Bioactivity of sol–gel derived organically modified silicates

Part I: In vitro *examination*

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Bioactivity was investigated for several organically modified silicates (Ormosils) prepared through sol–gel processes. Ca(II)-free samples were biocompatible only, but Ca(II)containing samples were bioactive and deposited apatite during immersion in a simulated body fluid. The ease of silanol (Si–OH) group formation on the ormosils was considered a predominant factor controlling the bioactivity, while the effect of dissolved Ca(II) ions to increase the degree of supersaturation in the simulated body fluid is secondary.

1. Introduction

Since Hench invented Bioglass® [1] in the early 1970s several glasses and glass ceramics have been developed [2–4] that exhibit bioactivity or can directly bond to living bones when embedded in human bodies. However, these ceramic materials are far from ideal since they are too hard, and so inferior to bone in mechanical strength and fracture toughness. Organic polymers, on the other hand, that are the mainstream materials employed for soft-tissue substitutes are non-bioactive and only biotolerant. They are surrounded by a fibrous tissue when embedded in the body. Thus materials are in demand that are not only bioactive but have bone-like mechanical properties, or at least are applicable to soft-tissue substitutes.

Studies on the bone-bonding mechanism of materials have indicated [5] that one essential is the spontaneous deposition of a layer of apatite, similar to bone in composition and crystallinity, on the surface when they are in contact with a blood plasma. Ohtsuki *et al.* [6] concluded that Ca(II) ions dissolved from the bone-substituting glass and glass ceramics and a layer of silanol (Si–OH) groups left on their surface are the key materials for apatite deposition: the Ca(II) ions increase the degree of supersaturation regarding apatite precipitation and the silanol layer provides sites for nucleation. Therefore it is expected that polymers having silanol groups or Ca(II) ions or those having both in their structure should exhibit bioactivity.

Mackenzie *et al.* [7] synthesized composite polymers "ormosils" from poly(dimethylsiloxane) (PDMS) and tetraethoxysilane (TEOS) through sol–gel processing. They consist of organic and inorganic components mixed at the nanometre level, and the mixing ratio of PDMS and TEOS controls their mechanical properties, e.g., rubbery to solid in elasticity. If a rubbery ormosil in which Ca(II) ions are dispersed can be synthesized through sol–gel processing it could be a bioactive soft tissue substitute. Jones prepared through sol–gel processing [8] similar composite polymers of SiO_2-poly(methyl methacrylate), which involved Ca(II) ions and exhibited bioactivity. Mechanical properties were unfortunately not described for the composite polymers, and it was uncertain whether they were rubbery or not. Thus we followed the process of Mackenzie *et al.* [7]. The goal of the present study is to derive experimental procedures to disperse Ca(II) ions in the ormosil and to examine the bioactivity of the obtained composite polymers.

2. Experimental

2.1. Sol–gel preparation of Ca(II)-containing ormosils

The starting materials were reagent grade calcium nitrate (Nacalai tesque, Osaka, Japan), TEOS (Nacalai tesque) and PDMS (Aldrich, 20c stokes fluid; average molecular weight $\approx$ 1500), while reagent grade 2-propanol and tetrahydrofurane (Nacalai tesque) were used for the solvents and hydrochloric acid (Nacalai tesque) was the catalyst. Mixing ratios of the raw materials are shown in Table I. TEOS (10 g) was charged to a mixture of 4.8 ml 2-propanol and 3.2 ml tetrahydrofurane and subsequently PDMS (5.9 g) was added to the resulting mixture and stirred (solution A). An appropriate amount of HCl solution (35%) diluted with distilled water was added to 8.0 ml 2-propanol, to which solution calcium nitrate was added and stirred (solution B). Solutions A and B were mixed and refluxed under stirring at 80 °C for 30 min. The mixture was then quenched to 25 °C by immersing in iced

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TABLE I Compositions of the ormosils (in molar ratio)

	TEOS	PDMS	HCl	H_2O	$Ca(NO_3)_2$
Rubbery ormosil	1	0.08[a]	0.3	3	–
Sample A	1	0.08[b]	0.1	3	0.01
Sample B	1	0.08[b]	0.1	3	0.05
Sample C	1	0.08[b]	0.05	3	0.05

Average molecular weight: [a] 1700, [b] 1500.

water. The solution obtained was poured into polystyrene containers, covered with a vinyldenechloride film and aged for gelation at 25 °C in an oven. The gels were dried at 25 °C for 1 to 2 weeks and subsequently at 40 °C for 48 h. After removal of the film, the gels were further dried at 60 °C in the oven for 48 h. Rubbery ormosils (TEOS/PDMS = 60/40 in weight) free from Ca(II) ions were prepared as described elsewhere [7].

