Heterocyclic methacrylates for clinical applications–further studies of water sorption

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The room temperature polymerizing system comprising poly(ethyl methacrylate)-tetra hydrofurfuryl methacrylate (PEM/THFMA) has potential in orthopaedic and dental applications, and earlier work has shown it to have unusual water absorption characteristics. This aspect has been studied in further detail, by studying the water absorption behaviour from some biological solutions, and the effect of the addition of an antibiotic (gentamicin). For comparison purposes, a parallel system whereby tetrahydrofuryl methacrylate was replaced by hydroxyethyl methacrylate (PEM/HEMA), was studied. In the case of PEM/THFMA, water uptake was substantially reduced when absorption was carried out from solutions (from about 30% in water to about 1.5% in solutions of higher concentrations), and the corresponding diffusion coefficient increased (by a factor of several hundred). The addition of gentamicin increased uptake, but the extent of increase also decreased in solutions. It was concluded that uptake was related to the osmolarity of the external solution, and also on the presence of osmotic sites within the polymer; hence the uptake process appears to be governed by chemical potential considerations. At the higher uptakes, there was evidence of water clusters. In marked contrast, the uptake by the PEM/HEMA system was independent of the osmolarity of the external solutions, presumably due to the hydrophilic nature of HEMA.

1. Introduction

Various studies have described the properties of polymeric systems based on heterocyclic methacrylates for clinical applications [1–6]. Pearson *et al.* showed the room temperature polymerizing system poly(ethyl methacrylate) (PEM)-tetrahydrofurfuryl methacrylate (THFMA) to have biocompatibility with respect to dental pulp comparable to a zinc oxide/eugenol cavity lining material [7]. Downes and co-workers and Reissis and co-workers have shown *in vivo* the same system to have potential for cartilage repair [8–11] and *in vitro* to be superior to poly (methyl methacrylate) systems for controlled drug release [12, 13]. Mcfarland *et al.* have discussed the system with respect to cell attachment [14] and Sawtell *et al.* for chondrocyte biocompatibility *in vitro* [15, 16].

The PEM/THFMA system proved to have high water absorption ($\sim 30\%$ at 37 °C), with strong evidence that the water was concentrated in clusters (cf. a hydrogel), and with an estimated low diffusion coefficient of $\sim 10^{-14}\ m^2\ s^{-1}$. It therefore seemed, *a priori*, that the above characteristics might be related to the water absorption behaviour.

However, all of the previous work on water absorption has been from distilled water at 37 °C. It therefore seemed logical to extend this work to biological solutions more relevant to clinical situations. This contribution describes measurements from artifical saliva (AS), phosphate-buffered saline (PBS), fetal calf serum (FCS) and culture medium (CM); the latter was included because it is used in the *in vitro* investigations of chondrocytes (cartilage cells). Attempts were made to include synovial fluid, but it decomposed within 7 days, far less than the time scale of the experiments. Similar measurements were carried out on a system whereby the THFMA was replaced by hydroxyethyl methacrylate (HEMA).

Because it has been found in a separate study that the presence of additives in the PEM/THFMA system can substantially influence uptake behaviour [13], the effects of three additives on this system have also been studied.

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2. Materials and methods

Two slightly different forms of PEM were used:

(i) A standard commercial PEM powder (Reference TS 1364 ex Bonar Polymers Ltd, Newton Aycliffe, Co. Durham UK.).
(ii) A modified version of TS1364 containing barium sulphate for radio-opacity (TS 1249/4).

These will be referred to as systems 1 and 2, respectively.

System 1 was prepared as follows: 0.9 g per 100 g of polymer powder of the following were added, respectively: chlorhexidine diacetate, genetamicin sulphate, and miconazole nitrate and blended for 3 h by ball milling. Specimens were prepared and tested for water uptake, as described previously, using a powder/monomer ratio of 5 g/3 ml. The THFMA monomer contained 2.5% of *N*,*N*-dimethyl-*p*-toluidine [5, 6].

System 2 was prepared as follows: using the same powder/monomer ratio as above, discs of approximately 15 mm diameter × 2 mm thickness were prepared in polythene moulds.

The discs were placed in bottles containing 100 ml of the appropriate solution in a thermostatically controlled water bath at 37 °C. Three discs were used for each series. Sorption and subsequent desorption methods were those described by Patel and Braden [6].

