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PHOSPHOENOLPYRUVATE-HOMO- AND COPOLYMERIZATION AND THE APPLICATION OF THESE POLYMERS AS DENTAL ANTICARIES AGENTS

A. R. ELMES, E. KHOSHDEL,* D. T. LITTLEWOOD and C. A. TAYLOR
Unilever Research, Port Sunlight Laboratory, Quarry Road East, Bebington, Wirral L63 3JW, U.K.

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Abstract—The first ever polymerization of the biological phosphorylating agent, phosphoenolpyruvate was carried out in our laboratory. This was achieved under mild conditions by employing an aqueous polymerization method and using a redox free radical initiator. Similarly, phosphoenolpyruvate was copolymerized with acrylic acid. These polymers were characterized by IR, GPC, ^{1}H and ^{31}PNMR spectroscopy. The results of *in vitro* anticaries studies indicate that these polymers significantly reduce tooth enamel demineralization compared with a placebo (water). © 1997 Elsevier Science Ltd

INTRODUCTION

Caries is a plaque disease wherein plaque bacteria generate acid from dietary carbohydrates. The acid formed demineralizes the tooth enamel and leads to the formation of cavities and eventual destruction of the tooth. Calculus is formed on the teeth when crystals of calcium phosphate begin to deposit in the pellicle and extracellular matrix of the dental plaque. These can eventually become sufficiently closely packed together for the aggregates to become resistant to deformation. Regular brushing aids in preventing a rapid build up of these deposits. However, regular brushing is not always sufficient to remove all of the calculus deposits which adhere to the teeth. The route by which calcium and orthophosphate ultimately give rise to a crystalline material called hydroxyapatite (HAP) is not well understood. It is generally considered that at above the critical saturation limit, the precursor to crystalline HAP is an amorphous or microcrytalline calcium phosphate.

There is a good correlation betwen the ability of a material to prevent HAP crystalline growth *in vitro* and its ability to prevent calcification *in vivo*. Generally, linear molecularly dehydrated polyphosphates such as alkali metal salts of tripolyphosphates, hexametaphosphates and pyrophosphates retard the HAP crystallization and hence help to prevent calcification [1]. Organic polymeric polyphosphates and polyphosphonates have also been considered as potential agents for the prevention of dental caries [2]. Indeed, these polymers adsorb on enamel surfaces in the form of a monolayer and inhibit calcium and fluoride ion transport, whereas phosphate ions are not effected. Several examples of such polymers include phosphonated polyethylene, poly(vinylphosphonate) (**I**) and poly(styrenephosphonate) (**II**), see Fig. 1.

This paper describes the homo- and copolymerization of a naturally derived phosphorous containing material namely, phosphoenolpyruvate (PEP, Fig. 2) and its anticaries benefits. This is to our knowledge, the first report on the purposeful polymerization of this interesting molecule.

Phosphoenolpyruvate is encountered in nature in plants where it is involved in the so-called C_4 cycle in carbon dioxide fixation (photosynthesis). An important function of PEP is its ability to phosphorylate biological molecules, for example, it is the ultimate agent for regeneration of ATP from ADP [3].

PEP is a unique compact multifunctional molecule. The presence of carboxylate, phosphate and unhindered vinylidine functionalities in this molecule makes it highly attractive from several points of view. Firstly, the vinylidine groups, under favourable conditions, should be capable of undergoing radical polymerization. Secondly, carboxylate and phosphate functionalities in polymeric PEP may induce good surface adhesion properties. Thirdly, the presence of the phosphate/carboxylate groups should provide favourable ion binding sites—especially for calcium ions. In addition, we may also expect such polymers to be biocompatible.

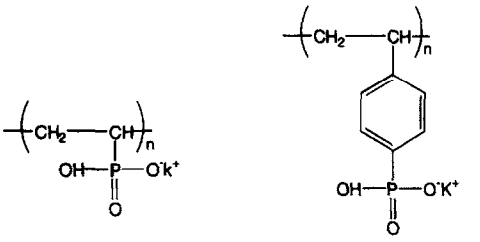
SYNTHETIC STRATEGY

Our strategy was based on having access to an efficient and commercially attractive route to phosphoenolpyruvate and an easy polymerization method operating under mild conditions.