2.2. Examination of bioactivity

Ormosils with and without Ca(II) ions were cut to $15 \times 10 \times 1$ mm in size, and the surface of specimens was polished with a # 1500 emery paper. Then they were gently rinsed with ethanol and dried. A simulated body fluid (denoted later as the Kokubo solution) was prepared following Ohtsuki *et al.* [6] by dissolving several inorganic salts such as NaCl, $NaHPO_4$, and $NaHCO_3$, and pH was adjusted to 7.25 with hydrochloric acid and tris(hydroxyaminomethane). The Kokubo solution contains the same inorganic components as human blood plasma in similar concentrations. It has been proved [9] that *in vitro* experiments with Kokubo solution can satisfactorily reproduce *in vivo* behaviour of implant materials. The specimens were kept in the Kokubo solution at 36.5 °C up to 30 days. The concentration of ions in the Kokubo solution was analysed by inductively coupled plasma photometry (Seiko Electronics, SPS7700), and the pH of the Kokubo solution was also monitored.

Specimens soaked in Kokubo solution were gently rinsed with distilled water and dried in an oven at 40 °C. Thin film X-ray diffraction measurements were made using a diffractometer (Rigaku, RAD-II) with attached thin-film apparatus whose incident angle was 1°. FT-IR spectra were measured with a JASCO FT-IR300 spectrometer taking 75° incident angle. Signals 1 μm depth could be obtained with both methods.

3. Results

Fig. 1 shows the thin-film X-ray diffraction (TF-XRD) patterns and FT-IR reflection spectra of the Ca(II)-free ormosil before and after soaking in Kokubo solution for 30 days. The IR peak assignment followed that in the literature [3, 10, 11]. The specimen after treatment gave X-ray diffraction patterns and IR spectra similar in profile to those before soaking; they gave no peaks that could be assigned to apatite. Thus the Ca(II)-free samples could not deposit the apatite layer and were non-bioactive. Fig. 2 shows the

