J Mater Sci: Mater Med (2007) 18:1141–1149
DOI 10.1007/s10856-007-0135-0

Preferential interactions of calcium ions in poly(2-hydroxyethyl methacrylate) hydrogels

Zainuddin · D. J. T. Hill · A. K. Whittaker · L. Lambert · T. V. Chirila

Received: 10 August 2005 / Accepted: 14 March 2006 / Published online: 1 February 2007

Abstract An investigation of the preferential interaction of calcium ions with oxygen atoms in poly (2-hydroxyethyl methacrylate) (PHEMA)-based hydrogels has been carried out. The formation of polymer–Ca complexes was achieved by exposing powdered or fully hydrated samples with 5 mM, 0.1–0.5 M, or saturated $CaCl_2$ solutions for certain periods of time. The characteristics of the polymer–Ca complexes were deduced from the effect of the solute on the equilibrium water content, and from NMR, atomic absorption and infrared spectroscopies. The absence of significant changes in the NMR chemical shift and infrared vibrational wavenumbers for the various functional groups confirmed that polymer complexation with Ca^{2+} ions involves only weak interactions, possibly electrostatic or ion–dipole interactions. Among the three types of oxygen atoms in PHEMA, hydroxyl oxygen atoms seem to be the most sensitive to the presence of Ca^{2+} ions. Complexation at the ester oxygen atoms was also evidenced by a new band in the infrared spectra at 1,550 cm^{-1}. On the other hand, there were no indications that the hydrophobic domains in the backbone and the methyl groups at the side chain of PHEMA interact significantly with Ca^{2+} ions.

Zainuddin (✉) · D. J. T. Hill
Department of Chemistry, The University of Queensland, Brisbane, QLD 4072, Australia
e-mail: z.zainuddin@uq.edu.au

A. K. Whittaker · L. Lambert
Centre for Magnetic Resonance, The University of Queensland, Brisbane, QLD 4072, Australia

T. V. Chirila
Queensland Eye Institute, 41 Annerley Road, South Brisbane, QLD 4101, Australia

Introduction

In a previous study [1], it was found that Ca^{2+} ions not only diffuse through poly(2-hydroxyethyl methacrylate) (PHEMA) hydrogel networks, they also interact with the polymer, leading to heterogeneous nucleation of precipitation of sparingly-soluble calcium phosphate (CaP) salts. CaP salts, particularly hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], are well known as the major component of bone and teeth. Investigation of the nucleation of calcium deposits within polymers is important in modeling the biomineralization processes, the development of novel biomaterial composites, and the study of mechanism and prevention of unwanted CaP deposition on implanted biomaterials.

Previous studies have shown that Ca^{2+} ions have an overwhelming preference to interact with oxygen atoms [2, 3], although such interactions may also take place with the hydrophobic groups or domains of the polymer. Most experimental studies on polymer complexation with Ca^{2+} ions have been performed in systems where there is a strong interaction between the Ca^{2+} ions and hydroxyl or carboxylic groups. Under these conditions, the polymer interaction with the Ca^{2+} ions can be sufficiently determined by analyzing infrared spectral or zeta potential data. For example, from analysis of infrared spectra, Elvira and Roman [4] revealed that the formation of stable Ca–polymer

complexes in polyacrylates containing aminosalicylic acid was due to ionic interactions between Ca^{2+} ions and hydroxylic or carboxylic groups. As a result of these interactions the polymer–calcium matrix became stiffer. Similarly, by measuring the zeta potential of the polymer particles in solution, Lubeck et al. [5] found that base-hydrolyzed PMMA formed stronger complexes than unmodified PMMA at high calcium concentration.

However, where the interactions between polymer chains and ions are weak, little is known about the site of interaction of Ca^{2+} ions with the different types of oxygen atoms in the polymer. In order to expand this understanding, we have investigated the complexation of Ca^{2+} ions with PHEMA hydrogels. The choice of PHEMA hydrogels was made because of their extensive use as biomaterials. Even though these hydrogels interact only weakly with Ca^{2+} ions, they have been reported to undergo extensive calcification when they are brought into contact with body fluids [6–9]. Additionally, Imai and Watanabe [10] observed an interesting phenomenon during the implantation of poly(methyl methacrylate) (PMMA) copolymers containing HEMA or *N*-vinylpyrrolidone (NVP) in rats. They found that the presence of NVP in the copolymers, P(MMA/NVP), did not promote calcification. On the other hand, the introduction of HEMA in the copolymers, P(MMA/HEMA), enhanced the amount of calcium deposited on the copolymers. It thus appears that Ca^{2+} ions selectively interact with specific sites in these polymers.

It has been known that NMR spectroscopy provides a powerful tool for detection of very specific motions and interactions of the polymer chains, and thus it has been utilized in the present work for the analysis of the interaction between Ca^{2+} ions and PHEMA hydrogels. Other techniques also utilized were flame atomic absorption (AA) and infrared spectroscopy and solute effect measurements. These techniques provided complementary results supporting the information obtained from solution and solid-state NMR measurements.