Monomer synthesis

Although phosphoenolpyruvate can be obtained from fine chemical suppliers, it is very expensive. However, an efficient and economically sound

*To whom all correspondence should be addressed.



(I) Poly(vinylphosphonate) potassium salt

(II) Poly(styrenephosphonate) potassium salt

Fig. 1. Polymeric phosphonates.

synthetic method for the production of the monopotassium salt is known [3]. This simple method which is shown in Scheme 1 involves only three steps. The first step involves the bromination of pyruvic acid with elemental bromine to obtain 3-bromopyruvic acid. Step two is the reaction of 3-bromopyruvic acid with trimethyl phosphite. The third step is simply the hydrolysis of the phosphate ester produced in the first step. Phosphoenolpyruvate may also be generated enzymatically from D-3-phosphoglyceric acid [4].

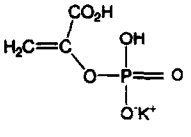
Polymer synthesis

We investigated an aqueous polymerization route from the outset. This was because the potassium salt of phosphoenolpyruvate (KPEP) is a water-soluble monomer and insoluble in many common organic solvents. It is also known that KPEP is thermally unstable is aqueous media (half-life at pH 7.0 of 98 days at 30°C [5, 6]. Therefore, we explored a mild redox initiated aqueous polymerization methodology. We selected a combination of sodium persulfate/bisulfite as a suitable redox initiator system. This system is convenient and particularly effective within the range 25–45°C.

EXPERIMENTAL

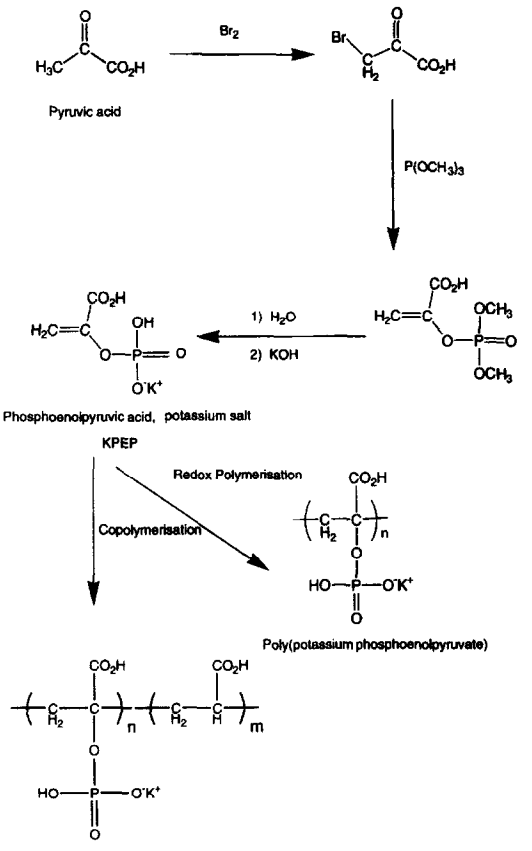
Reagents

Trimethyl phosphite and 3-bromopyruvic acid were purchased from Fluka and Lancaster Synthesis respectively. Phosphoenolpyruvic acid monopotassium salt (KPEP), 99%, was obtained from Aldrich and Fluka and used without further purification. KPEP was also synthesized in-house according to the method given in [3]. Acrylic acid was freshly distilled before copolymerization in the presence of an inhibitor (*t*-butyl catechol). Sodium fluoride was purchased from Sigma. All other reagents were used as received.



KPEP

Fig. 2. Phosphoenolpyruvate, potassium salt.



Scheme 1. Synthesis and polymerization of phosphoenolpyruvate.

Instrumentation

^1H and ^{31}P NMR were run in deuterium oxide solution using a Bruker 360 MHz spectrometer. The polymer molecular weights were determined by aqueous GPC using Hewlett Packard HP1090 liquid chromatograph fitted with a 30 × 7.5 cm TSK Gel linear GMPW column. Samples were measured against polyethyleneglycol standards. IR spectra were recorded as KBr discs using a Nicolet 1705X Fourier-Transform IR spectrophotometer with MCT detector and Nicolet 1280 processor. Sample pre-ultrafiltration was required to obtain the best NMR and FTIR spectra.

Monomer synthesis

Phosphoenolpyruvate, potassium salt (KPEP) was synthesized following the method given in the literature [3].

