Influence of a diene impurity on the molecular structure of phosphate-containing polymers with medical applications

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We have demonstrated that the unacknowledged presence of almost 30% diene impurity in some commercial phosphate monomers had not only a significant effect on the molecular structure (topology) of a series of synthesized polymers but the instability of the ester functionalities during these polymerizations resulted in unexpectedly complex co-polymer chemistry.

Phosphate-containing polymers have found many useful applications as coatings and fire retardants but it is in the medical area that much recent attention has been focused, hence interest in the synthesis and bioactivity of polymers containing phosphate groups is increasing.[1] The biological significance of phosphorus functionalities has motivated incorporation into biomaterials for bone and cartilage tissue repair and regeneration. One of the major challenges facing the development of new polymeric materials for biomedical applications is compliance with the strict approval protocols that must be satisfied before materials are authorised by organisations such as the Therapeutic Goods Administration and the Federal Drug Administration. Controlled polymerization reactions are highly desirable because they lead to, not only control over the desired structure, but also to the high level of reproducibility essential for the commercial production of polymeric biomaterials and devices. Since one of the prime purposes of introducing phosphate groups into polymeric materials is to enhance calcification as a prerequisite for good bone-bonding, good characterization of such polymers is critical to optimizing their bioactivity.

Two commercially available phosphate-containing monomers, the acrylate monacryloxyethyl phosphate (MAEP) and the methacrylate 2-(methacryloyloxy)ethyl phosphate (MOEP) (Fig. 1),† have been the monomers of choice in many significant studies.[2–5] Previously, both conventional free-radical homo- and copolymerization using the phosphate monomers were found to lead to the formation of insoluble crosslinked networks.[6–8] This was ascribed either to the presence of small amounts of phosphate diene impurities (an *in situ* crosslinker formed through transesterification) (Fig. 1) or to excessive chain transfer to the polymer.[6,7] In our study on polymerization of MAEP and MOEP leading to crosslinked gels we were able to gain insight into the origin of the crosslinking.[8] Since soluble polymers resulted after basic hydrolysis of the ester groups in the side chains, we were able to conclude that most, if not all, crosslinking occurs through the side chains (rather than through the polymer back-bone). Furthermore, using the Reversible Addition Fragmentation Chain Transfer (RAFT) polymerization process to synthesize PMAEP and PMOEP we demonstrated that the degree of crosslinking was inversely proportional to the molecular weight, leading to the conclusion that the mechanism of gel formation is *via* diene impurities.[8]

In this communication we present results that highlight the effects of the presence of an unprecedented amount of this diene on the PMOEP topology. In addition, the propensity of the ester groups to undergo cleavage during the polymerization reactions, leads to highly complex co-polymer chemistry. We demonstrate how by using a suite of techniques (elemental analysis, NMR and FTIR-ATR) we have been able to characterize the polymers formed.

The chemical suppliers of MOEP and most research groups using this monomer apply analytical techniques (*i.e.* elemental analysis, ^{1}H NMR, FTIR) for assessment of monomer purity which, as we will demonstrate, in this particular case are unable to detect the impurities present. Only one article has previously referred to a high percentage of diene impurity in MOEP but neither the supplier nor method of analysis was specified.[5] The elemental analysis of MOEP (Table 1) is in excellent agreement with the theoretical values and the ^{1}H NMR spectrum (data not shown) exhibited the expected signals with the correct integrations as expected for pure monomer.

The ^{31}P NMR spectrum of MOEP (Fig. 2(A)) shows three peaks at 0.91, 0.07, −0.74 ppm which based on the H-coupled spectrum (Fig. 2(B)) can be assigned to free-phosphate H_3PO_4 (24%); MOEP (51%) and diene (25%), respectively.‡ The presence of phosphoric acid in the monomer was confirmed by chemical phosphorus analysis by ICP-AES (determination of total phosphorous) and by the phosphomolybdate method (determination of free phosphate only) yielding 28 ± 1% free phosphate (out of total phosphorus) correlating well with the ^{31}P NMR data. The near 1 : 1 ratio of the impurities results in the elemental analysis for the MOEP–diene–H_3PO_4 mixture being virtually identical to the theoretical one for MOEP. Titration, elemental analysis, or ^{1}H NMR cannot detect either the diene or the free phosphate. In addition, without the infrared data for the

