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Poly(alkyl methacrylate) Tooth Coatings for Dental Care: Evaluation of the Demineralisation-Protection Benefit Using a Time-Resolved *In Vitro* Method

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Abstract: An *in vitro* method for the time-resolved quantification of acid-mediated tooth demineralisation has been developed and evaluated against putative non-permanent protective formulations based on a series of poly(alkyl methacrylate)s. Using a thermostatted carousel, dentally relevant substrates consisting of hydroxyapatite discs or sections of bovine teeth have been exposed to aqueous citric acid under controlled conditions, before and after being treated with the polymeric coatings. The dissolution of phosphate was monitored by the determination of ^{31}P by Inductively Coupled Plasma— Mass Spectrometry and by the spectrophotometric phosphovanadomolybdate method. Dose-response plots constructed for both groups of treated substrates have revealed that the coatings significantly reduce erosion rates but are less effective at inhibiting tooth demineralisation than the standard fluoride treatment. The approach has enabled an evaluation of the erosion-protection efficiency of each coating.

1. Introduction

Early stage dental erosion involves the reversible demineralisation of enamel through the dissolution of calcium hydroxyapatite (HA) and related substituted apatitic mineral phases by acids of non-bacterial origin [1-3]. In healthy individuals, the potentially deleterious effects of dietary acids on dental hard tissues are mitigated by the continuous production of saliva, whose inherent buffer capacity serves to raise pH in the oral cavity whilst at the same time diluting the acid challenge and promoting clearance [4]. Salivary proteins also play an important role in protecting teeth from acid insult, by being adsorbed selectively onto the tooth surface in the form of a tightly bound thin film. The salivary pellicle (thickness 0.1–1.0 µm depending on location and maturity) provides a significant barrier to acid attack on the underlying mineral surface [5,6]. The widespread use of fluoride in dentifrice formulations has been largely responsible for the marked decline in the incidence of dental caries in many populations over the last 40 years, but there are indications that this decline has slowed, while over the last 10 years dental erosion has become a clinical concern [7,8]. In particular, high levels of erosive wear have been observed in children and adolescents [9,10]. Risk factors include diet and social deprivation; but erosion and associated wear appear to be associated primarily with developed nations. Although consumption levels of acidic soft drinks has been positively correlated with increased levels of erosion, subjects exhibiting inappropriate consumption patterns are likely to be at highest risk [11,12]. Awareness by individuals and provision of timely advice by health care professionals are considered to be at least as important as therapeutic intervention in preventing progression of bulk tissue loss which in extremis can lead to complete loss of function [13].

The aetiology of dental caries requires the presence of bacterial plaque as one of several prerequisites, while dental erosion does not involve bacteria [9,14]. Early caries lesions form in the sub-surface of the tooth due to the presence of an overlying plaque layer; erosive lesions, by contrast, are surface defects formed by "top-down" demineralization [15]. Progression of early erosive lesions results in the formation of concavities, the appearance of which is accelerated by abrasive damage or by attrition-induced wear. Caries and erosion, however, do both involve acid-mediated demineralisation: in the case of caries the source is plaque acid, whilst in erosion the acid challenge derives from the stomach and/or from diet; acids associated with dental erosion are markedly more aggressive than plaque acids [12,15].

Fluoride is known to be highly efficacious in inhibiting the development of caries, and does so by substituting for -OH groups in HA, $Ca_{10}(PO_4)_6 (OH)_2$. This results in the formation of partially or fully fluoridated apatitic phases, $Ca_{10}(PO_4)_6F_x(OH)_{2-x}$, whose resistance to acid-mediated dissolution is much greater than that of the parent non-fluoridated apatite [16-19]. Fluoride also enhances remineralisation of early caries lesions by promoting mineral uptake as the less soluble fluoridated apatitic phase. The same fluoride chemistries apply in the case of dental erosion, but unlike dental caries, where there is a wealth of longitudinal clinical data supporting the efficacy of sodium monofluorophosphate and ionic fluorides, such as sodium fluoride (NaF) [20], evidence supporting the use of fluoride as an anti-erosion agent is currently limited to *in vitro* and *in situ* clinical studies [21]. The current view is that higher doses of fluoride may be required to achieve clinically significant levels of erosion control compared with that required in the prevention and management of dental caries [21-23]. The most convenient access to fluoridated medicaments is through the over-the-counter

availability of dentifrices and rinses. The levels of fluoride contained in these are dictated by the anti-caries monographs of regulatory agencies. In the absence of a specific anti-erosion monograph, and given the concerns for fluorosis associated with the use of higher levels of fluoride in children, technologies that augment the anti-erosion efficacy of medicaments containing fluoride at current concentrations (900–1,500 mg/kg for dentifrices and 90–450 mg/kg for mouth rinses), are of particular interest for future development.

In vitro erosion models have been effective in establishing the relative erosion potentials of foods and drinks, factors affecting these potentials, and the effects of specific interventions on the physicochemical properties of dentally-relevant substrates subject to acid insult. Data from such models indicate broad effects and trends, and the models are valuable tools for the screening of potential inhibitors of acid demineralisation. Due to the strict regulatory environment associated with the acquisition, processing, tracking, storage and disposal of human teeth, alternative substrates such as HA powder, beads or discs have been employed as non-biological enamel substitutes. Enamel and dentine specimens prepared from bovine incisors have also been widely used, because they can be acquired from the same stock at similar age (typically 30 months). Despite the differences between the histology of human and bovine teeth (the latter being more porous), the literature indicates a good correlation between the behaviour of human and bovine specimens in both caries and erosion models [24,25]. A simple means of determining the extent of erosive demineralisation is through the analysis of released phosphate using a single-point phosphovanadomolybdate assay. However, this spectrophotometric method does not (by definition) monitor the progression of erosion; furthermore, the assay is not specific to orthophosphate since linear condensed phosphates also react with the vanadomolybdate reagent. A pH-stat model has been used to monitor erosive demineralisation in real time, and to investigate the effect of specific interventions such as the pre-treatment of HA discs with fluoride, or modification of the erosive challenge itself by addition of film-forming polymers [26]. This model, however, may be difficult to employ for multiple specimens.

