Fourier-transform infrared spectroscopy study of an organic–mineral composite for bone and dental substitute materials

P. WEISS, M. LAPKOWSKI*, R. Z. LEGEROS‡, J. M. BOULER, A. JEAN, G. DACULSI

*Centre de Recherche Interdisciplinaire sur les Tissus Calcifiés et les Biomatériaux, Faculté de Chirurgie Dentaire, Place Alexis Ricordeau, 44042 Nantes cedex, France *Faculty of Chemistry, Silesian Technical University, ul. Ks. M. Strzody 9, 44-101 Gliwice, Poland ‡David B. Kriser Dental Center, College of Dentistry, Calcium Phosphate Research Laboratory, 345 East 24th Street New York, NY 100010-4086, USA*

A new injectable biomaterial for bone and dental surgery is a composite consisting of a polymer as a matrix and bioactive calcium phosphate (CaP) ceramics as fillers. The stability of the polymer is essential in the production of a ready-to-use injectable sterilized biomaterial. The purpose of this study was to detect possible polymer degradation which may have been caused by the interaction with the fillers using Fourier transform infrared spectroscopy. Composites containing CaP fillers (biphasic calcium phosphate, hydroxyapatite and peroxidized hydroxyapatite) and polymer (hydroxypropyl methyl cellulose) were prepared. To investigate the properties of the polymer, the inorganic and organic phases of the composite were separated using several extraction methods. The difficulty in separating the organic (polymer) from the mineral (CaP fillers) phases in the composite investigated in this study suggested the presence of strong interactions between the two phases. Spectra of extracted polymers showed new absorption bands of low intensities and indications that some chemical modifications of the original polymers have occurred. Results also indicated that the filler composition has an effect on the integrity of the polymer.

1. Introduction

Calcium phosphate biomaterials are used in bone repair, substitution or augmentation as an osteoconductive filler to achieve coalescence with bone [1–4]. However, several medical and dental applications of bone graft biomaterials require the biomaterials to be in an injectable form [5]. Such an injectable biomaterial was recently developed in our laboratory [6]. This biomaterial is a composite of a viscous phase consisting of a polymeric water solution (ionic cellulose ether) and calcium phosphate (CaP) ceramic fillers. The fillers are maintained in a viscous phase at the surgical site. This formulation could be modified to provide ready-to-use sterilized injectable material. Hydroxypropyl methyl cellulose (HPMC), the polymer employed, is a synthetic derivative from wood and cotton cellulose often used for food and drugs. It is prepared by reaction of mixtures of methyl chloride and propylene oxide with alkali cellulose [7].

Chemical changes in cellulose and cellulose derivatives are very complex. Many forms of degradation are possible owing to the composition of the medium, the pH, the temperature and/or the presence of different ions and oxygen [8, 9]. In our experimental conditions, two types of degradation of cellulose material can occur. First, alkaline degradation can produce ketone and carboxylic acids characterized by a chemical peeling-off mechanism involving the terminal monomeric unit [8, 10, 11]. Second, the presence of oxygen can affect the chain, producing lactones, ketones and carboxylic acid groups [9].

The purpose of this study was to investigate, using Fourier transform infrared spectroscopy (FTIR), the chemical changes or degradation in the polymer induced by the process of mixing with the calcium phosphate ceramic fillers and/or by sterilization.

Infrared (IR) spectroscopy is employed as a routine technique for determining the presence of functional groups and has been widely used in investigating biological and synthetic apatites and related calcium phosphates [12–15].

2. Materials and methods

2.1. Materials

The injectable material is a composite of a polymer and CaP ceramic fillers. The polymer used for this study was HPMC (Benecel® MP824 from

