

Calorimetric investigation of the precipitation of calcium monohydrogen phosphate in water/AOT/*n*-heptane microemulsions

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Abstract

The molar enthalpies of precipitation of calcium monohydrogen phosphate (CaHPO_4) in water/sodium bis(2-ethylhexyl) sulfosuccinate (AOT)/*n*-heptane microemulsions were measured at 25°C by a calorimetric technique as a function of the molar ratio R ($R = [\text{water}]/[\text{AOT}]$) at a fixed AOT concentration. Calorimetric data indicate the formation of CaHPO_4 nanoparticles encapsulated in the aqueous core of the AOT reversed micelles displaying a slow growing process with time. Their energetic state is initially different from that in bulk water approaching the value in water at longer times. The observed growing process results to be completely inhibited by the presence of small amounts of bis(2-ethylhexyl) phosphate acid (DEPA) leading to the formation of stable nanosized calcium monohydrogen phosphate particles coated by an oriented monolayer of chemically bonded DEPA molecules. After evaporation of the volatile components of the microemulsions, it was observed by transmission electron microscopy that CaHPO_4 nanoparticles embedded in the surfactant matrix are still present. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Recently, the production and the physico-chemical characterization of nanoparticles have become a very active field of investigations mainly because materials with new and interesting properties can be realized simply by reducing their size to the nanometer scale [1,2]. Size-dependence of the nanoparticle electronic structure, effects due to atoms located at the nanoparticle surface and huge increase of the ratio surface/volume (for example, about 30% of atoms are located

at the surface of a particle of 50 Å) are only some of the most cited peculiarities of nanoparticles responsible of their exotic behaviour [3,4]. Their synthesis gives the opportunity to obtain catalysts with high active surface [5,6], materials for optical applications [7,8] and to model or to mimic some biological functions such as the formation of solid constituents of human body (biomineralization) [9] or processes such as carbonate and phosphate formation in continental environments [10,11]. The interest to model biological or geological processes originates by the difficulties encountered in the analysis of real systems as a consequence of many uncontrolled and uncontrollable parameters influencing their behaviour or because it is not recommendable to perform experiments directly on the human or animal body or in the

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natural environment [12]. The interest to mimic biological or geological processes, on the other hand, originates by the hope to realize artificial solid components of the human body or to set processes for environmental improvements.

A simple way to model or to mimic some features of biomineralization processes such as crystal growth in confined space and near fluid interfaces is that based on the use of water in oil (w/o) microemulsions as solvent and reaction media [13–15]. This because these systems possess some structural aspects which are similar to that of cellular membranes such as ordered arrangement of amphiphilic molecules and spatially separated hydrophilic and hydrophobic nanoscopic domains. The size and shape of these domains are system specific allowing, in principle, a fine control of the nanoparticle size and shape. Among the various w/o microemulsions, the most frequently used is the water/sodium bis(2 ethylhexyl) sulfosuccinate (AOT)/*n*-heptane system since it is, in a wide composition range and without the need of cosurfactants, thermodynamically stable and constituted by nearly monodisperse spherical water nanodroplets coated with a monolayer of oriented AOT molecules (reversed micelles) dispersed in the organic solvent. The radius (r) of the reversed micelles, adjustable in the range 1.5–20 nm, is mainly controlled by the water to AOT molar ratio R ($R = [\text{water}]/[\text{AOT}]$) according to the equation [16]

$$r \text{ (nm)} = 1.5 + 1.8R, \quad (1)$$

whereas their dynamics is characterized by a wide variety of physical processes such as diffusion, shape fluctuations, intermicellar encounters and material exchange between neighbouring micelles [17,18]. Using this system, the synthesis of nanoparticles can be simply achieved at room temperature and in a few seconds by mixing two microemulsions, at the same R and AOT concentration, carrying inside the reversed micelles the appropriate hydrophilic reactants. This because the material exchange process occurring on a millisecond time scale allows hydrophilic reagents to come rapidly into contact and react [19], whereas the closed structure of AOT reversed micelles and their dispersion in a non polar environment could prevent nuclei agglomeration and bulk precipitation. Recently, it has been observed that also surfactant adsorption on the nanoparticle surface

could contribute to the inhibition of the nanoparticle growing process [20].

