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Growth of hydroxyapatite on silica gels in the presence of organic additives: kinetics and mechanism

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Abstract Synthetic hydroxyapatite was grown on surfaces of silica gels by immersing silica monoliths in a simulated body fluid at 37 °C. The gels were prepared by the sol-gel method and the drying process was controlled by using different additives (mono- and di-ethylene glycol, formamide and glycerin) to obtain large monoliths. The additives affect the growth of the apatite by changing the kinetic constants of chemical reactions on the silica surface. Surface areas and pore size distributions were determined, energy dispersion and FTIR spectra obtained, and scanning electron microscopy performed. The formation of the hydroxyapatite competes with the formation of crystalline calcium carbonate, but the results allow optimization of conditions for the growth of the former as a function of the type and concentration of the additive. For these optimal conditions, the mechanism and the order of the reaction were determined.

Keywords Hydroxapatite · Silica gels · Sol-gel method

1. Introduction and relation to previous work

Rapid growth of materials science and engineering makes possible more and more practical applications, including optics, construction, electronics biology, medicine, dentistry, cosmetics, etc. These capabilities arise because of our increased capabilities to control material morphology at the molecular level and thus meet specific purposes and demanding requirements [1–8]. This situation has been discussed in some detail by one of us [9]. We see this rapid growth in materials used in medicine,

where the relevance for human health is reflected in the high volume of the commercial demand.

Prostheses constitute an important class of bio-materials designed to fulfill specific requirements of the human body. Among them, the bone-like materials represent a high percentage of synthetic replacement parts for human beings [10–22]. To achieve a good performance as a bone replacement bio-material, we need to have surfaces of synthetic hydroxyapatite (HAp), which assure good bonding between the ceramic and the natural bone. Thus, it is relevant to study the growth of HAp on different substrates [20, 23–25]. These materials are produced by different synthetic routes, resulting in a wide variety of morphologies. Also, it is important to understand the kinetic mechanism for the production of synthetic HAp on such substrates.

A number of ceramic materials have been used traditionally as bio-materials, largely because they show high bio-compatibility, good resistance to corrosion and high wear resistance. However, the extant materials typically have low impact resistance and poor processability.

The sol-gel process for the preparation of ceramic materials was developed by Rustum Roy more than 40 years ago [26]. In the following decades further significant innovative procedures in this area were developed in the Roy laboratory [27–29]. The sol-gel method has a very wide range of applicability and considerable versatility. The versatility, however, requires optimization of the process leading to any predefined product. A number of simple applications of the sol-gel procedure, without a thorough analysis of the processes taking place, led to fragile materials with considerable shrinkage during drying. The use of additives may help to overcome these problems. However, the additives modify not only the material morphology but also the sol-gel hydrolysis and condensation reactions. Consequently, inappropriate additives can impede the growth of other phases on the surfaces of such sol-gel materials [30].

The drying process plays an important role in the fabrication of sol-gel materials because a substantial shrinkage takes place when the liquid phase is removed. The

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shrinkage is proportional to the volume of the evaporated liquid and is typically in the range of 30 to 70%. Moreover, the drying of the gels often produces high internal stresses, which can lead to fracture of the sample.

There exist several procedures which reduce the internal stresses and allow fabrication of monoliths of reasonable sizes (more than a few millimeters side length); e.g., hypercritical drying, freeze drying, and the use of chemical additives which control the evaporation. In the last case, the interfacial energy is reduced, diminishing the internal stresses. Additionally, this method reduces the shrinkage of the material. The most common drying control chemical additives (DCCA) are formamide (NH_2CHO) and mono- and di-ethylene glycol (MEG and DEG).

The use of additives increases the pore diameter r and results in a reduction in the capillary pressure P ($P \approx r^{-1}$) [4]; this is precisely how the additives stabilize the gel. Further, the additives reduce the pressure gradient within the gel, providing a path to diffuse out alcohol, water and unreacted tetraethyl orthosilicate, thus allowing a better control of the drying process.

Although glycerol has not been reported as an additive, we note that it contains three $(\text{OH})^-$ groups per molecule, which in principle can bond to the gel surface, reducing the capillary pressure. This reduction can be carried out through at least two different procedures: (a) by coating the gel with a thin film of glycerol which fills the pores and reduces the flow of volatile liquids from the gel; and (b) by modifying the surface tension between the pores and the volatiles, which also reduces the evaporation. Additionally, glycerol has the advantage of being well tolerated by living organisms.

On the other hand, formamide, the most common DCCA agent, produces very large and clear monoliths, but is highly toxic to living bodies; moreover, it is difficult to remove it completely from the gel.

The rate of gelation is also an important variable, because it controls, to some extent, the final mechanical performance of the material. This rate can be modified by using a catalyst. In the present case, the catalyst chosen for the gel preparation was hydrofluoric acid (HF), as the fluorine ion substitutes some $(\text{OH})^-$ groups on the silica surface, increasing the chemical stability of the material. Additionally, it is well known [31, 32] that, due to the nucleophilic attack of the fluorine ion, the gelation time can be drastically reduced, producing monoliths with a uniform pore size distribution.

Accordingly, in this paper the kinetic mechanisms for the growth of hydroxyapatite on silica gels as substrates and by using different additives to control the drying process to obtain monoliths of large sizes is reported. The role played by the additives on the growth of HAP was investigated and the kinetics of the chemical reaction determined.