0957–4530

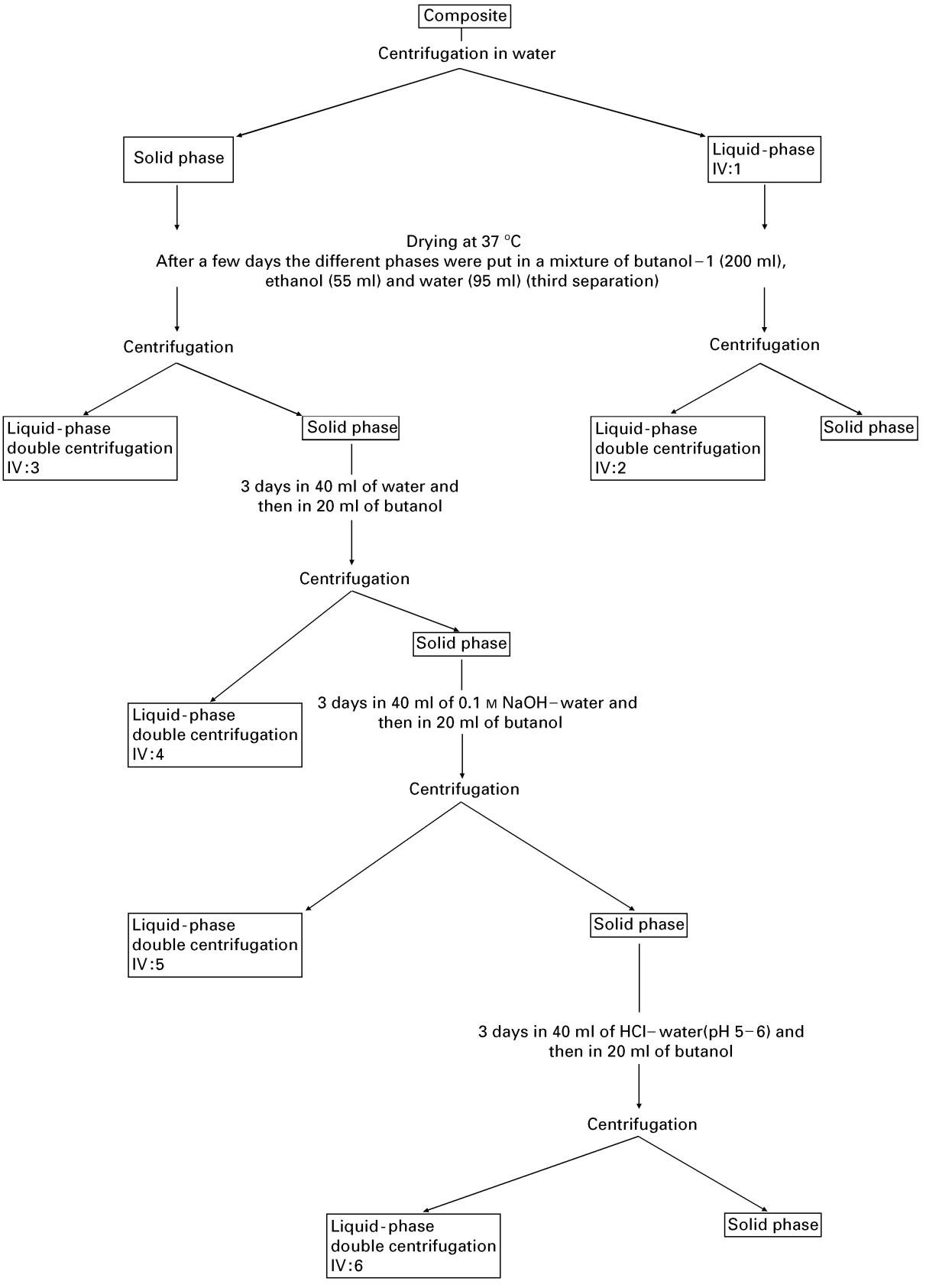

Figure 1 Diagram of the fourth separation (IV).

Aqualon™). The polymer solution (2 wt%) made using doubly distilled water was stirred for 3 days. The CaP fillers used included hydroxyapatite (HAP) ($Ca_{10}(PO_4)_6(OH)_2$), peroxidized hydroxyapatite (OHAP) and two preparations of biphasic calcium phosphate (BCP) (60% HAP and 40% β-tricalcium phosphate). The two preparations of BCP were as follows: BCP 1, commercial BCP produced by a sintering method; BCP 2, prepared in our laboratory according to the method of LeGeros and Daculsi [4] and LeGeros [16] (by precipitation of calcium deficient apatite and sintering). Because initial analyses of

some BCP 1 batches showed the presence of calcium oxide which can transform to calcium carbonate or calcium hydroxide [17], BCP 1 was modified by washing briefly in deionized water and sintering at 900 °C. The BCP 1 modified in this manner was designated BCP 1 W. All fillers were powdered and sieved at 80–200 μm.

The composite was prepared by mixing under air atmosphere 60 wt% CaP filler and 40 wt% of the polymer solution. The mixture was distributed in 20 ml aliquot into 50 ml glass bottles. All bottles were sealed at time T_0 and sterilized using an autoclave for 20 min at 121 °C according to pharmacopoeial recommendations.

2.2. Polymer extraction procedures

In order to obtain the FTIR spectra of only the polymer component of the composite, several extraction procedures (I–IV) using different solvents were used to separate the polymer from the filler (calcium phosphate materials). These procedures included the following.

I. Centrifugation in doubly distilled water: each phase (solid and liquid) was dried in an incubator at 37 °C.

II. Extraction with chloroform–water mixture at pH 6.2 (pH adjusted with HCl) (II-A) or at pH 12 (pH adjusted with 1 M NaOH solution) (II-B).

III. Extraction in a mixture of butanol-1 (200 ml), ethanol (55 ml) and water (95 ml): this formulation was previously used by Richards and Shephton [11] for paper chromatography in a study of alkaline degradation of polysaccharides.

IV. Successive extraction using different solvents (Fig. 1). This complex method was developed and employed after initial results using extraction methods I, II and III failed to extract the polymer completely from the fillers. After simple water centrifugation (IV : 1), alcohols were used to separate the mineral and organic phases (IV : 2 and IV : 3). The solid phase (Fig. 2d) was centrifuged, immersed in water for 3 days to dissolve part of the remaining polymer (IV : 4), treated by butanol and then centrifuged again. After centrifugation in the water mixture with butanol, the floculent masses were in the middle of the tube (Fig. 2b). The same operation was carried out with the solid phase (Fig. 2d) of this third centrifugation which was placed for 3 days in an aqueous basic medium (IV : 5). The last operation for the third separation was done in an aqueous acidic medium (IV : 6).

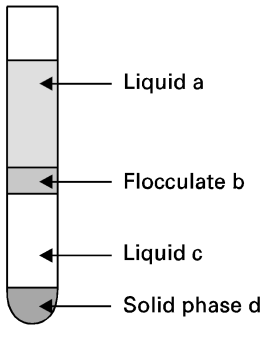

Figure 2 Different phases after centrifugation of composite with alcohol and water.

