Chemical Synthesis of Microskeletal Calcium Phosphate in Bicontinuous Microemulsions

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Supersaturated bicontinuous microemulsions have been used as organized reaction environments for the chemical synthesis of calcium phosphates with interconnected microskeletal architectures. Nucleation of apatite occurs within the nanometer-size water conduits of oil/water microemulsions stabilized with didodecyldimethylammonium bromide (DDAB). Subsequent growth results in localized microstructural changes such that the crystals attain dimensions that are larger than the microemulsion channels. Changes in the composition and texture of the microemulsions result in significant modifications in the inorganic architectures. Reticulated inorganic materials are formed from bicontinuous microemulsions that contain a liquid aqueous phase when stored at temperatures below the melting point of the oil phase (dodecane, tetradecane, or tetradecane/hexadecane). Similar experiments with DDAB microemulsions prepared as the high water containing cubic phase or with compositions close to edge of the bicontinuous phase boundary did not produce meshwork architectures. Calcium phosphates grown in tetradecane/water microemulsions stabilized with pentaethylene glycol dodecyl ether had an unusual sheetlike form.

Introduction

The chemical synthesis of inorganic materials with patterned architectures is of potential importance in catalysis, membrane and separation technologies, biomaterials science, and sensory devices. To date, much work has been accomplished in patterning zeolites and related materials at the nanometer scale.[1] More recently, mesoporous silicas have been prepared with hexagonal periodicities of around 5 nm.[2,3] In these materials, organic templates, such as quaternary amine molecules (zeolites) or cylindrical micelles (mesoporous silica), are used to organize the construction of soluble inorganic precursors. How might we extend the length scale of this patterning strategy? One possible approach is to use organic supramolecular assemblies that have preorganized architectures with micron-size dimensions. For example, biolipid tubules can be used to prepare fibrous iron oxide[4] and silica[5] composites, and helical arrays of gold particles.[6] However, these materials are not interconnected in three dimensions. The chemical construction of extended microscopic arrays of inorganic materials requires complementary templates, and recent studies have shown that self-organizing systems, such as oil/water foams[7] or vesicles,[8] can be used for inorganic patterning. The resulting inorganic materials exhibit unusual "organic" form, which in some cases bear a superficial resemblance to biominerals formed by marine algae such as diatoms[9] and coccolithophores.[7]

In this paper we describe another approach to the chemical synthesis of organized inorganic materials. Our strategy is based on the pattern replication of organized media (microemulsions) and is therefore similar to other approaches[7,8] but different in that the length scale of the complex inorganic architecture does not directly correspond to that of the microstructure of the templating agent. Our original intention was to use the nanoscale interconnecting network of water channels present in supersaturated bicontinuous microemulsions as a preorganized reaction system for the synthesis of calcium phosphate with high surface area and nanoscopic porosity. We chose to use oil/water microemulsions containing the surfactant didodecyldimethylammonium bromide (DDAB) because the phase diagram has been carefully studied and the bicontinuous region is stable over a relatively wide range of ternary compositions.[10,11] Small-angle X-ray and neutron scattering indicates that the continuous water channels are approximately 2 nm in size.[12,13] However, as described in a preliminary account of this work,[14] crystallization of calcium phosphate in the bicontinuous phase gave materials that were not commensurate with the microemulsion structure but exhibited a microskeletal or "reticulated" architecture comprising micron-size crystals.

Here, we describe in detail the types of calcium phosphate microstructures that can be obtained from supersaturated bicontinuous microemulsions and the effect of composition and storage temperature on these

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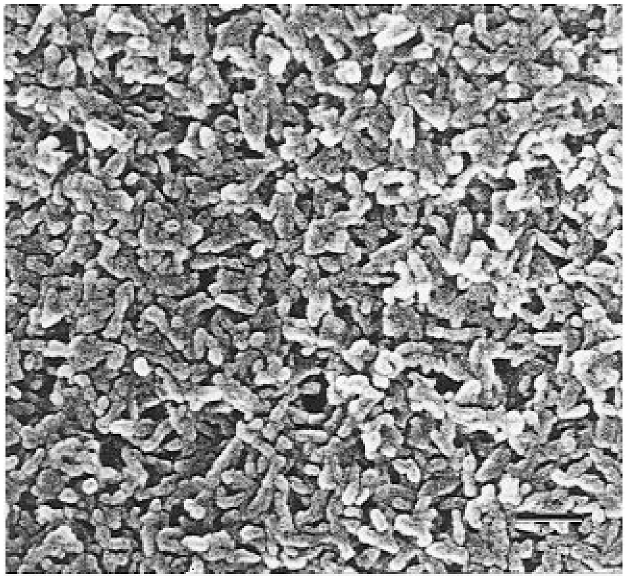

Figure 1. SEM micrograph of calcium phosphate material formed in the absence of microemulsions. Scale bar = 200 nm.

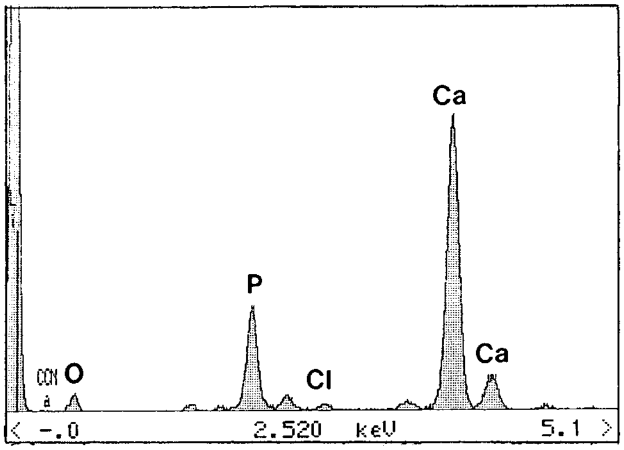

Figure 2. EDXA spectrum of washed calcium phosphate material extracted from DDAB bicontinuous microemulsions.

patterned materials. We also present electron microscopy data on materials extracted at different stages of development in order to elucidate the possible mechanisms responsible for the construction of these inorganic microskeletons. The results suggest that the growth of crystals in interconnected reaction environments that undergo localized reorganization (but not bulk phase separation) could be a promising approach to synthesizing complex inorganic patterns with length scales beyond the mesoscopic level.

Experimental Section

Materials. Didodecyldimethylammonium bromide [DDAB, $(C_{12}H_{25})_2(CH_3)_2NBr$; mp +160 °C] was obtained from Aldrich Chem. Co. and used as supplied. Pentaethylene glycol dodecyl ether [C12E5, $C_{12}H_{25}(OCH_2CH_2)_5OH$)] was obtained from Sigma Chemical Co. and was used without further purification. Dodecane ($C_{12}H_{26}$, mp −9.6 °C), tetradecane ($C_{14}H_{30}$, mp + 5.9 °C), and hexadecane ($C_{16}H_{34}$, mp +18.1 °C) were obtained from Aldrich Chemical Co. and were filtered through 0.2 μm membranes before use. Calcium chloride ($CaCl_2{\cdot}2H_2O$) and disodium hydrogen orthophosphate (Na_2HPO_4) were obtained from BDH and used as supplied.