The present paper can also be connected to our earlier work on poly(acrylic acid) + zinc diacetate composites [33]. We are also strongly interested in polymeric materials for a variety of applications [34–36]. We recognize

that combination of organic and inorganic constituents leads to composites with properties unobtainable if one is confined to inorganic or organic materials only. In the present case we modify the properties of an inorganic material by organic agents.

2. Experimental

Seven milliliters of tetraethyl orthosilicate (TEOS; reactive grade) (Aldrich, Milwaukee, WI) were mixed with 11 ml of ethanol (reactive grade; JT Baker, Phillipsburg, NJ) and 15 ml of distilled water, with strong agitation; the molar composition of the system TEOS:water:ethanol was 1:4:6. To catalyze the sol-gel reaction, 2 ml of HF (Baker) at 40% by wt. was added. Under these conditions the gelation occurs within 7 min.

Four different additives were added in turn to the system to control the drying process: formamide (reactive grade; JT Baker), MEG, DEG, and glycerin. Silica gels containing different concentrations of additives with respect to ethanol were prepared to find the optimal concentrations for the growth of HAP. The additives concentrations are summarized in Table 1.

The drying process was carried out at room temperature for one week in a saturated ethanol atmosphere; this was achieved by placing the samples in beakers covered with a plastic film containing a small hole (approximately 2 mm in diameter) which allows the elusion of ethanol gradually from the beakers. In all cases the monoliths underwent a dimensional shrinkage of 45%.

Simulated body fluid (SBF) was prepared by dissolving NaHCO_3 , KCl, $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, (JT Baker, reactive grade), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (reactive grade; Aldrich), $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, NaCl and Na_2SO_4 (reactive grade; Merck, Whitehouse Station, NJ) in distilled water; the concentrations of these salts are shown in Table 2. The concentrations of ions in SBF are quite close to that in the real body fluids [12].

After the drying process, the gels were carefully washed several times with distilled water to remove residues of ethanol, water, TEOS and TEOS oligomers that do not form part of the gel. Subsequently, the monoliths were cut into small cubes of 5 mm side length and immersed in SBF for 5 weeks at 37 °C. To keep the concentration of the ions constant, the SBF was replaced every week. To characterize the gels, samples were removed from the

Table 1 Type and concentration of additives used in the sol-gel process

Additive 1	Additive 2: ethanol (vol.%)
Formamide	40
MEG	35
DEG	35
Glycerin	5
	10
	15

Table 2 Concentrations of different salts used to prepare the SBF

Reactive	mmol/l	mg/l
NaCl	136.80	7994.59
NaHCO_3	4.20	352.84
KCl	3.00	223.65
K_2HPO_4	1.00	174.18
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	1.50	304.95
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	2.50	367.50
Na_2SO_4	0.50	71.02

SBF every week, washed with distilled water and dried at room temperature.

The surface areas and the pore size distributions of the monoliths were determined using BET analysis with a MicroMetrics (Londonderry, NH) ASAP 2000 apparatus.

The gel surfaces were characterized by using a scanning electron microscope JEOL (Peabody, MA) 5200; for this purpose all samples were carbon coated.

The determination of the chemical species in the samples was performed by elemental analysis using energy dispersion spectroscopy (EDS) with a JEOL model JEM 6400 scanning electron microscope. EDS experiments were performed on washed samples to take into account only the species that are chemically attached to the silica gel.

The crystalline structure of HAp was determined by using an Siemens (Madison, WI) x-ray D-5000 diffractometer with Cu-K α radiation. The 2θ angle was varied from 5° to 70° .

The IR spectroscopic characterization was performed in a Nicolet (Madison, WI) FT-Raman 910 apparatus.

3. Surface area pores and pore size distributions

Our BET results are summarized in Table 3. As can be noticed, the surface areas are not affected by the additive used to prepare the gels; the average surface area for all the additives is $360 \text{ m}^2/\text{g}$. It is important to note that this active surface area where the HAp grows is higher than in procedures reported in the literature. As a result, we have high process efficiency and high rate of deposition of the crystalline phase on the gel surface.

For the pore size distribution, the situation is different, in that a strong dependence on the type of additive used for the gel preparation is observed. For the case of MEG, the mean of the pore size distribution is significantly smaller than that of formamide. Apparently the pore size is controlled not only by the molecular size but also by the interfacial tension created by the corresponding additive. These differences may be important for some other specific properties. However, as will be discussed later, the growth of the HAp take place on the gel surface and thus the bulk pore size distribution is not relevant.

4. Energy dispersion spectroscopy (EDS)

A typical EDS spectrum for a sample with glycerin and with 5 weeks immersion time is shown in Fig. 1. Here, the intense peaks correspond to a high content of P and Ca in the sample, while the tiny peaks reflect the presence of small amounts of C, Na and Mg in the silica gel. The Si peak corresponds to the silica of the gel. Other EDS spectra we have obtained show similar features and therefore have been omitted here for brevity.