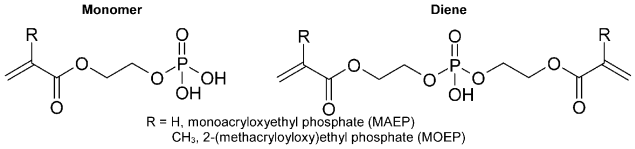

Fig. 1 Chemical structures of the monomer and diene.

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Table 1 Characterisation of MOEP and PMOEP

Sample experiment	Polymerization conditions[a]	%C	%H	%P[b]
MOEP		34.5	5.5	14.7
Sample I (soluble) PMOEP-RAFT[c]	CH_3OH 20 h @ 60 °C	38.3	6.0	9.9
Sample II (soluble) PMOEP-γ-aq.[d]	H_2O–CH_2Cl_2 3.75 h @ RT	32.4	6.1	15.2
Sample III (crosslinked) PMOEP-γ-org.[d]	H_2O–CH_2Cl_2 3.75 h @ RT	42.1	6.3	7.8
Sample IV (crosslinked) PMOEP-AIBN[e]	CH_3OH 3 h @ 60 °C	37.7	5.9	11.2
Theoretical:				
MOEP		34.3	5.3	14.8
MOEP–diene–H_3PO_4[f]		34.4	5.3	14.7
MOEP–diene[g]		38.8	5.6	12.5
Diene		44.9	6.0	9.3
Sample I[h]		40.4	5.8	11.2
Sample II[i]		35.4	5.4	13.9

[a] Solvent, time and temperature. [b] Obtained from ICP-AES. [c] [MOEP] = 0.96 mol L^{-1}, RAFT agent = cumyl dithiobenzoate (CDB), [RAFT] = 1×10^{-2} mol L^{-1}, [AIBN] = 2×10^{-3} mol L^{-1}, conversion = 74%. [d] [MOEP] = 0.48 mol L^{-1}, dose rate = 2.7 kGy h. [e] [MOEP] = 0.96 mol L^{-1}, [AIBN] = 2×10^{-3} mol L^{-1}, conversion = 96%. [f] Theoretical values calculated from the following ratios obtained from NMR, monomer : diene : H_3PO_4 = 50.8 : 24.9 : 24.2 (%). [g] Theoretical values calculated from the following ratios obtained from NMR, monomer : diene = 65.8 : 34.2 (%). [h] Theoretical values calculated from the following ratios obtained from NMR, PMOEP : PHEMA : P(diene) = 56.3 : 19.8 : 21.1 : 2.7 (%). [i] Theoretical values calculated from the following ratios obtained from NMR, PMOEP : PHEMA = 91.6 : 8.4 (%).

pure monomer this technique cannot be used to assess purity. These inadequacies have led to misleading information on supplier product web sites regarding purity and solubility.§

A selection of PMOEP soluble and crosslinked polymers from our studies (Table 1) were chosen for detailed analysis. The soluble polymers were prepared either by RAFT polymerisation in methanol (sample I)[8] or by gamma-induced polymerization in the aqueous phase (sample II) of a two-phase system (produced either from 1 : 1 water–CH_2Cl_2 or 1 : 1 : 1 water–methanol–CH_2Cl_2)[9] and the crosslinked polymers were produced either by gamma-induced polymerization in the organic phase (sample III) of the two-phase system or by AIBN initiated polymerization in methanol (sample IV).[8]

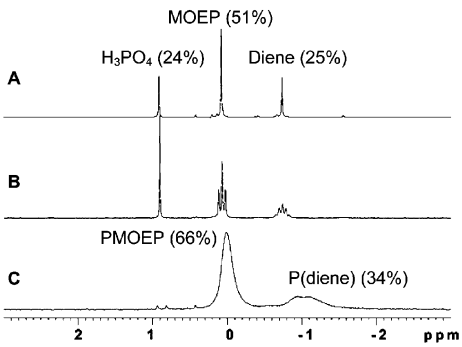

Fig. 2 ^{31}P NMR spectra of MOEP: (A) H-decoupled and (B) H-coupled, and sample I (C) H-decoupled, all in methanol-d_4.