Polymer synthesis

Homopolymerization of phosphoenolpyruvate. A small scale test-tube trial polymerization was first conducted as follows: KPEP (Fluka) (1.0 g, ~5 mmol) was dissolved in de-aerated deionized water (10 mL). Sodium persulphate (5 mg, 0.02 mmol), followed by sodium metabisulfite (2.5 mg, 0.01 mmol) was added to the solution and the tube contents heated at 40°C under a nitrogen atmosphere. After an hour some polymer precipitation was evident, leading to a precipitate after standing for several more hours. The polymer was separated by filtration, washed with a minimum of water, and acetone, then vacuum dried. A second aliquot of sodium persulfate (20 mg) and sodium metabisulfite (10 mg) was added to the aqueous filtrate at 40°C, whereupon more of the polymer precipitated over 3

Table 1. Polymer characterization data

Polymer number	Polymer yield (%)	Polymer molecular weights (GPC) M_n	M_w	D	Molar ratio of PO_4^{-2} (^{31}P NMR) Polymeric phosphate	*Ortho*Phosphate	Pyrophosphate
1	23	15,000	53,000	3.53	—	—	—
2	70	24,000	48,000	2.00	1.0	4.5	0.1
3	80	16,000	34,000	2.12	1.0	1.9	Trace
4	60	1800	2900	1.61	1.0	5.0	0.03
5	100	84,000	233,000	2.77	1.0	0.4	0.03

M_n = Number average molecular weight, M_w = weight average molecular weight, D = polydispersity.

days. This was isolated, washed and dried and combined with the first quantity (yield 0.23 g). The polymer was characterized by molecular weight, thermally and spectroscopically (see polymer number **1**, Table 1, also the Results and Discussion section).

Another polymerization based on the above method was performed on a rather larger amount of the commercially supplied KPEP (9 g, 44 mmol). The redox initiator system—sodium persulfate (0.2 g, 0.84 mmol) in water (5 mL), sodium metabisulfite (0.1 g, 0.53 mmol) in water (5 mL), was added in five aliquots to the stirred KPEP solution in water (70 mL) over 2 days, leaving the polymerization to proceed an additional 4 days. This resulted in a higher polymer yield (6.3 g, 70%) than the first attempt. However, to recover all of the polymer it was necessary to first partially evaporate the supernatant, then carefully add acetone to the concentrate to fractionally precipitate the soluble polymer. This larger amount of polymer was freeze-dried to conveniently obtain it in anhydrous form (see polymer number **2**, Table 1).

For subsequent polymerizations, the "in-house" synthesized KPEP was used. Homopolymerizations were performed on 10 g amounts as described for preparation polymer number **2**. After 4 days of polymerization, polymer number **3** (see Table 1) was obtained in 83% yield and polymer number 4 (see Table 1) was obtained in only 60% yield after 20 days.

Copolymerization of phosphoenolpyruvate with acrylic acid

KPEP was copolymerized with acrylic acid (entry 5) as follows: Acrylic acid (10 g, 139 mmol) was dissolved in de-aerated deionized water (100 mL) and 20% aqueous sodium hydroxide added to raise the pH to 5.0. KPEP (10 g, 48 mmol) was added followed by sodium persulphate (0.1 g, 0.42 mmol) and sodium metabisulphite (0.05 g, 0.26 mmol), and the solution was stirred and heated at 40°C under a nitrogen atmosphere. Two further equivalent initiator amounts were added after 18 and 24 hr and the polymerization left up to 2 days. The resulting syrupy solutionw as concentrated and the copolymer precipitated with acetone, filtered, redissolved, re-precipitated, then freeze-dried. The yield was 22 g (100%). Molecular weight and spectroscopic details are given in Table 1 (polymer number **5**).

Anticaries study—method and materials

The *in vitro* pH cycling model was used to assess the effect of the test agents on the demineralization of enamel, according to a previously described procedure [7].

A total of four teeth were used, sectioned into four slices and were exposed to the following treatments, for 15 cycles:

—distilled water;
—1% w/w phosphoenolpyruvate, monopotassium salt;
—1% w/w polyphosphoenolpyruvate, potassium salt;
—1000 ppm fluoride, as sodium fluoride.

