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Hydroxyapatite precipitation on a carboxylated vinyl chloride-vinyl acetate copolymer

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The kinetics of crystal growth of hydroxyapatite [$Ca_5(PO_4)_3OH$, HAP], the thermodynamically most stable calcium phosphate phase, on a commercially available carboxylated (1%) vinylchloride (83%) vinyl acetate (13%) copolymer was investigated at relatively low ionic strength, at 37 °C and pH 7.4. Stable supersaturated solutions were seeded with HAP seed crystals as reference and with the polymer in powder form. Well defined induction times preceded the growth of HAP on the polymeric substrate. The induction times measured were inversely proportional to the solution supersaturation. The rates of crystallization both on HAP and on the polymer were measured at conditions of constant supersaturation. HAP growth took place on both substrates and the parabolic dependence of the rates of crystal growth on the relative solution supersaturation suggested a surface diffusion controlled mechanism. The rates of HAP crystal growth on the polymeric substrate were found to depend on the amount of the seed material suggesting selective overgrowth of HAP. A possible explanation for this selectivity is the development of active growth sites through the formation of surface complexes of the calcium ions with the dissociated carboxyl groups. *© 2001 Kluwer Academic Publishers*

1. Introduction

The investigation of the possibility for using polymers as substrates for the selective deposition of sparingly soluble salts is of paramount importance both from the point of view of modeling of processes related to biomineralization [1–3] and for the design and development of novel biomaterials [4].

Hydroxyapatite ($Ca_5(PO_4)_3OH$, HAP) is considered to be the model compound for the inorganic constituent of bone and teeth. On the basis of total calcium and total phosphorus content, human blood serum is supersaturated with respect to a number of calcium phosphate salts, in the order of decreasing solubility: calcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$, DCPD), octacalcium phosphate ($Ca_4H(PO_4)_3 \cdot 2.5H_2O$, OCP), tricalcium phosphate ($Ca_3(PO_4)_2$, β-TCP), and HAP. Due to extensive complexation between the free calcium ions and the macromolecules present in biological fluids, such as proteins, enzymes, etc., at physiological conditions the actual supersaturation with respect to calcium phosphates is rather low, just sufficient for the formation of β-TCP and HAP. The formation of TCP at low temperatures has never been convincingly demonstrated [5–7]. In vitro crystallization experiments of calcium phosphates done at low supersaturations are therefore considered as better simulations for the biological mineralization processes [8, 9].

According to experimental reports, polymers inert with respect to the deposition of HAP, may be converted into substrates appropriate for the selective deposition of HAP by the introduction of phosphate groups on their surface. These groups are believed to function by providing the nucleation sites for HAP overgrowth, through Ca^{2+} ion accumulation [10–13]. An open question is whether the introduction of ionizable groups, other than phosphate, in the molecular structure of non nucleating polymers may also result in rendering the substrates suitable for HAP selective crystallization.

In the present work we have investigated the effectiveness of the presence of carboxylated groups on polymers for the induction of the overgrowth of HAP from aqueous supersaturated solutions. Experiments were done in which calcium phosphate supersaturated solutions were seeded with commercially obtained carboxylated vinyl chloride-vinyl acetate copolymer powders. The growth kinetics of HAP were measured by the highly sensitive, accurate and reproducible technique of constant solution supersaturation. The crystallization of HAP on HAP seed crystals was used as reference for the comparison of the effectiveness of the foreign substrate [14–17].

2. Experimental section

The commercially obtained carboxylated (1%) vinylchloride (83%) vinyl acetate (13%) copolymer [Aldrich, batch No 20, 030-1] was used as provided in powder form without further purification or drying.

HAP seed crystals were prepared from concentrated calcium nitrate and potassium phosphate as detailed elsewhere [18]. The specific surface area determined by nitrogen adsorption (multiple point B.E.T., Perkin-Elmer Model 212 D sorptometer) was found 52.0 m^2/g and 3.0 m^2/g for HAP and for the copolymer, respectively.