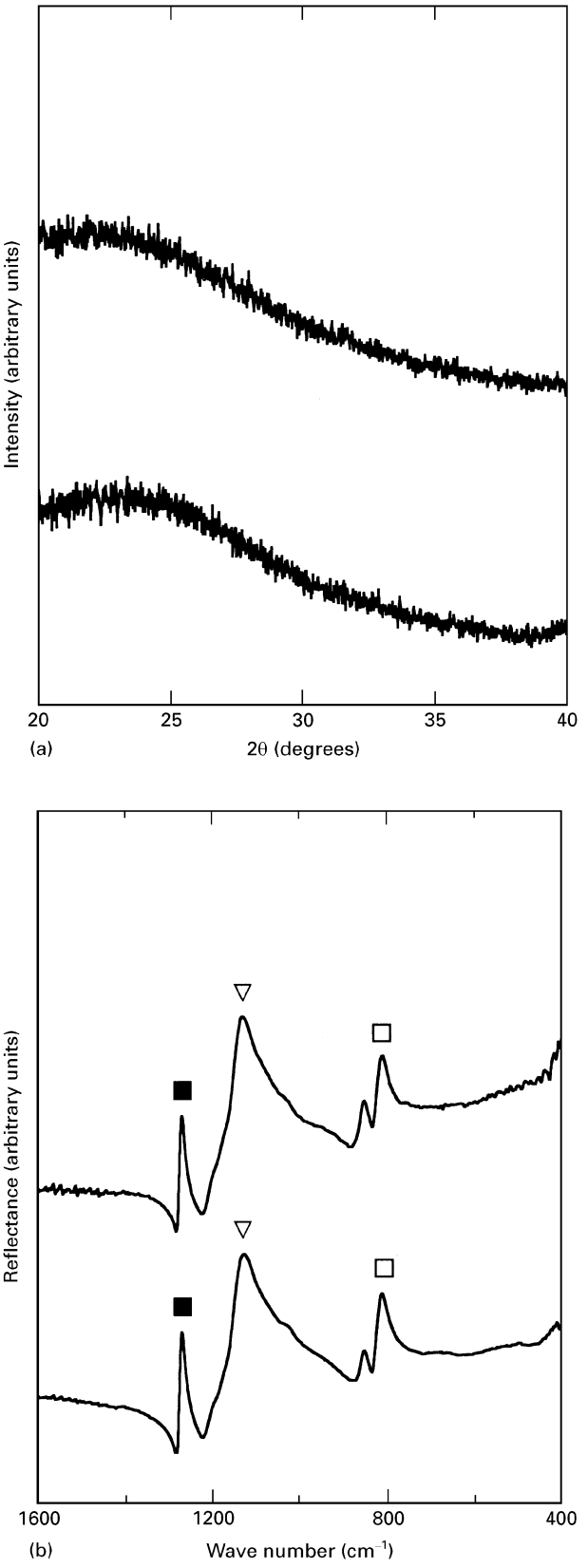


Figure 1 (a) Thin film X-ray diffraction patterns and (b) Fourier transform infrared reflection spectra for a rubbery ormosil (TEOS/PDMS = 60/40 (wt)) after soaking in Kokubo solution for 30 days. No apatite formation and hence no bioactivity is found. The infrared peaks are assigned as indicated. Lower traces, before soaking; upper traces, after 30 days soaking. ■ CH_3 def.; □ CH_3 rock and Si–C str.; ▽ Si–O str.

TF-XRD patterns and FT-IR spectra of the specimen having the compositions in Table I. A sharper TF-XRD peak at 26.0° was due to (0 0 2) diffraction of

apatite while a broader one at about 32° was an envelope of (2 1 1), (1 1 2) and (3 0 0) diffractions. An IR reflection peak at 1100 cm^{-1} was assigned to the transverse optical mode of Si–O stretching [10]. Peaks at 560 and 610 cm^{-1} were due to a P–O bending mode; 1060 and 1130 cm^{-1} peaks were assigned to P–O stretching [11]. The TF-XRD patterns indicated that sample B deposited the apatite layer after 30 days of soaking and sample C deposited it within only one day, whereas no apatite diffraction was observed for sample A after 30 days of soaking. The P–O bending and stretching peaks were observed for the samples that gave the diffractions of apatite. It seemed strange that sample A gave these IR peaks although exhibiting no apatite peaks in the TF-XRD patterns.

The concentrations of Ca(II), P(V) and Si(IV) ions in the Kokubo solution were plotted as a function of the soaking period in Fig. 3 for samples A, B and C. The values of pH of the Kokubo solution are also indicated. All samples showed a similar trend: the concentration of Ca(II) and P(V) ions decreased and that of Si(IV) increased with soaking time. The decrease in