RESULTS AND DISCUSSION

Monomer and polymer synthesis and characterization

Large laboratory scale (50–100 g) quantities of KPEP was easily prepared from 3-bromopyruvic acid and trimethyl phosphite in yields ranging between 50 and 70%. The in-house synthesized KPEP had m.pt (170°C), IR, ^1H and ^{31}P NMR virtually identical to that of the Aldrich/Fluka samples (m.pt 175°C).

An initial small scale (1 g) trial polymerization of Fluka KPEP with the redox initiator at 40°C was successful. Thus, the polymer [poly(KPEP)], was seen to precipitate from solution within an hour of initiation. Further polymerization was assisted by adding a second aliquot of initiator and leaving for 3 days. This gave 23% polymer after separation from unpolymerized monomer. It had a softening and charring temperature well above that on monomeric KPEP. Molecular weight data for this polymer (number **1**) is given in Table 1.

Comparison of the FTIR spectrum of the above polymer (Fig. 3) with that of the monomer (Fig. 4) showed the expected band broadening. The polymer showed a medium intensity band in the region 1160–1180 cm^{-1}, assignable to organophosphate P=O str. ^1H NMR of the polymer showed a single broad band around 2.2–4.0 ppm assignable to the backbone methylene groups. There was no indication of monomeric material.

We subsequently polymerized our in-house synthesized KPEP employing the above method. The best results were obtained by incremental addition of the redox initiator over several days. In this way higher yield (70–80%) were obtained. Generally, some hydrolysis occurs during the polymerization. The degree of hydrolysis is mainly determined by polymerization temperature and time. This led to the presence of 2-hydroxyacrylic acid segments in the polymer chains and formation of inorganic phosphate species. The latter was determined by ^{31}P NMR (see Table 1) and FTIR spectroscopy. In addition, the polymers show IR bands attributable to their carboxylate groups and to the presence of lactone bonds (1780 cm^{-1}). The latter probably results from the intramolecular condensation of chain hydroxyl and carboxylate groups.

An attempt to polymerize the dimethyl ester of PEP (intermediate) in an aqueous media using sodium persulfate/sodium bisulfite redox initiators was unsuccessful. Only a low yield of polymer which was difficult to purify was obtained. Similarly, fully neutralized KPEP did not homopolymerize in an aqueous media.