Experimental procedure

Synthesis of PHEMA-based hydrogels

2-Hydroxyethyl methacrylate (HEMA), stabilized with 300 ppm monomethyl ether hydroquinone (MEH), ethyl methacrylate (EMA), stabilized with 15 ppm MEH, styrene (St), stabilized with 10–15 ppm 4-*tert*-butyl catechol, and 1-vinyl-2-pyrrolidone (VP), stabilized with 0.01% NaOH, were all obtained from Sigma-Aldrich and purified by vacuum distillation (2–5 mmHg).

HEMA, or HEMA together with a comonomer, was mixed with initiator (dibenzoyl peroxide, 0.05 M) in a standard flask, and the resulting solution was poured into a silicone tube (silicone was used to allow easy removal of polymerized cylinders) and placed into a glass container that could be evacuated. After the container was purged with nitrogen for 1 h, it was gently evacuated to a pressure of 2–5 mmHg. Polymerization was carried out in a vacuum oven (150 mmHg) for 20 h at 50°C, followed by a post-cure treatment for 2 h at 80°C. This led to complete conversion of the monomer, as indicated by the loss of the NIR peak at 6,170 cm^{-1} which is characteristic of the monomer double bonds. After preparation, the cylinders were cut to 2.5 cm length and soaked in Millipore water to equilibrium swelling at 25°C. The EWC of the hydrogel cylinders was calculated from the weight of the hydrated sample at equilibrium and the weight of the dry polymer.

Solute effect measurements

After equilibration of the PHEMA hydrogel cylinders in Millipore water at 25°C, the samples were transferred to a 50 mM $CaCl_2$ solution. A relatively high concentration of calcium was used so as to provide a more marked change in the water content [11]. In this experiment, the change in weight was monitored during the salting out process.

Solution and solid-state NMR measurements

For the solution-state NMR measurements, a soluble sample of PHEMA (granules), purchased from Scientific Polymer Products Inc. with an average molecular weight of 300 kDa was ground to a fine powder and mixed homogeneously with anhydrous $CaCl_2$ in different proportions. The mixtures were transferred to glass tubes and then connected to a vacuum line at a pressure of ca. 10^{-2} Pa for 5 days. One gram of deuterated dimethylsulfoxide (DMSO-d_6) was introduced into each sample under nitrogen (in a glove box). Once the samples were completely dissolved, the glass tubes were again evacuated to ca. 10^{-2} Pa while the samples were frozen with dry ice for 4 days. The solutions were then transferred into NMR tubes and the lids were sealed with paraffin film under nitrogen. All samples were stored in a desiccator at room temperature prior to measurements.

For the ^{13}C solid-state NMR measurements, fully-hydrated PHEMA hydrogel cylinders were powdered using a freezer mill and then maintained in a 5 mM $CaCl_2$ solution for 3 days. Solid-state NMR measurements were made using a Bruker MSL300 spectrometer operating at 75.48 MHz. Samples were spun at the magic angle 54.7° from 2 to 3 kHz in 4 mm zirconium oxide rotors. Spectra were collected using a single pulse excitation sequence with high-power proton decoupling. The ^{13}C 90° pulse time was 4 μs, and the recycle delay was 5 s. Chemical shifts were referenced to adamantane as an external reference.

^{1}H and ^{13}C solution-state NMR spectra were obtained on either a Bruker AMX 400 or a Bruker DRX500 MHz spectrometer operating at 400.13/500.13 MHz for ^{1}H and 100.62/125.77 MHz for ^{13}C, respectively. Spectra were recorded using a 5 mm triple resonance z-gradient probe. Both ^{1}H and ^{13}C NMR spectra were acquired using a 45° and 90° pulse with a pulse time of 5 μs and 11.4 μs, and a recycle delay of 1 s and 10 s, respectively.

^{1}H T_1 relaxation times were measured on a Bruker DRX500 MHz spectrometer. The presence of water, even in very small quantities, in PHEMA solutions will result in chemical exchange between the PHEMA hydroxyl protons and water protons. Consequently, the intrinsic value of the ^{1}H T_1 relaxation time of hydroxyl protons will be shorter than the measured T_1 value, since the value of the ^{1}H T_1 for water protons will be longer than the T_1 of the hydroxyl protons. The observed T_1 of the hydroxyl protons is a weighted average of the T_1 values of the exchanging proton species. The T_1 for hydroxyl protons was measured with and without saturation of the water proton peak with a RF pulse with a power of 50–60 dB. Both T_1 values for hydroxyl protons under saturated and unsaturated water peaks were measured by the inversion recovery method with 8 scans and 4 dummy scans with a relaxation delay of 14 s.

The ^{1}H NMR spectrum of the hydroxyl group of HEMA monomer is a triplet. However, these separated lines are transformed into overlapping lines in the polymer (PHEMA) system due to its higher molecular weight and lower chain mobility, which leads to a short ^{1}H T_2 relaxation time. The two outer lines of the triplet arise from J-coupling and are expected to disappear if the two protons of the methylene group next to the hydroxyl groups are decoupled. In this experiment, J-homodecoupling was performed by irradiation of the methylene peak with a RF pulse with power of 45 dB. The ^{1}H NMR spectra were acquired using 32 scans and 2 dummy scans.