The solutions used were:

(i) distilled water (control);
(ii) PBS (pH 7.4);
(iii) FCS (Gibco, Paisley, UK);
(iv) CM (Dulbecco's modified Eagle's medium, 20% FCS, 2% HEPES, 1% glutamine, 100 units/ml penicillin/streptomycin (Gibco, Paisley UK), 0.85 mM ascorbic acid (Sigma Chemical Co., Poole, UK); sodium azide (0.03% w/v) was added to the FCS and CM in an attempt to stop decomposition.
(v) Artificial saliva (AS) dispensed in 50 ml containers, from Nycomed Saliva Orthana, A/S Orthana Kemisk Fabrik, Kastrup, Denmark (UK distributors: Nycome Ltd Birmingham).

2.1. Analysis of data

Uptake and desorption data were plotted as a function of $t^{1/2}$; if the plots were linear, then the following equation was used [17]

$$M_t/M_\infty = 2(Dt/\Pi l^2)^{1/2} \quad (1)$$

where M_t is the uptake at various time points, M_∞ is the uptake at equilibrium and $2l$ is the thickness of the specimen. The slopes of the linear plot(s) is obviously given by $s = 2(D/\Pi l^2)^{1/2}$, whence the diffusion coefficient (D) may be calculated. Equation 1 applies only to the early stages of diffusion; for the later stages, Equation 2 should apply [18]

$$M_t/M_\infty = 1 - 8\Pi^2 \sum_{n=0}^{n=\infty} 1/(2n+1)^2 \exp\left[-(2n+1)^2\Pi^2 Dt/4l^2\right] \quad (2)$$

The value of D obtained from the linear $t^{1/2}$ plot can be substituted into Equation 2, and the theoretical curve so obtained compared with experimental data. In those cases where the plots exhibited an intercept on the uptake axis (M_i), M_t was replaced by ($M_t - M_i$), and M_∞ by ($M_\infty - M_i$).

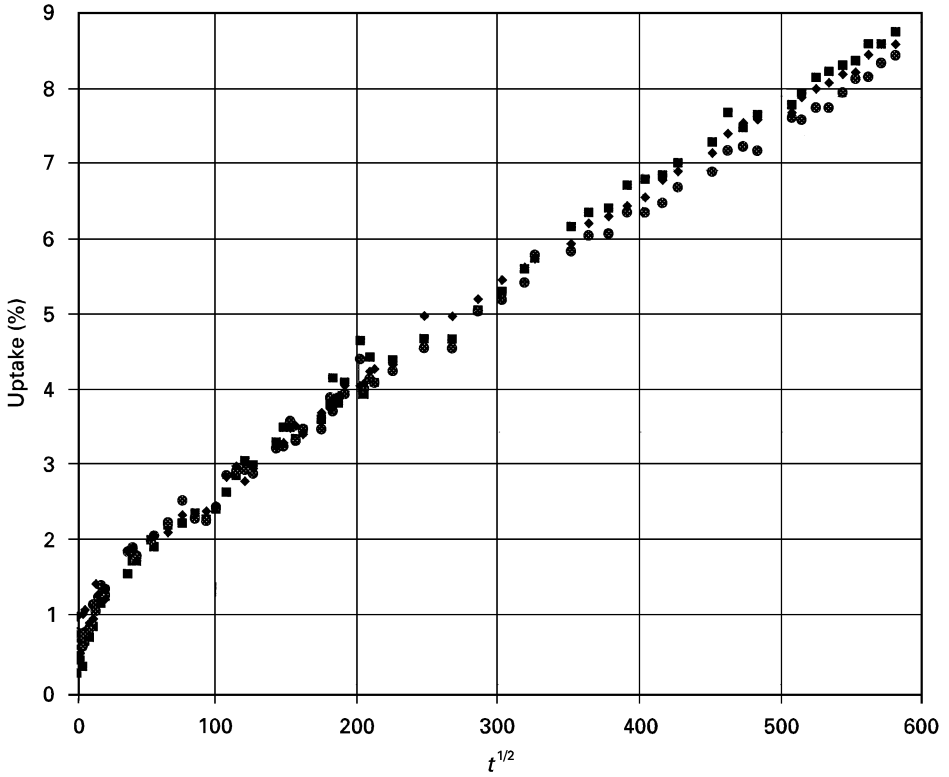

Figure 1 Plot of water uptake (%) by the PEMA/THFMA system from water as a function of $t^{1/2}$ ($min^{1/2}$) triplicate samples.