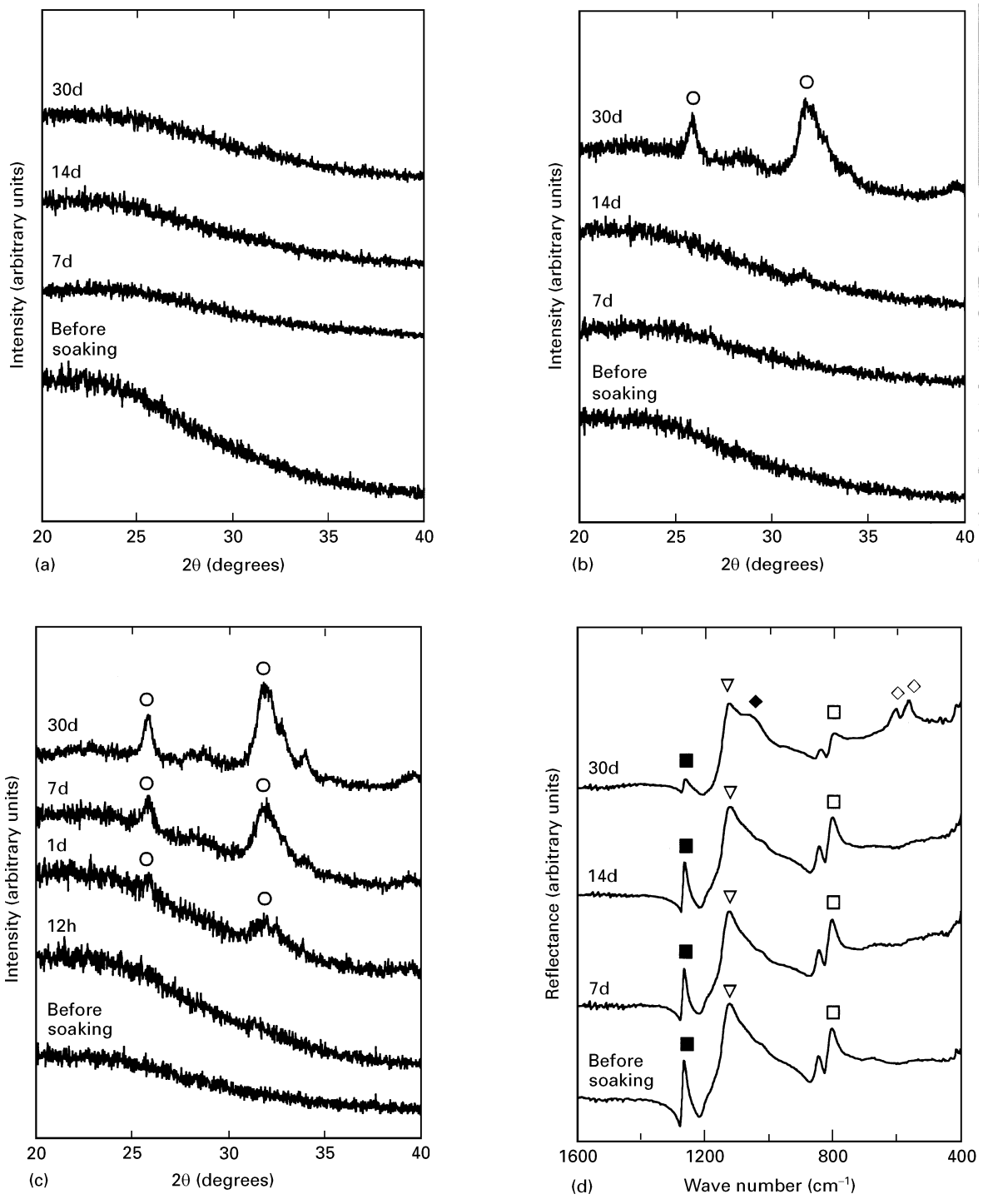
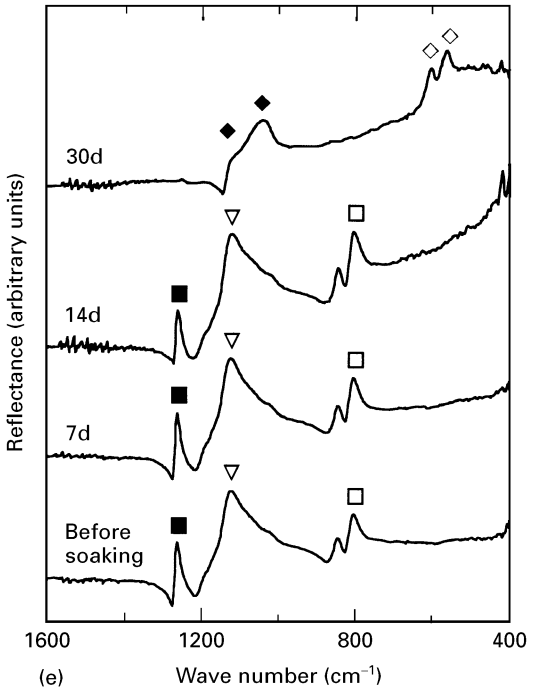


Figure 2 Thin film X-ray diffraction patterns (a), (b), (c) and infrared reflection spectra (d), (e), (f) for ormosil samples A, B and C, respectively, after soaking in Kokubo solution for up to 30 days. The 30-day specimen of sample A showed infrared peaks for P–O bending modes but gave no X-ray diffractions due to apatite. Peak assignments for X-ray diffraction, ○: apatite; for infra-red reflection, ■: δ (CH_3), □: δ (CH_3) and v (Si–C), ▽: v(Si–O), ◆: v (P–O), ◇: δ (P–O).