Driven by the need to develop a rapid and time-resolved experimental method, a thermostatted carousel has been used in the present study to provide a controlled environment in which citric acid-mediated erosion of HA discs and of pre-prepared sections of bovine teeth could be monitored by discontinuous sampling and subsequent determination of released phosphate. The phosphovanadomolybdate assay and the determination of ^{31}P by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) have been used for this analysis; the sensitivity and precision of these techniques are compared for this application. The protocol for the experimental method aims to facilitate the longitudinal monitoring of multiple erosion experiments (n = 12) that are conducted in parallel under identical conditions of temperature and stirring rate. The methodology is used to evaluate the anti-erosion efficacy of a series of biomedically relevant poly(alkyl methacrylate) coating materials [27].

2. Material and Methods

2.1. Substrates

A single batch of HA discs (diameter 12.5 mm, thickness 1.0–1.3 mm; *ca.* 0.46 g) was supplied by Himed HiMed, (Old Bethpage, NY, USA). Prior to use, discs (n = 6) were etched ×2 in 500 mL

of 10 g/L citric acid (adjusted to pH 3.5) for 30 min at room temperature. Excess acid was removed by rinsing with deionised (DI) water before storage in a humidified atmosphere.

Decay-free bovine incisors were sterilised by immersion in 50 g/L NaOCl for 2 min, followed by 300 mL/L H_2O_2 for a further 2 min. Thereafter, specimens were stored in 9 g/kg NaCl for a maximum of six weeks. Tooth sections (*ca*. 15 mm ×10 mm ×5 mm; 1.52 g) were cut from the buccal aspect using an Isomet™ 1000 precision saw (Buehler®, Düsseldorf, Germany). Since one larger face of each specimen consisted of exposed dentine whilst the other was unpolished enamel, some variability was to be expected both within and between the specimens [28,29]. The amorphous smear layer, formed by cutting, was removed by placing the tooth sections in 100 g/L citric acid (pH 2.2) for 2 min. After rinsing in DI water, the specimens were stored in sterile saline. Extracted human molars and pre-molars were stored in an aqueous solution of saturated thymol for up to two weeks after removal of the roots and pulp. Prior to use, teeth were thoroughly rinsed with DI water and visually examined for evidence of damage, decay and white spot lesions. Transverse sections of coronal dentine, 100 μm in thickness, were cut with an Isomet 1000 precision saw and etched (citric acid, 100 g/L; 2 min) to remove the smear layer. Specimens (bovine and human) were sputter-coated with a palladium-gold alloy before imaging using scanning electron microscopy (SEM) at an operating potential of 10 kV (Jeol JSM 6100, Welwyn Garden City, Hertfordshire, UK). Imaging of uncoated bovine specimens was performed by atomic force microscopy (AFM) in contact mode, using a MultiMode Nanoscope IV Scanning Probe Microscope (Digital Instruments, Santa Barbara, CA, USA).

Typical SEM images of transverse sections of human and bovine dentine are shown in Figures 1(a) and 1(b) and an AFM image from bovine dentine is shown in Figure 1(c). Tubule diameters in the bovine specimens (1.7–2.6 μm, *c.f.* lit. [30-33] 1.8–3.5 μm) were similar to those observed in human specimens (2.2–3.3 μm, *c.f.* lit. [30-33] 1.9–4.0 μm). Tubules in bovine specimens were less closely packed (3.1×10^4 mm^{-2}, *c.f.* lit. [30-33] 2.1×10^4–3.5×10^4 mm^{-2}) than those in human specimens (7.9×10^4 mm^{-2}, *c.f.* lit. [30-33] 2.1×10^4–8.3×10^4 mm^{-2}). AFM images, Figure 2, also established an optimum time of 2 min for exposure of freshly cut sections to citric acid (100 g/L), just sufficient for the removal of the smear layer and of debris within tubules. Longer exposures resulted in the formation of craters in the tubule structure whereas shorter exposures did not completely remove the smear layer.

Figure 1. Transverse sections of etched (100 g/L citric acid, pH 2.2, 2 min) human and bovine coronal dentine (deep layer): SEM images ×2,000 (**a**) human dentine, (**b**) bovine dentine; AFM image (**c**) bovine dentine.