Table 1. X-ray Diffraction Data for Microskeletal Calcium Phosphate and Standard Sample of Hydroxyapatite (ASTM Card 9-432)

d (Å)	*d* (Å) (std)	*hkl*
8.18	8.17	100
5.26	5.26	101
4.07	4.07	200
3.88	3.88	111
3.44	3.44	002
3.17	3.17	102
3.08	3.08	210
2.82	2.814	211
2.72	2.720	300
2.63	2.631	202
2.26	2.262	310
2.15	2.148	311
2.07	2.065	113
2.00	2.000	203
1.95	1.943	222
1.89	1.890	312
1.84	1.841	213
1.72	1.722	004,411

Instrumentation. Samples for scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were prepared by mounting small amounts of the mineralized material extracted by centriguation of the microemulsion phase. The mounted material was then carefully washed in hot hexane followed by air drying. Samples observed by SEM were gold-coated and examined with a JEOL 1200EX electron microscope operating at 120 kV with an attached scanning imaging device (ASID). Transmission electron microscopy was conducted with JEOL 1200EX and 2000FX electron microscopes operating at 120 and 200 kV, respectively. X-ray powder diffraction was obtained on a Phillips PW-1130 X-ray diffractometer fitted with a Debye−Scherrer camera using Cu Kα radiation. Infrared spectra of the samples were obtained using KBr disks on a Nicolet 510P Fourier transform infrared spectrometer.

Method. *Preparation of Supersaturated Calcium Phosphate Solution (CaP).* A metastable solution of calcium phosphate was prepared by mixing equal volumes of a 6.0 mM solution of calcium chloride with a 3.6 mM solution of disodium hydrogen orthophosphate and the pH adjusted to 7.4 by 0.1 M NaOH. Using a value for the solubility product for hydroxyapatite (HAP)[15], $K_{sp} = 10^{-59}$ (mol^9 L^{-9}), the supersaturation ratio (S), defined as the ratio of the concentration product to K_{sp}, was calculated as log $S \sim 32$. In the absence of a microemulsion phase, calcium phosphate precipitation from this supersaturated solution became visually apparent within 5 min after mixing.

Preparation of Calcium Phosphate Bicontinuous Microemulsions. *$C_{12}H_{26}$/DDAB/CaP:* In a typical experiment, 5 mL of freshly prepared supersaturated calcium phosphate solution was added dropwise to a stirred mixture of 10 g of DDAB and 10 g of dodecane at room temperature. On addition of sufficient solution the mixture transformed into a transparent, fairly viscous bicontinuous mixture. The mixture was then rapidly frozen by immersion of the vessel into liquid nitrogen and was stored at −25 °C for up to 21 days. After storage, the inorganic component was separated by the room temperature centrifugation of the liquid microemulsion at 10 000 rpm for 10 min. A small white pellet of material together with a small quantity of supernatant microemulsion was placed onto a SEM holder and carefully washed with hot hexane or acetone to remove all residual surfactant and oil. The samples were then air dried or oven heated to 200 °C for 25 min. XRD measurements were also made on the pelleted and washed inorganic material. For TEM, a drop of the microemulsion was placed onto a Formvar-covered copper grid and washed several times with hot hexane. SEM studies were also undertaken on inorganic material extracted by filtration of the microemulsion through a 0.2 μm cellulose nitrate membrane without solvent washing. The theoretical maximum yield of inorganic material is approximately 0.1 g/L of microemulsion.

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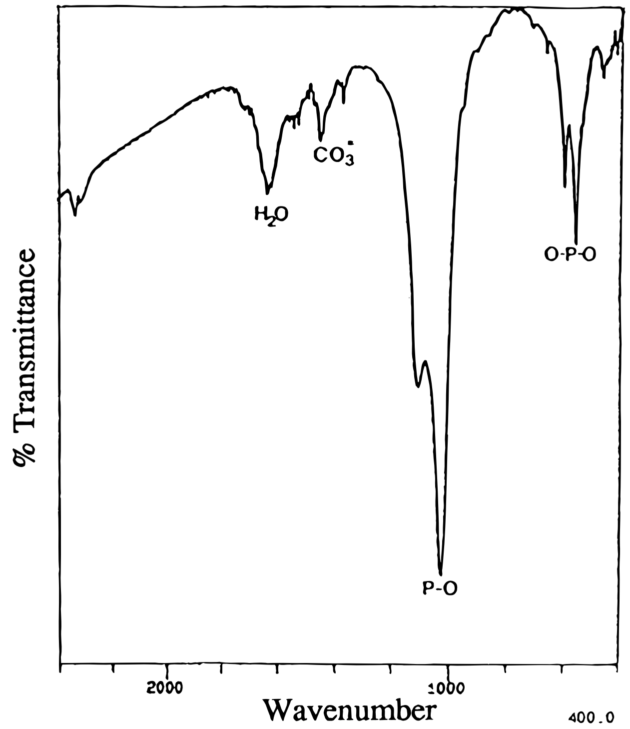

Figure 3. Infrared spectrum of washed calcium phosphate material extracted from DDAB bicontinuous microemulsions. The peaks are consistent with a carbonated hydroxyapatite.

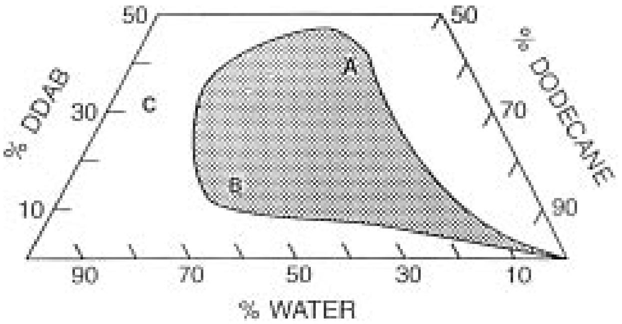

Figure 4. Phase diagram for DDAB/dodecane/water microemulsions.[16] Points A, B, and C refer to compositions used for the preparation of calcium phosphate microemulsions (see text). Point A corresponds to the bicontinuous region, B is at the edge of the phase boundary, and C is the cubic phase. Note that the phase diagram shown is for water and could be modified in the presence of supersaturated calcium phosphate solutions.

The above procedure was repeated but for different compositions in the phase diagram. First, a microemulsion with a composition corresponding to a region close to the edge of the bicontinuous phase was prepared by adding 2 mL of supersaturated solution to 1.1 g of dodecane and 0.55 g of DDAB. The mixture was frozen and stored at −25 °C for 14 days. Second, a supersaturated high water/low oil cubic phase was prepared by mixing 2.5 mL of supersaturated calcium phosphate solution with 0.25 g of dodecane and 1.2 g of DDAB. The mixture was stored at +25 °C. In both cases, the inorganic material was extracted after several days by centrifugation of the liquid mixture followed by solvent washing as described above.

$C_{14}H_{30}$/DDAB/CaP: A bicontinuous system employing tetradecane was prepared by adding dropwise 5 mL of freshly prepared supersaturated calcium phosphate solution to 4.2 g of DDAB and 7.5 g of tetradecane. The mixture was stored at +2 °C as a frozen oil for up to 14 days. The oil was melted and the inorganic component extracted as above.