Measurement of calcium concentrations by AA

After equilibration of the hydrogels in Millipore water at 25°C, they were maintained in a 5 mM $CaCl_2$ solution at 25°C for approximately one month. The amount of calcium accumulated in the hydrogels was determined using an AA spectrometer in two different procedures: (i) by measuring the calcium concentration in the $CaCl_2$ solution before and after calcification, and (ii) by leaching out the calcium from the hydrogels using 1 M HCl and then subjecting the solution to AA measurement. Both results were in good agreement. A calibration curve for Ca^{2+} ions was constructed over a concentration range of 2.5–20 ppm using a standard calcium chloride solution.

FT-IR measurements

Powdered polymer samples were exposed to a saturated calcium chloride solution for five days at room temperature, filtered and rinsed 5 times with Millipore water. The samples were then dried and diluted with KBr (1:50). The appropriate amount of this mixture was placed in a special die and a disk was pressed using approximately 10^4 kg pressure. The spectra were acquired in the region of 400–4,000 cm^{-1} using a Perkin-Elmer FTIR 1600 spectrometer at a resolution of 8 cm^{-1} and in 64 scans. The background for the KBr disk measurements was corrected using data for pure KBr.

Results and discussion

Solute effect

In a previous study [11] it was demonstrated that when dried PHEMA polymers were incubated for 2 weeks in solutions of $CaCl_2$ and Na_3PO_4, the degree of hydration or swelling of the hydrogel decreased with increasing solute concentration. This salting out effect is believed to contribute significantly to the spontaneous calcification of the hydrogels, due to local supersaturation of the calcium solution within the hydrogel matrix. It was suggested that the solute ions can promote the formation of physical crosslinks between two neighboring hydrophilic (polar) and hydrophobic (non-polar) groups of the polymer chains, thus tightening the network structure. As a result, a portion of the water is removed which is manifested globally as deswelling of the hydrogels. Based on this phenomenon, by changing the relative proportions of hydrophilic–hydrophobic groups in the polymer, evidence

for a preferential interaction of Ca^{2+} ions with specific sites in the polymer may be envisaged.

Figure 1 shows the effect of solute on the depletion of water in a series of PHEMA-based hydrogels. It can be seen that the rate of deswelling for the PHEMA-based hydrogels decreases with time in the following order: PHEMA > P(HEMA-co-EMA) > P(HEMA-co-St) > P(HEMA-co-NVP). This result suggests that the presence of Ca^{2+} ions in solution has the strongest affect on the pure PHEMA hydrogel, while P(HEMA-co-NVP) is the least susceptible to deswelling. PHEMA contains the largest proportion of oxygen atoms of the hydrogels studied, and therefore it may be expected to interact most strongly with the calcium ions. This effect of reduced deswelling with decreasing oxygen content is obeyed across the series of materials examined. However, in the P(HEMA-co-St) hydrogels, although the proportions of all three oxygen atoms in the copolymers have been reduced, the rate of deswelling in the presence of Ca^{2+} ions was not further reduced, and is even slightly higher than that of P(HEMA-co-NVP). This may be attributed to a balance between the effects of reducing the oxygen atom content and increasing the hydrophobicity of the polymer. Coleman et al. [12] reported that hydrophobicity has a propensity to induce interactions with Ca^{2+} ions due to the presence of negatively charged interface.

NMR analysis of the interaction of Ca^{2+} ion with the polymers

To assess in more detail the interaction of Ca^{2+} ions with the hydrogels, the ^{13}C NMR solution spectra of PHEMA in DMSO-d_6 in the presence of small amounts of added Ca^{2+} ions have been acquired (see Fig. 2). It can be seen in the expanded region of the spectra that upon the addition of Ca^{2+} ions there is a small increase in the chemical shift for the carbonyl carbon (4), and a small decrease for the methylene carbon (6) adjacent to the hydroxyl groups. These small changes suggest that the Ca^{2+} ions interact with the carbonyl and hydroxyl oxygen atoms of the polymer, most probably via electrostatic or ion–dipole interactions. Furthermore, as a result of these interactions, the local mobility of the polymer chains are restricted, leading to slight broadening of the peaks due to carbons 2, 4, 5 and 6 (see expanded areas). The ^{13}C MAS NMR solid-state spectra of the fully hydrated PHEMA (EWC ~34%) hydrogels (Fig. 3) show a similar trend in line broadening, particularly for carbon 4 and 6, although there is no observable change in the chemical shift in the presence of Ca^{2+} ions, likely due to the larger natural line widths in the solid state. The larger line width in solid-state ^{13}C NMR spectra is largely due to the presence of unaveraged chain conformations in the solid state [13] and thus it appears that the Ca^{2+} ions hold immobile a range of conformations. On the other hand, the increase in chemical shift for the carbonyl carbon (peak 4) in solution may be attributed to a change in the hydrogen bond environment of the carbonyl group due to the interaction between Ca^{2+} ions and the carbonyl oxygen of PHEMA. Hill et al. [14] in their study of intermolecular hydrogen bonding in a series of miscible polymer blends have found a correlation between the observed changes in carbonyl carbon chemical shift with changes in the hydrogen bond environment of the carbonyl group, as determined by NMR and IR spectroscopy.