Despite of their importance and the abundance of studies in bulk water [21,22], almost absent are studies on biomineralization processes carried out in w/o microemulsions. For this reason, taking into account the importance of calcium phosphates in the context of mineralized tissue formation and as major components of bones and teeth [22] and with the aim to shed some light on the microscopic processes involved in biomineralization carried out in confined space, we have recently undertaken an investigation on the synthesis of calcium phosphates in w/o microemulsions. In this preliminary paper, we report the results of a calorimetric investigation on the formation of calcium monohydrogen phosphate nanoparticles in water/AOT/*n*-heptane microemulsions. Nanoparticles were synthesized in situ by mixing two microemulsions obtained by dissolving in an AOT/*n*-heptane solution a 0.1 mol kg^{-1} aqueous solution of CaCl_2 and of Na_2HPO_4 , respectively. Since reagents and products are hydrophilic it must be anticipated that they are essentially located in the aqueous core of the AOT reversed micelles. Experiments were performed at a fixed AOT concentration ($[\text{AOT}] = 0.15 \text{ mol kg}^{-1}$) as a function of R . The effect of small amounts of bis(2-ethylhexyl) phosphate acid on the growing process of the nanoparticles was also investigated.

2. Experimental

2.1. Materials

Sodium bis(2-ethylhexyl) sulfosuccinate (AOT, Sigma 99%), bis(2-ethylhexyl) phosphate acid (DEPA, Sigma >95%), sodium phosphate (dibasic anhydrous, Sigma 99%), calcium chloride dihydrate (Carlo Erba 99%), and *n*-heptane (Merck >99%) were used as received. All the microemulsions were prepared by weight adding to a 0.15 mol kg^{-1} AOT/*n*-heptane solution, the appropriate amounts of pure water or of a 0.1 mol kg^{-1} CaCl_2 or Na_2HPO_4 aqueous solution.

2.2. Methods

Calorimetric measurements were performed at 25°C using a thermal activity monitor (TAM) pro-

duced by LKB (LKB 2277) equipped with a flow-mix cylinder (LKB 2277-204) and a titration cell (LKB 2277-402). As a standard procedure, two freshly prepared microemulsions, at the same R value and containing the hydrophilic reagents (CaCl_2 or Na_2HPO_4), were driven by two peristaltic pumps (Gilson, Minipuls 2) inside the calorimetric cell and the flow rates were determined by weight. Baseline was determined by mixing two microemulsions, at the same R and containing water, in the calorimetric cell. The estimated uncertainty in the experimental heat (Q) was $\pm 0.2\%$.

We performed two different calorimetric experiments:

1. In order to define the stoichiometry of the calcium phosphate, microemulsions were mixed at fixed total flow as a function of the mixing ratio S ($S = \Phi_p/\Phi_{ca}$), where Φ_{ca} and Φ_p (g s^{-1}) are the flow rates of the microemulsions containing the calcium chloride and the sodium phosphate, respectively (experiment a). It must be pointed out that, in our experimental conditions, S corresponds also to the molar ratio $[\text{Na}_2\text{HPO}_4]/[\text{CaCl}_2]$.
2. In order to study the growing process of the calcium phosphate nanoparticles, microemulsions were mixed at fixed S ($S = 1$) as a function of the residence time τ of the reactants in the calorimetric cell (experiment b). These experiments were also performed in the presence of small amounts of DEPA added to the microemulsions containing Na_2HPO_4 by varying the molar ratio X ($X = [\text{DEPA}]/[\text{Na}_2\text{HPO}_4]$) in the range $0.25 < X < 0.8$. In this case, baseline was determined by mixing in the calorimetric cell two microemulsions, at the same R and containing water and water plus DEPA, respectively. The residence time was varied by changing the total flow rate Φ ($=\Phi_{ca} + \Phi_p$) and calculated according to the following equation:

$$\tau \text{ (min)} = (vd)/(60\Phi), \quad (2)$$

where v is the “effective” volume of the calorimetric cell and d is the density of the water/AOT/ n -heptane microemulsions ($\cong 0.70 \text{ g cm}^{-3}$) [23,24]. The value of v (0.53 cm^3) was determined previously [25].