When a solvent such as alcohol is added to a water-soluble polymer such as HPMC, the polymer flocculates at the water–alcohol interface. This property allowed the separation of the polymer component of the composite from the fillers which remained at the bottom of the test tube (Fig. 2). All extracts were dried in a 37 °C incubator.

Extraction procedures were made before sterilization (T_0), and at three time periods after sterilization: immediately after sterilization (T_1), 1 month later (T_2) and 3 months later (T_3).

For control, the polymer alone was subjected to similar extraction separation procedures, I–IV.

TABLE I Average of the extractable pH of different fillers

Mineral fillers	pH
BCP 1	11
BCP 1 W	9–10
BCP 2	6
HA	8
OHAP	10

2.3. pH Measurements

1 g of the CaP ceramic fillers was suspended in 20 ml of doubly distilled water and the pH measured after 24 h (Table I).

2% polymer solutions was mixed with BCP 1, $Ca(OH)_2$ of NaOH and sterilized in an autoclave for 30 min at 121 °C (Table II). 2 ml of the mixture was added to 3 ml of doubly distilled water before measuring the pH. Each measurement was performed five times and the mean value calculated.

2.4. Fourier transform infrared analyses

FTIR analyses were performed on a Nicolet 20 SXC Spectrometer. 2 mg of dry sample were added to 300 mg of spectral-grade potassium bromide (KBr). The mixture was ground and pressed to form a transparent disc. The transmittance technique was used, and the samples were scanned 32 times for each spectrum.

The absorption bands in FTIR spectra were identified to determine the presence in the sample of the mineral and/or organic phases. The functional groups associated with the polymer HPMC are CH, CH_2, CH_3, C–O–C, O–H, carbonyl C=O due to oxidation and acid carboxyl salts $^-$O–C=O [18–22]. The functional groups associated with the mineral phase are O–H, P–O, P–O–H, C–O [12–16].

3. Results

3.1. pH

The different pH values for fillers in water are shown in Table I, and the pH values of polymer solutions are summarized in Table II.

Polymer solution alone showed that proton production occurred after sterilization. In basic media, protons were consumed, and this consumption was very high in BCP solution.

3.2. Fourier transform infrared analyses

3.2.1. Fourier transform infrared spectra of pure hydroxypropyl methyl cellulose

The absorption bands in the FTIR spectra (Fig. 3) of non-ionic cellulose ether, before and after sterilization were identical. C–O–C and C–O absorption bands of alcohol occur at about 1000 cm^{-1}, whereas CH, CH_2 and CH_3 absorption bands are found in the 1250–1460 cm^{-1} and 2850–2980 cm^{-1} regions. The absorption band at 1648 cm^{-1} is that of absorbed water (H–O–H). The broad absorption band in the 3500 cm^{-1} region represents hydrogen bonding in the polymer and absorbed water.

No change in the FTIR spectra were observed before and after sterilization and after extracting with chloroform or separation with solvents (butanol-1, ethanol and water) used in the extraction procedure III.

When the period of sterilization of the polymer was extended to 5 h, a small absorption band at 1727 cm^{-1} in the FTIR spectra of the extract appears. This band may have been an oxidation absorption band, C=O. For the same period of sterilization (5 h), the FTIR spectrum of one extract from the polymer solution with NaOH showed a low absorption band at 1596 cm^{-1}, representing carboxyl acid salts.

TABLE II pH of different media diluted with HPMC before (T_0) and after (T_1) sterilization

Medium	T_0	T_1	Proton consumption ($mol\,l^{-1}$)
Water	9.03	9.00	-6.64×10^{-11}
BCP (1 g/20 ml)	8.77	10.62	1.68×10^{-9}
0.1 M Ca $(OH)_2$	11.96	12.40	6.86×10^{-13}
0.1 M NaOH	11.62	12.48	2.06×10^{-12}
1 M NaOH	13.00	13.17	3.27×10^{-14}

3.2.2. Fourier transform infrared spectra of the composite

The spectrum for the composite mixture (Fig. 4) clearly shows the absorption bands of the mineral phase, and the organic absorption bands are visible in the 2900 cm^{-1} region. In this spectrum, it is very difficult to observe any changes in the properties of the polymer.

3.2.3. Fourier transform infrared spectra of the different mixture separations

3.2.3.1. Mineral and organic separation. The FTIR spectra of the composite show mainly the absorption bands of the mineral fillers (Fig. 4). Thus, the main purpose of the polymer extraction procedure was to separate the mineral and organic phases since mineral absorption bands overlap possible polymer absorption bands in the FTIR spectra.