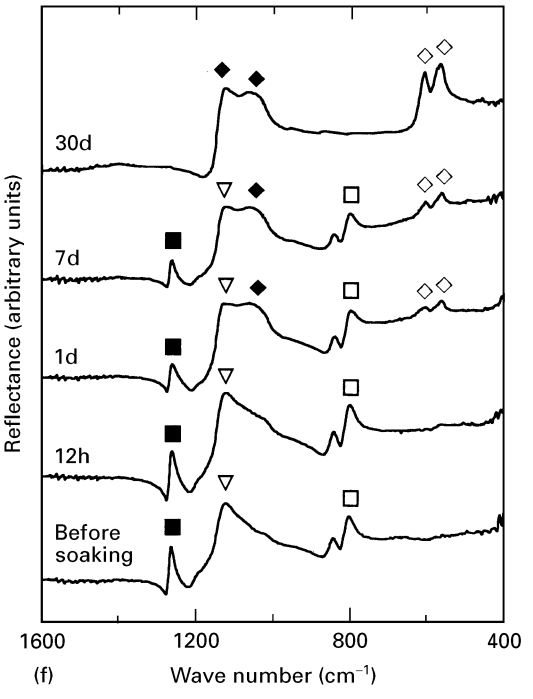


Figure 2 (*Continued*)

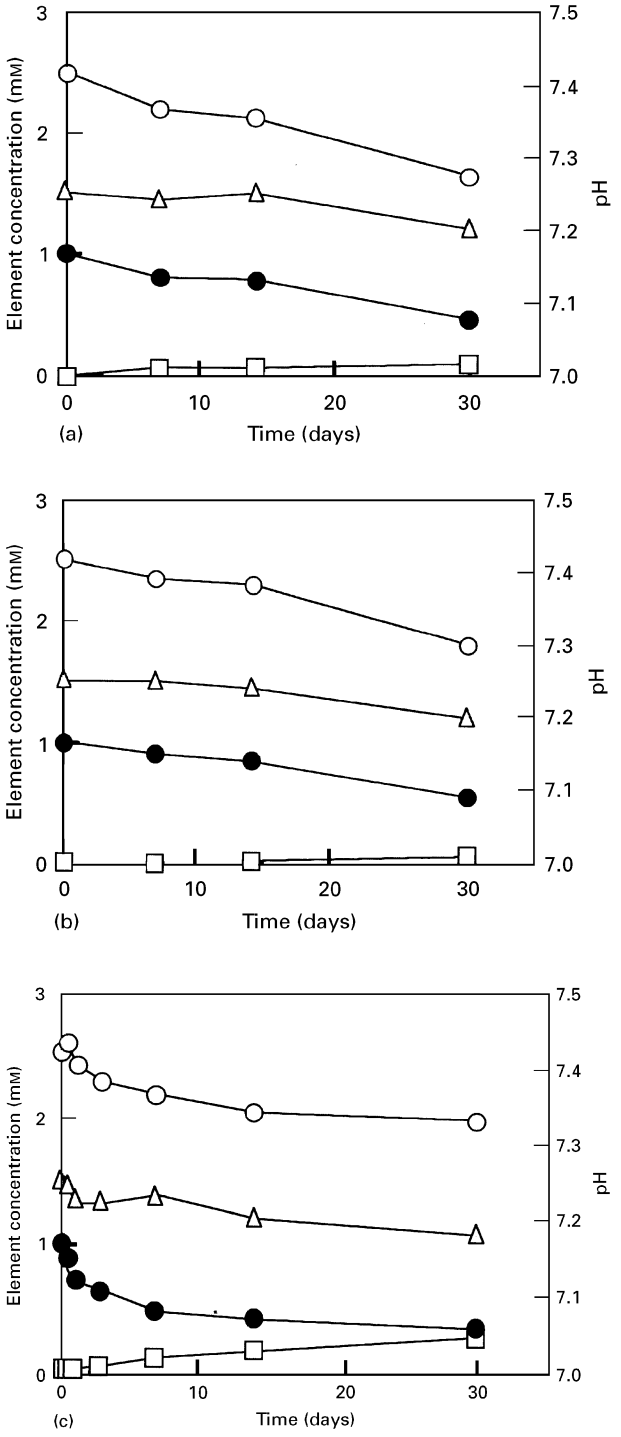


Figure 3 Element concentrations and pH values of the Kokubo solution as a function of the period of soaking: (a) sample A; (b) sample B; (c) sample C. □ Si; ●P; ○ Ca; △ pH.

Ca(II) and P(V) for samples B and C corresponded to the apatite precipitation confirmed by the TF-XRD patterns shown in Fig. 1.