Moreover, for comparison purpose, the heat due to the precipitation of calcium phosphate in bulk water

was also determined. These calorimetric experiments were performed using the titration cell, 1 g of an aqueous solution of CaCl_2 (0.1 mol kg^{-1}) was placed in the titration cell; the cell was inserted into the calorimeter and after thermal equilibration, small amounts ($50\text{--}100 \mu\text{l}$) of an aqueous solution of Na_2HPO_4 (0.1 mol kg^{-1}) were added by an injection cannula connected to a Hamilton syringe. The experimental heat, due to each addition, was evaluated from the area under the calorimetric signal.

In order to have more experimental evidences on the formation of calcium phosphate nanoparticles, some samples were observed with a transmission electronic microscope (Jeol 1220T operating at 120 kV). The samples were prepared on a carbon-copper grid by gentle evaporation of the volatile components of aged microemulsions (2–3 h after mixing).

3. Results and discussion

Fig. 1 shows the quantity Q/n_p , where Q indicates the experimental heat occurring when small amounts of a 0.1 mol kg^{-1} aqueous solution of Na_2HPO_4 containing n_p moles of the salt are added to 1 g of a 0.1 mol kg^{-1} aqueous solution of CaCl_2 , as a function of S . As it can be seen, the Q/n_p trend is characterized by two nearly constant parts separated by an abrupt change at $S = 0.3$ and followed by a decreasing trend

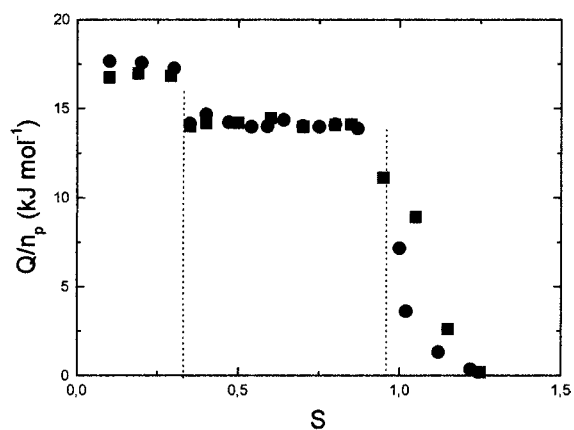


Fig. 1. Heat (Q/n_p) due to the precipitation of CaHPO_4 in water as a function of the molar ratio S ($S = [\text{Na}_2\text{HPO}_4]/[\text{CaCl}_2]$). Measurements have been duplicated (●: run 1; ■: run 2).

at $S > 0.9$. Taking into account that, depending upon the experimental conditions, calcium phosphate precipitates as calcium monohydrogen phosphate (CaHPO_4), octacalcium phosphate ($\text{Ca}_8\text{H}_2(\text{PO}_4)_6$) or hydroxyapatite ($\text{Ca}_5(\text{PO}_4)_3\text{OH}$), the observed behaviour can be rationalized in terms of the initial formation of $\text{Ca}_8\text{H}_2(\text{PO}_4)_6$ and/or $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ followed by the formation of CaHPO_4 at $0.3 < S < 0.9$ [26]. The formation of $\text{Ca}_8\text{H}_2(\text{PO}_4)_6$ and/or $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ is most probably favoured by the initial relatively high concentration of calcium ions in the aqueous medium, whereas at lower concentration, the formation of a more calcium-deficient phosphate becomes favoured. It is worth noting that this happens quite dramatically at $S = 0.3$. According to the above interpretation, a value of 14.5 kJ mol^{-1} for the molar enthalpy of precipitation of CaHPO_4 in bulk water can be estimated.