The change in ^{13}C chemical shift of the methylene group adjacent to the hydroxyl group of the PHEMA side chain suggests preferential interaction of the calcium ion with the hydroxyl group. It is therefore expected that changes in the hydroxyl region of the ^{1}H NMR spectra of the PHEMA solutions will be observed. In addition, the presence of Ca^{2+} ions may also be expected to affect the line shape and T_1 relaxation time of the hydroxyl protons. From Fig. 4, which shows the ^{1}H NMR spectra of pure PHEMA and PHEMA in the presence of 0.2 M $CaCl_2$, we observe that the peaks due to hydroxyl and water protons are shifted to higher chemical shift values, while the other peaks remained almost unaffected. An increase change in linewidth on addition of the calcium salt may indicate a change in dynamics of the polymer chain. The effect of increasing concentration of Ca^{2+} ions in solution on the chemical shift of the hydroxyl

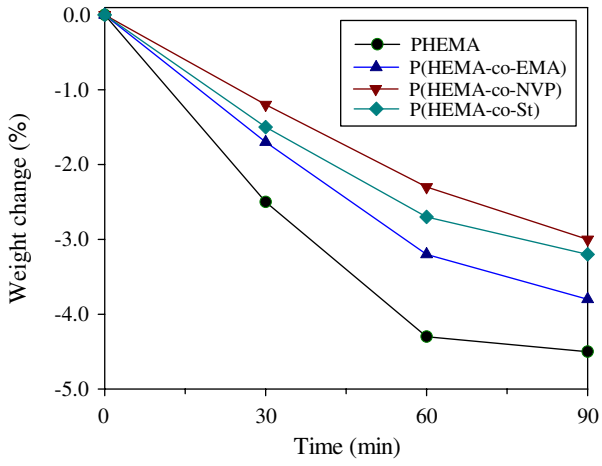

Fig. 1 Weight changes in the PHEMA-based hydrogels due to salting out effect of the 50 mM $CaCl_2$ solution at room temperature (25°C). The comonomer content was 10 mol%. Error in % weight changes is less than 1%