5. X-ray diffractometry

The X-ray diffractogram of the same sample as in Figure 1 is shown in Fig. 2. The main characteristic diffraction reflections of HAp are present. The broad peak centered

Table 3 Surface areas and pore size averages for the silica monoliths

Additive	Superficial area (m^2/g)	Average pore size (nm)
Formamide	340	16.7
MEG	389	9.6
Glycerin	358	10.6

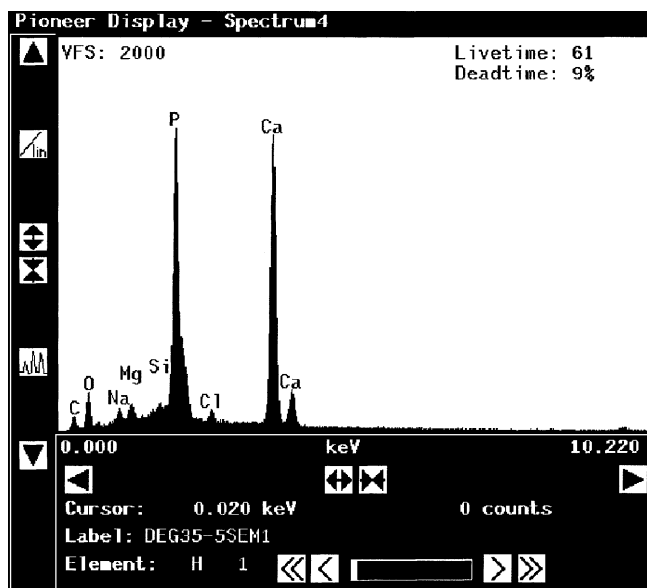


Fig. 1 EDS spectrum of a gel prepared with 10% glycerin and 5 weeks immersion time

at 23° corresponds to the amorphous silica gel. There is a strong reflection centered at 46° that corresponds to a different crystalline phase, namely calcium carbonate, which is also formed during this process. The calcium carbonate formation competes on the gel surface with the HAp formation; this issue will be addressed later on.

6. FTIR spectroscopy

FTIR spectra are shown in Fig. 3. It is possible to see the characteristic bands of HAp and those belonging to the gel, the additive (in this particular case, glycerin) and water. These results show that HAp was indeed grown on the surface of the silica gel. Similar results were obtained with the other additives. Thus, the use of these organic compounds allows the nucleation and growth of the HAp crystalline phase on the silica surface when is immersed in SBF. However, these additives also modify the chemical process, a topic to be discussed in what follows.

7. Scanning electron microscopy (SEM)

The SEM micrographs in Figs. 4, 5, 6, and 7 correspond to all the additives and to four different immersion times,

Fig. 2 X-ray diffractogram for the sample shown in Fig. 1

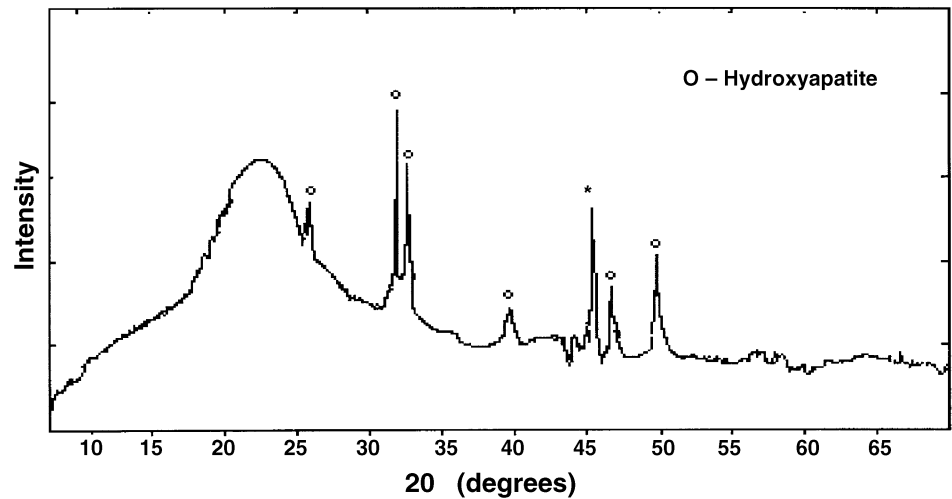
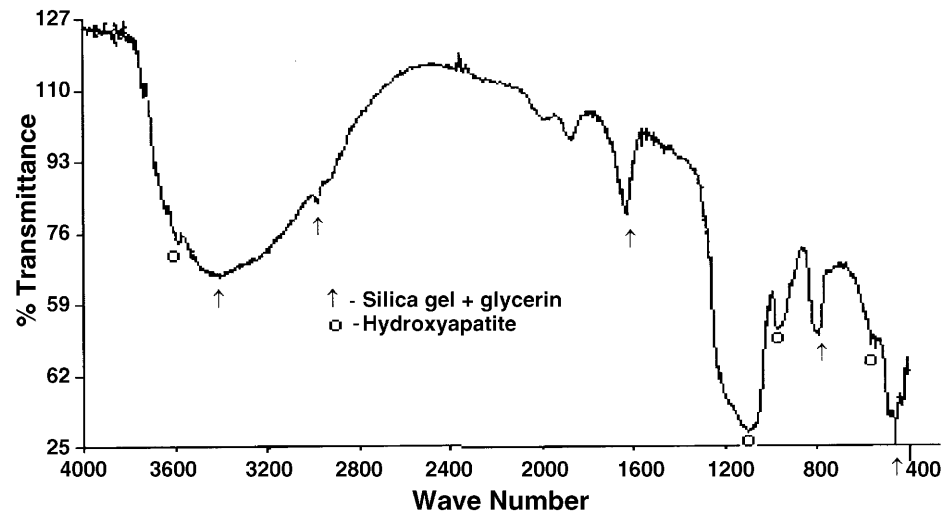


Fig. 3 FTIR spectrum for the sample shown in Fig. 1



namely 2, 3, 4 and 5 weeks. One can conclude from the micrographs that the amount of HAp on the gel surface increases with the immersion time. In all cases HAp begins to grow as small particles with a spherical shape. As the immersion time is increased, the size and number of these particles is also increased. Close to the end of the process, a variety of spherical particles with different sizes is observed.