The ^{31}P NMR spectrum of the soluble PMOEP (sample I) displays two bands, with the broad band at −1 ppm attributed to diene moieties incorporated into the polymer ("P(diene)", Fig. 2(C)), in a statistical amount. The use of a chain transfer agent (CTA) to suppress gel formation in the presence of a diene has been known since 1948, but Sherrington and co-workers were the first to use this approach to produce soluble branched polymers.[10] The successful use of a RAFT agent as the CTA was demonstrated by Perrier and co-workers.[11] In accordance with Sherrington's results this approach realised branched structures when a ratio of CTA to diene (brancher) of 1 or more was used. In our MOEP system, the CTA : diene ratio is much lower than 1 (1 : 25) and it is therefore more likely that the topology of sample I is closer to a nano- or micro-gel. Wang and Zhu[12] and Li and Armes[13] have independently reported using dienes with cleavable linkages to produce hydrolysed polymers with narrow PDIs and living character. The fact that our soluble PMOEP (sample I) showed a high PDI even after hydrolysis of the side chains is further evidence that a simple branched structure has not formed. The ^{31}P NMR spectrum for sample II (data not shown) shows only a single broad phosphorus peak indicating that no diene moieties are incorporated into this polymer. This is a particularly significant result since it provides a synthetic method for the production of linear PMOEP and suggests, that as a result of diene and diene-containing oligomer solubilities, they are partitioned into the organic phase in the two-phase system.

The degree of crosslinking in the PMOEP samples is reflected in the IR spectra (Fig. 3) as a consequence of the phosphate group's ability to participate in formation of strong hydrogen bonding interactions.[14] In sample II the P–OH band at ~976 cm^{-1} is broad and complex compared to the much sharper band at 980 cm^{-1} for sample III. Thus there is a correlation between the degree of crosslinking (resulting in the OH groups being less available for hydrogen bonding) and the broadness of the P–OH band. Hydrogen bonding effects are also evident in the C=O stretching region where the band at ~1723 cm^{-1} is much sharper for the crosslinked PMOEP (sample III) compared to either samples I or II. The relative intensities of the P–OH (976 cm^{-1}) and P–OC (1057 cm^{-1}) bands reflect the amount of diene in the samples indicating samples I and III contain similar amounts. The relative intensity ratio of the P–OH and CH_2 (1453 cm^{-1}) bands reflects the amount of phosphorus in the samples in agreement with %P obtained by elemental analysis (Table 1).

The ^1H NMR spectra of samples I and II display the expected resonances based on PMOEP (Fig. 4). However, distinct differences are observed for the two polymers: sample I displays resonances corresponding to unreacted unsaturated bonds originating from diene incorporation, while no such resonances are observed for sample II. This is in agreement with the ^{31}P NMR data. Both polymers display broad bands at 3.7–3.8 ppm indicating the presence of PHEMA. Since the amounts of PHEMA in these two polymers are different and correlate both with polymerization time and temperature, its presence is attributed to the acid catalysed hydrolysis of the phosphate esters. For both polymers, no cleavage of the side-chain at the C–O–C ester linkage appears to be occurring based on the ratio of $CH_2C(CH_3)$ of the backbone to the

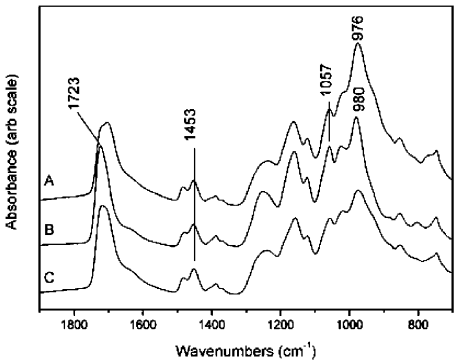

Fig. 3 FTIR-ATR spectra of PMOEP samples: (A) sample II, (B) sample III and (C) sample I. The 1800–800 cm^{-1} region of interest has been scaled to the CH_2 backbone band at 1453 cm^{-1}.