Let us now consider the formation of calcium phosphate in water/AOT/*n*-heptane microemulsions. Initially, some preliminary experiments were performed to test the stability of the samples obtained by mixing microemulsions containing Ca^{2+} ions with microemulsions containing HPO_4^{2-} ions. By visual inspection, it was observed that clear samples (at least within 4–5 days) were obtained at $R \leq 15$. This behaviour is completely different from that observed in water where precipitation occurs immediately. The appearance of an opalescence

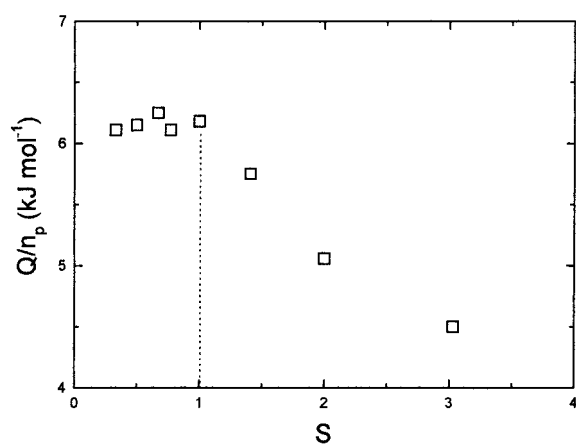


Fig. 2. Heat (Q/n_p) due to the precipitation of CaHPO_4 in water/AOT/*n*-heptane microemulsion at $R = 5$ ($R = [\text{water}]/[\text{AOT}]$) as a function of the molar ratio S ($S = [\text{Na}_2\text{HPO}_4]/[\text{CaCl}_2]$).