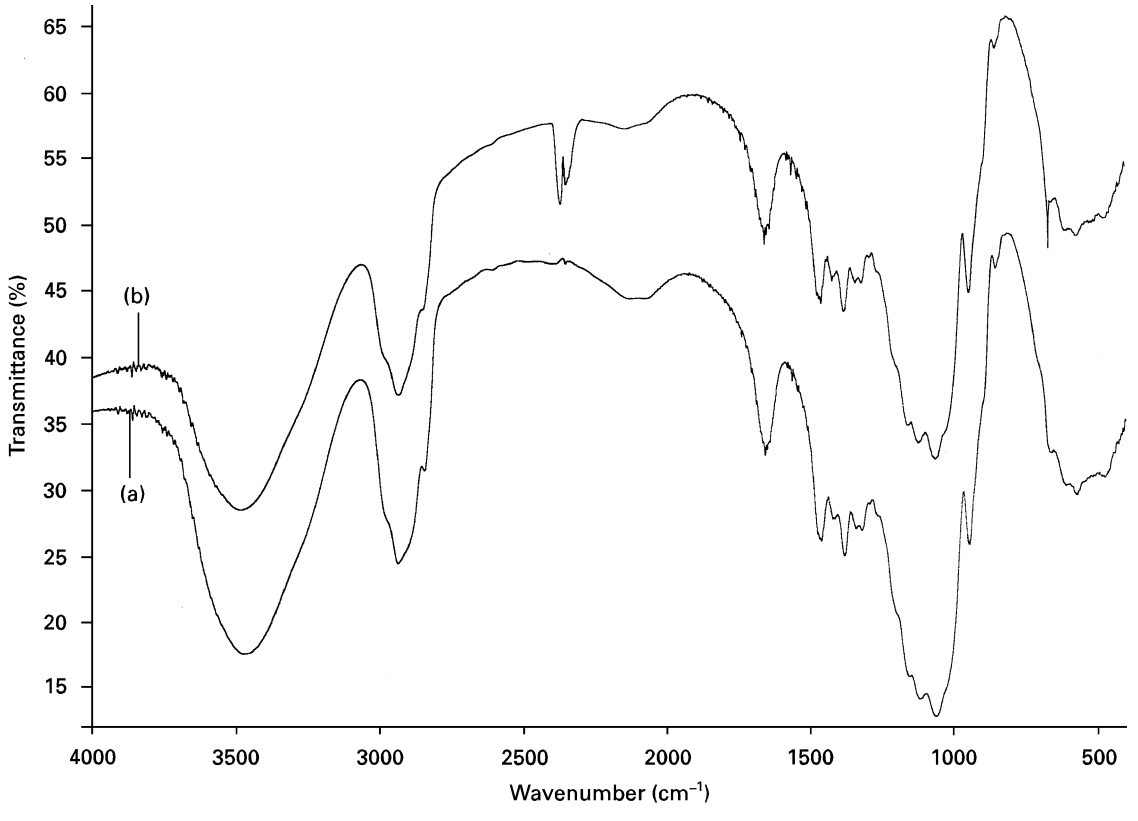

Figure 3 FTIR spectra of HPMC (a) before and (b) after autoclave sterilization (121 °C; 20 min).

3.2.3.2. Results for the separation in water medium (I). The FTIR spectrum of the air-dried liquid phase after water centrifugation of the BCP–HPMC composite showed polymer and mineral absorption bands before and after sterilization (Fig. 5). This method failed to separate the organic from the mineral phases and was not adequate to show any possible degradation of the polymer. The spectrum for the solid phase also showed the BCP absorption bands but not those of the polymer.

3.2.3.3. Results for the extraction with chloroform (II). The only sample for this separation was a HPMC–BCP composite at T_1. The FTIR spectra showed absorption bands of calcium carbonate in the basic medium in five extracts out of a total of 6 (II-B), and in one out of six extracts in the acidic medium (II-A).

3.2.3.4. Results for the extraction with alcoholic mixture (III). The absorption bands associated with calcium carbonate at 712, 870, 1420 and 1460 cm^{-1} were present in the FTIR spectra of the extract from the HPMC–BCP composite at T_0 and at T_3 but were not present in those at T_1. These absorption bands were observed in the spectra of the extract from the HPCM–OHAP composite but not in those of the HPMC–HAP composite.