$C_{14}H_{30}$+$C_{16}H_{34}$/DDAB/CaP: A bicontinuous system containing a binary mixture of alkane oils was prepared by adding 5 mL of freshly prepared mineral solution to 5.7 g of DDAB in 2.4 g of tetradecane and 1.2 g of hexadecane. The mixture was then stored as a frozen oil at either +2 or −25 °C for up to 21 days before extraction of the inorganic mineral as described above. The experiments were repeated with unfrozen microemulsions stored at +25 °C.

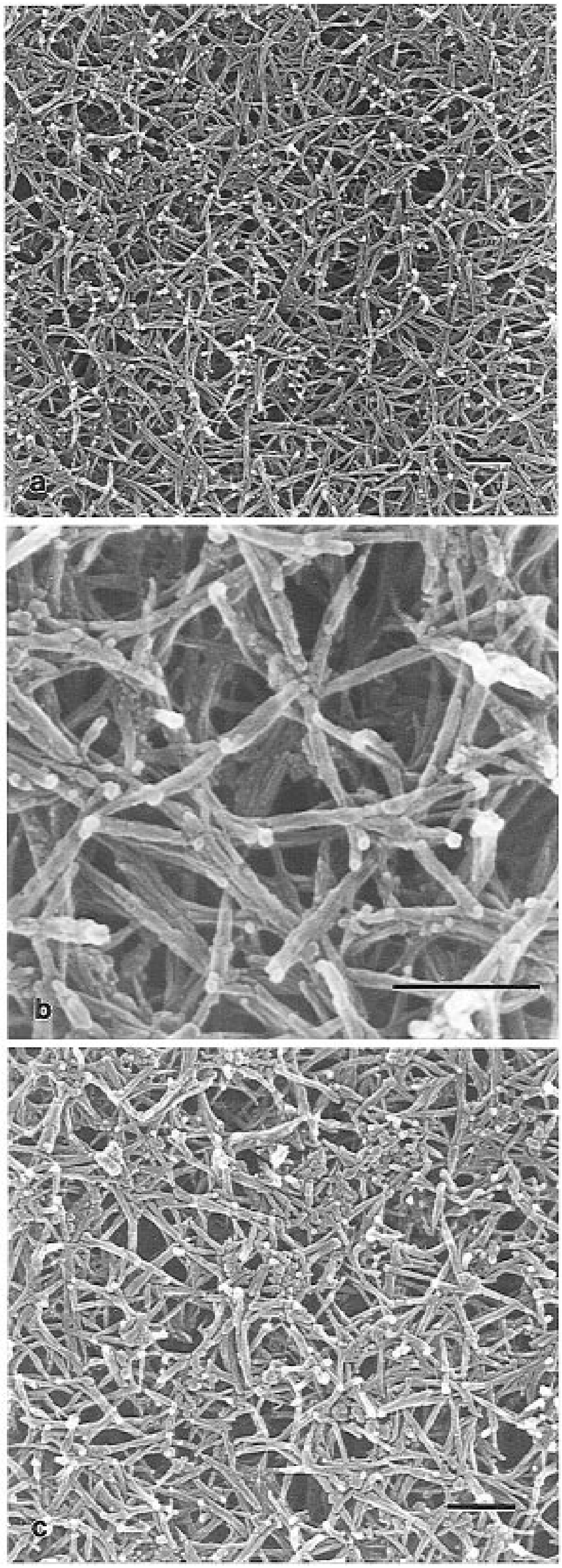

Figure 5. SEM micrographs of microskeletal calcium phosphate prepared in dodecane-containing DDAB bicontinuous microemulsions and stored at −25 °C for 10 days: (a) low magnification image after solvent washing; (b) high magnification image showing crystal connectivity; (c) after heating at 200 °C for 25 min. Scale bars in all micrographs = 500 nm.

Table 2. Formation of Calcium Phosphates in Microemulsions

alkane	oil (%)	sol (%)	DDAB (%)	storage temp (°C)	storage time (days)	pore size (μm)	wall size (nm)	construction unit
C12	40	20	40	−25	21	0.04−0.7	50−130	single-crystal needles
C12	30	55	15	−25	14	0.1−0.5	150	spherical crystals
C12 cubic	6	63	31	+25	7			nonorganized crystal bundles
C14	45	30	25	+2	14	0.1−2.5	100−250	organized bundles of needle crystals
C14	17	35	40	+2	21	0.5−2.5	50−130	single curved crystal needles
C16	8							
C14	17	35	40	−25	7−90			filamentous crystals (5−10 nm wide)
C16	8							
C14	17	35	40	+25	7	0.5−2	50−130	single straight crystal needles
C16	8							
C14	33	49	18 C12E5	+46	3	0.05−1.0		crystal sheets
C14	33	49	18 C12E5	+2	7	0.02−0.2		crystal sheets

$C_{14}H_{30}$/C12E5/CaP: In a typical experiment 0.67 g of C12E5 was mixed with 1.19 g of tetradecane and 1.76 g of freshly prepared calcium phosphate supersaturated solution was added. Storage of the microemulsion was carried out at +46 °C for 3 days, or by freezing in liquid nitrogen and storage at +2 °C for 7 days. The calcium phosphate mineral was separated by centrifugation and solvent washing of the liquid microemulsion as described for the DDAB systems.

Time-Dependent Studies. Crystallization within the microemulsion phases was studied by extracting inorganic material at various time intervals. A few drops of a mixed oil ($C_{14}H_{30}$/$C_{16}H_{34}$) bicontinuous microemulsion, stored at +2 °C (frozen) were removed at intervals and placed onto TEM grids. The grids were then carefully washed by repeated short immersion into warm hexane until oil and surfactant were removed and examined in the electron microscope. Corresponding SEM studies, in which a few milliliters of the microemulsion mixture were extracted by centrifugation at various time intervals, were also undertaken.

Results

In the absence of a microemulsion phase, a white precipitate of calcium phosphate was observed after 5 min, which quickly settled out of solution. SEM micrographs showed that the material was in the form of aggregates of small (<300 nm) crystals with no organized architecture (Figure 1). By contrast, bicontinuous microemulsions stored at temperatures to freeze the oil component but not the calcium phosphate supersaturated solution were optically opaque and showed no visual evidence of precipitation. Unfrozen supersaturated microemulsions were optically clear and showed no bulk precipitation up to 2 weeks after preparation.

In general, samples of inorganic materials recovered from microemulsions after room-temperature centrifugation and extensive washing in hot solvent were analyzed by energy-dispersive X-ray analysis (EDXA), X-ray diffraction, and infrared spectroscopy. For all materials prepared, the EDXA spectra showed peaks for Ca and P (Figure 2), and XRD data were consistent with a hydroxyapatite structure (Table 1). However, the 3570 cm^{-1} OH band for hydroxyapatite was absent from the IR spectra, indicating that the inorganic material was a defect or nonstoichiometric apatite. A band was observed in the carbonate region at 1466 cm^{-1} (Figure 3), but the uncharacteristic shape of this peak and the relative low intensity of the CO_3^{2-} peak at 880 cm^{-1} suggested that much of the intensity of the former was due to CH_2 groups arising from residual oil molecules. Small bands were observed at around 2900 and 1370 cm^{-1}, which confirmed the presence of alkanes. The data indicate that the calcium phosphate materials precipitated in the microemulsions were predominantly nonstoichiometric apatite with a minor carbonate impurity (abbreviated as "apatite" in the following sections). The washed inorganic materials were essentially free of surfactant, as shown by the absence of a Br peak in the EDXA spectra (Figure 2).