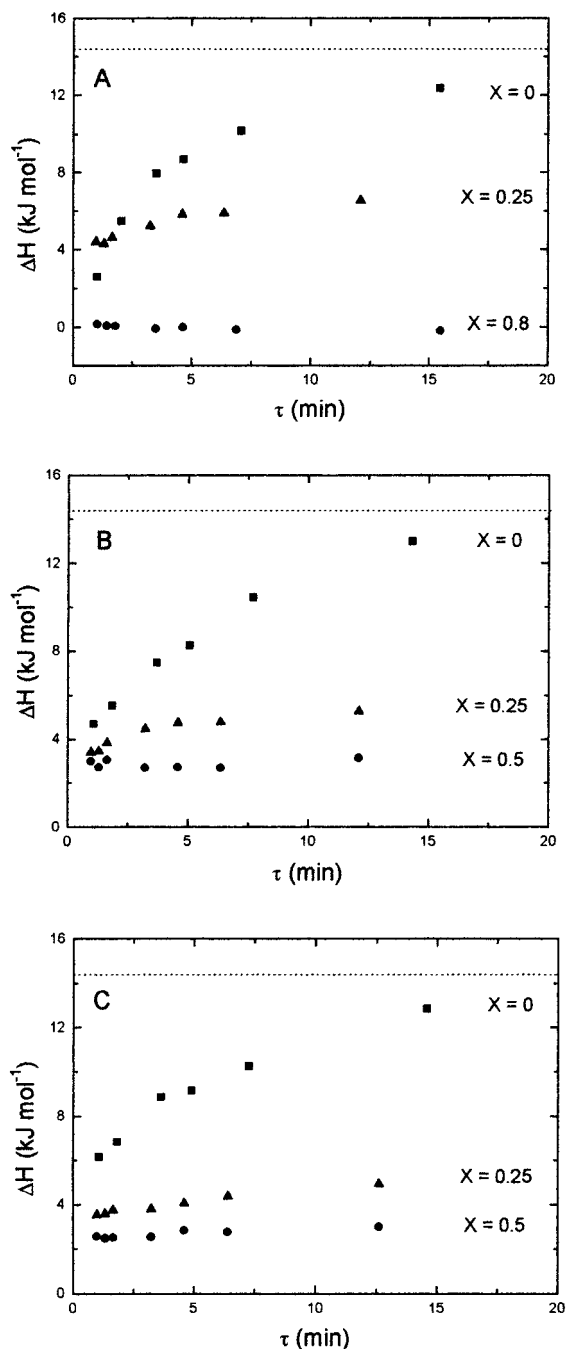


Fig. 3. Molar enthalpy (ΔH) of formation of CaHPO_4 nanoparticles as a function of the residence time (τ) at various molar ratios X ($X = [\text{DEPA}]/[\text{Na}_2\text{HPO}_4]$) and R ($R = [\text{water}]/[\text{AOT}]$): (A) $R = 5$; (B) $R = 10$; (C) $R = 15$. The dotted line indicates the ΔH value in water.

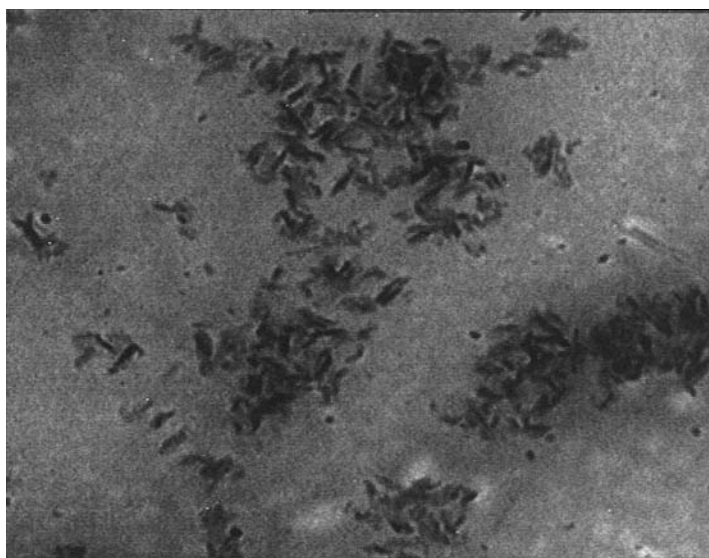


Fig. 4. Electronic micrograph of clusters of CaHPO_4 nanoparticles (average nanoparticle size: 72×17 nm) dispersed in the AOT matrix (sample obtained by a microemulsion at $R = 5$ and $X = 0$; magnification: $\times 72\,000$). ($R = [\text{water}]/[\text{AOT}]$ and $X = [\text{DEPA}]/[\text{Na}_2\text{HPO}_4]$).

in all the samples after several days can be, however, taken as a qualitative indication of the occurrence of a slow growing process of the nanoparticles. On the contrary, stable samples were obtained when small amounts of DEPA ($0.25 < X < 0.8$) were added

to the microemulsions containing HPO_4^{2-} ions before mixing.

Fig. 2 shows a typical result of the experiment a, i.e., the quantity Q/n_p versus S for the mixing of two microemulsions at $R = 5$ carrying inside the