As already pointed out, the use of additives modifies the morphology and the amount of HAp deposited on the silica surface. For example, for formamide, in the beginning of the immersion period two different particle sizes were observed. After 5 weeks, an average size of 4.5 μm was reached. For these samples, the SEM micrographs show that HAp particles appear from the very first week of immersion into the SBF, suggesting that a heterogeneous nucleation occurs on the gel surface. These particles show a fast growth rate at the beginning of the immersion. Later on, the process slows down, which is the reason why the final size does not exceed 4.5 μm .

For MEG, the growth of HAp was initially random, producing a wide particle size distribution. In this case,

the largest size reached was about 5 μm . For DEG, the situation was similar to the former case but a larger particle size was obtained (7.5 μm).

Finally, for glycerin, the initial number of HAp particles was smaller as compared to the other cases. However, we achieve here, with time, the largest particle size of 14.5 μm . In this case, the SEM micrographs show that the HAp particles appear after the second week of immersion, while all the particles have approximately the same size. This suggests that a heterogeneous nucleation occurs mainly in the SBF and, after these nuclei have reached some critical size, they are deposited on the gel surface. For this additive, the growth of HAp was a slow process, producing not only the largest particles but also more uniform sizes.

The size of the HAp particles depends strongly on the additive concentration. In Fig. 8, the case of glycerin at different immersion times is shown. The largest particle size was obtained for a glycerin concentration of 10% and 5 weeks of immersion time. It is important to notice that the particle size shows a maximum at a glycerin concentration of 10%, and increases monotonically with

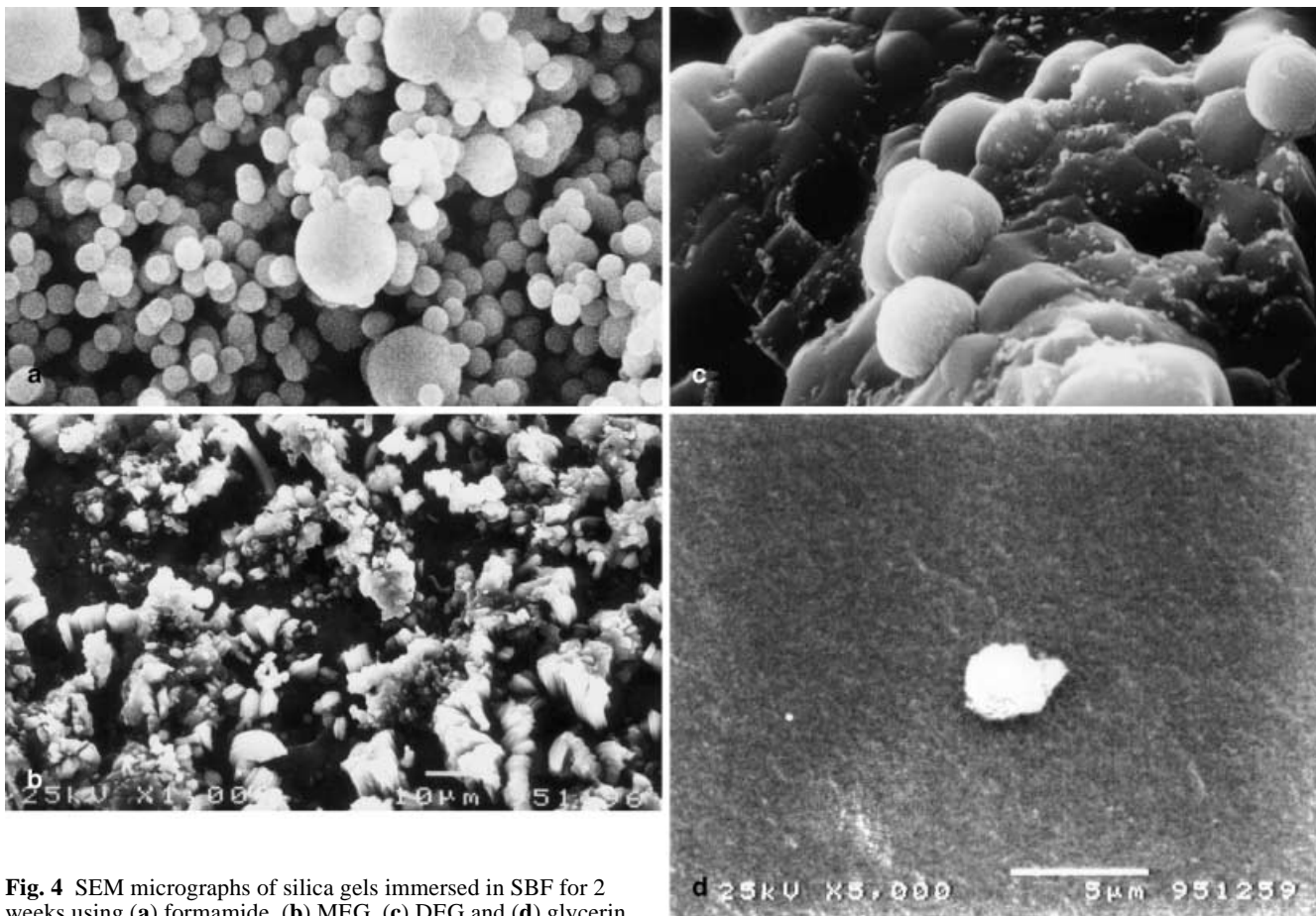


Fig. 4 SEM micrographs of silica gels immersed in SBF for 2 weeks using (a) formamide, (b) MEG, (c) DEG and (d) glycerin

the immersion time. One possible explanation for this behavior is the presence of the $(OH)^-$ groups of glycerin. An excess of this additive increases substantially the number of these groups where the HAp can be nucleated. However, this HAp is not chemically attached to the gel and apparently it was removed when the gel was washed.

