JOURNAL OF MATERIALS SCIENCE **39** (2004) 1861–1864

Spontaneous calcification of acrylic hydrogels in abiotic calcifying media and the relevance of ionic solute effects

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Polymers can undergo dystrophic calcification following their implantation in living tissues. The biomaterials-associated calcification involves the formation of calcium phosphate phases having compositions similar to that of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, but of poorer crystallinity. Calcification has a detrimental effect on the clinical success of the prosthetic devices currently in use, with the exception of orthopaedic implants where hydroxyapatite deposition is usually beneficial. Most previous calcification studies have been directed to the hydrophobic polymers, generally strong materials, either elastic or rigid. Many examples of clinical failure caused by calcification come from the field of artificial hearts and blood pumps [1–4].

Although mechanically weak, the hydrophilic polymers are also widely used in biomedical applications, especially the acrylic hydrogels such as those based on 2-hydroxyethyl methacrylate (HEMA), including the homopolymer (PHEMA) and copolymers. They are used as materials for contact lenses, of which spoliation with surface deposits is well documented [5, 6]. Calcium phosphate species are frequent components of such deposits, but quantitatively less significant than the associated organic matter. However, there is convincing experimental evidence, as recently reviewed [7], that PHEMA hydrogels (either as homogeneous gels or sponges) undergo calcification when placed inside living tissues. This process was explained by the existence of heterogeneous nucleation sites for the hydroxyapatite crystallization due to the presence of microscopic defects in the polymer, loose polymer particles and necrotic tissue debris, in combination with local disturbances in the metabolism of calcium [8, 9], a mechanism similar to that proposed for the calcification of hydrophobic polymers as cardiovascular implants [4, 10]. It is more difficult to explain the presence of calcium deposits *inside* the homogeneous hydrogels, as most of the potential nucleation centers precursors (e.g., tissue debris) are too large to penetrate the polymer network. This calcification pattern was noticed in a range of HEMA-based hydrogel ocular devices, such as contact lenses [11, 12] and intraocular lenses [13–15], but the proposed tentative mechanisms were rather speculative. An artificial cornea developed in our laboratories also showed circumstantial tendency for calcium deposition during clinical trials carried out in animals [16] and human patients [17], which prompted our interest in the mechanism. In a previous study [7], we have demonstrated the propensity of heterogeneous PHEMA hydrogels (sponges) to calcify by simple incubation in a metastable calcium phosphate solution, in the absence of any organic and biological components. It follows that the onset of calcification in hydrogels is independent of the physiological microenvironment and biological factors, which however may become significant later in the process. It also suggests that the mechanism of calcification in abiotic media is different from that in the presence of cells, tissues and plasma, and may be responsible for the deposit formation inside the homogeneous gels. We have hypothesized [7] a mechanism for calcification in abiotic aqueous solutions based on concentrating effects exerted by ionic solutes when the solution diffuses through the hydrogel network, leading to local supersaturation and spontaneous precipitation through homogeneous nucleation. Depletion of water as a solvent for ions may be caused by salting-out solute effects (deswelling) induced by any of the ions including calcium and phosphate themselves, when the equilibrium water content of the hydrogel becomes lower in solution than in pure water. Here, we evaluate the precipitation of calcium phosphate from a metastable solution in the presence of a *homogeneous* hydrogel (a transparent PHEMA gel), and investigate the solute effects of the participating ions.

High purity, sterile and nonpyrogenic water for injections, with osmolality zero (Viaflex®, Baxter), was used in all experiments. PHEMA disks were cut from sheets prepared by the polymerization of a solution of 70% HEMA in water, containing 0.35% crosslinking agent (ethylene dimethacrylate) and 0.2% initiator (equal amounts of 10% aqueous ammonium persulfate and N,N,N′,N′-tetramethylethylenediamine). The solution was poured between glass plates spaced at about 1.2 mm by a gasket, and kept for 24 h in an oven at 50 °C. Disks of 7 mm in diameter were cut and subjected to water extraction (Soxhlet) for 2 days, and stored in