3.2.3.5. Results for the sequential extraction (IV). The results are summarized in Table III.

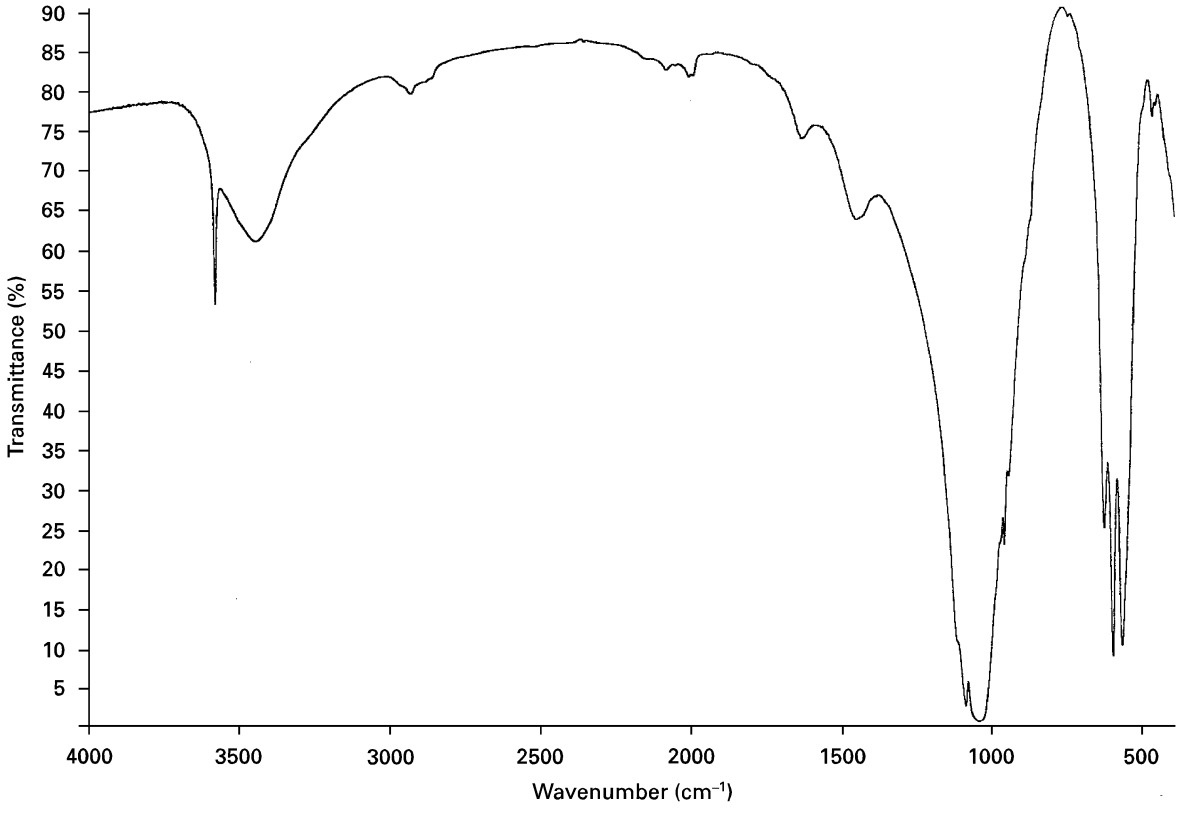

Figure 4 FTIR spectra of HPMC mixture with BCP, after drying in incubator.

TABLE III Number of samples in FTIR spectra with mineral peaks at the fourth extract of composite with HPMC and mineral fillers (BCP, HAP and OHAP), before sterilization (T_0), after sterilization (T_1), 1 month after sterilization (T_2) and 3 months after sterilization (T_3)

Filler	pH	Times	Number of samples with mineral peaks at the following separation stages in the following separation solutions						Total
			IV-1 Water	IV-2 Mixture of alcohols + water	IV-3 Mixture of alcohols + water	IV-4 Butanol + water	IV-5 Butanol + alkaline solution	IV-6 Butanol + acidic solution	
BCP 1	11	T_0	Yes		Yes	Yes	Yes	No	4/5
		T_3		Yes	No	Yes	Yes	Yes	4/5
BCP 1 W	9–10	T_2		Yes	Yes	Yes	Yes	No	4/5
BCP 2	6	T_1		No	No	No	Yes	No	1/5
HAP	8	T_3		No	No		Yes	No	1/5
OHAP	10	T_0	Yes		Yes	Yes	Yes	No	4/5
		T_3		No	No	Yes	Yes	Yes	3/5
Total			2/2	2/5	3/7	5/6	7/7	2/7	

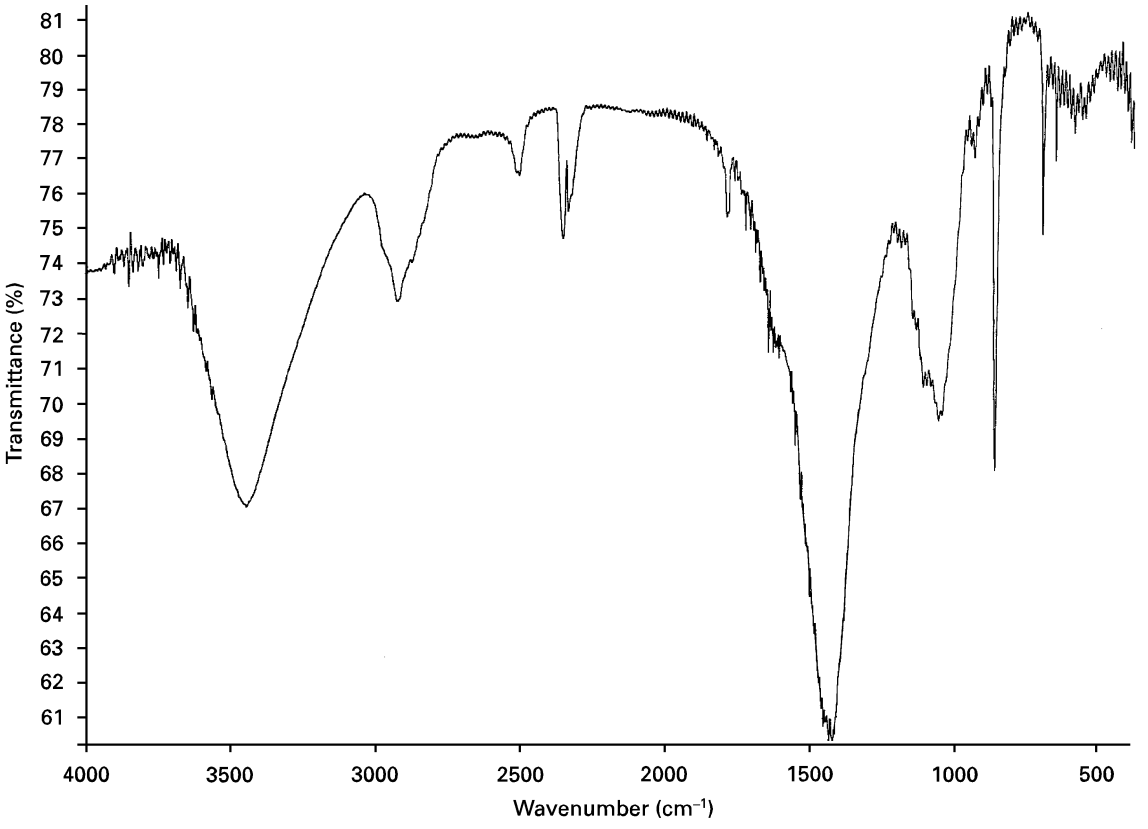

Figure 5 FTIR spectra of dried liquid phase of water separation of the BCP + HPMC at T_0.

