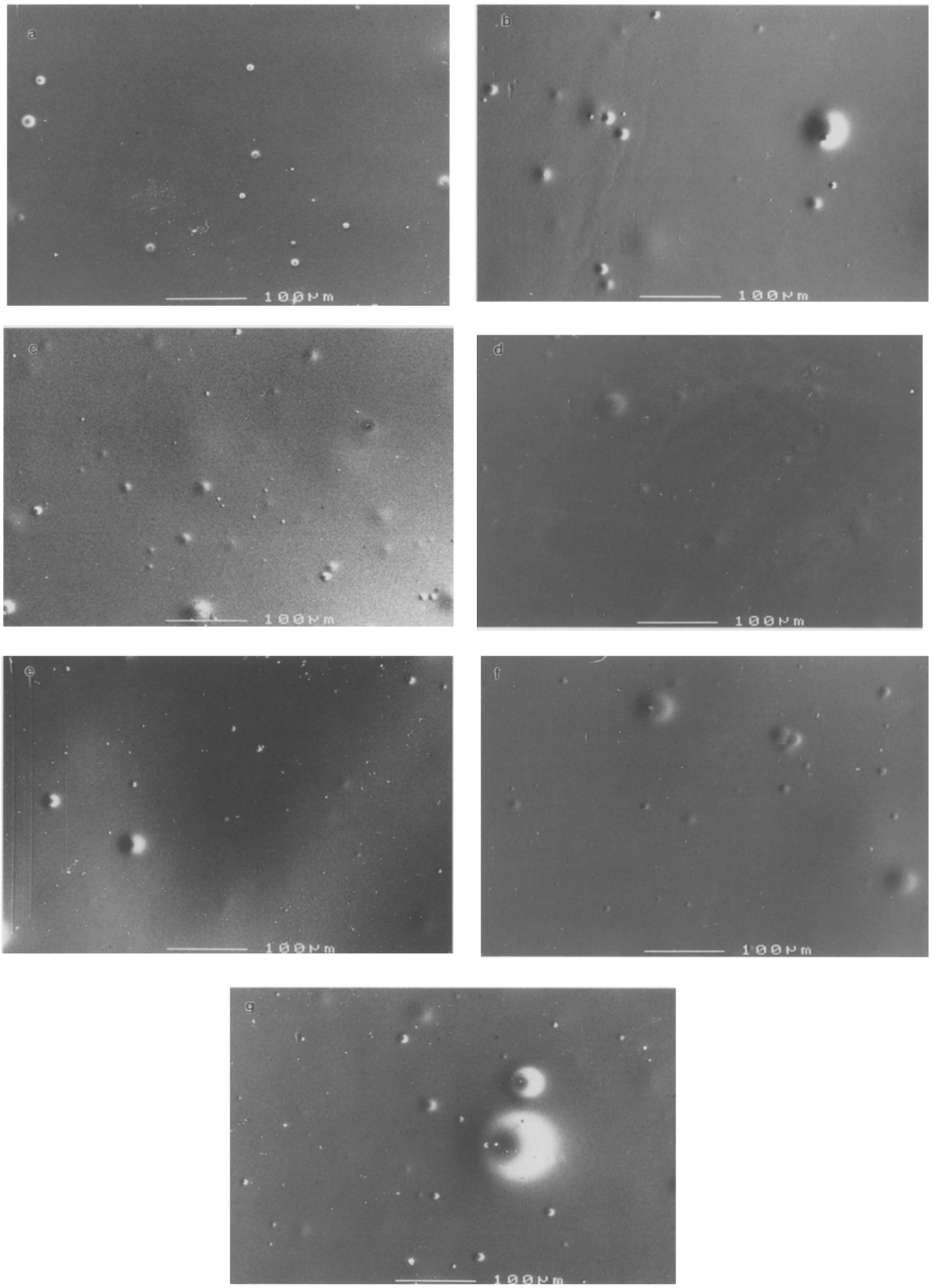

Figure 1 Scanning electron micrographs of the surfaces of PEM gelled with HEMA/nBM in the ratios (a) 10/90, (b) 30/70, (c) 50/50, (d) 60/40, (e) 70/30, (f) 80/20 and (g) 90/10. All the combinations demonstrated smooth undulating surfaces with small convex protrusions

Table 1 Advancing contact angles: PEM/HEMA–nBM

Polymer formulation (HEMA/nBM)	Mean (°)	SD (°)
10/90	83	±0.63
30/70	82	±1.32
50/50	81	±0.48
60/40	78	±0.81
70/30	78	±1.71
80/20	77	±0.87
90/10	76	±0.75