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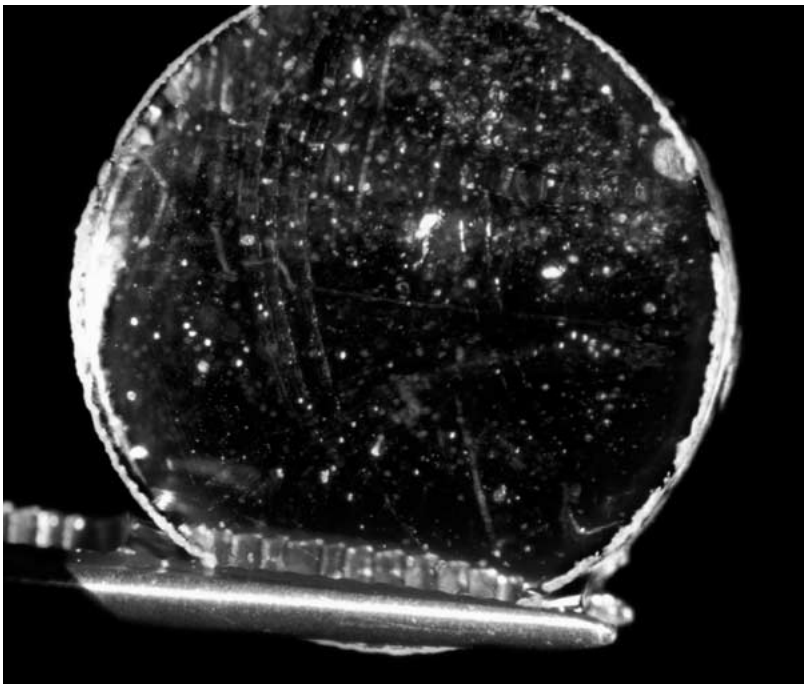

Figure 1 Distribution of calcific deposits in hydrated PHEMA following one-week storage in a metastable calcifying solution.

water. For the calcification experiments, solutions of $CaCl_2$ (2.57×10^{-3} mol L^{-1}) and Na_3PO_4 (1.54×10^{-3} mol L^{-1}) were separately prepared. After adjusting in each the pH to 8 with HCl, equal volumes of each solution were mixed to result in a metastable calcifying medium with a ratio $[Ca^{2+}]/[PO_4^{3-}] = 1.67$ (as in hydroxyapatite) and pH 7.3. Six hydrated PHEMA disks were each immersed in vials containing 3 mL of the calcifying medium and incubated in a shaker at 150 cycles/min for 1 week. All disks presented white deposits at the end of the experiment. Sediments could be also seen on the bottom of vials. The deposits that persisted after the disks were washed with a water jet were distributed randomly onto and inside the disk, being denser along the edges (Fig. 1). Clusters that penetrated below the surface of polymer can be seen in Fig. 2. Staining with alizarin red confirmed the presence of calcium in the deposits.

The solute effects of the ions upon hydration of PHEMA was measured by keeping dried polymer specimens in salt solutions of various concentrations for 2 weeks, and then plotting the degree of swelling S (the weight ratio of the hydrated and dried PHEMA specimen) against the solute concentration C_s. In assessing solute effects, we had to consider that the calcifying medium in this case is a complex system that can

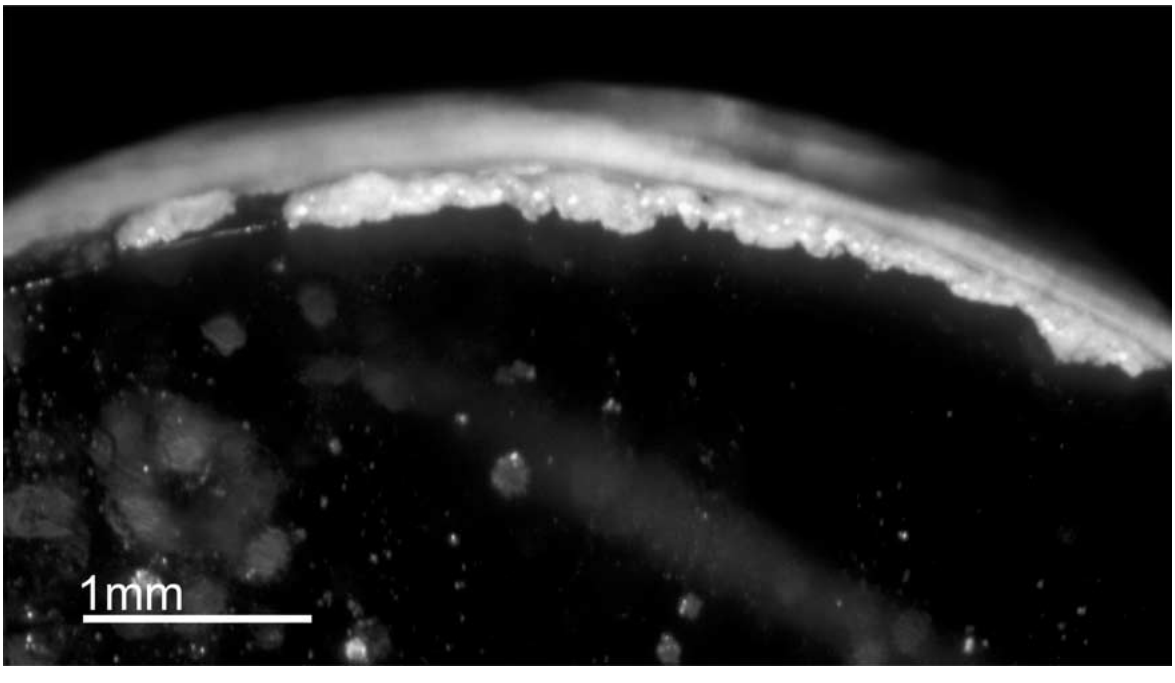

Figure 2 Presence of crystals in the bulk of hydrogel.