The adsorption of Ca^{2+} on the copolymer was studied by suspending 0.03 g of powdered copolymer in 0.013 dm^3 of solutions with various Ca^{2+} concentrations ranging from 3×10^{-4} to 6×10^{-3} mol dm^{-3}, at 37 °C and pH = 7.40. Past the equilibration period, (ca. 20 h), the suspensions were filtered through membrane filters (0.2 μm, Millipore) and the filtrates were analyzed for calcium by atomic absorption spectrometry (AAnalyst 300). The quantitative analysis of the total phosphate in solution was done spectrophotometrically (Hitachi, U-2001) using the appropriate calibration curve [18].

All precipitation experiments were done at 37.0 ± 0.2 °C in a 0.250 dm^3 double-walled glass reactor thermostated with circulating water from a water bath. Stock solutions of calcium nitrate and sodium dihydrogen phosphate were prepared from solid reagents (Merck puriss) using triply distilled, CO_2-free water. Standardization was carried out as described elsewhere [6]. Prior to standardization, all solutions were filtered through membrane filters. The supersaturated solutions were prepared in the glass reactor by mixing equal volumes (0.1 dm^3 each) of calcium and phosphate solutions. Sodium nitrate was used for the adjustment of ionic strength. The solution pH was adjusted by the slow addition of standard sodium hydroxide under nitrogen atmosphere which was ensured by bubbling water vapor saturated prepurified nitrogen (Linde Hellas) through the supersaturated solution. All experiments were conducted at pH 7.40. Following pH adjustment, all solutions in this work were stable at least for 3 days, as indicated from the constancy of pH and from the solution composition. Past a degassing period of 4 h for each experiment, a quantity of 0.015 g and 0.2 g in the case of HAP and powdered copolymer, respectively, was introduced into the supersaturated solutions. The seeds added were dispersed completely in the magnetically stirred supersaturated solutions. Precipitation started immediately after the introduction of HAP seeds, whereas the lapse of easily measurable and reproducible (better than 10%, mean of four experiments) induction times followed the addition of the powdered copolymer in the solutions. The precipitation process, accompanied with proton release, triggered the addition of calcium nitrate, sodium dihydrogen phosphate and sodium hydroxide titrants from two mechanically coupled burettes of a modified automatic titrator controlled by a personal computer so as to keep the solution supersaturation constant [14–17]. The precipitation process was monitored by a glass/ saturated calomel pair of electrodes (Metrohm) standardized with NBS standard buffer solutions before and after each experiment. The constancy of calcium and phosphate concentrations throughout the course of precipitation was confirmed by analysis of samples withdrawn periodically from the working solution. The samples withdrawn were filtered under vacuum through membrane filters (millipore 0.22 μm) in order to separate the solids from the aqueous phase. The filtrates were analyzed for calcium and phosphates. The solids were air dried. Next, they were examined by powder X-ray diffraction (XRD, Phillips 1830/40), Fourier transform infrared spectroscopy (FTIR, Bruker), differential scanning calorimetry (DSC, TA Instruments 2920) and scanning electron microscopy (SEM, JEOL ISM 5200).

3. Results and discussion

The solution composition is very important in determining the supersaturation which is an absolute prerequisite for the growth of salts in supersaturated solutions. The polymer tested contained ionisable groups which through the interaction with the solution components would possibly cause changes of the concentration of the HAP constituent ions in the solution.

The process of selective growth of HAP on the polymer is a heterogeneous process. The polymeric substrate provides the active sites necessary for the nucleation of HAP. The growth of a mineral on a foreign substrate requires therefore overcoming a free energy barrier. In the case in which the substrate is of the same nature as the overgrowth this energy is minimal and as a rule a salt grows on seed crystals of the salt introduced in its supersaturated solutions. This fact is used for the investigation of the crystal growth of sparingly soluble salts in supersaturated solutions (seeded growth technique) [19]. In the present work, the growth of HAP on the polymeric substrate by heterogeneous nucleation was investigated in supersaturated calcium phosphate solutions. The rates of HAP formation on the polymer were compared with the rates of HAP growth on synthetic HAP seed crystals. The latter experiments were used as reference. HAP seed crystals is the most favorable substrate for the formation of HAP. Any substrate, other than HAP, should be compared with HAP with respect to the kinetics of formation of this salt.