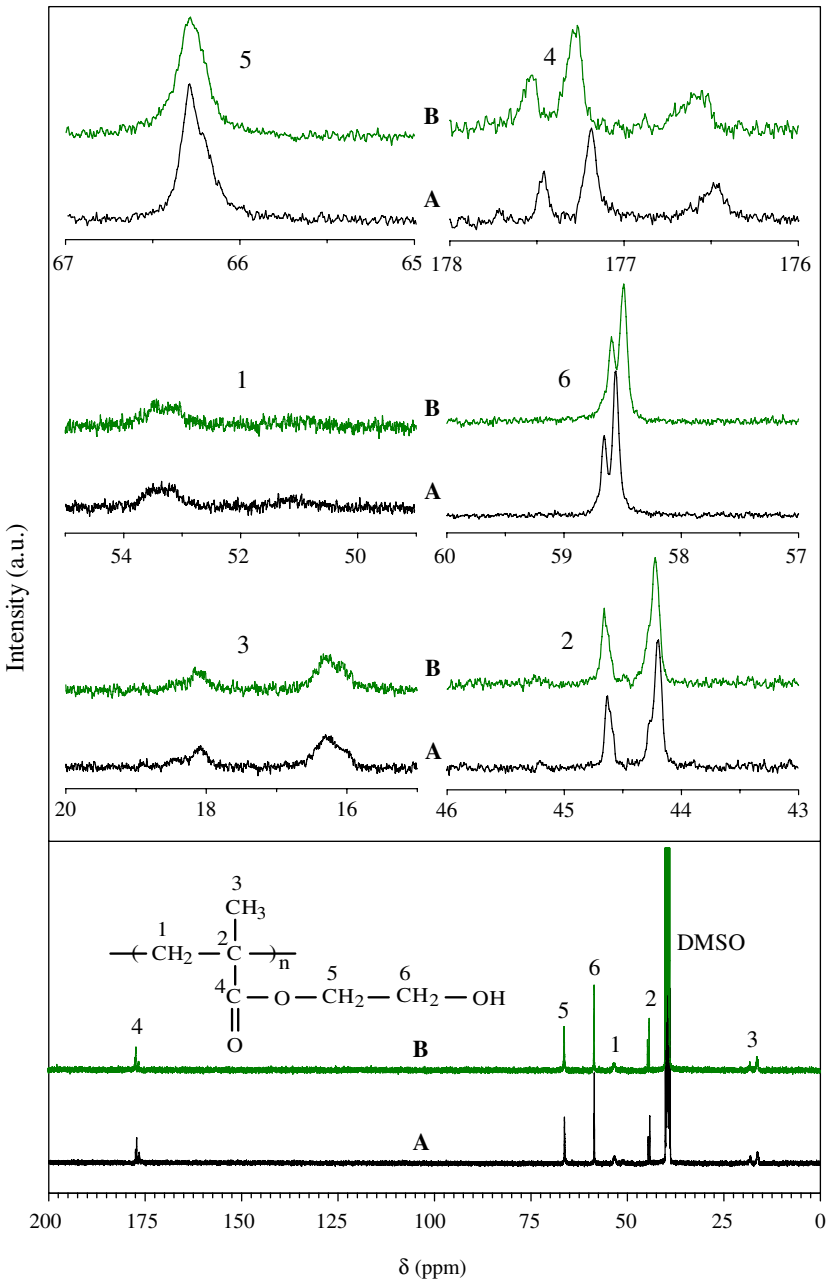

Fig. 2 ^{13}C NMR spectra of 300 kDa PHEMA (**A**) and PHEMA + 0.2 M $CaCl_2$ (**B**) in DMSO-d_6 solutions measured at room temperature. Expansions of the spectra are also presented

peaks is also evident in Fig. 5. The increase in chemical shift must largely be attributed to interactions of the hydroxyl oxygen and Ca^{2+} ions. While it was not possible to completely remove all water from the PHEMA solutions, and hence the effects of chemical exchange between the hydroxyl and the water protons cannot be eliminated, a higher probability of rapid chemical exchange of the hydroxyl protons would be expected to lead to a *decrease* in the chemical shift of the hydroxyl peak to bring it closer to the water peak. In fact there is an increase in the chemical shift of both peaks with increasing calcium concentration.

A close observation of the hydroxyl peaks in Fig. 5 reveals that upon increasing the calcium concentration there is a gradual change in the line shape. There are at least three overlapping lines under the hydroxyl peak of PHEMA. The three lines are most clearly resolved in the spectrum of the 0.2 M Ca^{2+} solution (Fig. 5C). The two lines that appear as shoulders are due to J-coupling with the methylene proton next to the hydroxyl group. However, in all spectra the hydroxyl peak is asymmetric, even after removing J-coupling (Fig. 6). This is a strong indication for the presence of a number of distinct environments of the hydroxyl

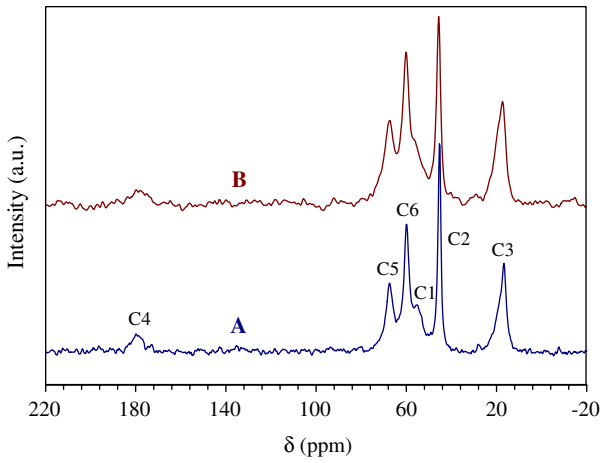

Fig. 3 CPMAS ^{13}C NMR spectra of fully hydrated PHEMA (**A**) and PHEMA + 0.2 M $CaCl_2$ solution (**B**) measured at room temperature

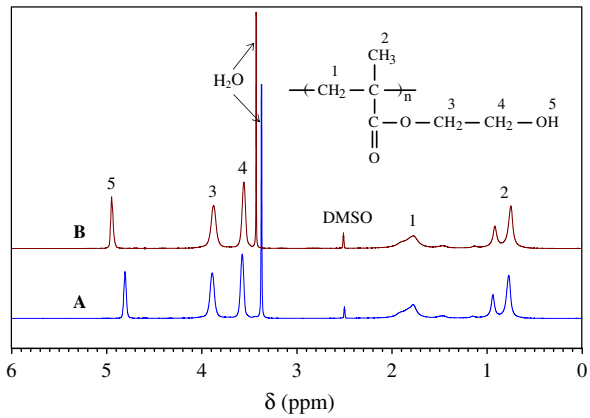

Fig. 4 Effect of Ca^{2+} ions on the ^1H NMR spectrum of PHEMA (300 kDa) in DMSO-d_6 solution at room temperature. (**A**) Without $CaCl_2$ and (**B**) 0.2 M $CaCl_2$

protons, in which the local environment is sufficiently altered so different isotropic shifts are observed. The peaks additional to the main triplet may have different coupling constants from the main peak, due to the possibility of exchange averaging of the J-coupling [15]. In such self-decoupling the J-coupling constant of the hydroxyl proton is reduced by chemical exchange with the uncoupled protons on water molecules.