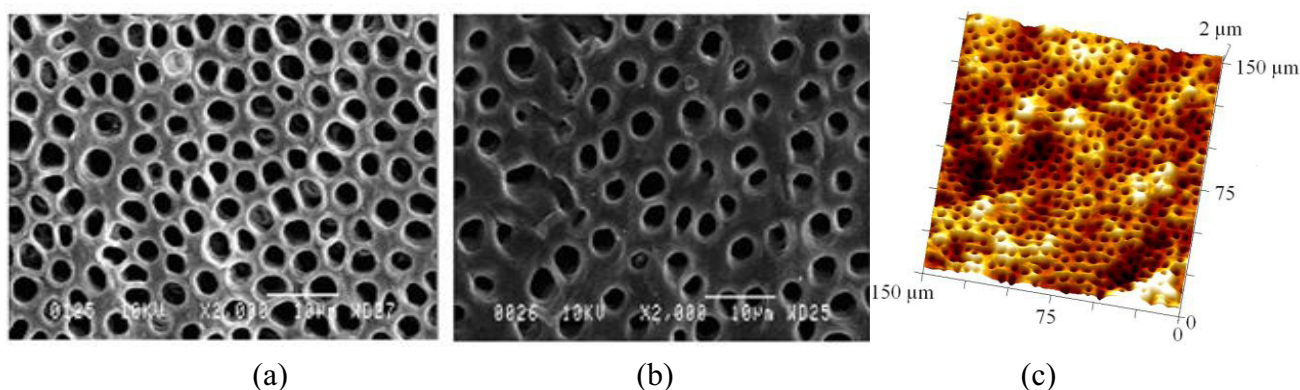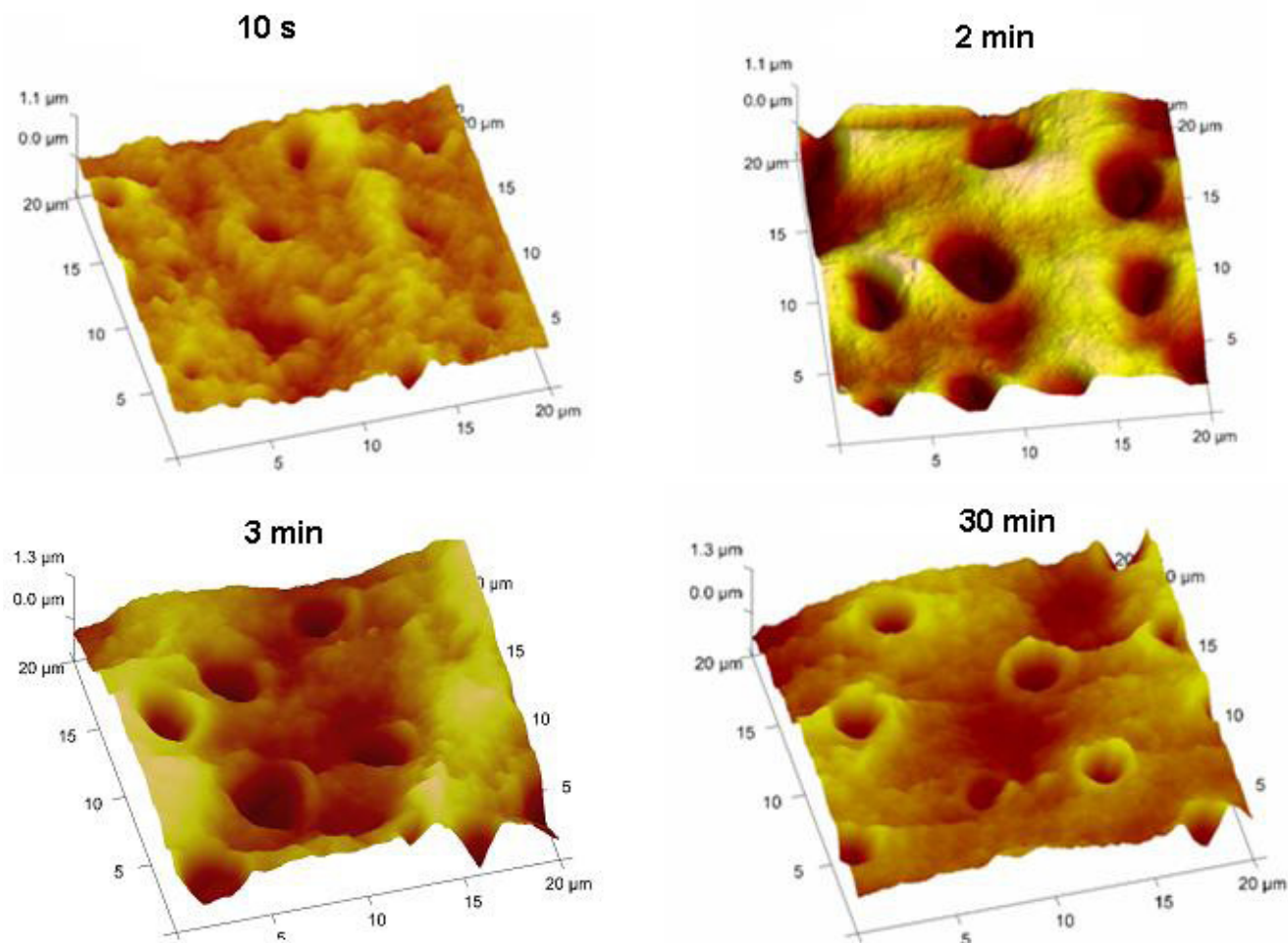(a)   (b)   (c)

Figure 2. AFM images of bovine dentine (different areas) pre-etched (100 g/L citric acid, pH 2.2) for 10 s, 2 min, 3 min and 30 min.

2.2. Polymer Emulsions and the Coating of Specimens

Poly(alkyl methacrylate)s were supplied by Sigma-Aldrich (Poole, Dorset, UK or Schnelldorf, Germany). The generic structure of these polymers and associated acronyms are shown in Figure 3, and their key physicochemical parameters summarised in Table 1. PBMA and PEMA were obtained as solids whilst POMA, PLMA, PHMA, PHxMA were supplied as solutions in toluene. Pure samples of solid polymers were recovered by repeated precipitation from cooled methanol.

Emulsions (28 g/kg) were prepared by adding each polymer (450 mg) in solution (dichloromethane (PEMA) or diethyl ether (PBMA) or low-boiling petroleum ether, 3.0 g) drop-wise (2 min) into aqueous sodium dodecyl sulphate (SDS, Acros Organics, 5 g/kg, 15 g) under sonication (Ultrasonic Processor, amplitude 37%, 7 min). The organic solvent was evaporated from each batch by stirring at *ca.* 5 K above the solvent boiling point for 4–6 h. To compensate for the concurrent evaporation of water, 500 µL aliquots of DI water were added at 30 min intervals (after cooling to ambient temperature) to give a final mass of 15.45 g. To determine the dry mass, the aqueous phase was removed following separation by centrifugation at 4,000 rpm for 5 min. Particle sizes were determined using a Coulter N4MD Sub-Micron Particle Analyser (Beckman Coulter, High Wycombe, Buckinghamshire, UK) with multiple scattering angle detection and size distribution analysis yielding

the diameters and SDs indicated in Table 1. HA discs and bovine tooth specimens were coated by immersion (2 min) in the appropriate polymer-containing emulsion, followed by rinsing with DI water.