$C_{12}H_{26}$/DDAB/CaP microemulsions: Microemulsions formed from compositions at three different positions in the reported phase diagram for pure water[16] were investigated (Figure 4). In preparations involving frozen-oil microemulsions, stored at −25 °C, (that is, 15 °C below the melting point of dodecane) the mixtures were initially soft in texture suggesting the presence of supercooled, but not frozen, water. However, the microemulsions appeared to harden after 1 week, indicating gradual freezing of the water component after this period.

In general, the inorganic material was extracted from the microemulsions in the form of dispersed clumps of calcium phosphate, often several hundred microns across on the SEM stub. Crystals extracted after 10 days from a dodecane bicontinuous microemulsion ($C_{12}H_{26}$:CaP:DDAB = 1:0.5:1 by weight; point A in Figure 4) were organized as meshworks of relatively straight apatite needles approximately 0.5 μm in length and 50−130 nm in thickness (Figure 5a, Table 2). The basic constructional unit comprised outgrowths of single crystals from localized centers (Figure 5b) which in some micrographs appeared to contain small amounts of granular material. The crystals were interconnected by random intergrowth, giving rise to a macroporous material with pore sizes up to 0.7 μm (Table 2). Some micrographs gave the impression that the architectures were partially collapsed onto the SEM holder, suggesting that the intercrystalline connections were possibly fragile. However, heating the material at 200 °C for 25 min revealed unchanged networks (Figure 5c).

Similar experiments but with microemulsions prepared with a composition close to the edge of the bicontinuous phase region ($C_{12}H_{26}$:CaP:DDAB = 2:3.6:1 by weight; point B in Figure 4), gave strikingly different results. Under these conditions, interconnected chains of spherical particles with diameters up to 100 nm were extracted after 5 days (Figure 6a). After 10 days, relatively uniform spherical particles of poorly crystalline apatite approximately 150 nm in diameter were imaged by SEM (Figure 6b). No meshwork architectures were observed.

Experiments undertaken with the high water, low oil cubic phase of dodecane microemulsions ($C_{12}H_{26}$:CaP:

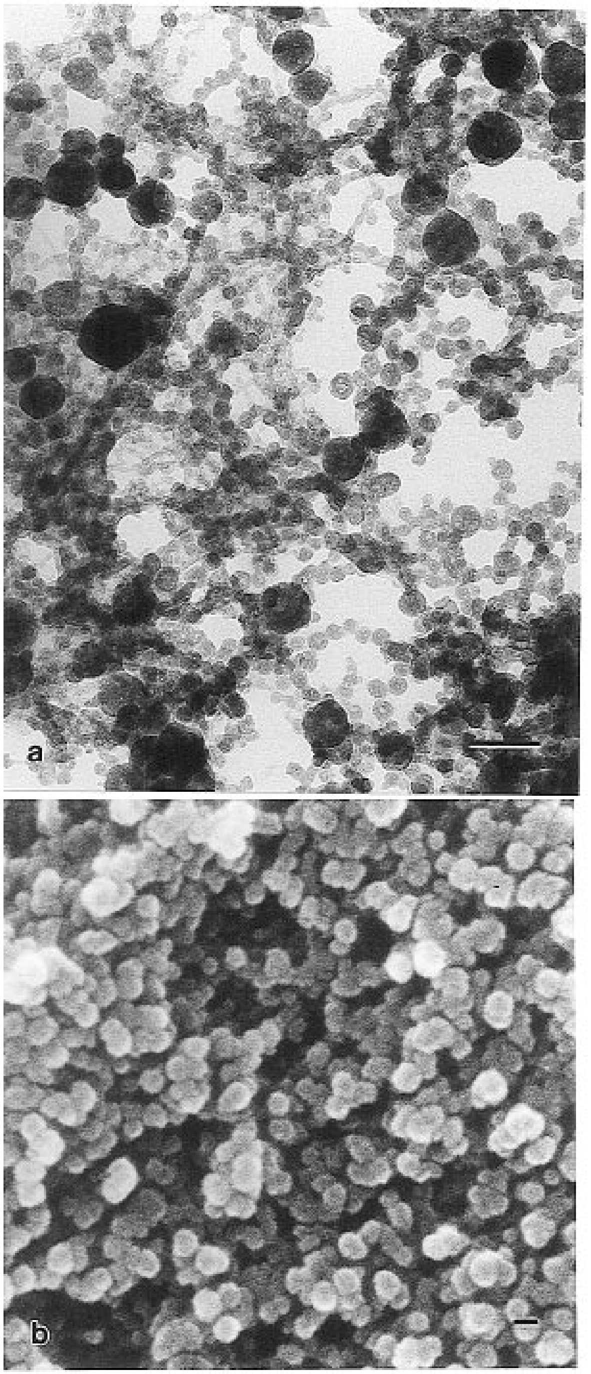

Figure 6. Calcium phosphate prepared in a dodecane-containing DDAB microemulsion with a composition close to the edge of the single phase boundary: (a) TEM image of spherical particles extracted after 5 days at −25 °C; (b) SEM micrograph of material extracted after 10 days −25 °C. Scale bar = 100 nm in both micrographs.

DDAB = 1:10:5 by weight; point C in Figure 4) resulted in an optically clear viscous supersaturated gel at +25 °C. The inorganic material was extracted after 3 days in the form of 100 nm aggregates of intergrown platelike crystals (Figure 7a). After 7 days, SEM micrographs showed bundles of 500 nm size apatite crystals that were not interconnected (Figure 7b).

$C_{14}H_{30}$/DDAB/CaP microemulsions: Supersaturated bicontinuous microemulsions containing frozen oil but liquid water channels were prepared by storing tetradecane-based mixtures above 0 °C but below the oil melting point of +5.9 °C. A typical composition studied