Figure 9 shows graphically the dependence of the particle size as a function of the immersion time for glycerin at different concentrations. As mentioned, for 10% of glycerin the largest particle size is obtained. It is important to remark that the largest concentration of glycerin used (15%) results in the smallest particle size. This indicates an inhibition effect of the chemical reaction between the silica gel and the metal ions, which are the first to react with the active groups on the gel surface. This effect is more pronounced in the case of glycerin, with its three $(OH)^-$ groups per molecule, than with other additives. The competition between the growth of HAp either on the silica gel or in the glycerin molecules has to be taken into account because it will eventually reduce the efficiency of the HAp coating process on the gel surface.

8. Kinetics of the HAp growth

From Fig. 9, a parabolic dependence of the particle size on the immersion time is observed for all concentrations of glycerin used. The continuous curves in the figure correspond to a parabolic fitting of the experimental data. On this basis, it is possible to obtain the rate of particle growth as a function of time, a function which is obviously linear. This is shown in Fig. 10 for all glycerin concentrations. This result is fairly important because it predicts a first-order kinetic mechanism for the growth of HAp when glycerin is used as the additive:

$$\frac{dR_{\text{HAp}}}{dt} = gt + a \quad (1)$$

where R_{HAp} is the radius of the HAp particle, g is a function of the additive concentration only and a depends also on the additive concentration.

To determine the kinetics for HAp formation, the concentration of Ca^{2+} ions in the SBF was titrated by using EDT and black of erichromium as the indicator. The results are shown in Fig. 11, where the relative concentration of Ca^{2+} ions, represented as $-\ln c_{\text{Ca}}/c_{\text{Ca}0}$, was plotted as a function of the immersion time; $c_{\text{Ca}0}$ pertains to the concentration of the ions at the beginning of the reac-