Figure 3. Poly(alkyl methacrylate)s. n = 1 poly(ethyl methacrylate), PEMA; n = 3 poly(butyl methacrylate), PBMA; n = 5, poly(hexyl methacrylate), PHxMA; n = 11, poly(lauryl methacrylate), PLMA; n = 15, poly(hexadecyl methacrylate), PHMA; n = 17, poly(octadecyl methacrylate), POMA.
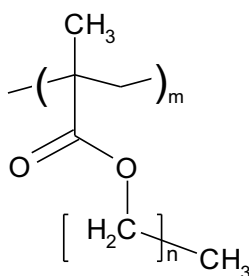

Table 1. Poly(alkyl methacrylate)s: surface energy (γ) [34]; glass transition temperature and average relative molar mass (T_g, *RMM*; data supplied); latex particle sizes (*d*) with standard deviation (sd).

Polymer	Code	*RMM*	γ/mJ/m^2	T_g/°C	*d* (sd)/nm
Poly(ethyl methacrylate)	PEMA	515,000	33.6	65	197 (8)
Poly(butyl methacrylate)	PBMA	337,000	28.8	20	74 (12)
Poly(hexyl methacrylate)	PHxMA	400,000	23.1	−5	94 (3)
Poly(lauryl methacrylate)	PLMA	576,400	19.1	−70	91 (7)
Poly(hexadecyl methacrylate)	PHMA	200,000	-	15	91 (5)
Poly(octadecyl methacrylate)	POMA	170,000	17.6	−100	73 (14)

2.3. Acid Demineralisation In Vitro

The demineralisation experiments were performed using a 12-station synthesis carousel supplied by Radleys Discovery Technologies (Saffron Waldon, Essex, UK). Untreated pre-weighed HA discs or bovine enamel sections were individually placed on a wire mesh support at a fixed distance above the stirrer bar in a reaction tube containing 15.0 mL of 10 g/L citric acid adjusted to pH 3.75 thermostatted to 37 °C. The baskets were then lowered so that the specimens were fully immersed in the erosive medium; typically 4-6 specimens were employed for each experiment. Samples (100 µL aliquots) for the determination of dissolved phosphate were taken at 5 or 10 min intervals up to 40 min. Specimens were then removed, rinsed with DI water, and immersed in the test treatment (28 g/kg polymer latex; 120 s, 20 °C), DI water negative control or one of a range of benchmark control treatments comprising 250–2,000 mg/kg fluoride as NaF. In the latter case exposure times of both 30 s and 120 s were employed. Together with a fresh portion of erosion medium (15.0 mL), the treated specimens were then returned to a cleaned tube in the reaction carousel. Discontinuous sampling and subsequent analysis of phosphate and phosphorous by spectrophotometry or ICP-MS respectively were used to create plots of dissolution as a function of time and thus dissolution rate. The efficacy of the treatment to inhibit erosive demineralisation was determined by using each specimen as its own control. Using a

one-way ANOVA with a post hoc Fisher test statistical analysis of the results was performed to compare the effects of the putative protective treatments with that of the fluoride control.

2.3.1. Determination of Dissolved Phosphate with Vanadomolybdate Reagent

Samples (100 µL) of phosphate-containing erosion medium were transferred to a 96-well microplate. Using a multipipette for simultaneous additions to all wells, samples were mixed with 100 µL of vanadomolybdate reagent (VWR, Lutterworth, Leicestershire, UK) and allowed to stand for 5 min, after which the absorbances were measured (Wallac Victor^2 1420 Multilabel Counter, 450 nm, 1 well per second). Using the same procedure for standard solutions (KH_2PO_4 > 99%, dried) a calibration plot over the phosphorus concentration range 10–60 mg/L was linear (R^2= 0.9996) but with a significant positive intercept (no reference cell). This calibration was used for calculating concentrations of phosphorus in the erosion media. A further calibration using a 1 cm pathlength cell with matched reference in a dual-beam spectrophotometer over the concentration range 0.1–2.3 mg/L was linear through the origin yielding values for A450 in the range 0.002 to 0.068; R^2 = 0.9999. Allowing for a minimum net absorbance of 0.005, the lowest reliable estimation level was *ca.* 0.2 mg/L in 1 cm cells or 0.5 mg/L using the microplate reader.