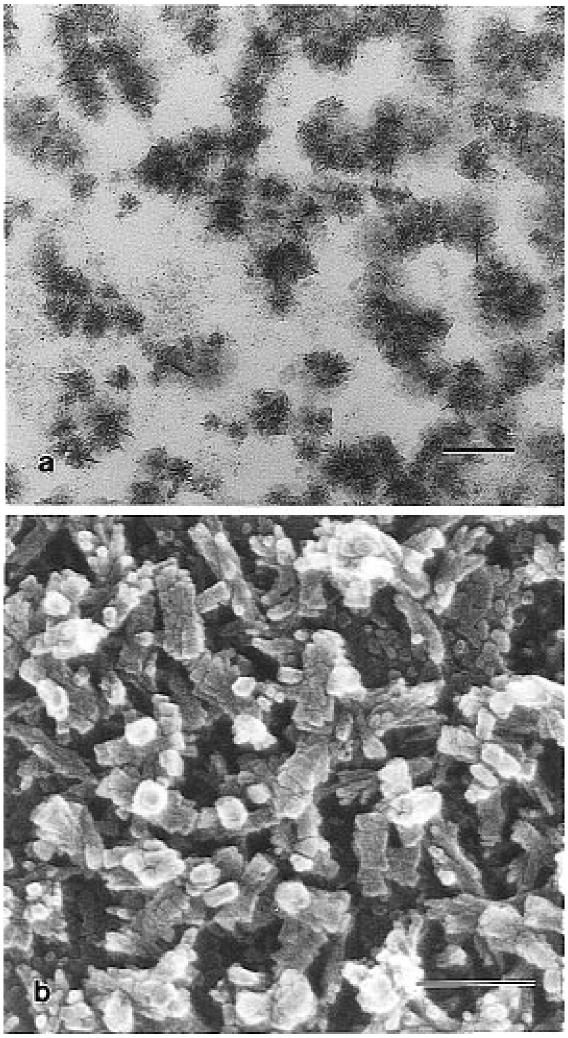

Figure 7. Calcium phosphate prepared in a high water, low dodecane-containing cubic phase DDAB microemulsion: (a) TEM image of intergrown clusters extracted after 3 days at +25 °C; scale bar = 100 nm; (b) SEM micrograph of material extracted after 7 days at +25 °C. Scale bar = 500 nm.

was $C_{14}H_{30}$:CaP:DDAB = 1.8:1.2:1, by weight. The relatively low water content gave a reasonably firm texture at +2 °C, although these microemulsions remained softer in texture than the frozen dodecane systems stored at −25 °C. The resulting microemulsions with their associated mineral phase remained free of bulk precipitation for several weeks.

After 14 days, the extracted inorganic material was in the form of an irregular meshwork of interconnected apatite crystals (Figure 8a). SEM micrographs gave the impression that the framework was only loosely held together and often disrupted during sample preparation. Although this architecture was similar in form to that extracted from the dodecane-based microemulsions, the interconnecting walls were thicker (100−250 nm), being composed of micron-long bundles of thin needlelike crystals rather than single-crystal building blocks (Figure 8b). A corresponding increase in the pore dimensions beyond 1 μm was observed in the structures obtained from tetradecane-containing microemulsions (Table 2).

$C_{14}H_{30}$ + $C_{16}H_{34}$/DDAB/CaP microemulsions: To increase the firmness of the frozen tetradecane microemulsion stored at +2 °C, a mixed oil system of tetradecane and hexadecane (mp +18.1 °C) was used

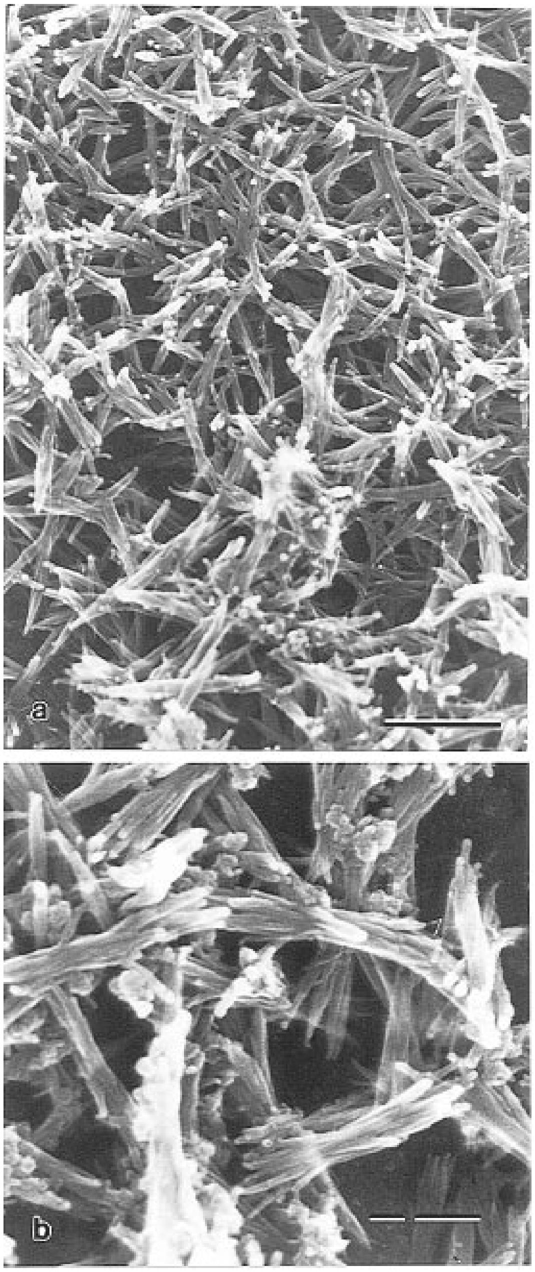

Figure 8. SEM micrographs of microskeletal calcium phosphate prepared in tetradecane-containing DDAB bicontinuous microemulsions and stored at +2 °C for 14 days: (a) low magnification image showing reticulated framework; scale bar = 1 μm; (b) high-magnification image showing interconnecting walls consisting of bundles of HAP crystal. Scale bar = 500 nm.