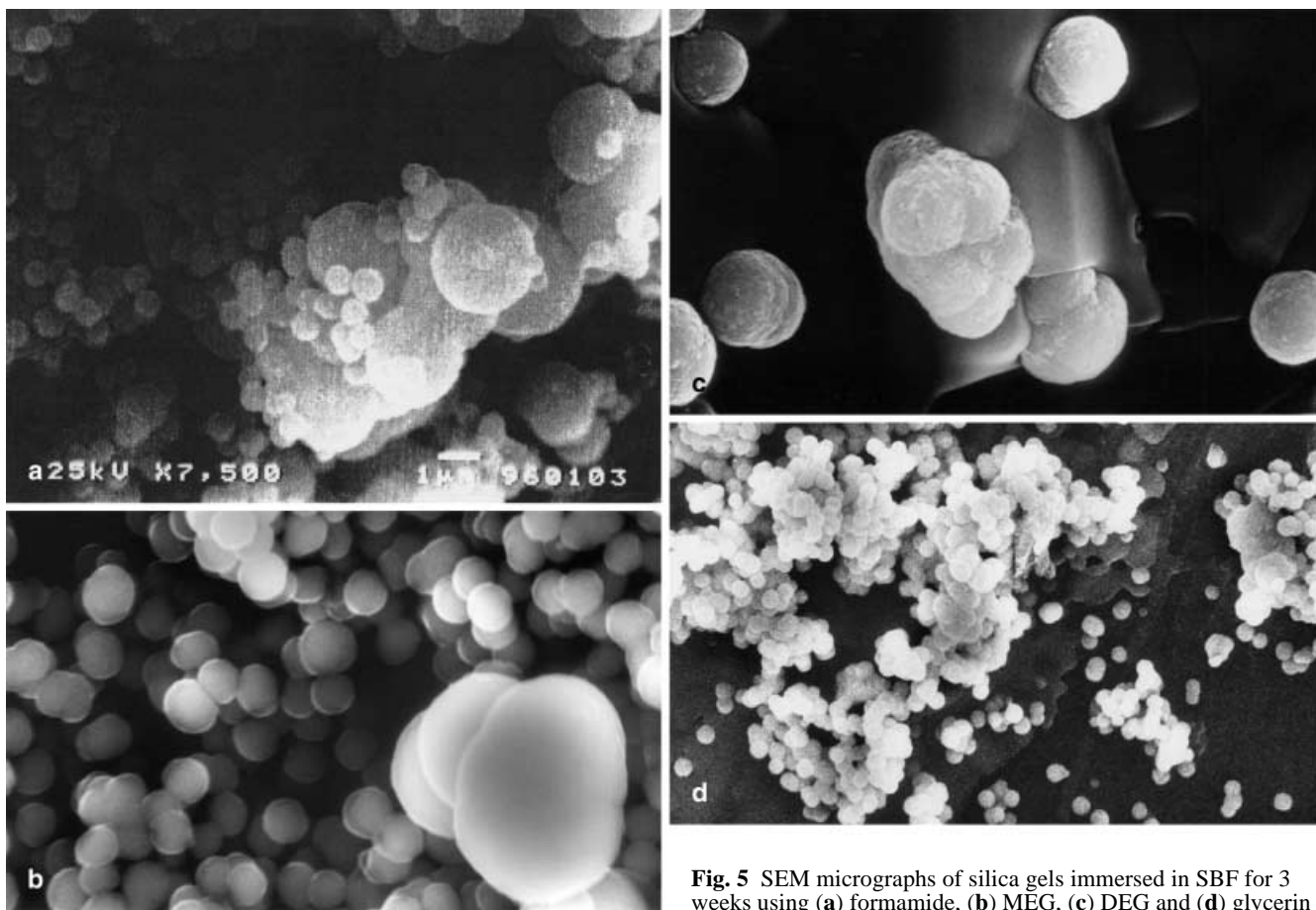


Fig. 5 SEM micrographs of silica gels immersed in SBF for 3 weeks using (a) formamide, (b) MEG, (c) DEG and (d) glycerin

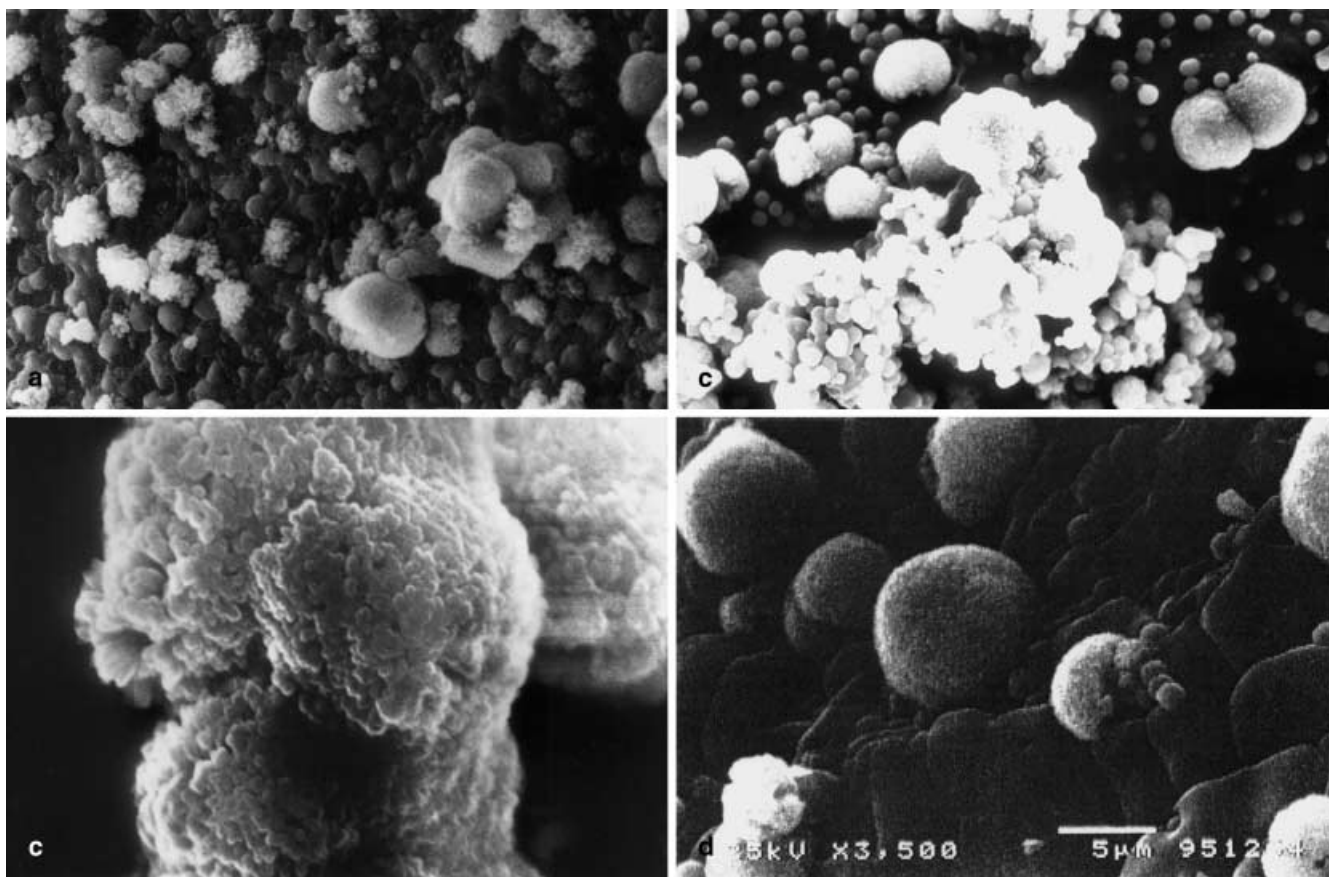


Fig. 6 SEM micrographs of silica gels immersed in SBF for 4 weeks using (a) formamide, (b) MEG, (c) DEG and (d) glycerin