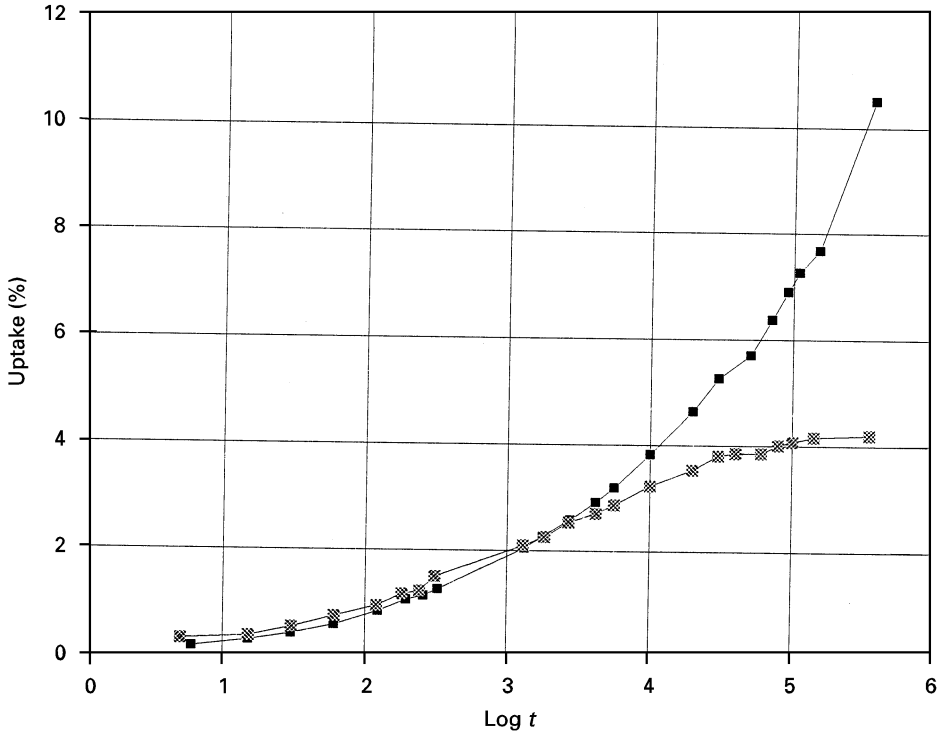

Figure 2 Plot of water uptake (%) of the PEM/THFMA system containing gentamicin as a function of log time (min) from (a) water (■); (b) AS (▩).

3. Results

The uptake of the PEM/THFMA system in water is shown in Fig. 1. It will be noted that there is only a very small linear $t^{1/2}$ region, the process is very protracted, and the uptake is high. Fig. 2 compares the water uptake of the PEM/THFMA polymer system, containing gentamicin in water and AS. Fig. 3, shows the early stages of the uptake data on a $t^{1/2}$ plot for the PEM/THFMA system itself and containing gentamicin, immersed in AS. Uptake and desorption data for the three additives in the PEM/THFMA system, in AS and PBS, and PEMA/HEMA in all solutions gave good linear $t^{1/2}$ plots, from which diffusion coefficients could be obtained; Fig. 4 gives the data for the

