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Waterborne Acrylic-Casein Nanoparticles. Nucleation and Grafting

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Summary: Hybrid nanoparticles containing proteins have a technological interest because they attempt to achieve improved properties with respect to the single materials by chemically linking both components. In this article, the batch emulsion polymerization of methyl methacrylate in the presence of varied concentration of casein and tert-butyl hydroperoxide as initiator was investigated. A detailed characterization of the molecular microstructure and morphology of the hybrid nanoparticles allowed the identification of two competitive particle formation mechanisms. Compatibilized nanoparticles were produced at the beginning of the polymerization, while uncompatibilized particles could be generated by a second way of nucleation, which is promoted by the ungrafted protein and depends on its concentration.

Keywords: casein; emulsion polymerization; hybrid nanoparticles; poly(methyl methacrylate)

Introduction

Natural proteins, as casein, have been widely used for long time in paper coatings, adhesives, glues, and paint binders, because of their reduced environmental impact, high degradability, good stained acceptance, finishing glazed aspect and good substrate penetrability.[1–2] However, the casein films present low wet rub resistance, susceptibility to microbial attack, and poor mechanical properties.[1] Therefore, the casein has been replaced in many applications by synthetic polymers, which provide better mechanical properties, but resigning the good penetration and adhesion to substrates, and the low environmental impact that a natural product offers.

The synthesis of hybrid latexes containing proteins has gained technological and biomedical interest because of the attempts to modify and improve the properties of natural substances by incorporating synthetic polymers, such as acrylates.[3] For instance, acrylic/casein nanoparticles could be potentially useful in immunodiagnostic testing, biosensors, gene treatment, controlled release of drugs and other biological agents, colloidal nanocatalyst, enzyme immobilization, thermal laser imaging and water-borne coatings and adhesives. The adequate balance between the application properties of casein and those of synthetic polymers is expected to be achieved when they form a compatible system, i.e., when both materials remain chemically linked. Therefore, in order to develop a successful polymerization strategy, the mechanisms involving grafting must be well understood. This requires a complete characterization of

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the molecular microstructure that includes the quantification of the fractions corresponding to the synthetic polymer, the ungrafted protein, and the grafted copolymer containing the two components chemically bonded.

The high water solubility of casein makes the emulsion polymerization process the best alternative for the synthesis of these hybrid nanoparticles compared to other processes like miniemulsion or suspension polymerizations. Graft copolymerization of casein with acrylic monomers, acrylamide and styrene has been previously studied in aqueous media using persulphate initiators.[4–9] In such works, the effect of the concentrations of initiator, monomer and casein on the grafting extent, measured as the fraction of synthetic polymer containing grafted protein, was studied. It was found that the fraction of grafted polymer increased as the concentrations of initiator, monomer and casein were augmented. The grafting of methyl methacrylate (MMA) onto casein was also promoted by the redox initiation system casein-potassium ditelluratocuprate(III), producing high levels of grafted polymer.[10] A one-step procedure for synthesizing particles of poly(methyl methacrylate) (PMMA) with grafted water-soluble polymers containing amino groups was proposed by Li et al.[11] It consists in initiating the polymerization according to a redox reaction between an alkyl hydroperoxide and the amino group of the water soluble polymer.[12] Then, the propagation of the radicals on the amine nitrogen of the water-soluble polymers initiates the graft polymerization. The amphiphilic macroradicals generated in situ can self-assemble to form polymeric micelle-like microdomains, which promote the emulsion polymerization. This procedure was employed to produce PMMA/casein nanoparticles with core-shell morphology.[13] They achieved around 40–50% of grafting efficiency, defined as the weight percentage of PMMA branches with respect to the polymerized MMA. However, this variable did not indicate the fraction of casein that was incorporated to the PMMA, or in other words, the amount of unreacted casein.

In this work, the emulsion polymerization of MMA in the presence of casein was investigated with the objective of revealing the mechanism involved in the production and growth of acrylic-protein nanoparticles, in the light of the complete molecular microstructure of the hybrid material, the resulting particle morphology, and its size distribution. As proposed by Li et al.,[11] a redox initiation system that involves *tert*-butyl hydroperoxide (TBHP) and amine groups of casein was adopted. MMA was preferred over other acrylic monomers because it produces simple linear homopolymers (in absence of casein) which simplify the characterization of the hybrid acrylic-casein latexes. The compatibilization extent, which includes the grafted fraction of both casein and polymerized MMA, was evaluated for different monomer/casein and casein/TBHP formulations.

Experimental Part

Materials

Technical grade casein from bovine milk (Sigma) and MMA (containing $\leq$30 ppm of hydroquinone methylether as inhibitor, Aldrich) were used. The employed initiator was TBHP (Aldrich) and the buffer was sodium carbonate (Na_2CO_3, Cicarelli). Phosphotungstic acid (PTA, Fluka) and formvar® (polyvinyl formal, Fluka) were used for preparing TEM samples. Other used reagents were tetrahydrofuran (THF, J.T. Baker) and sodium dodecyl sulphate (SDS, Anedra). All the reagents were used as received without any kind of purification. Distilled and deionized water was used throughout the work.