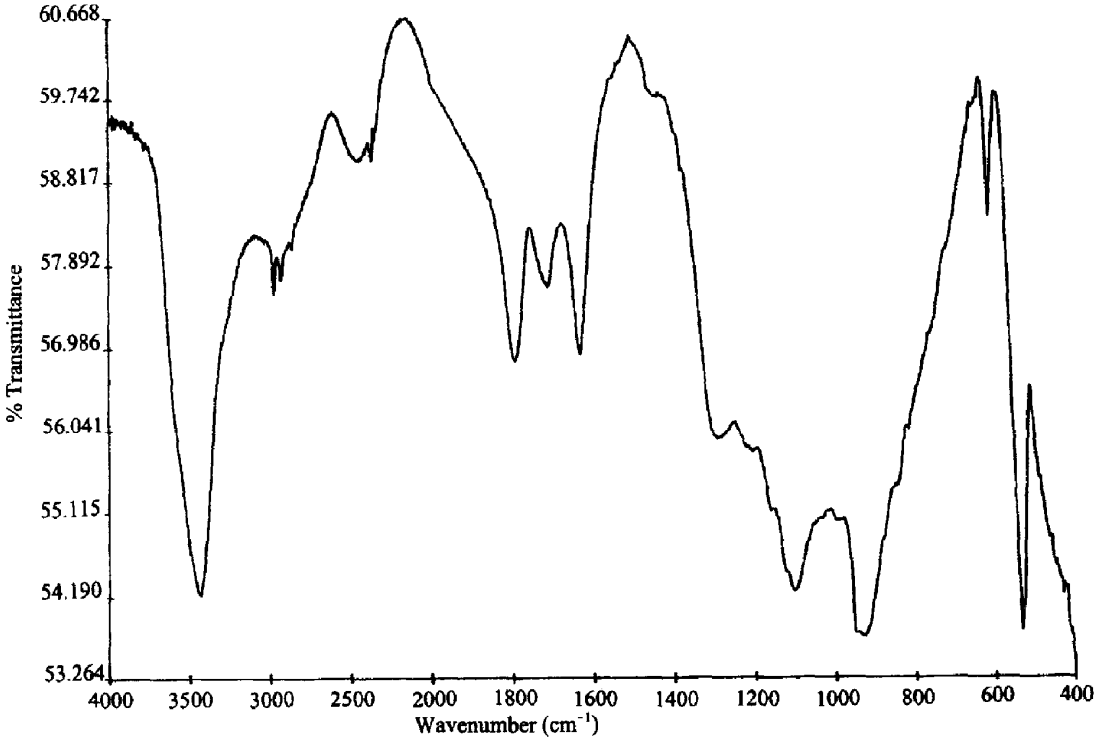

60.668
59.742
58.817
57.892
56.986
56.041
55.115
54.190
53.264
% Transmittance
4000 3500 3000 2500 2000 1800 1600 1400 1200 1000 800 600 400
Wavenumber (cm-1)

Fig. 3

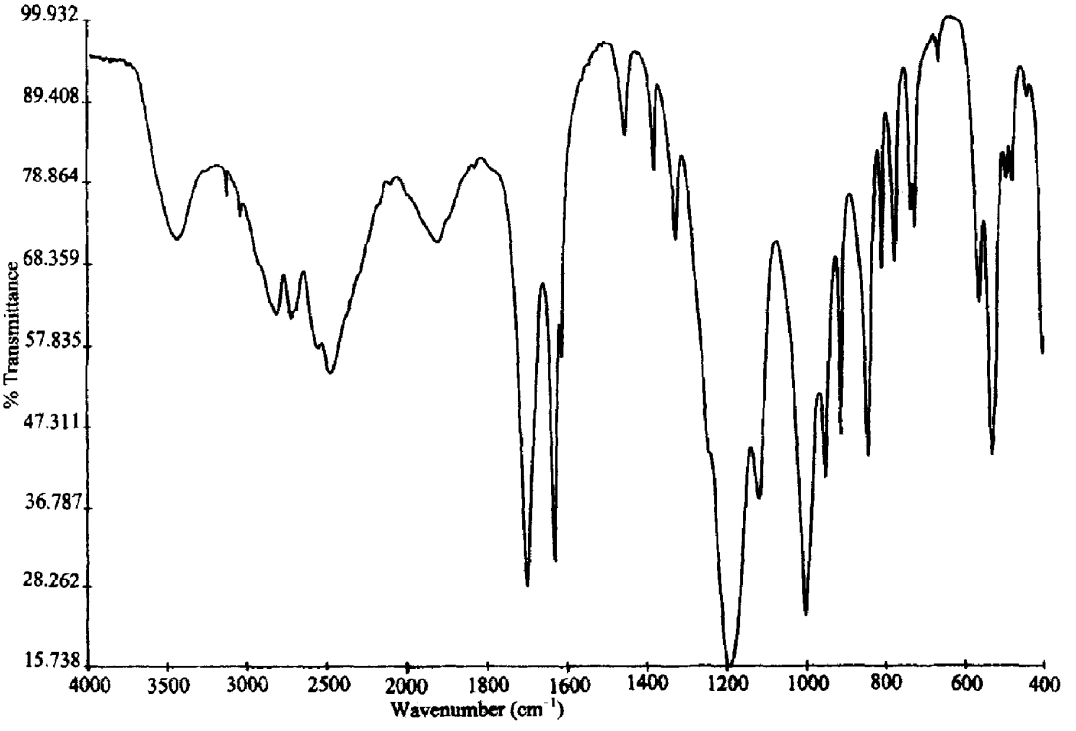
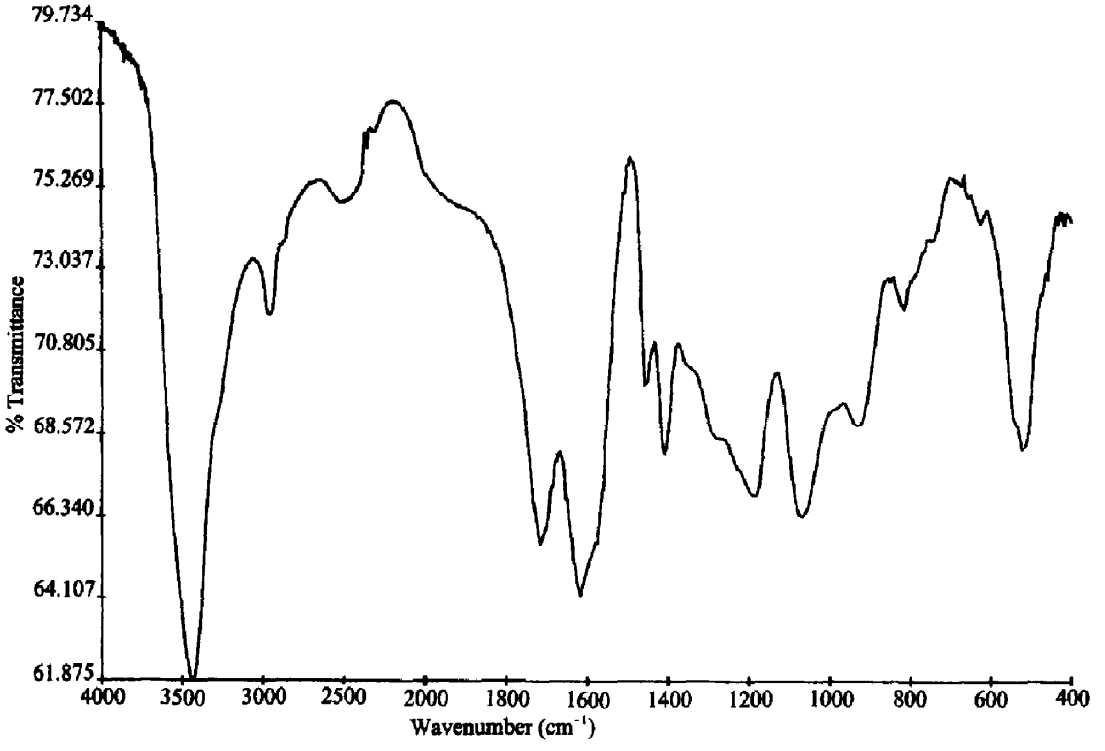