For the calculation of the solution supersaturation, the Ca^{2+} uptake from the supersaturated solutions by the suspended copolymer with the dissociated carboxyl groups was investigated. The adsorption isotherm obtained is shown in Fig. 1. No adsorption was found

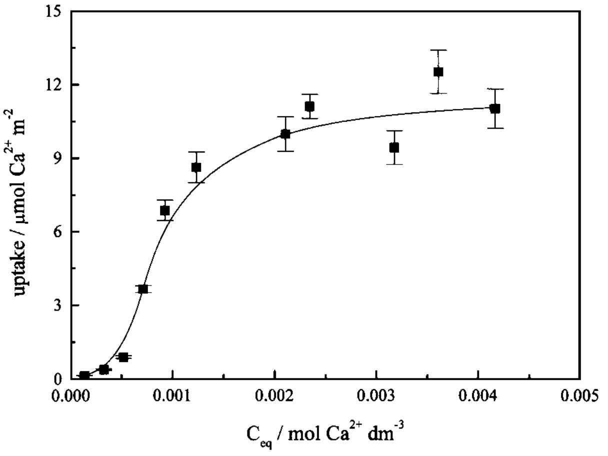


Figure 1 Adsorption of Ca^{2+} ions on the carboxylated (1%) vinylchloride (83%) vinyl acetate (13%) copolymer per unit surface area of the substrate as a function of the equilibrium concentration; pH = 7.4, t = 37 °C, ionic strength 0.01 M $NaNO_3$.

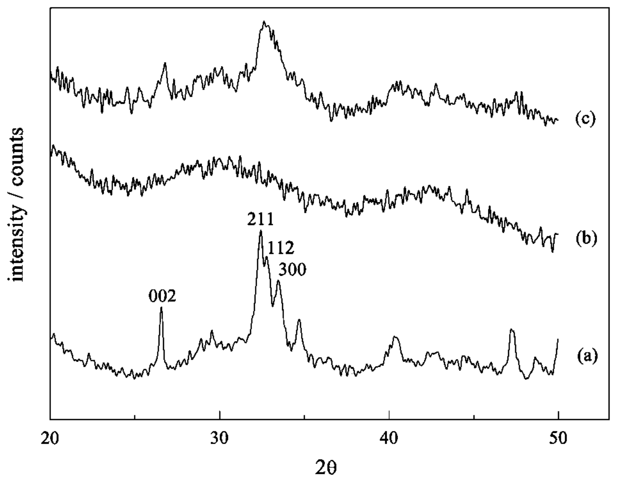


Figure 2 X-ray diffraction patterns corresponding to: (a) HAP synthetic seed crystals, (b) carboxylated copolymer and (c) copolymer with HAP overgrowth.

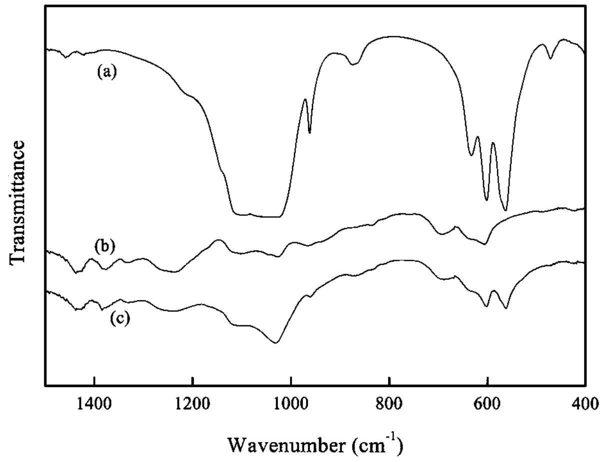


Figure 3 Infrared spectra of (a) HAP synthetic seed crystals, (b) carboxylated copolymer and (c) HAP deposited on the copolymer.