3.2.4. Fourier transform infrared study of polymer degradation

3.2.4.1. Results for the extract with chloroform (II). FTIR analysis showed the presence of the COO^- absorption band in the region 1563–1667 cm^{-1} in the FTIR spectrum of one of the six extracts of the BCP composite at T_1 and the presence of C=O absorption bands in five extracts in basic medium and only one in acidic medium (Fig. 6). The C=O absorption band at 1690 cm^{-1} attributed to polymer oxidation. The C–O–C absorption bands of the polymer were present in the FTIR spectra of all the extracts.

3.2.4.2. Results for the extraction with alcoholic mixture (III). For alcohol separation, acid carboxyl salts (absorption bands at 1560 cm^{-1}) appeared after sterilization with BCP and HAP (Fig. 7a and d). The C=O absorption bands at 1730 cm^{-1} occurred in the FTIR spectra of extracts from all composites at T_1 and T_2 (Fig. 7c and d). At T_2, one of the extracts for the HPMC–BCP composite displayed a major carboxylic absorption band at 1596 cm^{-1} (Fig. 8).

3.2.4.3. Results for the sequential extraction with alcoholic media (IV). Table IV shows that carboxyl groups appeared only in the FTIR spectra of extracts at T_3. The intensities of the absorption bands were very low in all the spectra.

The FTIR spectra of four extracts from the composite with BCP 2 at pH 6 showed carbonyl absorption bands at T_1, whereas the BCP 1 (pH 11) composite showed these absorption bands in the spectra of only two extracts at T_3 and only one at T_0.

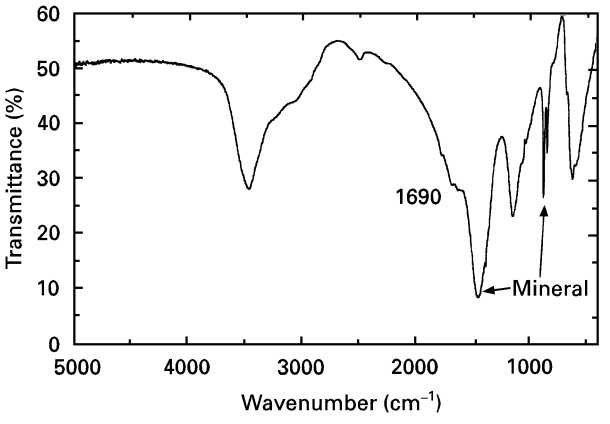

Figure 6 FTIR spectrum of the HPMC–BCP composite extraction at T_1 with chloroform–1 M NaOH solution (II-B).

The carbonyl absorption bands were present in the FTIR spectra of two extracts from the HAP composite at T_3 and in the spectra of four extracts from OHAP composite at T_0 and T_3.

Some modifications were observed in the absorption bands in the FTIR spectra of the extracts. However, one spectrum of one extract from HPMC–OHAP composite at T_3 indicated considerable modification. These spectra display very high absorption bands in the CH, CH_2 and CH_3 regions. A carbonyl absorption band occurred at 1739 cm^{-1} (Fig. 9). The same spectrum of one extract from HPMC–BCP composite at T_1 was observed for the extraction with chloroform (II). These extract materials were present in very small quantities.

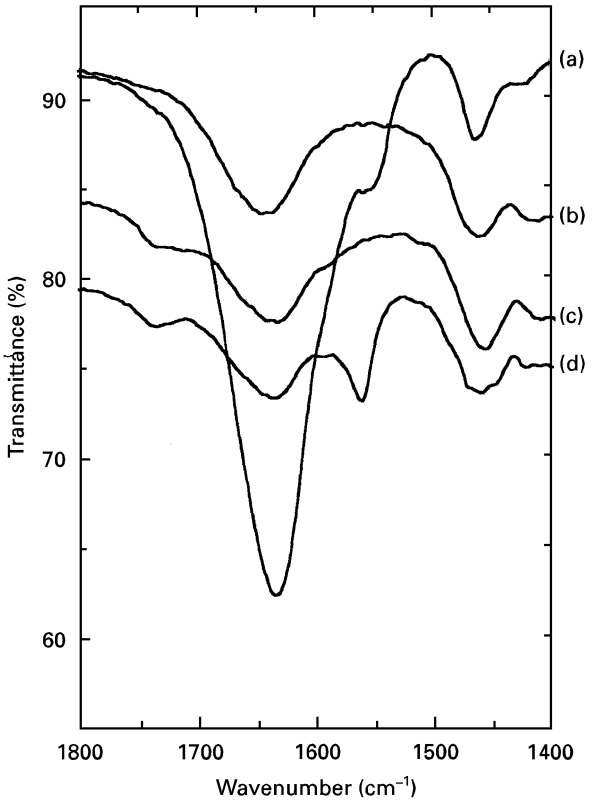

Figure 7 FTIR spectra of the third extract with an alcohol mixture from the following (a) BCP–HPMC composite, T_1; (b) third separation of HPMC alone; (c) HAP–HPMC composite, T_2; (d) OHAP–HPMC, T_2.