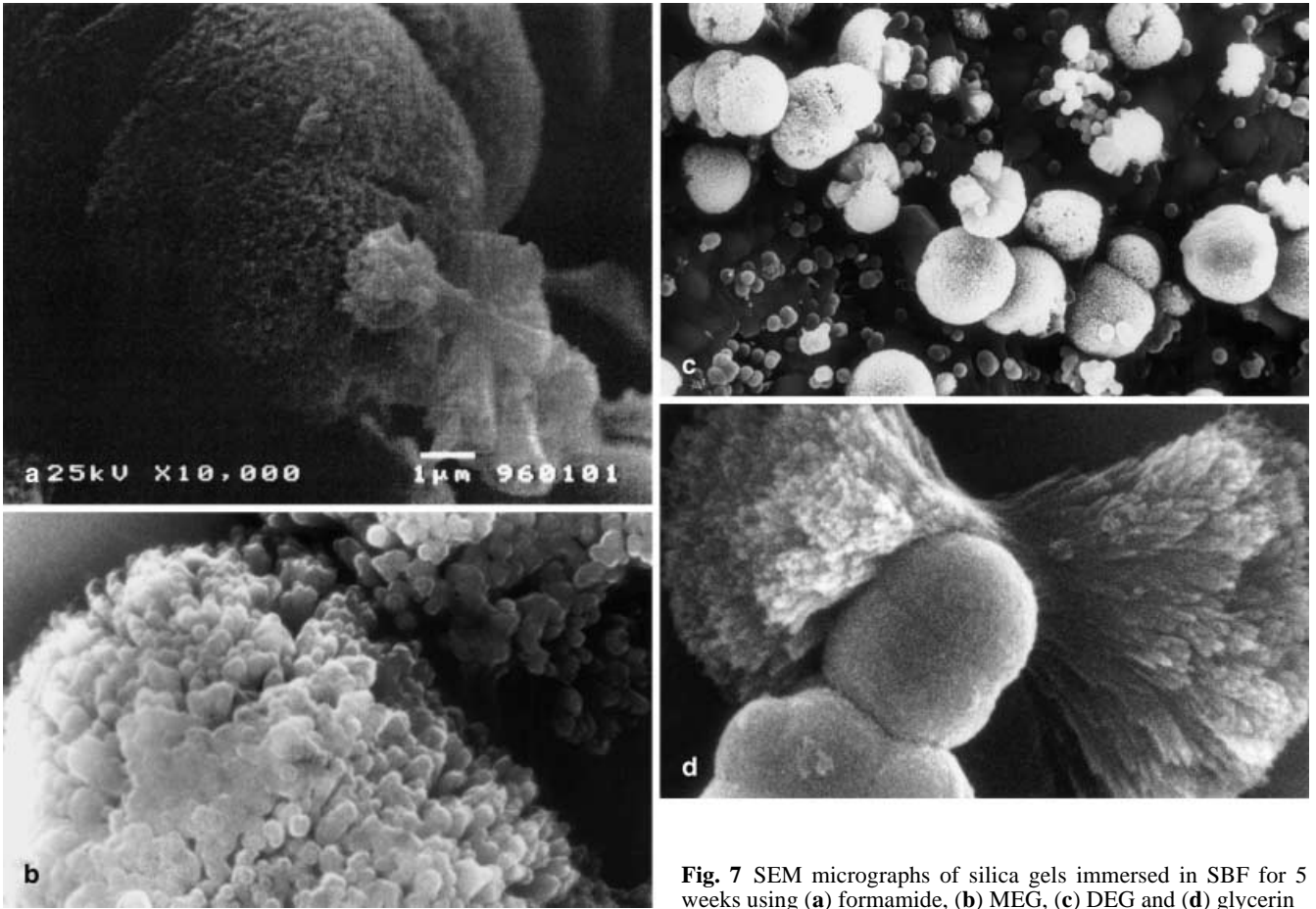


Fig. 7 SEM micrographs of silica gels immersed in SBF for 5 weeks using (a) formamide, (b) MEG, (c) DEG and (d) glycerin

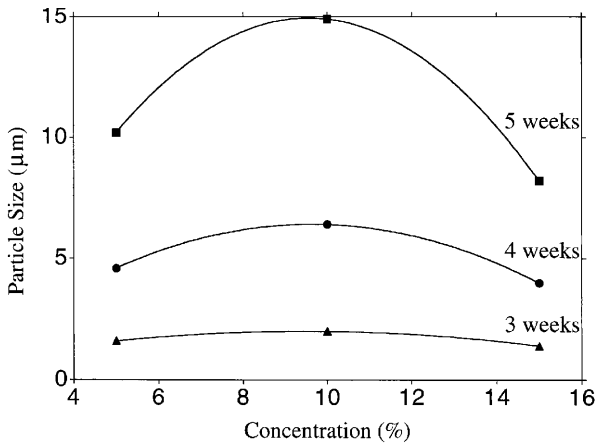


Fig. 8 Particle size of HAp as a function of glycerin concentration for different immersion times

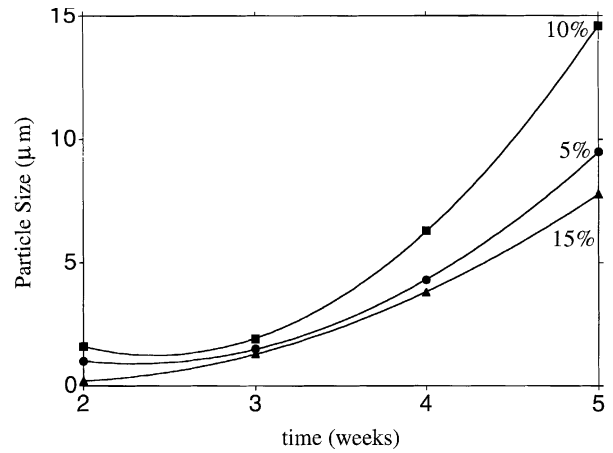
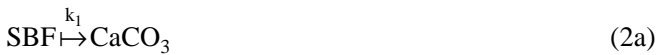


Fig. 9 Particle size of HAp as a function of immersion time for different glycerin concentrations

tion. The data are fitted to a straight line (the continuous line in the figure).