CH_2CH_2 of the side-chain. Based on the integrals of the assigned peaks in the ^1H NMR spectra a full description of the polymer chemistry can be obtained (Fig. 4).

The elemental analyses data (Table 1) reveal that vastly different amounts of phosphorus are incorporated into the different polymers. This stems from differences in both the amount of diene copolymerization and the extent of ester cleavage occurring. The elemental analyses data also strongly supports our NMR analysis of polymers I and II. Where the two crosslinked polymers III and IV are concerned it is clear that

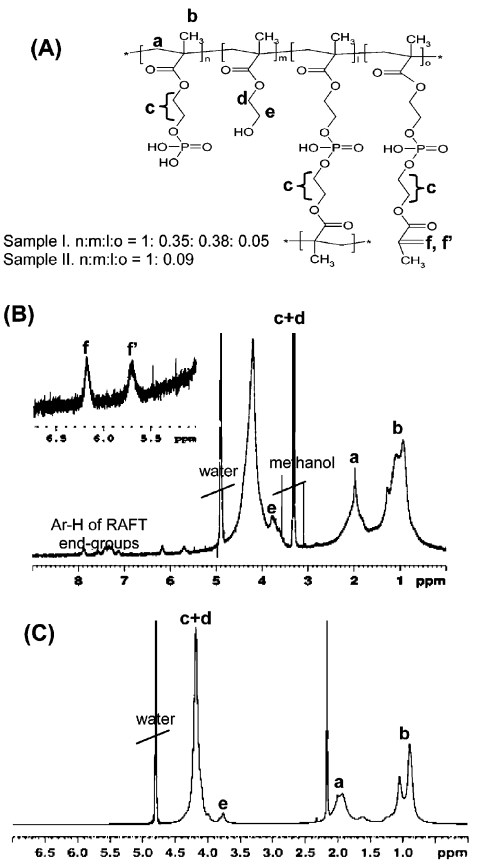

Fig. 4 Proposed structure (A) and ^1H NMR spectra of PMOEP samples in Table 1 (ratios calculated from ^1H and ^{31}P NMR) for (B) sample I in methanol-d_4 and (C) sample II in D_2O.

they contain large amounts of diene involved in the crosslinking as evident from the high C%. In particular, significant amounts of PHEMA and/or PMAc moieties are incorporated into sample III as can be seen from the very low P%.

As part of our strategy for using surface modification to improve the surface bioactivity of commercially available polytetrafluoroethylene (PTFE) membranes for cranio-facial reconstruction, we used γ-irradiation to make a series of PTFE-g-PMOEP and PTFE-g-PMAEP membranes.[9,15] Based on the findings presented here, and extrapolating from the homopolymer data, we now have a better understanding of the structure of the graft-copolymers produced previously as well as those produced in the two-phase system. Hence, we are better able to explain our earlier *in vitro* mineralisation results as affected by polymer topology (molecular structure) and composition. These results are currently in preparation for publication.

The full characterization of synthetic polymers is always of great importance, not least when these polymers are destined for tissue repair applications. This study has demonstrated that only through the use of a suite of analytical techniques is it possible to discover large amounts of a diene and phosphoric acid impurities in commercial MOEP and MAEP monomers. This previously unacknowledged high level of impurity in starting monomers was shown to further complicate both the synthesis and characterization of these polymers. Despite this fact, soluble polymers were successfully synthesized using either a CTA or a two-phase solvent system, with the latter method allowing synthesis of linear polymers.

Notes and references

† As correctly pointed out in Chirila's review,[1] various names and acronyms for these two monomers are found in the literature. For the sake of consistency and because it is the one most commonly used, we will continue to use MAEP and MOEP (the latter sold commercially as ethylene glycol methacrylate phosphate, EGMP).

‡ A similar ^{31}P NMR spectrum was obtained for the monomer MAEP.

§ Currently, no information regarding MOEP purity is available on supplier websites, although the percentage purity for commercially available MAEP is indicated as being ~97%.

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