Polymerization

Polymerizations of MMA in the presence of variable amounts of casein and TBHP were carried out in a 0.5 L jacketed reactor equipped with thermostatic bath, digital thermometer, condenser, stirrer, N_2 inlet and sampling device. The polymerizations

were carried out as follows. Casein was first dissolved at 50 °C into the reactor in a water solution of pH = 11 containing 0.4% weight based on water of Na_2CO_3. At pH higher than 10.0 the casein solubility is maximum and a loose micelle structure is obtained due to a reduced association of molecules by hydrophobic interaction.[14] Then, the solution temperature was raised up to 80 °C and the monomer was loaded. The resulting dispersion was purged with N_2 for 30 min before injecting the initiator (TBHP). Samples were withdrawn along the polymerization at a regular time. Table 1 presents a general recipe.

Characterization

MMA conversion (x) was measured by gravimetry. The average particle diameter (d_p) was determined by dynamic light scattering, using a Brookhaven BI-9000 AT photometer at a detection angle of 90°. Particle size distribution (PSD) was determined by capillary hydrodynamic fractionation employing a CHDF2000 (Matec Applied Sciences) equipment. The molecular architecture of the casein/acrylic hybrid latexes was mainly characterized by determining the fraction of casein grafted to the acrylic polymer (casein grafting efficiency, CGE), and the fraction of the polymerized MMA that contains grafted casein (acrylic grafting efficiency, AGE).

The CGE was determined from Eq. 1 by correlating the weight of the grafted casein with the loaded casein. To separate the ungrafted casein from the latex a procedure of multiple centrifugation and redispersion was applied as follows: i) the diluted latex at 1% of solids content was centrifuged at a relative centrifugal force (RCF) of 25000 g during 4 hours; and ii) the supernatant was separated and the pellet was redispersed with a 1 wt % SLS solution and shook for overnight, to promote the desorption of the ungrafted casein from the particles. The procedure of centrifugation/separation/redispersion was repeated several times until no casein was detected in the supernatant.

All supernatants from each repeated centrifugation were analyzed by UV absorbance spectroscopy and the ungrafted casein concentration was obtained by combining the characteristic peak area at 280 nm with a calibration of casein concentration. Then, the weight of grafted casein was obtained as the difference between the loaded and ungrafted casein.

$$CGE = \frac{\text{Weight of Grafted Casein}}{\text{Weight of Loaded Casein}} \cdot 100 \quad (1)$$

The AGE is defined as the ratio between the weights of grafted PMMA and the total polymerized MMA, as follows:

$$AGE = \frac{\text{Weight of Grafted PMMA}}{\text{Weight of Total Polymerized MMA}} \times 100 \quad (2)$$

To determine the AGE, the ungrafted PMMA was soxhlet extracted with THF during 24 h from the latex sample, dried in an oven at 60 °C and weighted before and after the extraction. The morphology of latex particles was studied by means of transmission electron microscopy (TEM), using a TECNAI G2 20 TWIN (200kV, LaB6). To this effect, 1 mL of a diluted dispersion of latex (around 0.01 wt % of solids content) was stained with 20 μL of a 1 wt% aqueous solution of PTA, during 30 min. Positive staining of the amine component of casein with PTA was used.[15] The dark region corresponds to the casein. A drop of the stained diluted latex was placed on copper grids covered with

Table 1.
General recipe for the synthesis of PMMA/casein latex.

Reagent	Amounts (pphm)[a]
MMA	100
Casein	3–50
TBHP	0.20, 0.35
Na_2CO_3	5.332
H_2O	1333

[a] pphm: parts per hundred monomer.

formvar® and dried at room temperature. Micrographs were taken at different magnifications depending on particle size.

Results and Discussion

Effect of the Casein Concentration

Table 2 summarizes the experiments carried out with varied amounts of casein, while maintaining constant the amounts of the other reagents (Table 1). Note that in all reactions the casein concentration was much higher than the critical micellar concentration (0.1 mg/mL, determined by surface tension measurements at room temperature and at the same buffer concentration used in the polymerization experiments). The final values of x, d_p, CGE, AGE, and the weight of grafted casein per hundred grams of MMA (g-casein, %wbm) are also presented in Table 2.

The evolution of x and d_p along the polymerizations are shown in Figure 1. When casein concentration was increased from 3 up to 25 pphm, both the initial polymerization rate and the final x increased, while the final d_p decreased. According to Li et al.,[11] this behaviour was rather expected because the reaction is started by the presence of amino and tert-butoxy radicals, in turn produced by interaction of the hydroperoxide molecules with the amino groups present in the casein backbone. Therefore, the larger the amount of casein, the greater the quantity of amino groups available for interacting with the TBHP. As a consequence, the radical concentration and the number of nucleated polymer particles were increased.