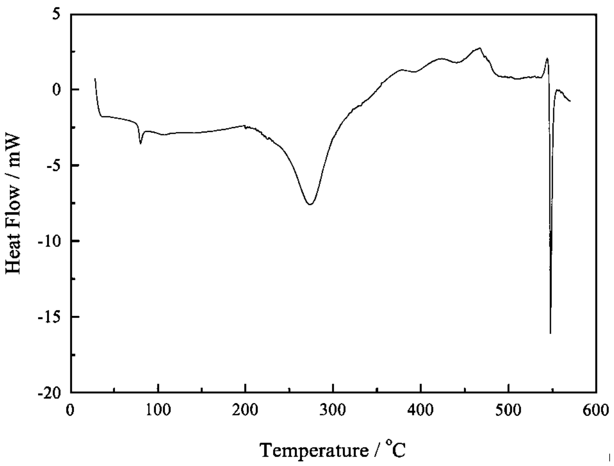


Figure 4 Differential scanning calorimetry results of the composite copolymer-HAP formed in calcium phosphate supersaturated solutions.

for the phosphate ions upon equilibration with the substrate.

In both series of experiments HAP was found to form exclusively on the seed solid particles introduced in the supersaturated solutions, as may be seen from the X-ray diffractograms and the FTIR spectra shown in Figs 2 and 3 respectively. The characteristic diffraction peaks of HAP (Fig. 2a) corresponding to the 002, 211, 112 and 300 reflections [20] can be easily distinguished in the XRD pattern of the composite polymer-HAP material (Fig. 2c). Moreover, the bands at 1032, 962, 602, 561 cm^{-1} corresponding to P-O asymmetrical and symmetrical stretching vibrations and O-P-O bending vibrations of the phosphate group [21], and at 631 cm^{-1} corresponding to rotating vibrations of OH group of HAP [22] (Fig. 3a), which are not present in the initial polymer seed particles (Fig. 3b), were found in the spectrum of the composite HAP-polymer solid obtained after the completion of the crystal growth process (Fig. 3c).

Additional evidence for the formation of HAP on the copolymer substrate was obtained by thermal analysis of the composite precipitates. Differential scanning calorimetry measurements yielded the results shown in Fig. 4. The endothermic changes at 274 and 547 °C correspond to the copolymer degradation and HAP decomposition, respectively. More specifically the sharp peak at ca. 550 °C may be ascribed to the conversion of the HPO_4^{2-} present on the HAP surface to pyrophosphate and water release [23]

$$2HPO_4^{2-} \rightarrow P_2O_7^{4-} + H_2O$$

The peak therefore at 547 °C provided additional evidence for the presence of HAP in the composite polymer-mineral material formed in the supersaturated solutions. Morphological examination of the precipitated HAP on the amorphous copolymer by scanning electron microscopy revealed the formation of microscopic needle-like crystalline HAP on the amorphous polymer (Fig. 5).

The driving force for the formation of HAP from a supersaturated solution is the change in Gibbs free energy, ΔG, for the transfer from the supersaturated solution to equilibrium:

$$\Delta G = -\frac{R_g T}{\nu} \ln \frac{\text{IP}}{K_s^o} \tag{1}$$

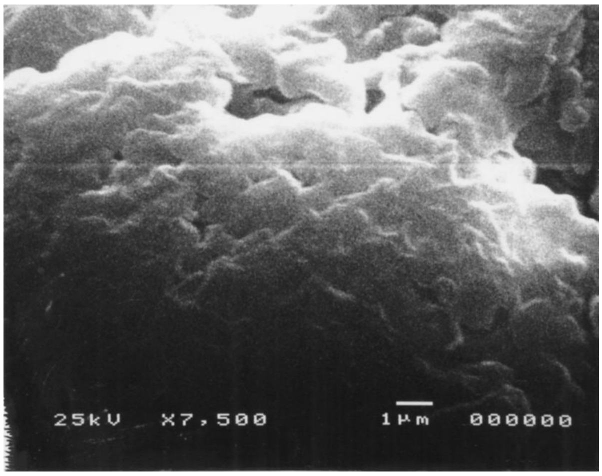
In Equation 1 IP is the ion activity product of HAP: $(\text{Ca}^{2+})^5\ (\text{PO}_4^{3-})^3\ (\text{OH}^-)$, K_s^o its solubility product, ν the number of ions, R_g the gas constant and T the absolute temperature. The ratio IP/K_s^o represents the degree of supersaturation, Ω, and was computed by using the computer code HYDRAQL [24], which is a free energy minimization program taking into account all equilibria in the solution, mass balance and electroneutrality conditions. The relative solution supersaturation, σ, is defined as:

$$\sigma = \Omega^{1/\nu} - 1 \tag{2}$$

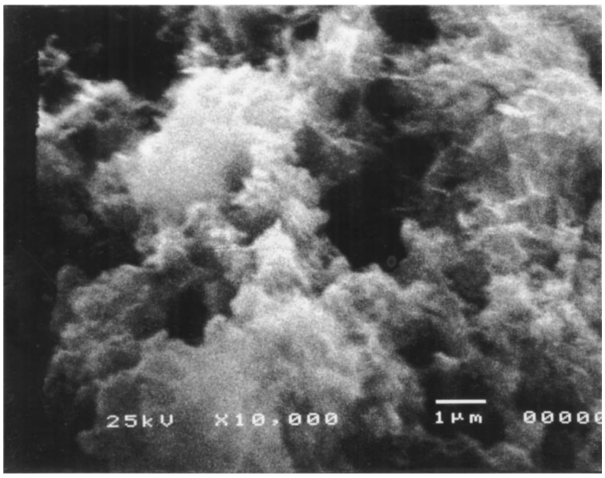
The growth of HAP on the tested polymer took place past the lapse of induction times, τ, which were inversely proportional to the solution supersaturation. The induction time reflects the time needed for the formation of stable supercritical nuclei on the substrate [13]. No induction times were obtained when pure HAP was used as a substrate (seeded growth). The initial solution conditions, the induction times and the

TABLE I Experimental conditions for the crystallization of HAP on different substrates at conditions of constant supersaturation, at pH = 7.40 and $t = 37\,^{\circ}$C: Ca_t, total calcium; P_t, total phosphate. Ionic strengths were adjusted by $NaNO_3$

Substrate	Ca_t (10^{-4} M)	P_t (10^{-4} M)	[$NaNO_3$] (10^{-3} M)	ΔG/KJ mol^{-1} DCPD	OCP	TCP	HAP	τ/s	R (10^{-8} mol/min m^2)
HAP	1.50	0.90	1.50	2.51	1.99	0.86	−3.44	-	4.2
HAP	1.25	1.20	2.00	2.34	1.82	0.75	−3.38	-	4.0
HAP	1.56	0.93	1.56	2.34	1.74	0.62	−3.53	-	6.0
HAP	2.00	1.25	2.00	1.92	1.37	0.27	−4.05	-	5.5
HAP	1.56	1.50	2.50	2.00	1.39	0.25	−3.83	-	4.9
HAP	1.87	1.80	3.00	1.73	1.04	−0.09	−4.18	-	7.6
HAP	2.50	1.50	5.00	1.73	0.96	−0.28	−4.32	-	15.0
HAP	2.50	1.50	2.50	1.63	0.82	−0.44	−4.46	-	17.0
copolymer	4.00	2.40	8.00	1.08	0.09	−1.26	−5.19	2080	1.2
copolymer	4.50	2.70	9.00	0.91	−0.12	−1.50	−5.41	1700	1.3
copolymer	5.00	3.00	5.00	0.64	−0.47	−1.92	−5.78	1060	7.4
copolymer	5.50	3.30	5.50	0.51	−0.64	−2.11	−5.95	680	15.9
copolymer	6.50	3.90	6.50	0.29	−0.94	−2.45	−6.25	240	36.1



(a)



(b)

Figure 5 Scanning electron micrographs of (a) copolymer substrate and (b) HAP deposited on the copolymer.

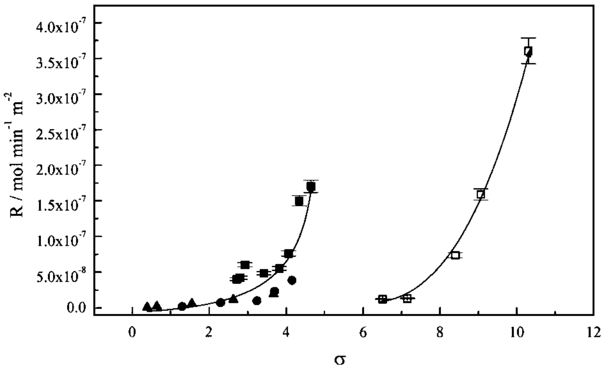


Figure 6 Dependence on the relative solution supersaturation with respect to HAP, of the rates of crystal growth of HAP on different substrates: (■) on HAP seed crystals, (□) on the copolymer and (▲ and ●) on HAP seed crystals (the data have been obtained from refs. 27 and 28, respectively).

rates of HAP formation, R, obtained are summarized in Table I.