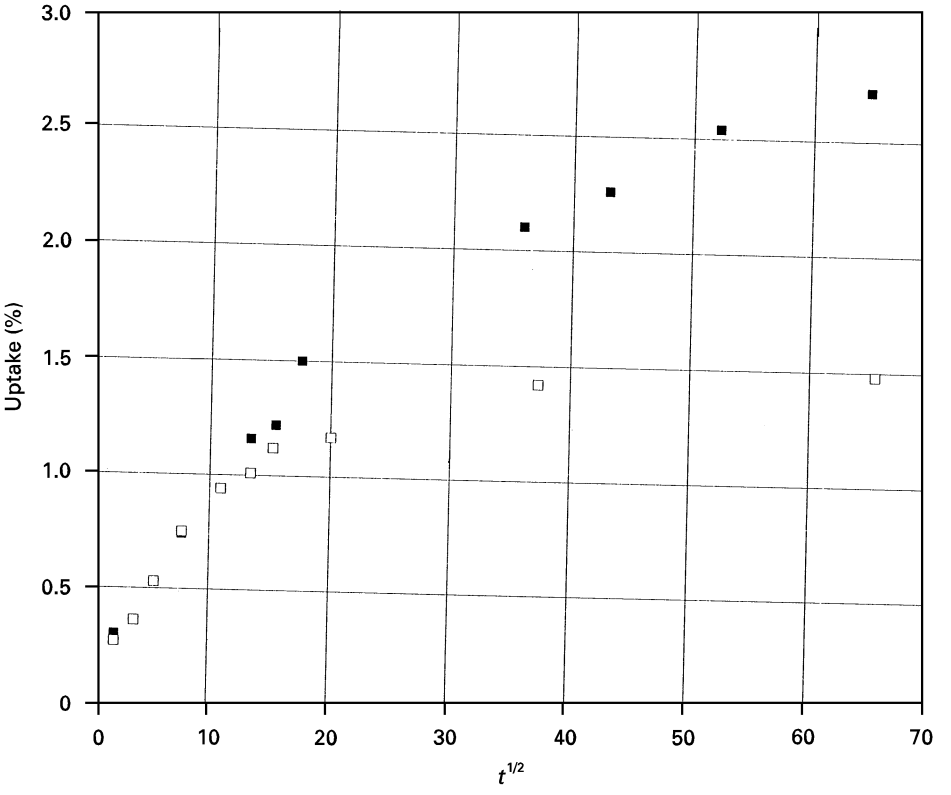

Figure 3 Water uptake (%) of the PEM/THFMA system from AS as a function of log time (min): (a) with no additive (■); (b) when containing gentamicin (□).

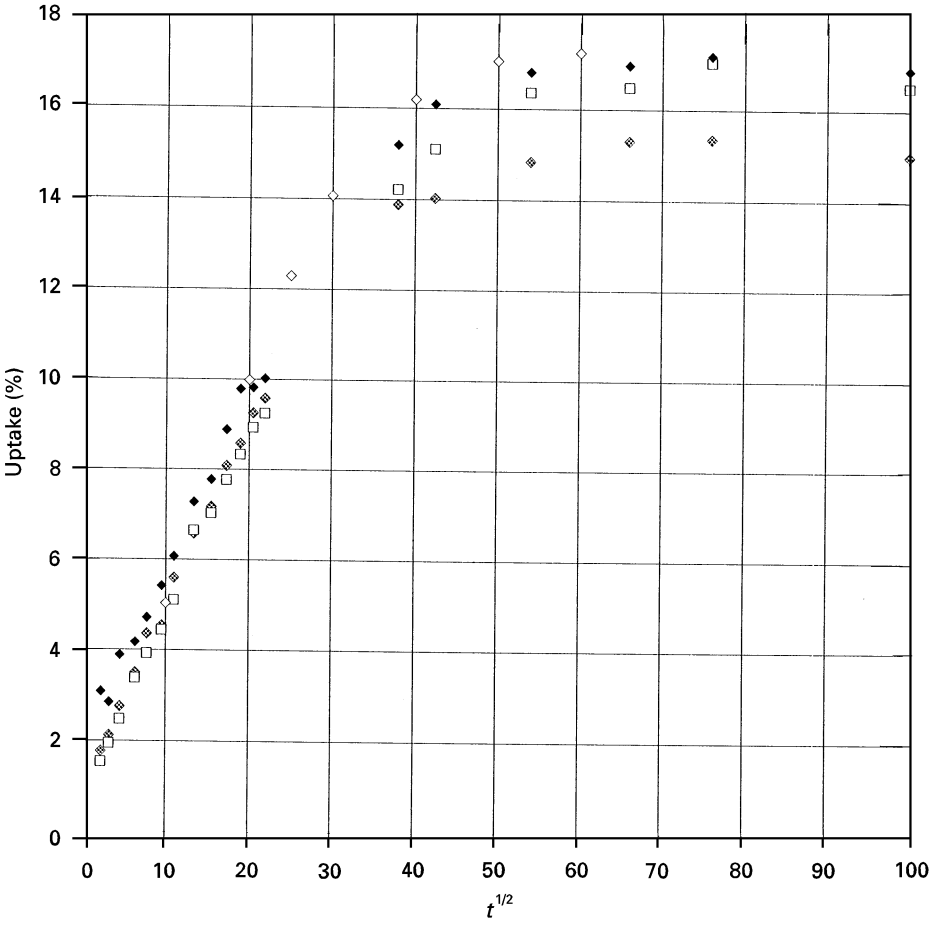

Figure 4 Water uptake (%) of the PEM/HEMA system triplicate samples as a function of $t^{1/2}$ ($min^{1/2}$). (◇), theoretical predictions of Equation 2.