The consumption of Ca^{2+} from the SBF does not only produce HAp, but also creates calcium carbonate. These are simultaneous first-order reactions that can be written as:



where k_1 and k_2 are the respective reaction constants.

The rates of reaction for reactants and products are given by

$$r_{\text{SBF}} = -\frac{dc_{\text{SBF}}}{dt} = k_1 c_{\text{SBF}} + k_2 c_{\text{SBF}} = (k_1 + k_2) c_{\text{SBF}} \quad (3a)$$

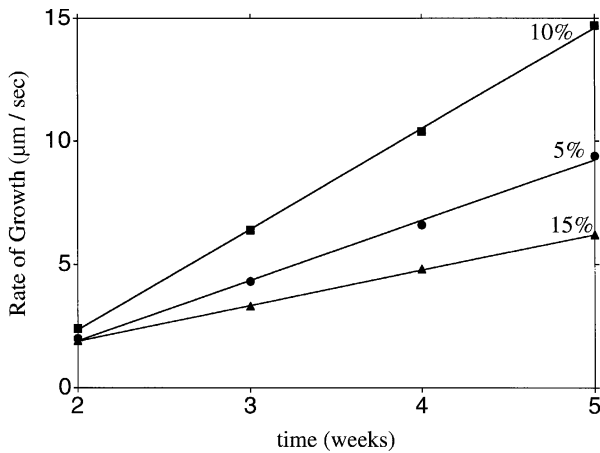


Fig. 10 Rate of growth of HAp as a function of immersion time for different glycerin concentrations

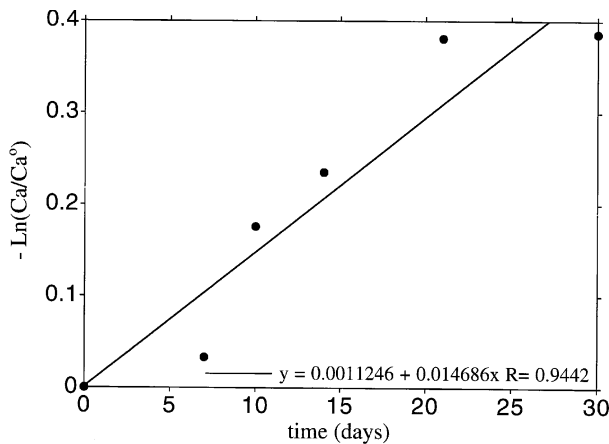


Fig. 11 Dependence of the concentration of Ca²⁺ ions in the SBF on immersion time

$$r_{\text{CaCO}_3} = -\frac{dc_{\text{CaCO}_3}}{dt} = k_1 c_{\text{SBF}} \quad (3b)$$

$$r_{\text{HAp}} = -\frac{dc_{\text{HAp}}}{dt} = k_2 c_{\text{SBF}} \quad (3c)$$

After integrating equation (3a) one obtains:

$$-\ln\left(\frac{c_{\text{SBF}}}{c_0}\right) = (k_1 + k_2)t \quad (4)$$

This equation fits well the experimental data shown in Fig. 11, where the sum of the reaction constants, $k_1+k_2=1.946\times 10^{-7} \text{ s}^{-1}$, can be easily determined. It was not possible, however, to find exactly the reaction constant for the HAp formation k_2 alone, since the precise concentrations of the products would need to be known. At the same time, it is clear that there are two simultaneous first-order reactions that consume calcium ions from SBF: one that produces calcium carbonate and another that produces HAp.

Nevertheless, it is possible to make a rough estimation of k_1 and k_2 based on the relative size of the corre-

sponding diffractogram peaks shown in Fig. 2. The approximate results so obtained are then $k_1=0.769\times 10^{-7} \text{ s}^{-1}$ and $k_2=1.177\times 10^{-7} \text{ s}^{-1}$. This indicates that the reaction involving the growth of HAp on the silica surface is preferred over the formation of calcium carbonate. A precise knowledge of the rate constants k_1 and k_2 would allow understanding of under what circumstances which of these reactions is dominant, making possible the optimization of the formation of HAp on the silica surface.

9. Concluding remarks

Synthetic hydroxyapatite was grown on the surface of silica gels prepared by the sol-gel method and modified with different additives. The fastest growth rate of HAp on the silica surface was obtained with glycerin at a concentration of 10%. It was also found that simultaneous first-order chemical reactions were taking place, one that produces HAp at the rate k_1 , and another that corresponds to the formation of a crystalline phase of calcium carbonate at the rate k_2 . For the case of glycerin, the reaction constant for the HAp formation was determined; it predicts that this reaction is preferred with respect to the formation of calcium carbonate.

Needless to say, the constants k_1 and k_2 would be different for other concentrations of glycerin, and still different for other additives. Thus, a particular combination of experimental techniques and computational procedures should enable the optimization of HAp formation on the silica surfaces.

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