A clearer picture of the effect of calcium on the chemical shift of the hydroxyl peak can be obtained by homonuclear decoupling of the adjacent methylene protons. As shown in Fig. 6A, irradiation of the methylene proton peak produces a symmetrical hydroxyl peak, with some evidence of an underlying signal. The effect of addition of Ca^{2+} ions is clearly demonstrated in Fig. 6B–D where a systematic change in the line shape of the hydroxyl peak with increasing the

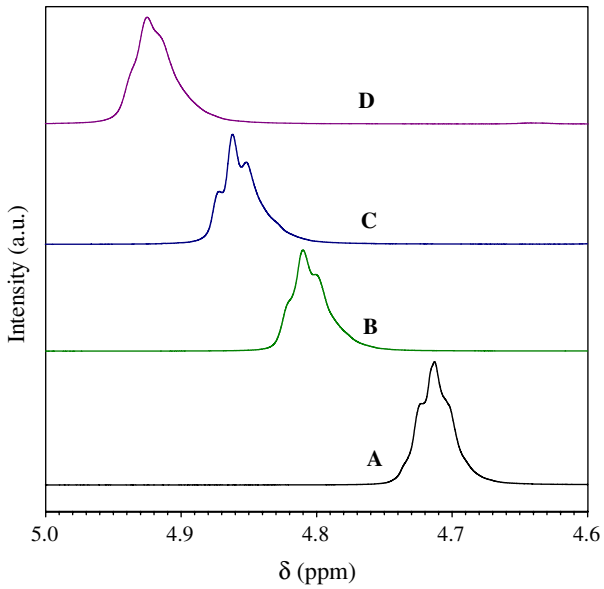

Fig. 5 Effect of Ca^{2+} ions on the hydroxyl peak of PHEMA (300 kDa) in DMSO-d_6 solution at 320 K. (**A**) Without $CaCl_2$, (**B**) 0.1, (**C**) 0.2 and (**D**) 0.5 M $CaCl_2$

concentration of Ca^{2+} ions is observed. As the amount of calcium ions increases there is a progressive increase in chemical shift, and the appearance of additional peaks, which taken together indicate interactions between the Ca^{2+} ions and the hydroxyl group of PHEMA.

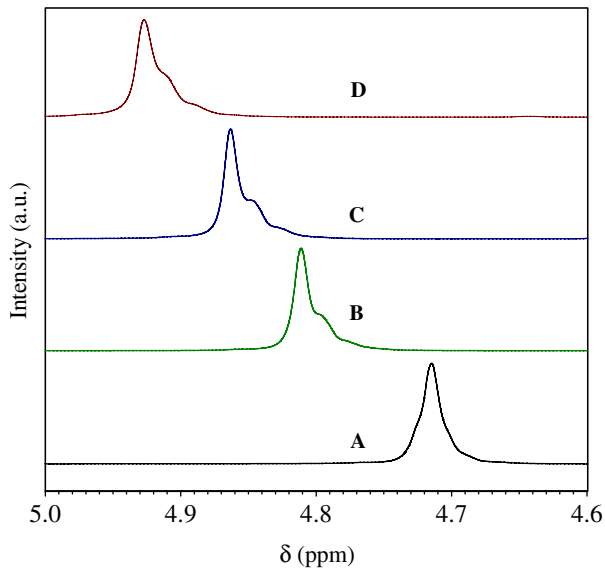

Fig. 6 Changes in hydroxyl peaks of PHEMA (300 kDa) in DMSO-d_6 solution in the presence of Ca^{2+} ions during the homodecoupling of hydroxyl-methylene protons (CH_2 protons next to hydroxyl group) at 320 K. (**A**) Without $CaCl_2$, (**B**) 0.1, (**C**) 0.2 and (**D**). 0.5 M $CaCl_2$

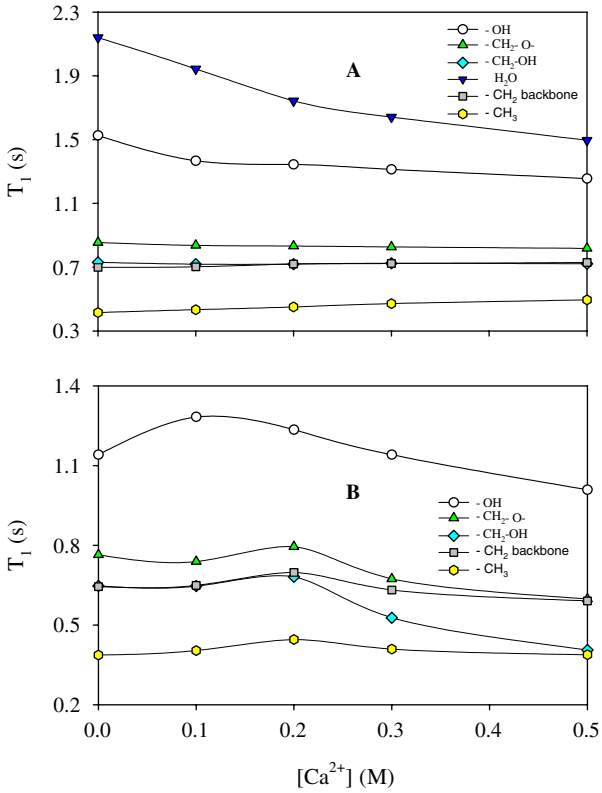

Fig. 7 ^{1}H T_1 relaxation times for PHEMA (300 kDa) in DMSO-d_6 solution at various [Ca^{2+}] without (**A**) and with (**B**) irradiation of water peak at 320 K

In addition, changes in the ^{1}H T_1 relaxation times provide evidence for an interaction between Ca^{2+} ions and the ester oxygen atoms of the PHEMA. This can be through Fig. 7B, where a significant change in the T_1 of the methylene proton next to the ester oxygen is observed when the concentration of Ca^{2+} ions was increased. It should be noted here that a change in T_1 for the methylene proton in the presence of the Ca^{2+} ion could only be observed when the water peak was saturated. Furthermore, the T_1 values for the backbone and methyl protons are relatively unaffected by the presence of Ca^{2+} ions, suggesting that these chain functionalities do not interact with Ca^{2+} ions.