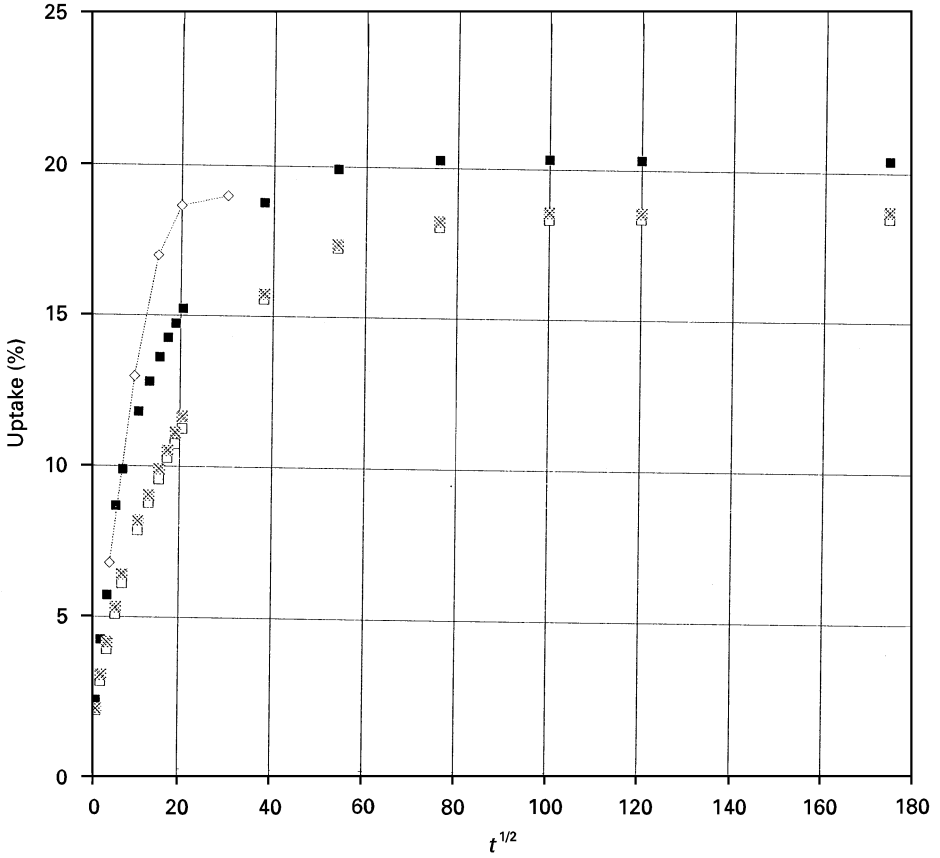

Figure 5 Water desorption data corresponding to the system in Fig. 4.

TABLE I Absorption behaviour of the PEM/HEMA system

Immersion liquid	Equilibrium (%)		Diffusion coefficient (10^{-11} m^2 s^{-1})	
	Gain	Loss	Absorption	Desorption
Water	19.32 (3.56)	18.3 (3.33)	1.48 (1.02)	4.96 (2.58)
PBS	22.85 (5.78)	20.9 (1.9)	1.53 (1.46)	4.57 (1.11)
CM	23.84 (4.15)	20.56 (1.62)	1.37 (1.37)	3.94 (0.72)
FCS	17.64 (4.55)	17.96 (2.27)	1.35 (0.78)	3.03 (1.37)

Figures in parentheses are the range.

TABLE II Absorption behaviour of the PEM/THFMA system

Immersion liquid	Equilibrium (%)		Diffusion coefficient (10^{-11} m^2 s^{-1})	
	Gain	Loss	Absorption	Desorption
[6]				
Water	~30	—	0.0015	0.08
PBS	2.82 (0.44)	3.06 (0.25)	0.59 (0.18)	0.97 (0.57)
CM	3.48 (1.08)	3.75 (1.27)	1.5 (0.6)	1.44 (0.71)
FCS	2.81 (1.06)	3.27 (0.62)	0.63 (0.43)	1.22 (0.79)

Figures in parentheses are the range.

TABLE III Ratio of desorption/sorption diffusion coefficients

Immersion liquid	PEM/HEMA	PEM/THFMA
Water	3.38	53.3
PBS	2.29	1.64
CM	2.88	0.96
FCS	2.24	1.93

TABLE IV Absorption behaviour from AS of the PEM/THFMA system containing various additives

Additive	Equilibrium uptake (%)	Diffusion coefficient (10^{-11} m^2 s^{-1})
Miconazole	2.3	0.49
Gentamicin	4.2	0.19
Control	1.55	0.82
Chlorhexidine	2.84	0.35

absorption of water by PEMA/HEMA, and Fig. 5 the corresponding desorption process. Equilibrium uptake and diffusion coefficient data are summarized in Tables I and II, the results being the average of three samples, each of which was subjected to three sorption/desorption cycles; the exception was PEM/THFMA in FCS, where the long times involved resulted in decomposition of the FCS. Table III summarizes the ratios of diffusion coefficients in desorption to those in sorption, and Table IV the effect of additives on uptake in AS.