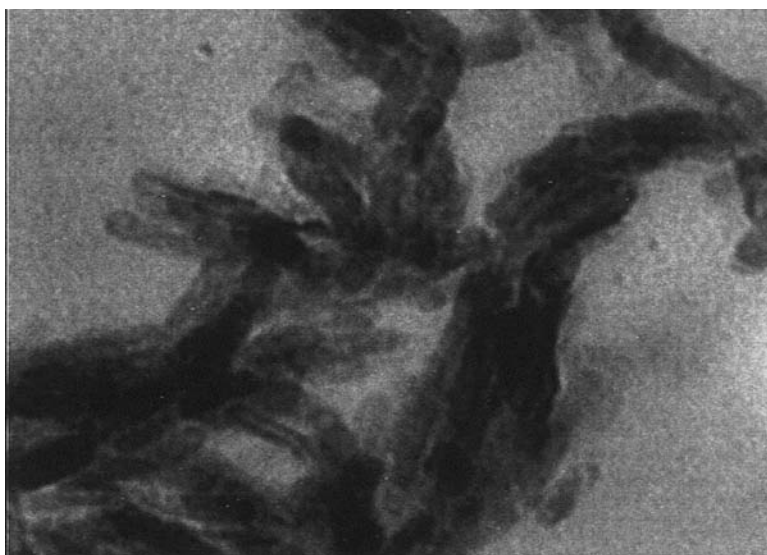


Fig. 5. Electronic micrograph of a cluster of CaHPO_4 nanoparticles (average nanoparticle size: 68×20 nm) dispersed in the AOT matrix (sample obtained by a microemulsion at $R = 10$ and $X = 0$; magnification: $\times 360\,000$). ($R = [\text{water}]/[\text{AOT}]$ and $X = [\text{DEPA}]/[\text{Na}_2\text{HPO}_4]$).

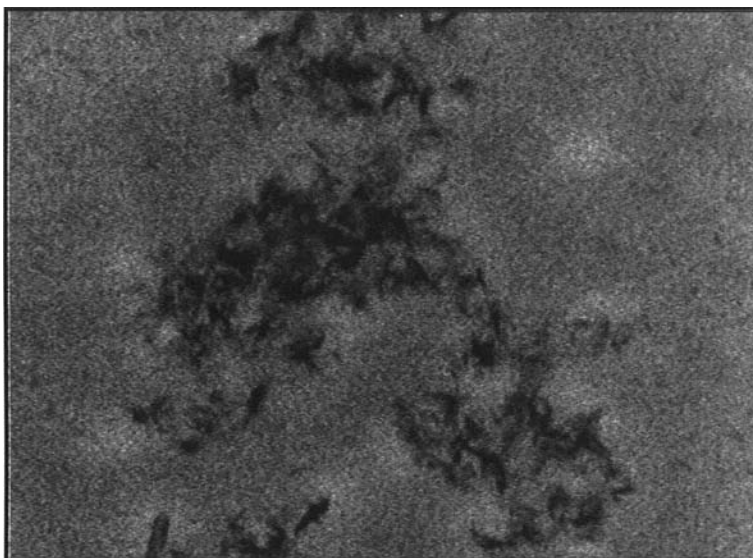


Fig. 6. Electronic micrograph of clusters of CaHPO_4 nanoparticles (average nanoparticle size: 95×15 nm) dispersed in the AOT matrix (sample obtained by a microemulsion at $R = 15$ and $X = 0$; magnification: $\times 72\,000$). ($R = [\text{water}]/[\text{AOT}]$ and $X = [\text{DEPA}]/[\text{Na}_2\text{HPO}_4]$).

micellar core a 0.1 mol kg^{-1} aqueous solution of Na_2HPO_4 and of CaCl_2 , respectively. Taking into account that these data are obtained at a constant total flow, namely, at constant residence time τ of reactants in the calorimetric cell, the abrupt change

of the slope at $S = 1$ indicates that inside the aqueous nanodroplets it is formed calcium monohydrogen phosphate (CaHPO_4). This is a quite general conclusion, since the same behaviour was also observed at $R = 10$ and 15 .