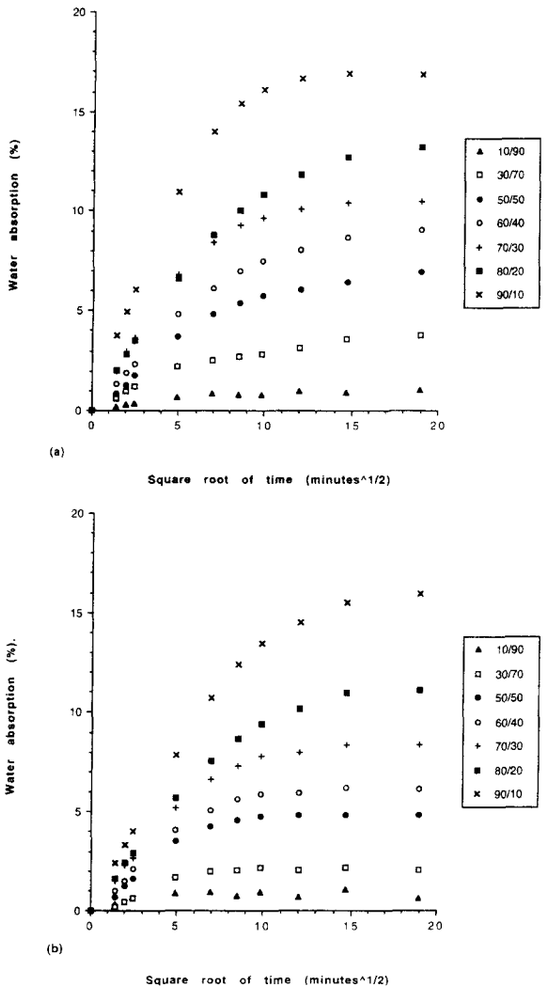

Figure 2 Plot of percentage water uptake *versus* $t^{1/2}$ for PEM gelled with HEMA/nBM (10–90% HEMA) for (a) distilled water and (b) PBS

simple diffusion. From the initial linear portions of the $t^{1/2}$ graphs, diffusion coefficients were calculated as described previously[7]; these values are given in *Table 2*. The EWC of the polymers, calculated from these data, increased with the mole fraction of HEMA, which varied between 0.5 and 16% (*Figure 4*).

Water desorption

The pattern of water desorption was similar for all ratios of polymer samples, except for the 10/90 combination showing again linear $t^{1/2}$ plots (*Figure 5*). A direct relationship between water uptake and water desorption was apparent from both distilled water and

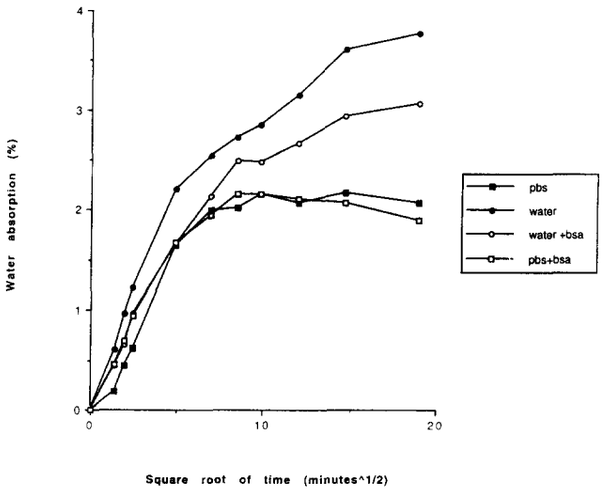

Figure 3 Plot of percentage water uptake *versus* $t^{1/2}$ for PEM gelled with HEMA/nBM (30/70) for water and PBS with and without the addition of BSA

Table 2 Calculated diffusion coefficients for polymers of HEMA/nBM mixtures gelled with PEM

	Diffusion coefficient (D, $\times 10^{-8}$ cm^2 s^{-1})					
	Sorption		Desorption		D^d/D^s	
HEMA content (%)	H_2O	PBS	H_2O	PBS	H_2O	PBS
10	6.90	30.60	–	–	–	–
30	2.05	24.43	15.57	27.15	7.6	1.1
50	5.55	12.07	15.47	14.4	2.8	1.2
60	3.53	9.6	9.33	11.8	2.6	1.2
70	8.07	13.33	6.3	8.14	0.8	0.6
80	5.02	7.13	8.48	6.63	1.7	0.9
90	7.35	3.24	8.20	8.87	1.1	2.7