The uptake of water from FCS and CM by system 2 was more complex, exhibiting an intercept on the ordinate in both sorption and desorption, and sometimes high experimental scatter, as shown in Figs 6 and 7. A diffusion coefficient value could still be calculated from the linear portion of the curve, although with less precision.

4. Discussion

4.1. PEM/HEMA system

Table I shows equilibrium uptake to be sensibly independent of the medium of immersion. There is quite a range of diffusion coefficients, both in sorption and desorption, with no clear correlation evident. Application of Equation 2 shows the experimental data lags slightly behind the theoretical plot, both in sorption and desorption. If D decreases with concentration (c), better agreement would have been expected in desorption. Poly(HEMA) itself is hydrophilic, which would be expected to have D increasing with c. However, the present system is partially heterogeneous.

4.2. PEM/THFMA system

Systems 1 and 2, where direct comparisons could be made, gave congruent data, and will not be further separately identified. Fig. 1 shows the uptake of the system in water to be very high, with only a very small linear $t^{1/2}$ region, as observed previously by Patel and Braden [6]. Fig. 2 shows that gentamicin enhances water uptake, but uptake when immersed in AS is dramatically reduced, and equilibrium attained very much quicker. Also the $t^{1/2}$ plots of both the PEM/THFMA itself, and when containing gentamicin in AS, are linear (Fig. 3) This transpires to be true for absorption from all the other solutions. Table II shows equilibrium uptake to be reduced from ~30%, to 2–5%, and the diffusion coefficients to increase by a factor of ~1000. Table III shows generally that $D_a > D_s$ for all systems, generally symptomatic of D decreasing with c. Figure 8 shows the data fits a common D–c plot to a first approximation; some data for immersion in NaCl are added for comparison. This graph is readily explained qualitatively. At higher uptakes, water clusters develop, the filling of which either delays the ingress process or possibly it is

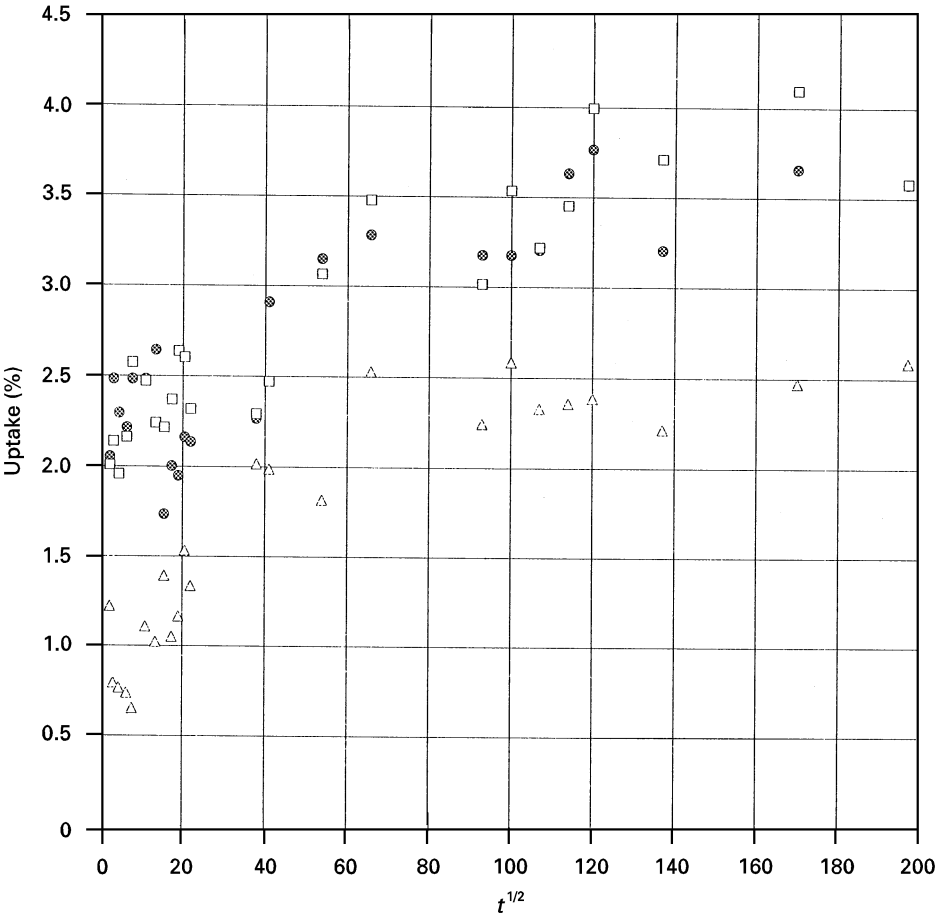

Figure 6 Water uptake of the PEMA/THFMA system from CM (triplicate samples).