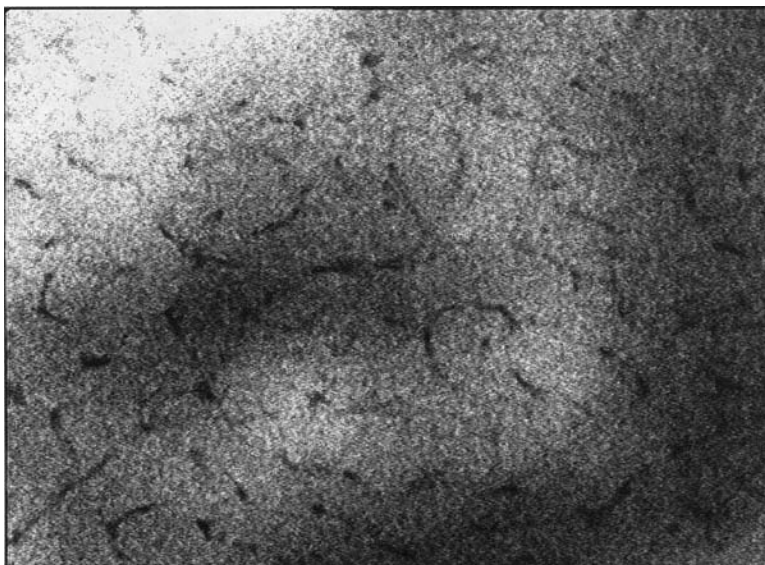


Fig. 7. Electronic micrograph of quite randomly distributed CaHPO_4 nanoparticles (average nanoparticle size: 63×7 nm) dispersed in the AOT matrix (sample obtained by a microemulsion at $R = 15$ and $X = 0.5$; magnification: $\times 144\,000$). ($R = [\text{water}]/[\text{AOT}]$ and $X = [\text{DEPA}]/[\text{Na}_2\text{HPO}_4]$).

Fig. 3 shows the plots of the experimental molar enthalpy (ΔH) of formation of CaHPO_4 as a function of τ at various R and X values and at $S = 1$ (experiment b). Moreover, for comparison, it has been also reported the molar enthalpy of precipitation of CaHPO_4 in bulk water. In absence of bis(2-ethylhexyl) phosphate acid, enthalpy values increase with τ trending, at long times ($\tau > 30$ min), to the value observed in water. This behaviour is a strong evidence of a nanoparticle growing process (reaction and nucleation, in fact, should occur at a smaller time-scale) occurring with a time-scale longer than in bulk water and probably caused by the physical adsorption of surfactant on the nanoparticle surface [27]. Moreover, it can be noted that the enthalpy values do not display a significant dependence on R , i.e., the nanoparticle size and its evolution with time is independent of the micellar radius. This because an increase of R involves opposite effects, i.e., it induces an increase of the nanodroplet size and at the same time a decrease of the nanodroplet concentration. All these findings have been confirmed by the electron micrographs of samples obtained from microemulsions after 1–2 h of ageing (see Figs. 4–6). Independently of the R value, the typical rod-like crystallites of calcium monohydrogen phosphate [28] reached nearly the same size, similar to that observed in the early stages of precipitation in water, displaying in all the samples a marked tendency to cluster [22,29,30], however, it is worth noting that the observed topological distribution of the crystallites may be considered frozen in the calcium monohydrogen phosphate/surfactant composite, whereas in water the crystallite clustering and growth is an unlimited process.

A different calorimetric behaviour is observed in the presence of small amounts of DEPA (see Fig. 3). At $X > 0$, in fact, quite constant trends are obtained independently of the R values. This behaviour strongly suggests that the nanoparticle growing process is inhibited by DEPA most probably as a consequence of the formation of a chemically bonded monolayer of oriented molecules on the nanoparticle surface. This effect seems to be more effective by increasing X . This conclusion is also supported by the electron micrograph of a sample at $R = 15$ and $X = 0.5$ (see Fig. 7). In fact, quite smaller and unclustered rod-like nanoparticles are found in these conditions.

4. Conclusion

From calorimetric and TEM results, the following conclusions can be drawn: (i) in the absence of DEPA, CaHPO_4 nanoparticles are stable only in the CaHPO_4 /surfactant composites where the nanoparticles are spatially separated and their diffusive motion is frozen; (ii) in presence of appropriate amounts of DEPA, CaHPO_4 nanoparticles are stable both in w/o microemulsions and in the composite as a consequence of the formation of a chemically bonded monolayer of oriented DEPA molecules on the nanoparticle surface. These DEPA coated CaHPO_4 nanoparticles are a novel nanomaterial whose properties and potential applications deserve to be investigated.

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