The experimental conditions were selected so that the only phase which may be formed was HAP without the intermediate formation of unstable precursor phases. This was verified by the constancy of solution composition throughout the precipitation process. The rate of HAP formation was found to increase with supersaturation and the kinetics were interpreted according to the phenomenological Equation 3 [14, 25, 26]:

$$R = k_g f(s)\sigma^n \qquad (3)$$

In Equation 3 k_g is the precipitation rate constant and $f(s)$ a function of the active growth sites on the substrate. In both series of experiments in which either HAP or the polymer were used as substrates, the rates of HAP formation (normalized per unit surface area) did not depend on the amount of the solid substrate used to induce HAP crystallization. The exponent n in Equation 3 is the apparent growth order. The value of this exponent is considered to be indicative of the mechanism of the crystallization reaction. The kinetics data were fitted according to Equation 3. The curve obtained, shown in Fig. 6, suggested a parabolic dependence of the rates as a function of the solution supersaturation. This dependence in agreement with earlier reports on the seeded growth of HAP on synthetic HAP seed crystals, suggested a surface-controlled spiral growth mechanism [14]. The rate of stirring did not have any effect on the measured rates of crystallization suggesting that the mass transport did not contribute significantly to the precipitation mechanism. Moreover, doubling or tripling the amount of the solid introduced increased the rate of HAP deposition by two or three

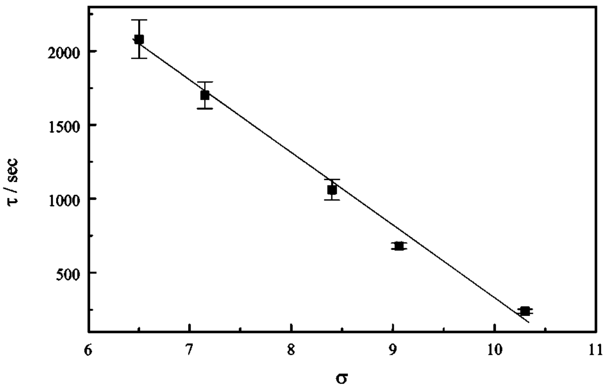
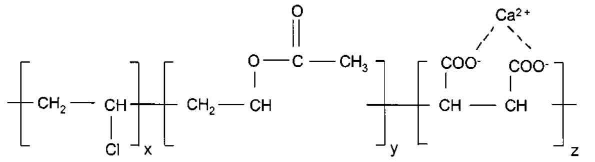


Figure 7 Induction time preceding the precipitation of HAP on copolymer seeds as a function of the relative solution supersaturation; pH = 7.40, $t = 37\,^{\circ}\mathrm{C}$.

times respectively, suggesting that crystal growth took place exclusively on specific active sites on the surface of the substrate. The induction time preceding precipitation of HAP on the copolymer was found to decrease with increasing supersaturation (Fig. 7).

Potentiometric titrations of the copolymer suspensions have shown that at the working solution conditions (pH = 7.40) the carboxyl groups are ionized, facilitating electrostatic interactions with the free calcium ions [29]. The mechanism most likely for the initiation of HAP nucleation on the carboxylated copolymer is through binding of the calcium ions at the ionized carboxyl groups:



Increased calcium ion concentration on the polymer may cause accumulation of phosphate ions and in turn locally enhanced supersaturation which may signal the onset of HAP formation at these sites.

It should be noted that calculations on a model carboxylated polymer containing carboxylate sticker groups, have shown that there is a critical concentration of the carboxyl groups at which the fracture energy for the polymer-solid interface is attained [30]. According to the adsorption data obtained in the present work (Fig. 1) and the concentrations of calcium in the supersaturated solutions the extent of complexation of the free calcium ions with the suspended polymer did not affect the solution supersaturation. Surface complexation, may provide the appropriate local geometry for matching the overgrowth with the substrate [31].