2.3.2. Determination of ^{31}P by ICP-MS

The analysis of dissolved phosphate as ^{31}P by ICP-MS necessitated the elimination of $[^{14}N^{16}O^{1}H]^{+}$ and/or $[^{15}N^{16}O]^{+}$ from the experimental protocol [35] by using HCl (analytical grade for trace analysis) rather than HNO_3, and HPLC grade water for all procedures. All glassware and equipment was cleaned in 30 g/L HCl and subsequently rinsed ×3 with DI water. Each 100 µL sample was added to 14 g of 6 g/L HCl. Using an Agilent ICP-MS 7500 operating in standard mode with argon as carrier gas, a linear calibration (R^2 = 1.000) was obtained for ^{31}P using acidified (6 g/L HCl) phosphate solutions (Spex CertipreP 1,000 ppm ^{31}P, Fisher Scientific; diluted to 1–110 µg/L). Allowing for the background response, the lowest reliable estimation level of ^{31}P in the diluted samples was *ca.*5 µg/L.
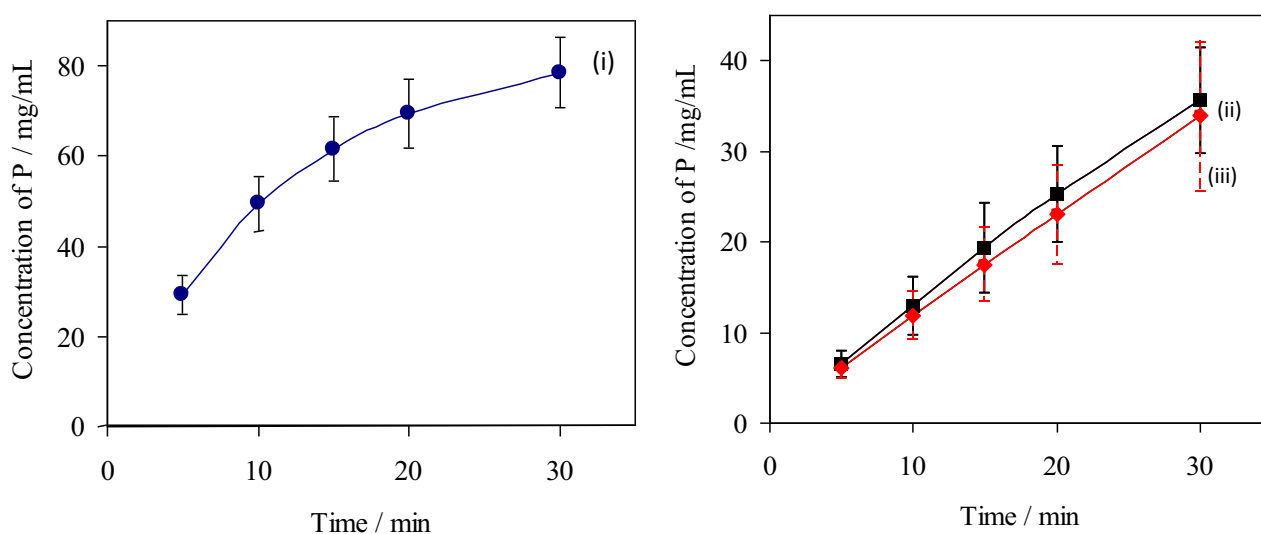
3. Results

3.1. Comparison of Analytical Methods

The rates of erosion of individual untreated dental specimens by citric acid (10 g/L, pH 3.75; 37 °C) have been measured by analysis of dissolved phosphorus, for comparison with rates obtained under the same conditions but after exposure of the same specimens to a putative protective treatment: the "*in vitro* demineralisation method". These data have been used to assess the variability of specimens and to compare the two methods of analysis of aqueous samples for dissolved phosphate, the data are summarised graphically in Figure 4.

For HA discs, the initial rates of erosion were almost constant (0 to 10 min: as observed 1.24 ± 0.08 mg/L min, relative to the mass of the specimen 0.041 ± 0.002 mg/g min, relative to the area from the dimensions 5.6(±0.3) × 10^{-5} mg/mm^2 min), decreasing by *ca.* 15% over each complete time profile (20 to 30 min: 1.06 ± 0.01 mg/L min, 0.035 ± 0.001 mg/g min, 4.8(±0.1) × 10^{-5} mg/mm^2 min). For bovine tooth sections the initial rates of dissolution were higher (8.0 mg/L min estimated from a

tangent to curve (i) at the origin, Figure 4; average over 0 to 5 min 5.8 mg/L min, 0.058 mg/g min, 1.6×10^{-4} mg/mm^2 min). In all experiments the highest total amounts of dissolved phosphorus after 30 min were equivalent to 4.6($\pm$0.9)% of the mass of the HA specimens and 2.5($\pm$0.3)% of the mass of the of bovine tooth specimens. The corresponding proportions reacted of citric acid were estimated respectively as 6($\pm$1)% and 11($\pm$1)%.

> **Figure 4.** Measurements of phosphorus released as phosphate by erosion with citric acid (10 g/L, pH 3.75; 37.0 °C) of untreated dental specimens: (i,●) bovine dentine (ICP-MS), (ii,■) hydroxyapatite discs (ICP-MS), and (iii,♦) hydroxyapatite discs (phosphovanadomolybdate complex absorbance); n = 15, error bars represent standard deviations.

In comparing the erosion results from the two methods (15 HA specimens each), Figure 4 lines (ii) and (iii)), the errors were similar for results from both analytical methods (coefficients of variation, cv, 16–26% for ICP-MS and 18–25% for the phosphovanadomolybdate method). The mean values were consistent to within 5–11%, such that pairs of data at each time point did not differ significantly ('t' test for null hypothesis, p < 0.01). The errors in data were smaller for the results for bovine tooth specimens (cv 10–15%).

3.2. Effectiveness of Fluoride as Erosion-Inhibiting Agent

To compensate for the variability in the erosion rates of dental specimens under controlled conditions, the effects of protective treatments have been assessed *in vitro* by comparing measurements made before and after their application. Dissolution by citric acid (10 g/L, pH 3.75; 37 °C) was monitored by sampling at intervals over 30 min. The method was evaluated by using it to measure the effects of treating specimens with fluoride ion. Dose-response plots (n = 4 for each experiment) were constructed for HA discs that had been immersed (30 s or 120 s) in water or in aqueous sodium fluoride (fluoride ion concentrations 250–2,000 µg/g).

Treatment with fluoride ion (300 µg/g, 120 s) reduced the rate of erosion by 40 ± 10% over 0 to 5 min, increasing to 70 ± 20% over 20 to 30 min, Figure 5(a), reflecting the considerably reduced rate of erosion of the treated sample. Inhibition was increased by the use of higher concentrations of

fluoride ion but the effect of using the longer treatment exposure time was much greater (Figure 5(b)). The proportional reductions in sampled phosphorus concentrations at each time point show lower variability (cv 8–22%) than the absolute values (cv 20–32% both before and after treatment).