4. Discussion

The fact that sample A showed the same responses as B and C when soaked in Kokubo solution suggested that it deposited amorphous calcium phosphates or an apatite layer which was too thin to give detectable TF-XRD intensities. The Si(IV) ions in the Kokubo solution were dissolved from the samples since the as-prepared Kokubo solution did not contain them. Possible origins were the silicate blocks in the composites derived from TEOS, composite blocks due to copolymerization between TEOS and PDMS, and PDMS monomers. It was considered that the silica blocks among those species gave the Si(IV) ions because the Si–O bonds in the copolymer blocks or PDMS monomer, if present, were unlikely to react with the Kokubo solution to release Si(IV). If the silica blocks in sample C are more reactive with Kokubo solution, sample C leaves more hydrated silanol groups on the surface in a shorter soaking time. Thus it supplies more nucleation sites for apatite, deposits the apatite layer in the shorter period, and is found

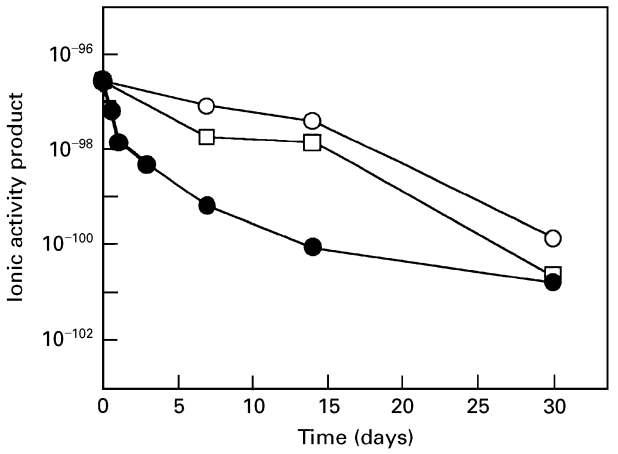


Figure 4 Ionic activity product for sample A (□), B (○) and C (●) regarding apatite (Equation 1) in Kokubo solution. The product for all samples considerably exceeds the solubility constant K_{sp} of apatite, about 5×10^{-118}, indicating a greater driving force for apatite precipitation.

most bioactive. Here the question arises as to why Ca(II)-free ormosils are not bioactive. We consider at this moment that silica blocks without Ca(II) ions are less reactive with Kokubo solution, and that they could not provide sufficient apatite nucleation sites. Li *et al.* reported [12] that porous silica gels from TEOS with poly(ethyleneglycol) as the solvent were bioactive. Thus the Ca(II)-free ormosils could be bioactive if silica blocks had an atomic arrangement similar to those gels.

The equation for apatite precipitation

$$5Ca^{2+} + 3PO_4^{3-} + OH^- \rightarrow Ca_5(PO_4)_3OH \quad (1)$$

demonstrates that dissolution of Ca(II) ions from the materials increases the degree of supersaturation in the Kokubo solution, which is already supersaturated regarding apatite precipitation and hence makes it much easier for apatite to be precipitated. The effect of the dissolved Ca(II) ions can be evaluated by the ion activity product in the Kokubo solution [6]. Fig. 4 shows the product for each sample as a function of soaking time. The greater is the product the easier is apatite precipitation. Sample C has the smallest values of ion activity product among the Ca(II)-containing ormosils because of rapid consumption of the phosphate ions (shown in Fig. 3). Samples A and B have greater values of product than sample C, that is, have a greater driving force for apatite formation. Still they are less bioactive than sample C. One could make much account of the role of Ca(II) ions dissolved from the specimen on apatite precipitation if there concentration increased at the earlier stage of soaking and then decreased. The reason is that the decrease can be attributed to a sudden increase in the degree of supersaturation that is caused by dissolution. However, Fig. 4 shows a rather smooth decrease in concentration. On these bases it is concluded that the ease of silanol formation on the surface of sample C is a predominant factor in the better bioactivity for the sample although the effect of dissolved Ca(II) ions cannot be ruled out.

5. Conclusion

Several organically modified silicates (ormosils) with or without incorporation of Ca(II) ions were prepared using sol–gel processes. Their bioactivity was investigated by measuring thin film X-ray diffraction patterns and IR reflection spectra for the specimen before and after soaking in a simulated body fluid (Kokubo solution). The soaking could not precipitate apatite on the surface of Ca (II) free samples, indicating that they were only biocompatible. However, Ca(II)-containing specimens deposited apatite during immersion in the Kokubo solution and were bioactive. The ion activity product in Kokubo solution decreased monotonously with soaking time. Thus the ease of silanol group formation on the ormosils was considered a predominant factor controlling the bioactivity, while the effect of dissolved Ca(II) ions to increase the degree of supersaturation in the simulated body fluid could not be neglected.

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