In the heterogeneous nucleation of HAP on the polymeric substrate the free energy change for the formation of the mineral phase nuclei (and hence the macroscopic rate of their formation) depends both on the solution supersaturation (energy for the formation of the bulk phase) and on the interfacial tension of the new phase (energy for the formation of the new embryo) [32]. The rate of heterogeneous nucleation, according to the classical nucleation theory is [33]:

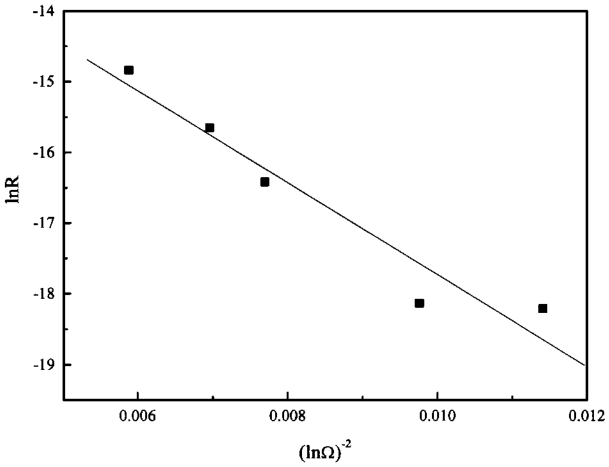


Figure 8 Dependence of the rates of HAP overgrowth on the carboxylated copolymer on $1/(\ln\Omega)^2$.

$$R = A\exp\left(\frac{-\beta\gamma^3\upsilon^2}{k^3T^3}\frac{f(\theta)}{(\ln\Omega)^2}\right) \tag{4}$$

where A a constant, β is a shape factor (32 for spheres), υ is the molecular volume of the HAP overgrowth (= formula weight/$\rho \times 9 \times 6.023 \times 10^{23}$), k is Boltzmann's constant and $f(\theta)$ a function of the contact angle between the nucleating embryo and the substrate. $\theta = 0^{\circ}$ corresponds to perfect matching between the crystal lattices of the substrate with the overgrowth and $\theta = 180^{\circ}$ to complete mismatch. ρ represents the density of HAP [34].

The data obtained from the kinetics of HAP overgrowth on the copolymer were fitted to the logartithmic form of Equation 4. The fit was satisfactory and yielded the straight line shown in Fig. 8. From the slope of this line a value of 123 mJ m^{-2} was calculated. Interfacial tension values obtained from the fitting of the rates measured as a function of the solution supersaturation are summarized in Table II. The interfacial tension value determined in the present work is in good agreement within the range of the values published in the literature. These values are typical for sparingly soluble salts. The low surface tension values may be an indication for the presence of impurities which may catalyse nucleation. Since however there is valid criticism concerning the application of Young's equation to solid-liquid systems [39] the values obtained by fitting kinetics data should be used with caution and for comparison reasons.

TABLE II Interfacial tension values for HAP reported on the literature, based on kinetics data

Substrate	Interfacial tension mJ m^{-2}	Reference
Titanium oxide	39	[33]
Hydroxyapatite	80	[34]
Carboxylated copolymer	123	This work
Dicalcium phosphate dihydrate	152	[26]
Collagen	70	[35]
Phospholipids	55	[36]
Poly-p-phenylene terephthalamide	93	[12]

4. Conclusions

In the present work, it was shown that polymers containing carboxylate groups are effective nucleators of HAP. HAP crystallized selectively on these substrates in calcium phosphate supersaturated solutions following a surface diffusion controlled mechanism. Induction times decreasing with increasing supersaturation were measured. The results obtained suggested that the nucleation centers for the HAP growth are formed by surface complexation of the ionized carboxyl groups with the free calcium ions. The dependence of the rates of HAP formation on the polymer tested yielded an interfacial energy for the new phase of 123 mJ m^{-2} comparable to the values obtained from the analysis of kinetic results reported in the literature and within the range expected for sparingly soluble salts.

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