Figure 5. (**a**) Release of phosphorus (ICP-MS) from HA specimens (n = 4) into citric acid (10 g/L, pH 3.75; 37.0 °C) (i) before treatment, (ii) after immersion (120 s) in aqueous NaF (F⁻ = 300 µg/mL); (**b**) Fluoride dose response (37 °C) of HA discs as determined by phosphate analysis (n = 4, bars represent standard deviations): treatment time 30 s, fluoride ion concentrations (µg/g) A = 2,000, B = 1,000, C = 250, D = 0 (phosphovanadomolybdate spectrophotometry); treatment time 120 s, fluoride ion concentrations (µg/g) E = 300 (ICP-MS)—direct comparisons were made in two stages (i) between groups A, B, C and D (for different fluoride concentration after 30 s treatment), and (ii) between groups C and E for (for different treatment times at fluoride concentration of 300 µg/g).

(a)

(b)
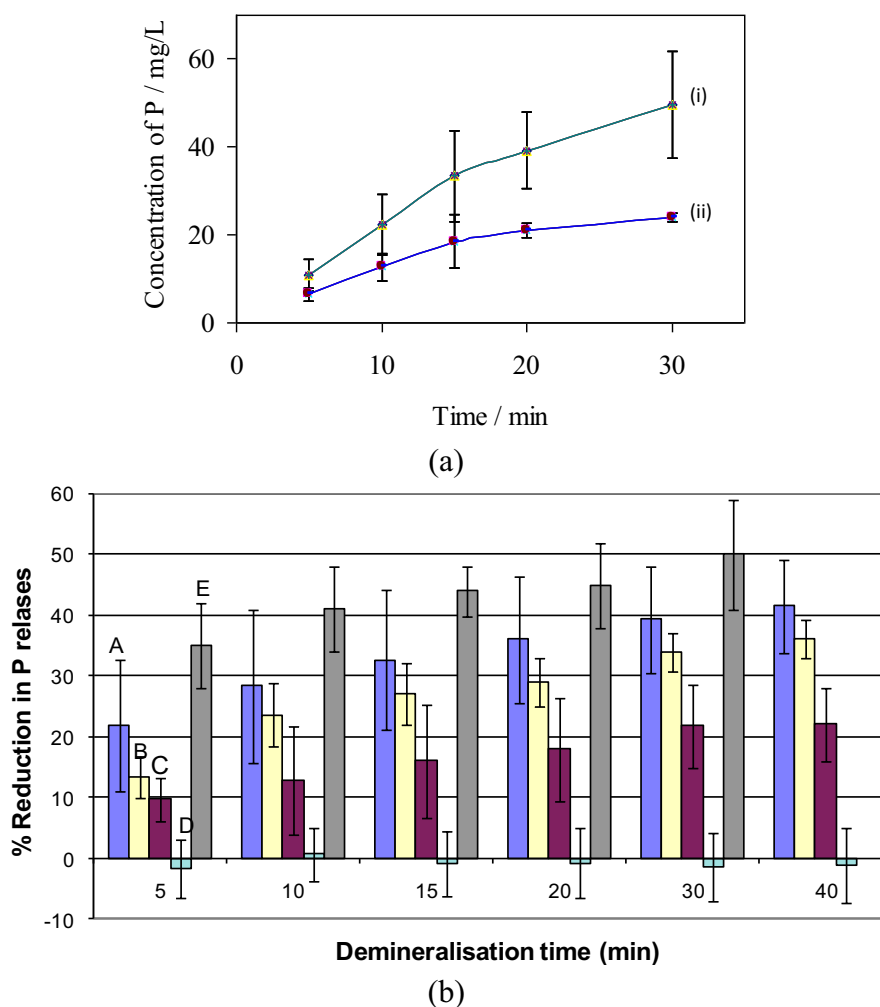
3.3. Effectiveness of Poly(alkyl methacrylate) Coatings as Anti-Erosion Agents

Poly(alkyl methacrylate)s were selected as putative erosion-protection coatings because of their established good film-forming characteristics and of their affinity for the tooth surface [36]. For systematic investigation, the study employed a homologous series of polymers with glass transition temperatures in the range −100 to +65 °C and surface energies in the range 17–34 mJ/m², Table 1. For

each specimen the inhibition of erosion was assessed from the relative initial rates of dissolution of phosphorus (average over 0–5 min) before and after coating. Relative rates of dissolution over 20–30 min were also obtained.

For HA specimens, erosion was slightly enhanced by pre-immersion in water, Table 2. Relative to this, significant inhibition of erosion was shown by specimens coated with PBMA (32% initially, decreasing to 22%) and PHxMA (24% initially, decreasing to 20%), Figure 6(a,b) and Table 2. Erosion also appeared to be inhibited by coating with PHMA (*ca.* 9% initially) but the standard deviations were too large for the effect to be significant. The polymers PLMA and POMA formed coatings with very slight inhibiting effects while any effect of PEMA coatings was undetectable. For comparison, the inhibition by fluoride ion (300 µg/g) was 43% over 0–5 min increasing to 73% over 20–30 min, Figure 5(a).