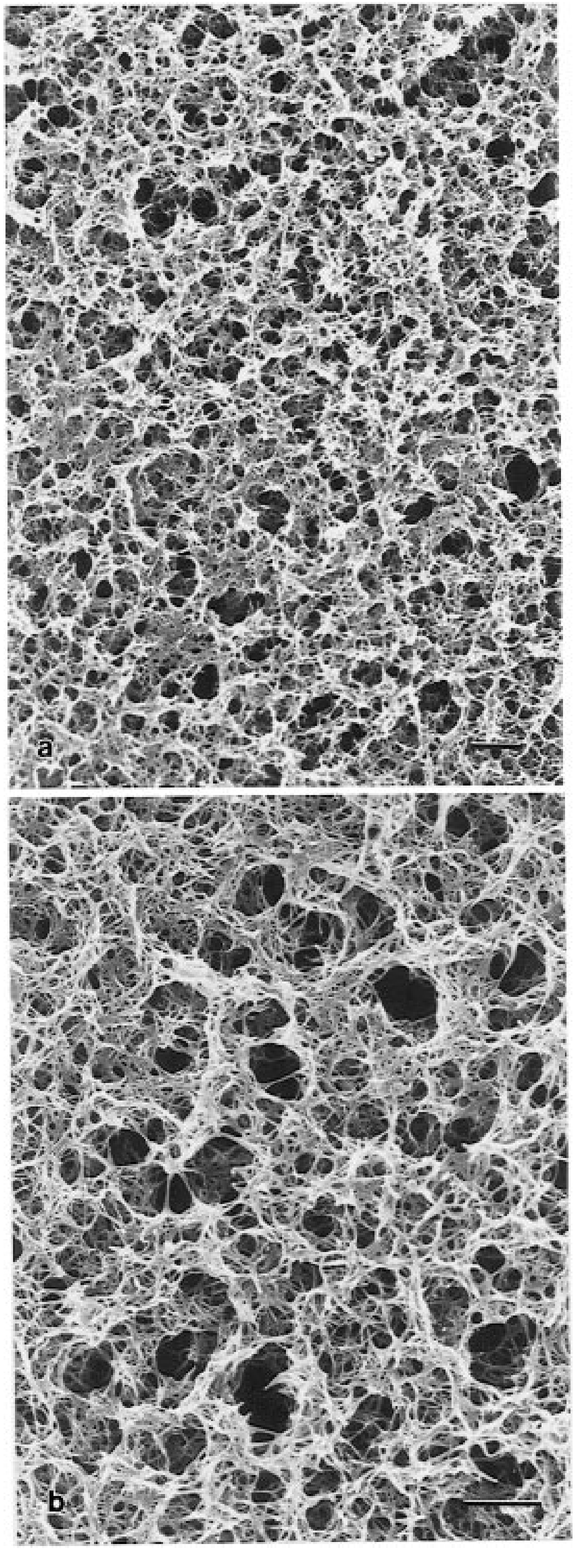

Figure 9. SEM micrographs of microskeletal calcium phosphate prepared in tetradecane/hexadecane-containing DDAB bicontinuous microemulsions and stored at +2 °C for 21 days: (a) low magnification image showing the extensive reticulated framework; (b) higher magnification image showing the complex meshwork architecture. Scale bars = 2 μm in both micrographs.

(Table 2). The inorganic architecture produced at +2 °C after 21 days had a complex microskeletal form that appeared the most elaborate and continuous of all the samples investigated (Figure 9). This framework was constructed from an irregular array of single interconnecting apatite crystals, that were often over a micron in length and sometimes curved. The pore size distribution was very heterogeneous, with some pores being mesoscopic (<10 nm) while others were 2−3 μm in dimension. Many of the larger pores were associated with curved cylinders of crystal meshworks and were possibly artifacts of the washing and drying processes.

The importance of microemulsion texture was investigated by studying inorganic architectures produced from supersaturated bicontinuous phases of the same composition but stored at lower or higher temperatures than the system described above (Table 2). A preparation of the mixed oil system stored for 1 week at −25 °C (that is, 31 and 43 °C below the melting points of tetradecane and hexadecane, respectively) produced highly elongated apatite crystals with lengths of 100−

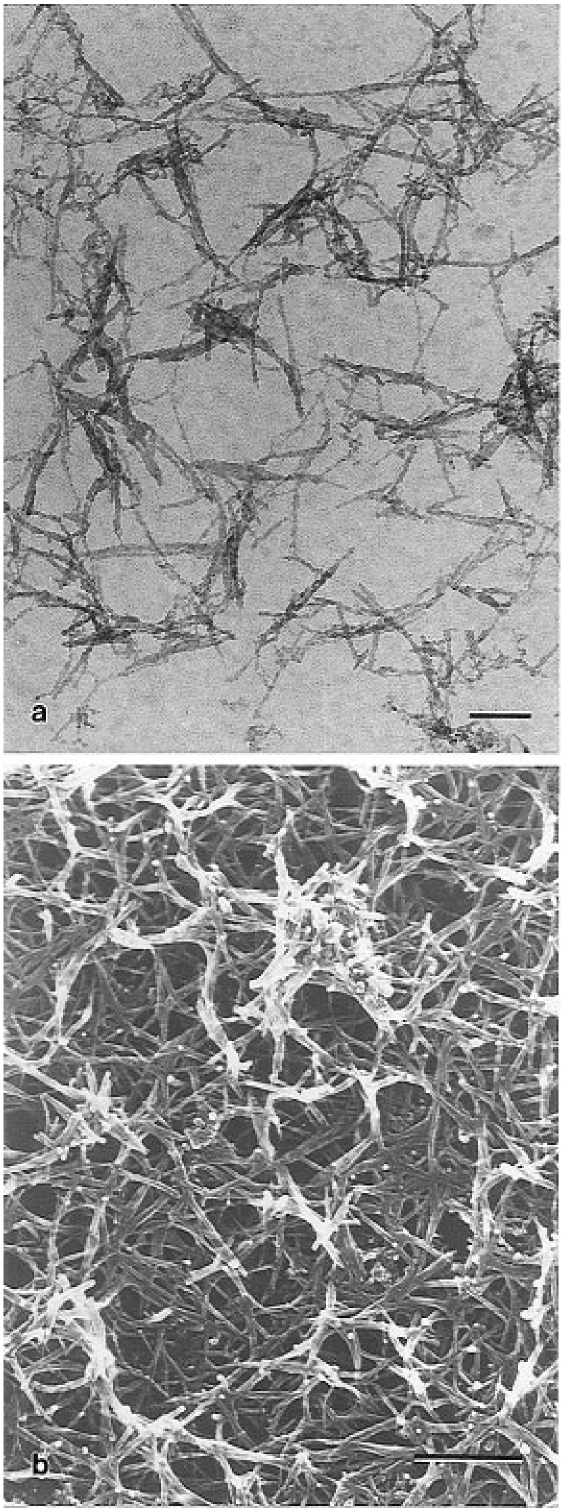

Figure 10. (a) TEM image of calcium phosphate crystals extracted from tetradecane/hexadecane-containing DDAB bicontinuous microemulsions after storage at −25 °C for 7 days; scale bar = 100 nm. (b) SEM micrograph of reticulated calcium phosphate prepared in tetradecane/hexadecane-containing DDAB bicontinuous microemulsions and stored at +25 °C for 21 days. Scale bar = 1 μm.

300 nm and widths within the range 5−10 nm (Figure 10a). Crystal growth did not proceed beyond this stage, and no open-framed architectures were observed even after storage for several months. Conversely, the same supersaturated microemulsion stored unfrozen at +25 °C produced intact reticulated structures composed of straight interconnected crystals together with areas of extensive aggregation (Figure 10b). These architectures were not as continuous as those formed in frozen oils at +2 °C and were often partially collapsed in SEM preparations.

The growth and assembly of reticulated calcium phosphate structures from supersaturated tetradecane/hexadecane/DDAB microemulsions stored at +2 °C (frozen oil) was investigated by electron microscopy analysis of products extracted at various time intervals. At +2 °C, the initial stages of precipitation (2−6 h), were associated with branched filamentous strings of aggregated mineral particles, 1−5 nm in size (Figure 11a). In some TEM images these highly convoluted chainlike structures extended to micron dimensions in length even though their constituents were nanoscale particles. However, these aggregates were not present after 4−6 days at +2 °C but were replaced by needlelike apatite crystals (100−500 nm in length) (Figure 11b). Initially, these crystals were discrete and unconnected, but further growth (10 days) resulted in a loosely held interconnecting array of relatively straight needles in association with 100 nm spherulites (Figure 11c). The final stage of framework construction (2−3 weeks at +2 °C) was characterized by an increased curvature and continued lengthening and thickening of the crystalline walls (Figure 11d).