TABLE IV Number of samples with particular organic peaks in FTIR spectra at the fourth extract of composite with HPMC and mineral fillers (BCP, HAP and OHAP), before sterilization (T_0), after steriliztion (T_1), 1 month after sterilization (T_2) and three months after sterilization (T_3)

	T_0	T_1	T_2	T_3
Presence of COO peaks				
BCP 1	0			1
BCP 1 W			0	
BCP 2		0		
HAP				1
OHAP	0			3
Presence of C=O peaks				
BCP 1	1			2
BCP 1 W			3	
BCP 2		4		
HAP				2
OHAP	4			4

4. Discussion

The presence of calcium oxide as an impurity in BCP1 was responsible for its strong alkaline pH and for the presence of $CaCO_3$ in the FTIR spectra. The changes with the polymer alone were very slight.

The FTIR spectra of the composites did not permit analyses of some changes in the polymer. The absorption bands of the BCP were too strong and overlapped those of the polymer. It was necessary to separate the polymer from the mineral phase in order to study any chemical changes which may have occurred.

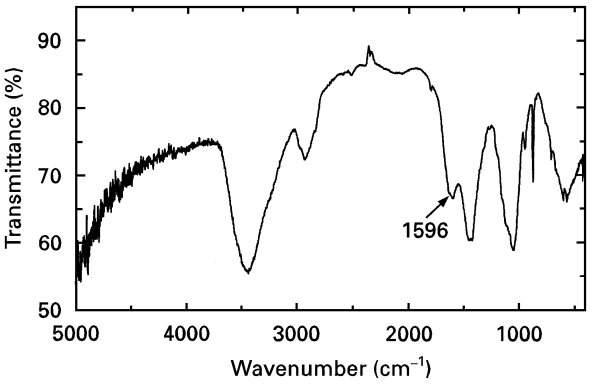

Figure 8 FTIR spectrum of the third extract of HPMC–BCP at T_2.

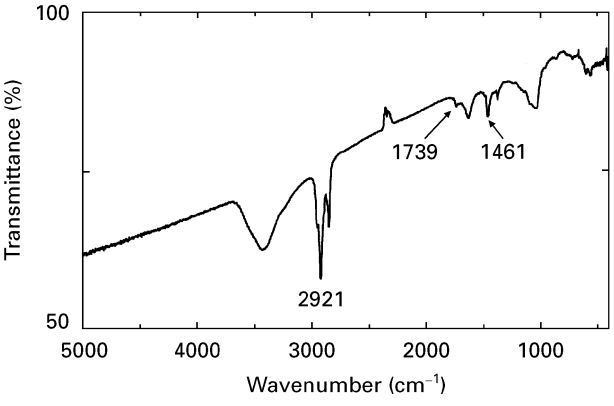

Figure 9 FTIR spectrum of the sixth extract (IV : 6) (liquid a + flocculate b) of the sequential extraction of the HPMC–HAP composite, 3 months after sterilization (T_3).

The first aim of this study was to monitor the separation between the polymer and calcium phosphate fillers using FTIR. Extraction with water was not sufficient to separate the mineral from the organic phases as evidenced by the persistent presence of the absorption bands of calcium carbonate. Extraction with acidic chloroform seemed more efficient than extraction with alkaline chloroform. Extraction with alcohol was more efficient in separating the polymer from the HAP mineral than from the BCP mineral.

The fact that six extractions in different media were equired to remove all the polymer from the CaP fillers confirm the strong interaction between polymer and mineral particles.

All the experiments showed that separation was better in acidic than alkaline media, owing to the greater dissolution of the CaP filler in acidic solutions, suggesting that the electrostatic interactions along the polymer (hydrogen and acidic bonding) were probably cancelled out by the presence of protons.

Vapour sterilization appeared to promote H_3O^+ capture by the polymer, resulting in an increase in the pH of different sterilized polymer solutions. Indeed, the pH increased with alkaline medium after sterilization, whereas cellulose alkaline degradation was previously found to decrease with increasing pH through the action of acid products [23]. In our experimental conditions, the temperature could have modified the

aqueous environment of the polymer, with H_3O^+ occurring along the macromolecule and thereby increasing the pH. In fact, protons were consumed by the polymer during sterilization in alkaline media.

Although the separation of the organic and mineral phases was not perfect, the FTIR analysis of the polymer extracts showed slight changes in the characteristics of the absorption bands. These changes were visible only in the spectra of the composite after extraction and are not due to the extraction process since polymer controls were not modified. Only the polymer subjected to 5 h sterilization showed few chemical modifications. With the extracts of the composites, some spectra show absorption bands of carbonyl or carboxyl groups.

The presence of carboxyl groups was observed in the spectra of extracts from alkaline pH and of those with oxidizing fillers such as OHAP. These results are comparable with those in the literature, although this study could not show where these groups were along the cellulose polymer chain or in a degradation residue.

According to Nevell [9], oxidation can transform the polymeric chain without decreasing the degree of polymerization. Without oxygen, the degradation under a nitrogen atmosphere occurs by a peeling-off mechanism at the end of the polymeric chain. In this latter case, degradation decreases the degree of polymerization. This study could not determine the type of carboxyl group or whether it was located on the polymer, on a residue or on both.

All the spectra of sample extracts showed low intensities of the carboxyl absorption bands. Even though this result may be attributable to experimental conditions, it indicates that the presence of carboxyl groups was relatively rare, occurring only in alkaline mixture after sterilization, especially in oxidizing media (Table IV).

The presence of carbonyl groups in the spectra was independent of the pH of the medium pH owing to the fillers. In the spectra of the third extract, these absorption bands appear after sterilization, as in the fourth extract these bands were visible before sterilization with BCP1 and OHAP. This last separation was more complete.