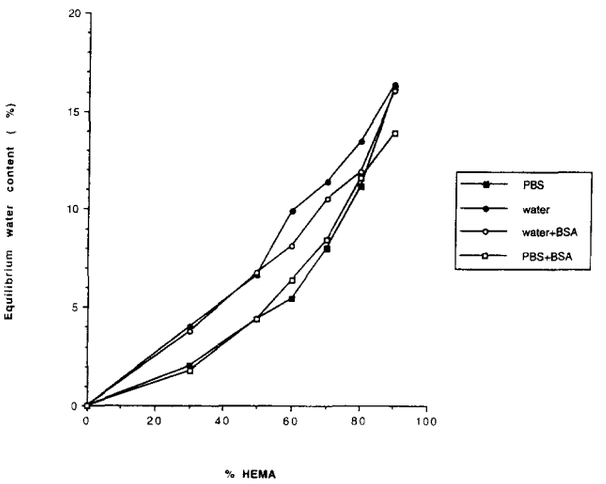

Figure 4 Equilibrium water content of PEM gelled with HEMA/nBM (30–90% HEMA)

PBS. In distilled water D^d/D^s for samples with high levels of HEMA was close to 1, in agreement with previous work. Higher values of D^d/D^s were calculated for samples with higher nBM contents. With PBS, D^d/D^s remains close to 1 across the range of polymer compositions.

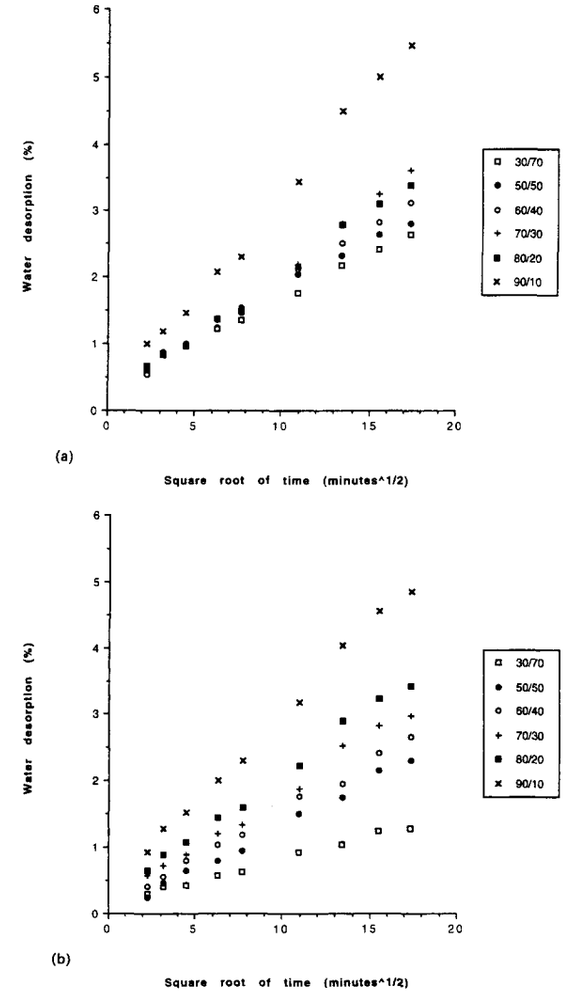

Figure 5 Plot of percentage water desorption *versus* $t^{1/2}$ for PEM gelled with HEMA/nBM (10–90% HEMA) gelled with PEM for (a) distilled water and (b) PBS

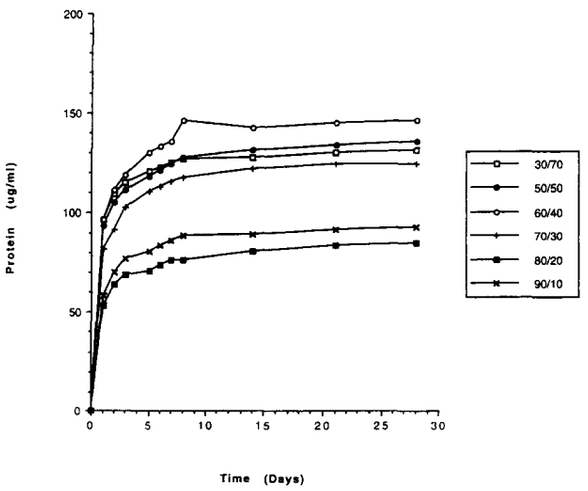

Figure 6 Accumulative release of protein from the various polymer systems of PEM gelled with HEMA/nBM mixtures. Note that the pattern of release is similar for all systems

Protein release

All the polymer systems released albumin into the PBS at 37°C (*Figure 6*). The pattern of albumin release was similar for all polymer combinations, with the exception of the 10/90 (HEMA/nBM) combination, with a rapid release of protein in the first day, followed by a slower continuous release for the duration of the experiment. There was an inverse relationship between the amount of albumin released and the HEMA content of the copolymer system.