$C_{14}H_{30}$/C12E5/CaP microemulsions: TEM examination of the inorganic material extracted from a supersaturated C12E5-containing bicontinuous microemulsion stored at 46 °C for 1 day showed the presence of interwoven plates which were shown by electron diffraction to be apatite (data not shown). After 3 days, a network of interconnecting mineral plates was visible by SEM (Figure 12a). The calcium phosphate material was meso- and macroporous, although some of the larger pores may have been artifacts induced by sample preparation. By comparison, frozen supersaturated C12E5/tetradecane microemulsions (7 days at +2 °C) gave lamellar networks of 20−100 nm apatite crystals but the material was more consolidated (Figure 12b).

Discussion

The results presented in this paper indicate that under certain experimental conditions microskeletal forms of calcium phosphate can be synthesized within supersaturated bicontinuous microemulsions. Because both the nanoscopic aqueous and oil channels present in these microemulsions are believed to rapidly fluctuate, disconnect, and reform,[10,11] we chose alkane oils that could be frozen without concomitant freezing of the water phase, in the expectation that an immobilized oil component might facilitate replication of the interconnected reaction spaces. An interesting feature of the recovered inorganic architectures is that they outwardly resemble the envisaged structure of the corresponding microemulsion reaction mixture. Thus, disordered reticulated frameworks were produced in the interconnecting network of the DDAB-containing bicontinuous microemulsions, whereas rounded particles were precipitated in microemulsions with compositions close to the boundary for phase separation. At this boundary, the rodlike water conduits begin to disconnect into chains of spherical micelles before macroscopic phase separation.[17] Similarly, a sheetlike apatite was formed from the interconnected lamellar microstructure of a tetradecane/water/C12E5 bicontinuous microemulsion.[18]

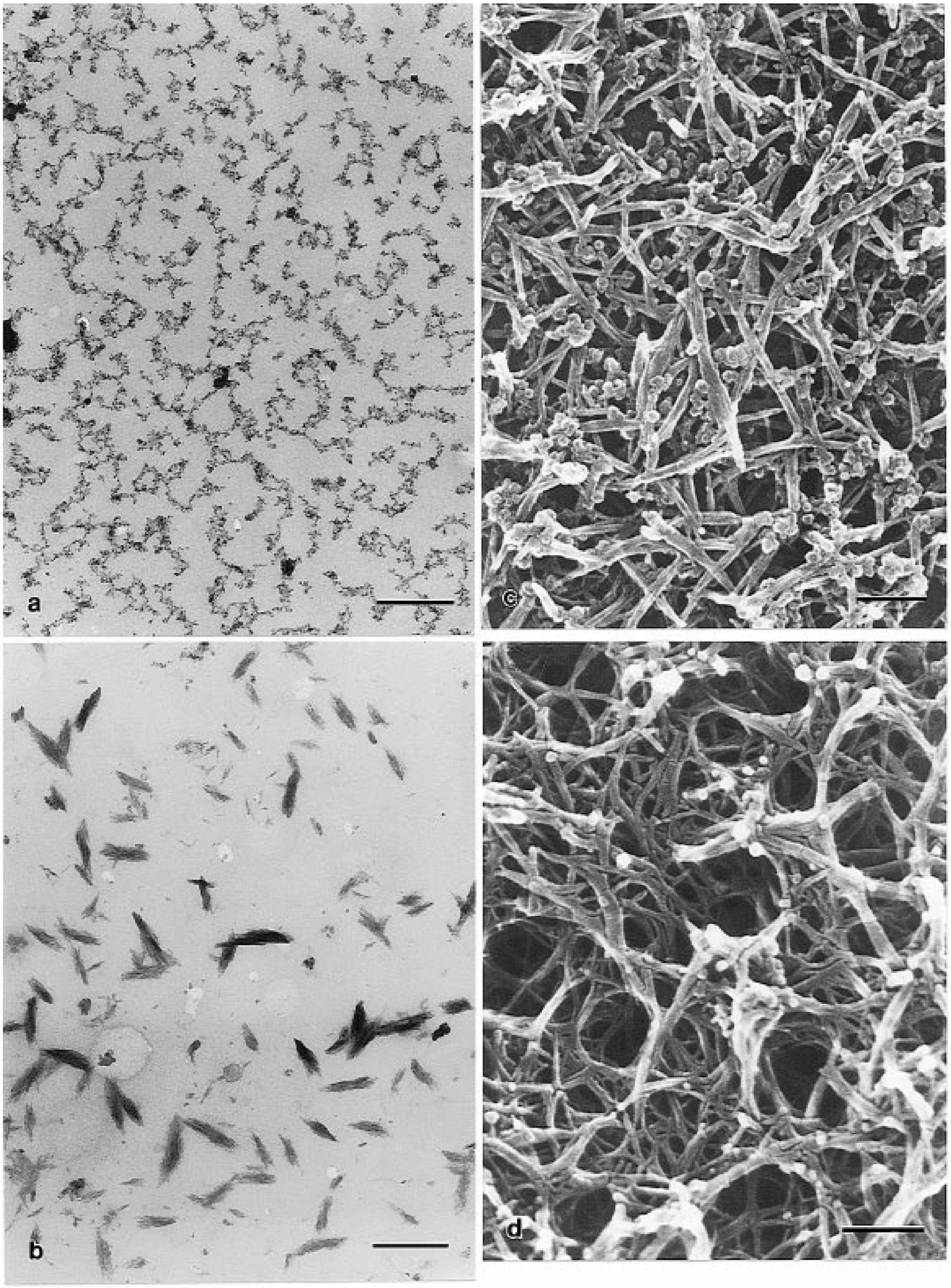

Figure 11. Growth of microskeletal calcium phosphate in tetradecane/hexadecane-containing DDAB bicontinuous microemulsions at +2 °C. TEM images of material extracted after (a) 5 h and (b) after 5 days. SEM images of inorganic material formed after (c) 10 days and (d) 2 weeks. Scale bars in all micrographs = 500 nm.

It seems clear, therefore, that the composition and phase structure of the microemulsions have a profound influence on the general morphological features of the associated inorganic mineral but that the fidelity of this pattern replication is low because although there is morphological correspondence, the length scales of the reaction media and inorganic products are very different. For example, the widths (50 nm) and lengths (500 nm) of the apatite crystals are clearly incommensurate with the 2 nm water channels considered to be present in a DDAB-containing bicontinuous microemulsion.[12,13] Similar observations have been reported for silica[19,20] and poly(methyl methacrylate) polymerization[21] within