With the HPMC–OHAP composite, the chemical reaction is expected to have originated from the mixture because of the oxidizing power of the fillers.

Results of the FTIR analyses demonstrated the effect of the fillers and of the media on polymer degradation. Only the composite produced with BCP (pH 11) and OHAP have shown degradation after sterilization.

5. Conclusion

Results from this study demonstrated the importance of the medium and type of CaP ceramic filler in causing modification of a cellulose polymer after sterilization and in long-term conditions. These results indicate that preparation of the polymer–CaP filler composite did not involve a simple mixing of organic (polymer) and inorganic (CaP fillers) phases but a complex reaction between the two phases.

The results of degradation appeared to be slight since most FTIR spectra displayed low absorption bands or no absorption bands of chemical modification. The most important degradation was shown in alkaline conditions and with oxidizing agents. With HAP or BCP without calcium oxide, the FTIR analysis did not show detectable chemical modifications of the polymer. However, the modification of the degree of polymerization may be below the detection limit in this experiment.

The difficulty in completely separating the polymer from the mineral filler experienced in this study suggested a strong interaction between the two phases. The present study suggests that biocompatibility and toxicity should be studied at the time of polymer production, after sterilization and after long-term incubation, especially if modifications of the properties of the polymer are possible.

Acknowledgements

We thank J. L. Lacout, Toulouse, for supplying the HAP and OHAP, and S. Lefrant, Laboratoire de Physique Cristalline, Institut des Matériaux de Nantes, 2 rue de la Houssinière, 44072 Nantes Cédex 03, France, for making the FTIR measurements possible.

This work was supported by Centre National de la Recherche Scientifique grant EP 59 and Institut National de la Santé et de la Recherche grant CJF 93/05.

References

1. M. JARCHO, *Clin. Orthop.* **157** (1981) 257.
2. K. DE GROOT, "Bioceramics of calcium phosphate" (CRC Press, Boca Raton, FL, 1983).
3. B. NERY, R. Z. LEGEROS, K. LYNCH and K. LEE, *J. Periodont.* **63** (1992) 729.
4. R. Z. LEGEROS and G. DACULSI, in "Handbook of bioactive ceramics, calcium phosphate and hydroxyapatite ceramics", edited by T. Yamamuro, L. L. Hench and J. Wilson Hench (CRC Press, Boca Raton, FL, 1990) pp. 17–28.
5. N. PASSUTI, F. MILLOT, A. DUPRAZ, P. WEISS, G. GRIMANDI, J. DELECRIN and G. DACULSI, *Innov. Tech. Biol. Med.* **16** (1995) 20.
6. G. DACULSI, P. WEISS, J. DELECRIN, G. GRIMANDI, N. PASSUTI and F. GUERIN, International Patent WO 95/21634 (17 August 1995).
7. E. K. JUST and T. G. MAJEWICZ, in "Encyclopedia of polymer science and engineering", Vol. 3, edited by J. K. Kroschwitz, H. F. Mark, N. M. Bikales, C. G. Overberger and G. Menges (Wiley, New York, 1985) p. 226.
8. T. P. NEVELL, in "Cellulose chemistry and its applications", edited by T. P. Nevell and S. H. Zeronian (Ellis Horwood, Chichester, West Sussex 1985) p. 223.
9. T. P. NEVELL, in "Cellulose chemistry and its applications", edited by T. P. Nevell and S. H. Zeronian (Ellis Horwood, Chichester, West Sussex, 1985) p. 242.
10. M. H. JOHANSSON and O. SAMUELSON, *J. Appl. Polym. Sci.* **19** (1975) 3007.
11. G. N. RICHARDS and H. H. SHEPHTON, *J. Chem. Soc.* (1957) 4492.
12. R. Z. LEGEROS, J. P. LEGEROS, O. R. TRAUTZ and E. KLEIN, *Dev. Appl. Spec. B* **7** (1970) 3.
13. C. C. REY, H. M. KIM and M. J. GLIMCHER, *J. Bone Miner. Res.* **10** (1995) 1577.
14. F. APFELBAUM, H. DIAB, I. MAYER and J. D. B. FEATHERSTONE, *J. Inorg. Biochem.* **45** (1992) 227.
15. R. Z. LEGEROS, "Calcium phosphates in oral biology and medicine", Monographs in Oral Sciences, Vol. 15, edited by H. Myers (Karger, Basel, 1991).

16. R. Z. LEGEROS, *J. Dent. Res.* **65** (1986) 292.
17. R. Z. LEGEROS, Private communication, 1994.
18. N. B. COLTHUP, L. H. DALY and S. E. WIBERLEY, "Introduction to infrared and Raman spectroscopy" (Academic Press, New York, 3rd Edn, 1990).
19. H. HATAKEYAMA, C. NAGASAKI and T. TUGURI, *Carbohydr. Res.* **48** (1976) 149.
20. C. J. POUCHERT, "*The Aldrich library of infrared spectra*" (Aldrich, Gllingham, Dorset, 1970).
21. D. FENGEL and M. LUDWIG, *Papier, Darmstadt* **45** (1991) 45.
22. R. T. O'CONNOR, E. F. DUPRE and E. R. McCALL, *Anal. Chem.* **29** (7) (1957) 998.
23. G. MACHELL and B. N. RICHARDS, *J. Chem. Soc.* (1960) 1924.

Received 29 August
and accepted 8 October 1996