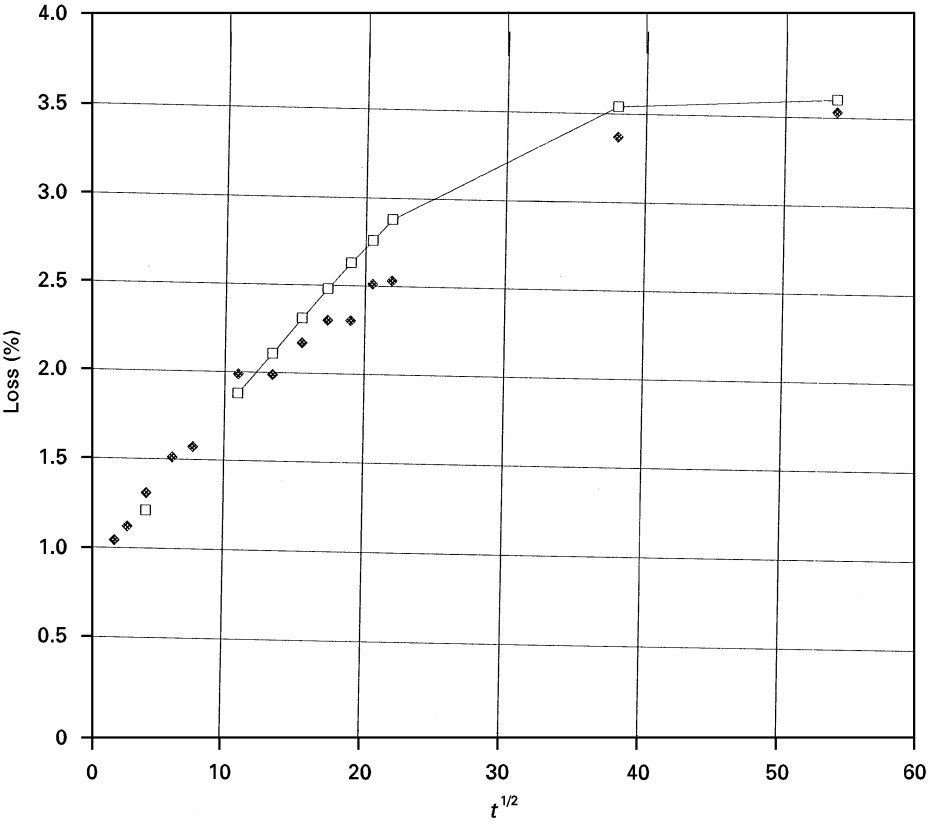

Figure 7 Water desorption data corresponding to the system in Fig. 6; open squares, theoretical data from Equation 2.