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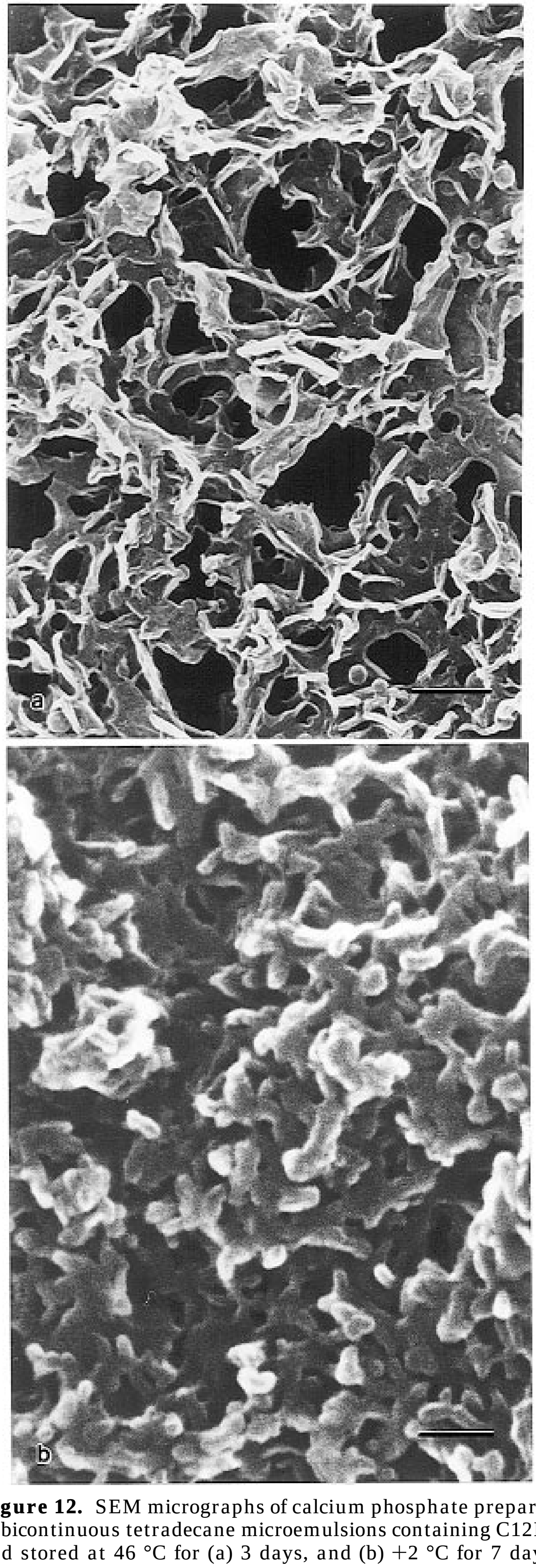

Figure 12. SEM micrographs of calcium phosphate prepared in bicontinuous tetradecane microemulsions containing C12E5 and stored at 46 °C for (a) 3 days, and (b) +2 °C for 7 days. Scale bars = 500 and 200 nm, respectively.

DDAB bicontinuous microemulsions. In both cases, materials with larger structures than the microemulsion network are formed within the organized media.

TEM images of samples recovered at early stages of crystallization suggest that the bicontinuous structure is essentially intact during calcium phosphate nucleation. The crystals are nanometer in size and develop initially as long strings of particles indicative of constrained nucleation within the water conduits of the microemulsion. However, subsequent growth of the mineral particles appears to induce changes at the surfactant−water interface and larger discrete apatite crystals are formed. Significantly, small-angle X-ray scattering studies of silica[20] or organic polymerization[21] in DDAB microemulsions indicate that the nanoscopic bicontinuous structure is retained between modified regions containing the larger scale microstructures of the deposited materials. Thus we speculate that the growing apatite crystals locally disrupt the water channels but that most of the bicontinuous microstructure remains intact. One possible mechanism for the local mobilization of water molecules could be surface hydration of the growing crystals. Also, there might be local collapse and spreading of the water-filled channels onto the inorganic surfaces without inducing long-range reorganization of the microemulsion. The inorganic particles would no longer be rigidly confined to the space delineated by the initial water channels. Instead, reorganized domains of growing crystals together with their hydrated sheath of DDAB molecules could be interspaced between regions of the unmodified bicontinuous phase.

The formation of an interconnected framework from these initially discrete apatite particles suggests that the crystallization process remains spatially constrained at the microscopic level during maturation. In this regard, the degree of microscopic flexibility in the oil phase surrounding the developing crystals seems to be important. Three observations support this working model. First, different inorganic structures are obtained with a softer textured microemulsion of pure tetradecane compared to a C14/C16 mixture, when both are stored with frozen oil components at +2 °C. The former gives a loosely connected network of crystal bundles while the presence of the longer chain alkane results in a highly interconnected architecture. The tetradecane is only supercooled by 4 °C, whereas the hexadecane component in the mixed oil microemulsion is 16 °C below its melting point. Thus, it is feasible that the bicontinuous matrix which lies interspersed between the growing apatite needles in the C_{14}/C_{16} microemulsion contains a higher proportion of solid oil (hexadecane) than in the tetradecane system. This provides a more effective spatial constraint in the development and intergrowth of the inorganic crystals.

Second, when the C_{14}/C_{16} oil mixture is stored unfrozen at +25 °C, the lower viscosity of the medium gives rise to straight interconnected crystals and crystal aggregates. Interestingly, when the mixed oil microemulsions are stored with a hardened texture at −25 °C, no interconnected inorganic network develops presumably due to the extreme rigidity of the oil components. It is also probable that the water component freezes at this temperature which could have a significant effect on determining the morphology of the apatite crystals. However, reticulated materials were produced

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from dodecane microemulsions stored at −25 °C. This oil melts at a significantly lower temperature (−10 °C) compared with the C_{14} and C_{16} oils and could possibly remain supercooled long enough for inorganic nucleation to take place.

A third observation that supports a mechanism of microscopic reorganization of the microemulsions during crystal growth is that experiments involving a viscous cubic phase microemulsion produced apatite crystals which were not organized into a framework structure. The high proportion of water to oil in the cubic phase produces a microstructure with relatively thin alkane-containing walls.[22] Even though the medium is viscous, the crystals appear to rupture the boundaries delineated by the oil molecules, which presumably are not rigid enough to spatially constrain crystal development at the microscopic level.

Although much emphasis has been placed on the role of the oil component in the restructuring of the reaction media, it is clear that the nature of the inorganic phase is important. In particular, it seems probable that the needlelike crystal habit of apatite facilitates the formation of the interconnected framework within the reorganized microemulsions by providing effective interlocking and interlinking of the architecture at relatively early stages of formation. We have attempted similar experiments involving the precipitation of CdS but no reticulated architectures were formed for these nanosized spherical particles. Interestingly, attempts to produce framework structures from needlelike crystals of gypsum ($CaSO_4 \cdot 2H_2O$) were also unsuccessful;[23] in this case, the crystal needles grew to millimeter dimensions regardless of the structural organization of the microemulsion reaction medium. These preliminary results suggest that the kinetics of crystallization may be an important factor in determining whether microskeletal inorganic materials can be produced from bicontinuous microemulsions.

Finally, the low yield (0.3 mg/mL of H_2O) of the reticulated calcium phosphates formed from DDAB-containing microemulsions is a constraint on the use of these materials, for example, as macroporous artificial bone implants. The yield is limited because the bicontinuous microemulsion becomes unstable at higher ionic concentrations of calcium and phosphate. Thus, only a very small volume fraction (ca. 0.008%) of the water channels could be replicated through mineralization. One possible strategy to increase the yield involves continuous growth of the apatite crystals within the microemulsion by slow dissolution of a more soluble calcium phosphate, present as a preformed mineral pellet in the reaction medium. Experiments are currently underway using brushite (DCPD, $Ca(HPO_4) \cdot 2H_2O$) as the source of Ca^{2+} and phosphate ions to increase the yield and extension of the interconnected apatite architectures.

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