Effect of reducing the number of O atoms on binding of Ca^{2+} ions

The results described in the previous section have indicated that Ca^{2+} ions interact preferentially with the oxygen atoms of PHEMA. Hence, it would be expected that by reducing the relative number of oxygen atoms in the polymer the extent of binding of Ca^{2+} ions may be reduced. Thus to investigate further the role of oxygen atoms on interactions with Ca^{2+} ions, a series of copolymers of HEMA with 10 mol% of either St, EMA or NVP were prepared. After exposing the copolymers to a 5 mM $CaCl_2$ solution, the extent of binding of Ca^{2+} ions was measured by AA and the results are presented in Table 1.

By incorporating EMA, which has one oxygen less than HEMA, into the copolymer P(HEMA-co-EMA) the extent of binding of Ca^{2+} ions is reduced by about 4%. On copolymerizing HEMA with NVP, instead of EMA, so reducing the oxygen content of the comonomer further, the amount of Ca^{2+} ions attracted to the copolymer is further significantly reduced. However, replacing all three oxygen atoms by incorporating St as the comonomer did not further reduce the binding of Ca^{2+} ions over that for NVP in the copolymer. The significant reduction in calcium binding observed on the introduction of pyrrolidone groups into the copolymer may be due to stereochemical structural factors, which do not allow the pyrrolidone rings to adopt a conformation for interaction with Ca^{2+} ions. A similar trend has also been observed for the extent of calcification for the copolymers of HEMA with 2-ethoxyethyl methacrylate (EEMA) which has an additional oxygen atom [16]. Overall it is generally observed that the extent of calcium ions binding is reduced by reducing the oxygen content of the polymer.

Infrared spectra of Ca–PHEMA copolymers

To further investigate the interactions between Ca^{2+} ions and the oxygen atoms of PHEMA, an FTIR analysis was performed on the PHEMA copolymers after treatment with saturated $CaCl_2$ solutions. The wide scan infrared spectra for all of the calcified copolymers are presented in Fig. 8.

As would be anticipated from the NMR results, there are no noticeable shifts in the position of the peaks in the vibrational spectrum arising from interactions between Ca^{2+} ions and the polymer. The only change observed was that of the presence of a new peak at around 1,550 cm^{-1}, which appears in all of the infrared spectra of the calcified samples. This peak has been attributed to the electrostatic/ion–dipole interaction between Ca^{2+} ions and O atoms of the ester sidechain of PHEMA, as illustrated in Fig. 9.

These results are hence in good agreement with the results obtained from ^{1}H NMR which indicated that complexation of Ca^{2+} ions can occur at the ester oxygens of PHEMA. On the other hand, because the broad nature of the H_2O absorption masks the hydroxyl peak of PHEMA in the copolymer, no PHEMA hydroxyl peak is resolved in the FTIR spectra

Table 1 Effect of reducing the number of oxygen atoms on binding Ca^{2+} ions to the polymer at room temperature (25°C). [Ca^{2+}] = 5 mM

Polymer hydrogels	Mole fraction of HEMA	EWC_{ss} (wt.%)	[Ca^{2+}] ($\mu g/g_{Polymer}$)	Reduction (%)
PHEMA	1.0	33	490	–
P(HEMA-co-EMA)	0.9	31	470	4
P(HEMA-co-NVP)	0.9	36	330	32
P(HEMA-co-St)	0.9	29	350	29

(it should appear at around 3,600 cm^{-1}). Consequently, any interaction between the Ca^{2+} ions and the hydroxyl oxygen atoms cannot be probed via infrared spectroscopic measurements.

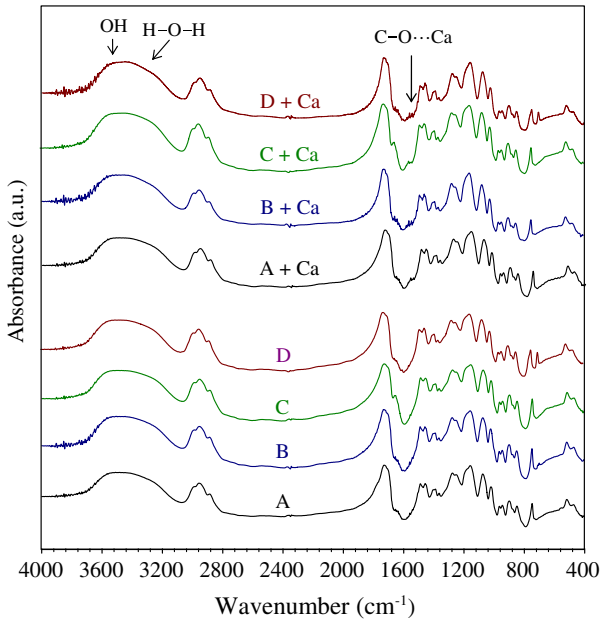

Fig. 8 FT-IR spectra of PHEMA-based copolymers before and after treatment with saturated $CaCl_2$ solution. (**A**) PHEMA, (**B**) P(HEMA-co-EMA), (**C**) P(HEMA-co-NVP) and (**D**) P(HEMA-co-St)