Figure 6. Release of phosphorus (as phosphate) from dental specimens exposed to citric acid (10 g/L, pH 3.75; 37 °C): untreated specimen - - - -, treated specimen. (**a**) HA discs treated with PBMA (28 g/L, 120 s; ICP-MS), (**b**) HA discs treated with PHxMA (28 g/L, 120 s; ICP-MS), (**c**) Bovine teeth sections treated with PBMA (28 g/L, 120 s; phosphovanadomolybdate), (**d**) Bovine teeth sections treated with PHxMA (28 g/L, 120 s; phosphovanadomolybdate).
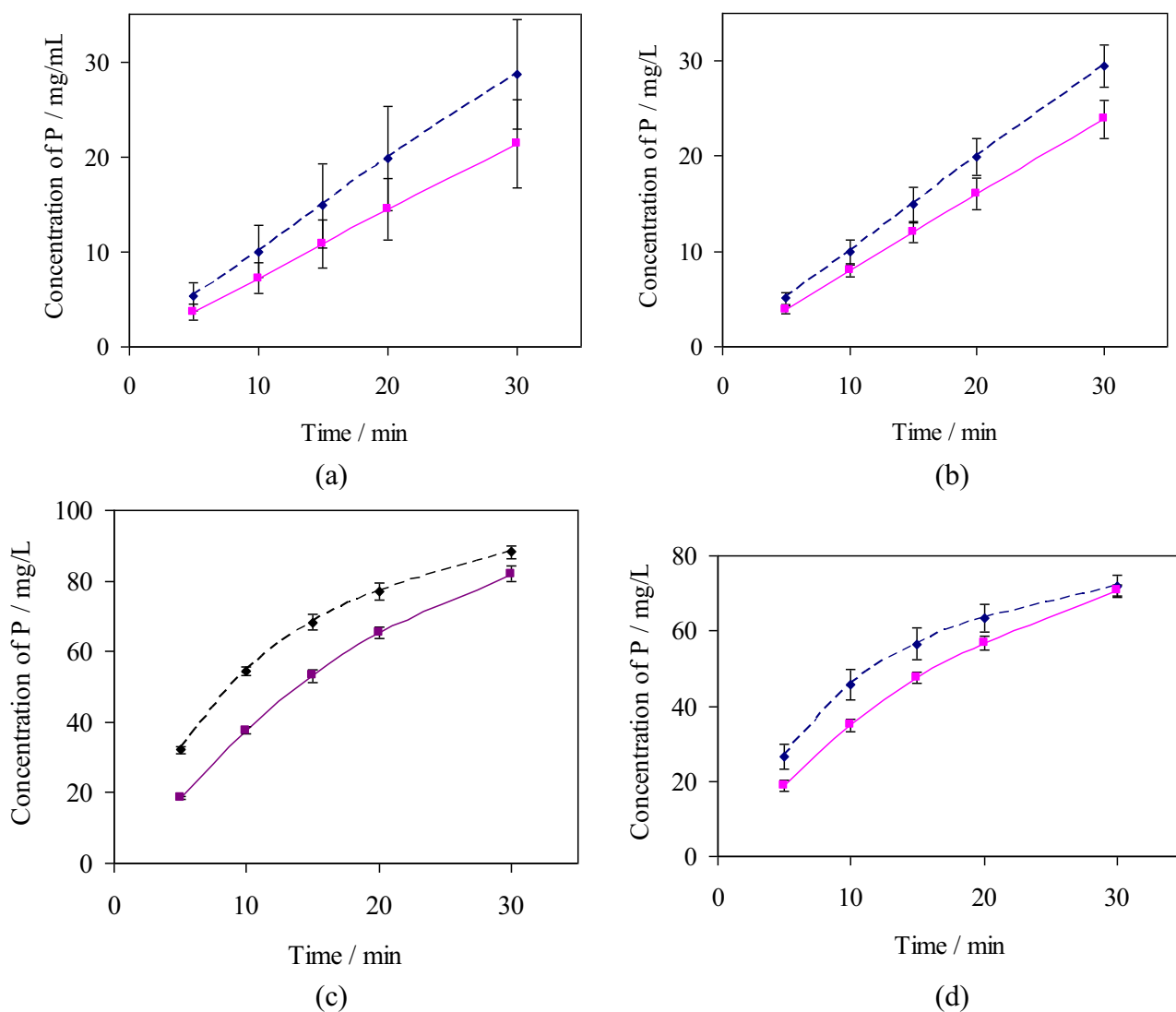

(a)

(b)

(c)

(d)

Table 2. Reduction in initial rate of release of phosphorus (as phosphate, 0–5 min) from treated dental specimens relative to the untreated controls (n ≥ 4) in erosion experiments over 30 min.

Specimen	Treatment (120 s)	Fractional reduction in initial rate of erosion (SD)
HA	300 µg/g F⁻	0.38 (0.07) [a]
	Water	−0.05 (0.07)
	PEMA	−0.02 (0.07)
	PBMA	0.27 (0.07)[a]
	PHxMA	0.19 (0.08)[a]
	PLMA	0.05 (0.10)
	PHMA	0.09 (0.10)
	POMA	0.02 (0.08)
Bovine tooth section	300 µg/g F⁻	0.37 (0.04)
	Water	0.19 (0.02)
	PBMA	0.35 (0.02)[b]
	PHxMA	0.23 (0.05)[c]
	300 µg/g F⁻ + PBMA	0.35 (0.01)[b]
	(i) PBMA (ii) 300 µg/g F⁻	0.35 (0.02)[b]
	300 µg/g F⁻ + PHxMA	0.33 (0.06)[b]

For HA specimens [a] denotes that the mean value is significantly different ($p < 0.01$) from that of the water control; for bovine tooth sections [b] denotes that the mean value is not statistically different ($p < 0.05$) from the fluoride control; [c] denotes that the mean value is not statistically different ($p < 0.05$) from the water-treated control (one-way ANOVA with a post hoc Fisher's test).

In erosion tests using bovine tooth sections prior to treatment, Figure 6(c,d), dissolved phosphorus concentrations initially increased rapidly, reaching 40 mg/L after 10–15 min, but dissolution progressively slowed thereafter. Following measurements to establish baseline erosion rates [37], immersion of the specimens in water (120 s) appeared to inhibit erosion considerably, Table 2. The inhibition (fractional reduction in initial erosion rate) due to PBMA was similar to that given by fluoride ion (Table 2) and to that found using HA discs (F⁻ 250 µg/g, 30 s; Figure 5(b)). Treatment with PHxMA also apparently inhibited erosion but the effect was not significantly greater than the inhibition by water. The effects of combined or sequential treatments with fluoride and PBMA or PHxMA were very close to those for single treatments with fluoride, Table 2. Longer term effects (10–30 min) could not be assessed from the measurements made.