99.932
89.408
78.864
68.359
57.835
47.311
36.787
28.262
15.738
% Transmittance
4000 3500 3000 2500 2000 1800 1600 1400 1200 1000 800 600 400
Wavenumber (cm-1)

Fig. 4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77.502
75.269
73.037
70.805
68.572
66.340
64.107
61.875
% Transmittance
4000
3500
3000
2500
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm⁻¹)

Fig. 5

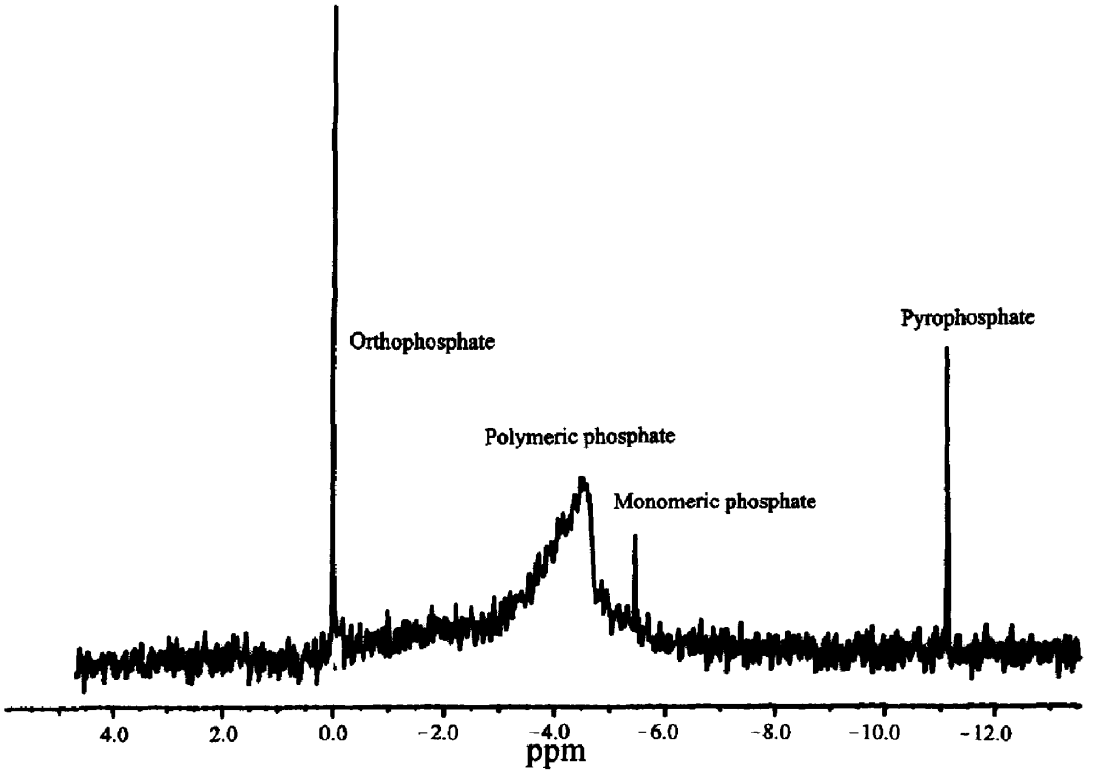
Orthophosphate
Polymeric phosphate
Monomeric phosphate
Pyrophosphate
4.0
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
ppm