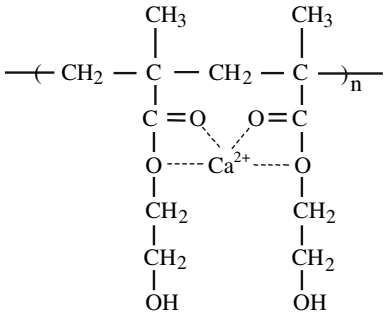

Fig. 9 Calcium complexation proposed at ester side chain of PHEMA

Conclusions

On the basis of the salting out effect, NMR, atomic absorption and infrared analyses, the interactions between Ca^{2+} ions and PHEMA copolymers can be characterized as follows:

The salting out effect indicated that Ca^{2+} ions interact mainly with the oxygen atoms of the polymer, rather than with the hydrophobic domains. This was evidenced by the decrease in the depletion of water in the copolymer as the relative number of oxygen atoms in the HEMA copolymers was reduced.

Among the three types of oxygen atoms present in PHEMA copolymers, C=O, C–O and OH, the hydroxyl oxygen seems to be the most sensitive for complexation with Ca^{2+} ions. The main reason for this conclusion is that the hydroxyl group experienced the most significant changes in the NMR line shape and T_1 relaxation time in the presence of Ca^{2+} ions.

Acknowledgments This research was supported in part by a grant from the Australian Research Council. Support from The University of Queensland through a postgraduate research scholarship for Z is also acknowledged. The authors thank Dr. Tri Le for assistance during the NMR experiments.

References

1. ZAINUDDIN, T. V. CHIRILA, D. J. T. HILL and A. K. WHITTAKER, *J. Mol. Struct.* **739** (2005) 199
2. B. A. LEVINE and R. J. P. WILLIAMS, "The chemistry of calcium ion and its biological relevance." In *The Role of Calcium in Biological Systems*, edited by L. J. Anghileri and A. M. Tuffet-Anghiler (Boca Raton: CRC Press, 1982), vol. 1, p. 3
3. J. K. JAISWAL, *J. Biosci.* **26** (2001) 357
4. C. ELVIRA and J. SAN ROMAN, *J. Mater. Sci. Mater. Med.* **8** (1997) 743
5. C. R. LUBECK, M. C. CHIN and F. M. DOYLE, "Purification, synthesis, modification and characterization of fillers and nanofillers." In *Proceeding of the Symposium on Functional Fillers and Nanoscale Minerals*, February 2003 (Cincinnati, OH, United States, 2003), p. 95
6. Y. IMAI and E. MASUHARA, *J. Biomed. Mater. Res.* **16** (1982) 609
7. I. CIFKOVA, M. STOL, R. HOLUSA and M. ADAM, *Biomaterials* **8** (1987) 30
8. L. SPRINCL, J. KOPECEK and D. LIM, *Calc. Tiss. Res.* **13** (1973) 63

9. Z. VOLDRICH, Z. TOMANEK, J. VACIK and J. KOPECEK, *J. Biomed. Mater. Res.* **9** (1975) 675
10. Y. IMAI and A. WATANABE, In *Proceeding of 5th International Society of Artificial Organs' World Congress*, 1985, edited by Y. Nose, C. M. Kjellstrand and P. Ivanovich (Cleveland, Ohio: Isao Press, 1986), p. 994
11. T. V. CHIRILA, Z. GRIDNEVA, D. A. MORRISON, C. J. BARRY, C. R. HICKS, D. J. T. HILL, A. K. WHITTAKER, and ZAINUDDIN, *J. Mater. Sci.* **39** (2004) 1861
12. D. L. COLEMAN, H. HWE-CHUEN, D. E. DONG and D. B. OLSEN, ''Polymer properties associated with calcification of cardiovascular devices.'' In *Biomineralization. Chemical and Biochemical Perspective*, edited by S. Mann, J. Webb and R. J. P. Williams (New York: Vch, 1989), p. 157
13. D. L. VANDERHART, W. L. EARL and A. N. GARROWAY, *J. Magn. Reson.* **44** (1981) 361
14. D. J. T. HILL, A. K. WHITTAKER and K. W. WONG, *Macromolecules* **32** (1999) 5285
15. R. K. HARRIS, *Nuclear Magnetic Resonance Spectroscopy* (England: Longman Group, 1986), p. 1
16. T. V. CHIRILA, D. A. MORRISON, Z. GRIDNEVA, A. J. A. GARCIA, S. T. PLATTEN, B. J. GRIFFIN, ZAINUDDIN, A. K. WHITTAKER and D. J. T. HILL, *J. Mater. Sci.* **40** (2005) 4987