4. Discussion

4.1. Comparison of Analytical Methods

The precision, consistency, the scatter of experimental data and the lower detection limits have been compared for the two analytical methods. In both methods samples were taken using 100 µL micropipettes. In the phosphovanadomolybdate method micropipettes were also used for adding the complexing reagent, whereas in ICP-MS each sample was diluted with a precise, relatively large, amount of aqueous HCl. Absorbances were measured to ±0.001, giving precision in the range 0.2–2%.

In ICP-MS, counting rates were reproducible to <2%; other contributions to error arising from procedures are likely to be relatively small. The observed errors in the HA disc erosion experiments (cv, 16–26%; similar for the two methods) were much greater than 2%, while the differences (5–11%) between comparable mean analytical values were not significant. Therefore up to 8% of the variability may be attributed to the HA discs. For erosion experiments using bovine tooth specimens, the errors were smaller–indicating that these specimens were less variable than the HA discs. As used for these experiments, there was little to choose between the two analytical methods, which have different potential problems of interference. Samples for ICP-MS were, however, diluted by 1:140 whereas for the phosphovanadomolybdate method the dilution factor was 1:2. Therefore, allowing also for the minimum detection levels obtained, the ICP-MS method would be more sensitive by a conservative factor of *ca.* 40. This method could be used for measuring much lower levels of dissolved phosphate, for example from erosion experiments either over shorter time scales or using smaller dental samples.

4.2. Poly(alkyl methacrylate) Barrier Coatings

New agents or technologies incorporated into dentifrices and/or mouth-rinses in future may play an important role in enhancing the anti-erosion efficacy of the formulation. Ideally, these technologies should be fluoride-compatible since anti-caries activity is a key benefit of modern dentifrices and rinses, and the available *in vitro* and *in situ* studies suggest fluoride may be clinically relevant as an anti-erosion active. Although the polymeric materials investigated here are intended to form protective barrier coatings that protect the underlying mineral from acid attack, the established *in vitro* and *in situ* anti-erosion efficacy of fluoride effectively dictates its adoption as the positive/benchmark control in anti-erosion studies. The homologous series of poly(alkyl methacrylate)s investigated in the present study are characterised by a range of glass transition temperatures, and surface energies of deposited films; both exhibit decreasing trends with increasing pendent-chain length. With the exception of PEMA, the relative efficacy of each coating as an inhibitor of acid erosion of HA discs was also lower for the polymers with longer pendent chains, as indicated in Table 2. The behaviour of PEMA may be due to the poor film-forming characteristics of this polymer. Its T_g is higher than the temperature used for film deposition and its latex particle sizes exceeded 100 nm (Table 1), which is the maximum for the deposition of good quality films [36].

Data from the citric acid-mediated dissolution of untreated HA discs showed a progressive general decrease in erosion rate of 10–20% over each 30 min assay as shown in Figure 5(a). Since erosion might have been expected to accelerate due to a progressive increase in accessible area as the external surface became pitted, this decrease probably reflected the reducing aggressiveness of the challenge as the citric acid was consumed and calcium and phosphate were released. Throughout each assay the dissolution of HA discs after their treatment with PBMA or PHxMA was significantly less (p < 0.01) than that of the corresponding controls (before treatment). These consistent and statistically significant results identify PBMA or by PHxMA as potentially useful anti-erosion agents that may offer additional efficacy benefits to fluoride-based formulations, or new fluoride-free formulations.

That the initial rate of erosion relative to dimensions was approximately three times higher for bovine tooth sections than for HA discs is consistent with the tooth sections' greater exposed surface area, which was associated with the corrugated surface of a cut dentine surface together with the

porosity associated with the tubules (Figure 2). Pre-etching of the enamel is also likely to have increased its surface area. Additional factors are possible, however, since the dissolution of biological HA is dependent on the molar ratio of calcium to phosphorus in the sample [38] and also because biological apatite is both more soluble and susceptible to partial transformation to other phosphate phases [39]. Further, the size and perfection of HA crystals may influence the rate of erosion, since smaller or less perfect crystals are more susceptible to dissolution [40]. Finally, the presence in biological apatite of carbonate ions and of other impurities may impact upon solubility [41,42]. The greater susceptibility of dentine to erosive demineralisation is well known and has been attributed to a combination of its porosity and its relatively small crystallites of HA [29]. Although the experiments showed that *ca.* 11% of the available citric acid had been consumed over the course of each assay (30 min), the decrease in the rate of erosion during the control measurements appeared to exceed what might have been expected from the changes in composition of the liquid phase. The rapidly declining rate of erosion of dentine (Figure 6(c,d)) may be due to the gradual build up of a collagen mat on the surface. This would explain the apparent inhibiting effect of water: after the control measurements the specimens would have become partially protected. The suitability of bovine- tooth specimens to act as their own controls in these experiments therefore becomes questionable. As a consequence, data obtained using these substrates may only be treated as indicative of trends in dissolution behaviour. Nevertheless, significant inhibition by PBMA of citric acid mediated erosion is confirmed. Treatments with both fluoride and a polymer coating produced similar inhibition to that with fluoride alone. Therefore although enhanced inhibition was not shown, neither was there any indication of incompatibility between the treatments.

5. Conclusions

Towards the development of an *in vitro* technique for the time-resolved quantification of acid-mediated tooth demineralisation, a method that monitors the release of phosphate from dental specimens has been used to evaluate, in comparison with the standard fluoride treatment, the relative efficiencies of protective coatings formed from a series of poly(alkyl methacrylate)s. To facilitate reproducibility and the control of experimental conditions, up to 12 dissolution assays were performed at the same time using a thermostatted carousel with automated sampling. Using HA discs and sections of bovine tooth, significant inhibition of demineralisation was shown by coatings of PBMA and PHxMA. The degree of protection offered by these two coatings was lower than that conferred by 300 mg/kg fluoride, and the present study indicated no significant improvement in erosion protection associated with combining the two treatments.

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