Fig. 6

Table 2. Mean calcium demineralization rates (CDR) and % reduction in demineralization for each treatment

Treatment	Mean CDR ($\mu g/mm^2/hr$)	Mean % reduction in demineralization
Water	0.35 (0.04)	—
1% PEP (K Salt)	0.37 (0.03)	−12 (0.1)
1% Poly(PEP) (K Salt)	0.28 (0.01)	18 (7.1)[a]
1000 ppm F (as NaF)	0.18 (0.04)	51 (4.5)[a]

Figure in parentheses represent the standard deviation.
[a]Indicates a significant reduction in demineralization ($\alpha = 0.05$) compared with the water control.

Copolymerization of KPEP with acrylic acid

Copolymerization of a 50:50 weight ratio mixture of KPEP with partly neutralized acrylic acid gave a 100% conversion to a high molecular weight phosphate containing copolymer (Table 1, number **5**). The 1780 cm^{-1} lactone band was notably absent in its IR spectrum (Fig. 5).

^{31}P NMR (Fig. 6) provided structural and quantitative compositional information on the polymers. Peaks were assignable to polymer and inorganic orthophosphate species as well as monomeric KPEP and pyrophosphate. The molar ratios of these components are listed in Table 1. The copolymer with acrylic acid has the highest ratio of polymer derived organic phosphate to inorganic phosphate.

Anticaries studies

The mean calcium demineralization rates (CDR) and % reduction in demineralization for these materials were measured against water (control) and fluoride (positive control). These figures for each treatment are shown in Table 2. It is evident that fluoride (positive control) and polyphosphoenolpyruvate reduced the demineralization of enamel significantly ($\alpha = 0.05$) compared with the water control. The effect of the polyphosphoenolpyruvate was approximately one third of the fluoride activity.

It is proposed that polyphosphoenolpyruvate is able to interact with the cationic sites of the pellicle coated tooth surface. Once adsorbed, this creates a diffusion barrier to the loss of calcium and phosphate which are extracted during the acid dissolution process of the cycling treatment. The effect of fluoride in reducing enamel demineralization is due to the incorporation of fluoride into the hydroxyapatite of enamel forming fluorapatite, which is more resistant to acid dissolution, due to its lower solubility characteristics [8].

Since polyphosphoenolpyruvate and fluoride inhibit enamel demineralization by different mechanisms then it may be expected that a combination of these two agents could give rise to a synergistic effect. Interestingly, the monomer, phosphoenolpyruvate, did not inhibit enamel demineralization, but actually caused enhancement and this may be a reflection of the ability of this agent to extract calcium ions from the enamel surface.

CONCLUSIONS

Phosphoenolpyruvic acid was polymerized under mild conditions by employing an aqueous polymerization method and using a redox free radical initiator. Similarly phosphoenolpyruvate was copolymerized with acrylic acid. The anticaries study indicate that these polymers significantly reduce tooth enamel demineralization compared with a placebo (water). The activity ascribed to polyphosphoenolpyruvate was approximately one third of the fluoride activity. In contrast to the polymer, the monomer, phosphoenolpyruvate enhanced demineralization.

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