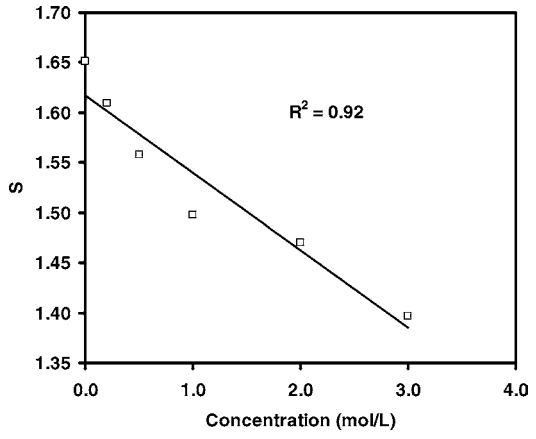

Figure 3 Solute effect of calcium chloride on the swelling of PHEMA.

contain transiently a maximum of four pairs of ions i.e., $Ca^{2+} \cdots Cl^{-}$, $Na^{+} \cdots PO_4^{3-}$, $Na^{+} \cdots Cl^{-}$ and $Ca^{2+} \cdots PO_4^{3-}$, the latter progressively separating as insoluble crystals. The few available studies of solute effects on the hydration of PHEMA [18–21] indicate that NaCl induces a salting-out effect. $CaCl_2$ was also reported [20] to elicit a salting-out effect upon hydration of PHEMA, and we have now confirmed this (Fig. 3). We also determined here for the first time that Na_3PO_4 induced a salting-out effect in PHEMA (Fig. 4). The solute effect of $Ca_3(PO_4)_2$ could not be determined because of its extremely low solubility, and mixed solutions of $CaCl_2$ and Na_3PO_4 precipitated at any concentration higher than 5×10^{-4} mol L^{-1} in the presence of hydrogel. At the very low concentrations at which the solutions were still clear the measured values of S consisted of a random distribution around the value of S in pure water.

Our experiments proved that all ionic species involved in the calcification of PHEMA in a metastable solution of $CaCl_2$ and Na_3PO_4 induced salting-out effects. As solutes can affect only the non-covalent crosslinks (i.e., the hydrophobic interactions) in a hydrogel network, a salting-out effect is usually interpreted as additional association promoted by the solute between the hydrophobic groups in any two close polymer chains, which further stabilizes the secondary structure of PHEMA and leads to the tightening of the network. As a result, a portion of water is removed locally from the tighter network, which is manifested globally as deswelling of the hydrogel. On the other hand, the calcium ion has a great tendency to chelate to oxygen atoms [22], and there are 3 oxygen atoms in each structural unit of PHEMA available for chelation. Once attached to the network, a calcium ion complex can play the role of a nucleation center for opportunistic crystallization. The spontaneous precipitation of additional calcium and phosphate ions may be then triggered where there is depletion of water due to a salting-out effect. We showed here that the contribution of solute effects to the spontaneous calcification of hydrogels is a plausible hypothesis.

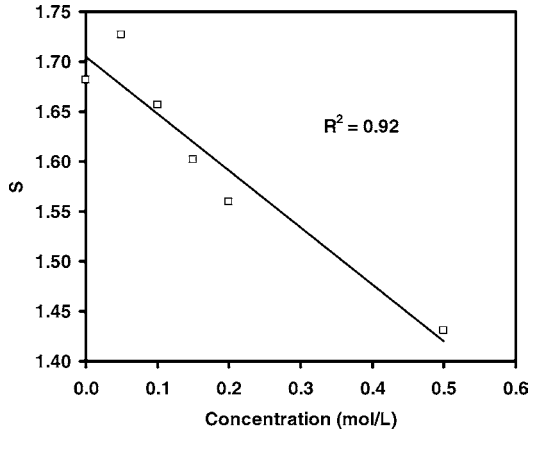

Figure 4 Solute effect of sodium phosphate on the swelling of PHEMA.

This research was supported by grant from the Australian Research Council.

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Received 18 June
and accepted 26 September 2003