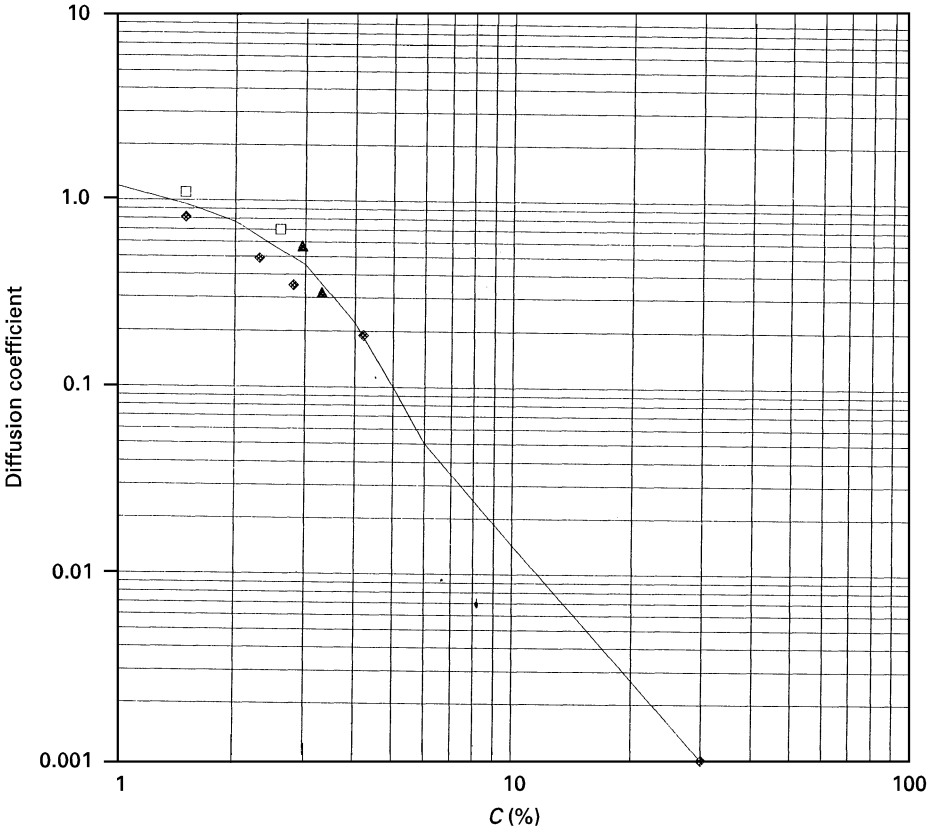

Figure 8 Variation of the diffusion coefficient (D) (10^{-11} m^2 s^{-1}) with equilibrium uptake C_0 (%).

a secondary process in addition to diffusion through the polymer network. These findings are of obvious clinical importance, because behaviour is critically dependent on the external medium, including the uptake kinetics. Hence, drug release will be a complex process, depending as it will on the effect of the drug on water uptake of the system, and its interrelationship with the external solution.

From a fundamental viewpoint, the critical role of the external solution, points to an osmotic process within the PEM/THFMA system itself. The source of this osmotic process is not clear at this stage, but the implication is that the theoretical treatment of the process should be based on the modified form of Fick's law based on chemical potential (μ)

$$F = -Dc/RT\ \partial\mu/\partial x \tag{3}$$

as has been successfully done for elastomeric materials.

The high uptake in water itself, and the resulting sample opacity may indicate some form of failure, as observed by Brook and van Noort [19] in PMMA bone cements, and Bucknall *et al.* in epoxy resins [20], although the PEM/THFMA system is ductile rather than brittle.

Application of the Muniandy and Thomas theory [21] which uses a chemical potential approach shows that

$$(C_W - S)/C_i = \rho_W/\rho_i(\lambda^3 - 1) \tag{4}$$

where C_W is the total concentration of water in the polymer, and S is the solubility of water in the polymer; that is, $C_W - S$ is the concentration of water in the clusters. In Muniandy and Thomas's work, C_i was the concentration of water soluble inclusions or additives. λ is the extension ratio of the polymer consequent on cluster formation, and ρ_i, ρ_W are the densities of the inclusions and water, respectively. This theory successfully explained the over-riding role of water-soluble impurities in the water uptake of elastomers, due largely to the high compliance of elastomers.

If this theory is to be extended to the PEM/THFMA system itself, C_i must represent the concentration of the as yet unidentified sites of cluster formation, obviously a major restraint on the use of the theory. Equation 4 can be re-written as

$$(C_W - S)/\rho_i = V_i(\lambda^3 - 1) \tag{5}$$

where V_i is the volume concentration of clustering sites; this is of course as yet unknown. If S is taken as the limiting water uptake in concentrated solutions (i.e. -0.03), and C_W as 0.3, $(C_W - S)/\rho_i$ is 0.27. The yield strain of the polymer system [4] is $\sim$3% (the formation of voids suggests yielding) i.e. $\lambda = 1.03$. Substituting some hypothetical values for V_i gives

V_i	$(C_W - S)/\rho_i$
0.001	9.27×10^{-5}
0.01	9.27×10^{-4}
0.1	9.27×10^{-3}

Clearly the values predicted above are at least two orders of magnitude below the experimental values. This suggests that the high water uptake values are accommodated by local failure at the clustering sites,

either by substantial yielding or failure; further studies are needed on this process.

4.3. Desorption data

The presence of an intercept on the ordinates of some desorption plots may be the rapid water desorption from the layer deposited during sorption.

4.4. Effect of additives on the PEM/THFMA system

This has been studied in AS, and it can be seen that the additives increase water uptake to varying degrees. Again this is in general accord with the role of an underlying osmotic process, the key factor being the difference in osmolarity between the external solution and that within the clusters within the polymer.

5. Conclusions

The water uptake behaviour of the PEM/HEMA system is sensibly independent of the osmolarity of the external solution. In marked contrast, the water uptake of the PEM/THFMA system is critically dependent on the osmolarity of the external solution, ranging from 30% in distilled water, to $\sim$2.5% in some solutions, and on the presence of water-soluble additives. There is an even greater effect on the apparent diffusion coefficient, which increased by $\sim$1000-fold over the same range. These observations are strongly suggestive of an osmotically driven process. In the case of biological solutions, there is evidence of the deposition of a surface layer from the solution.

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