DISCUSSION

In this study we have demonstrated it is possible to produce a series of stable copolymers comprising PEM/HEMA/nBM. The failure to produce a fully polymerized system at 10/90 HEMA/nBM may be due to the system being starved of benzoyl peroxide, present in the PEMA powder, with the lowered ratio of PEMA in the system, as nBM is less reactive than HEMA. Scanning electron microscopical examination of the surfaces of this copolymer series showed similar, relatively featureless, topographies throughout the series. This would tend to discount topography as an influencing factor on variations in the properties of the copolymers in the series as seen in related systems[21].

The measured contact angles for these materials are quite high, indicating the polymers are relatively hydrophobic. Initial surprise was expressed at the narrow range within which the contact angles of these copolymers fell. However, on further consideration, although the ratio of HEMA to nBM changes greatly through the polymer series, the contribution of HEMA (v/v), which is moderately hydrophilic[5], remains relatively low compared to the combined, relatively hydrophobic, components nBM and PEMA.

Lydon and colleagues[5] have shown a relationship between surface properties and bulk properties of copolymers with respect to cell adhesion. In particular, EWC was shown to be related to interfacial behaviour. In this study both the rate and amount of water uptake at equilibrium were directly related to the percentage of HEMA in the system, with equilibrium occurring after approximately 3 weeks. The EWC of the polymers increased with the mole fraction of HEMA. The maximum EWC remained less than 16% therefore, the incorporation of HEMA into the copolymer system increased the water uptake of the polymers without producing true hydrogels, which by definition possess EWCs over 20%[5]. This implies that they do not produce water-swollen networks like hydrogels. It is thought that this restriction on swelling is conferred by cross-linking within the system. Uncontrolled swelling of hydrogels, whilst an advantage for biomaterial applications such as contact lenses, makes them unsuitable for the applications considered here. The slight swelling and increased water uptake of this copolymer system, however, is considered advantageous to provide a good polymer–tissue interface at the site of implantation and reducing the risk of loosening. It is also thought that uptake of biological fluids at sites of tissue damage may be beneficial to healing due to the enhanced availability of wound healing factors. The EWCs calculated for this system fit favourably into the range of copolymer EWCs seen to support cell attachment[5].

The reduced water uptake observed in PBS indicates an osmotic effect, which has significance when considering physiological systems. This effect decreases with increasing HEMA content, which correlates well with the observed absence of such an effect with hydrogels (EWC > 20%).

Diffusion coefficients were generally greater for desorption, a common property of glassy polymers. However, water was absorbed and subsequently desorbed to zero in all experimental regimes, indicating an absence of protein blocking or barrier formation[7].

Table 2 shows that, for water, the ratio of D^d/D^s is large for higher levels of nBM, but decreases with increasing HEMA content. As already pointed out, $D^d > D^s$ is symptomatic of clustering of water within the polymer. However, this ratio is approximately unity when PBS is the medium, strongly suggesting that the clustering is osmotically driven; furthermore, *Table 2* shows that in PBS at high nBM levels the diffusion coefficients are high, again reflecting the suppression of clustering.

This copolymer system can be used to deliver protein. Incorporation of BSA into the system demonstrated that, with the exception of the 10/90 (HEMA/nBM) combination, protein was released in a dose-dependent manner. Surprisingly, protein release was reduced with increased HEMA in the copolymer system. This was thought to be due to a binding interaction between HEMA and the protein. This may possibly be facilitated through hydroxyl groups of the copolymer system interacting with the amino terminal of proteins. The above finding differs from earlier work with PEMA/HEMA polymer systems where the release of the water-soluble albumin from the polymer system was high due to the relatively high diffusion coefficients and water uptake by systems gelled with HEMA[21].

This study has demonstrated that the incorporation of HEMA into a PEMA/nBM copolymer system produces a controlled system sensitive to ionic but not protein changes. The resulting copolymer system possesses bulk and surface properties which have previously been shown to favour mammalian cell adhesion and spreading. A potential for tissue fluid uptake was demonstrated, whilst maintaining a more stable structure than a hydrogel, suggesting potential applications at sites of tissue repair requiring stability. This is further supported by the lack of shrinkage and low exothermic polymerization temperature relative to polymer systems currently in use such as PMMA. This system also provides a favourable model polymer system for further work using biological fluids and cells to investigate properties influencing cell adhesion and their mechanism of action.

ACKNOWLEDGEMENT

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