Following this reasoning, more grafted casein and PMMA would be expected as the amount of casein in the formulation is increased. Furthermore, the casein grafting efficiency would increase along the reaction. However, as shown in Table 2, the amount of grafted casein increased up to 12 pphm of casein in the formulation and then decreased, while the AGE continuously decreased with the protein content. Furthermore, the fraction of grafted casein remained constant along the polymerization (Figure 2). This last observation was independent of the amount of casein used, showing that casein grafting only occurs at the beginning of the polymerization. Note that despite casein incorporation stops at low MMA conversion, the final AGE is

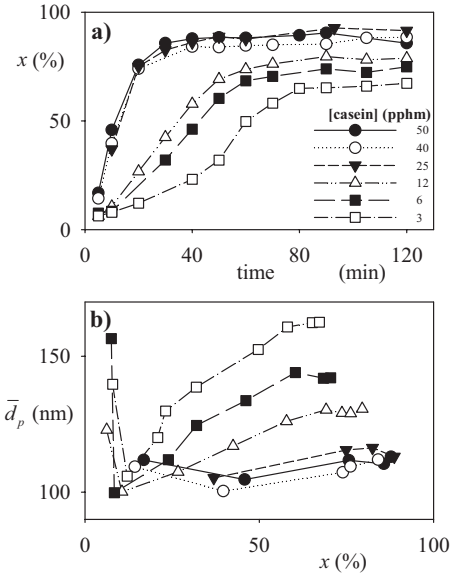

Figure 1.
MMA polymerization in the presence of varied casein concentrations. Evolution of (a) conversion and (b) particle diameter.

Table 2.
MMA polymerization in the presence of varied casein concentrations.

	Cas 3	Cas 6	Cas 12	Cas 25	Cas 40	Cas 50
Casein (pphm)	3	6	12	25	40	50
x (%)	68	75	79	91	88	89
dp (nm)	162	142	132	120	114	113
AGE (%)	87	81	74	68	58	53
CGE (%)	90	71	56	26	9	5
g-casein (%wbm)	2.7	4.3	6.7	6.5	3.6	2.5

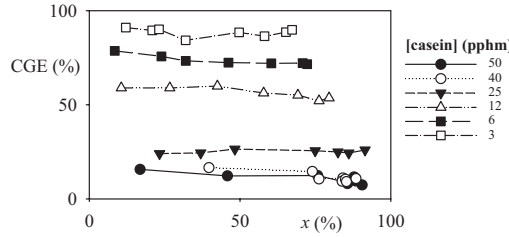

Figure 2.
MMA polymerization in the presence of varied casein concentrations. Evolution of CGE with conversion.

higher than 50% independently of the casein concentration (Table 2), indicating that as polymerization proceed acrylic polymer is grafted on the initially incorporated casein.

In order to get more insight into the mechanism governing those reactions, the evolution of the number of particles (N_p) was calculated for each polymerization. These estimations were carried out from d_p measurements of latex samples in absence of free-casein. To this effect, the free casein was first removed by several centrifugation and re-dispersion steps with a SLS solution. Figure 3 shows that N_p increases along the polymerization, being the particle generation much more significant for higher casein concentrations.

According to the mechanism proposed by Li et al.,[11] particles are produced by the self assembly of amphiphilic-grafted radicals containing the protein, and this step is promoted by the casein grafting. This mechanism could explain the results of the experiments performed at low casein contents, in which the new particle production is not significant after a monomer conversion above 20–30%; however, it is unable to explain the results of the reactions performed with high casein contents. The new particle generation along the reaction together with the constant fraction of grafted casein indicates that a second way of nucleation is present along the process, which becomes more important as the casein concentration in the reaction media is increased.

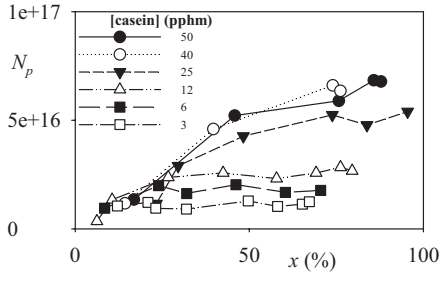

Figure 3.
Particle generation along the MMA polymerization in the presence of varied casein concentrations.

The resulting particle morphologies obtained by TEM (Figure 4) and the PSD measured by CHDF (Figure 5) with different casein concentrations for the final latexes gave more light to this hypothesis. In the cases of lower casein concentration (3–12 pphm, Figures 4.a-b), where the grafted fractions of acrylic and casein are high, most of the particles contain casein in their surface (showed as a dark shell or peel for the PTA positive staining of amine groups). Notice that particles with the lowest casein concentration are not fully overlaid with casein. On the other hand, for the latexes of higher casein contents (25–50 pphm), where the ungrafted fraction of both casein and PMMA is significant and the particle generation by the second way of nucleation is considerable, the TEM pictures of Figure 4. c-d show: i) large particles partially covered by a dark peel that corresponds to protein, and ii) small particles that do not present the casein peel on their surface. These observations are in agreement with the measured PSD for latexes synthesized with different casein concentration. For the lowest casein

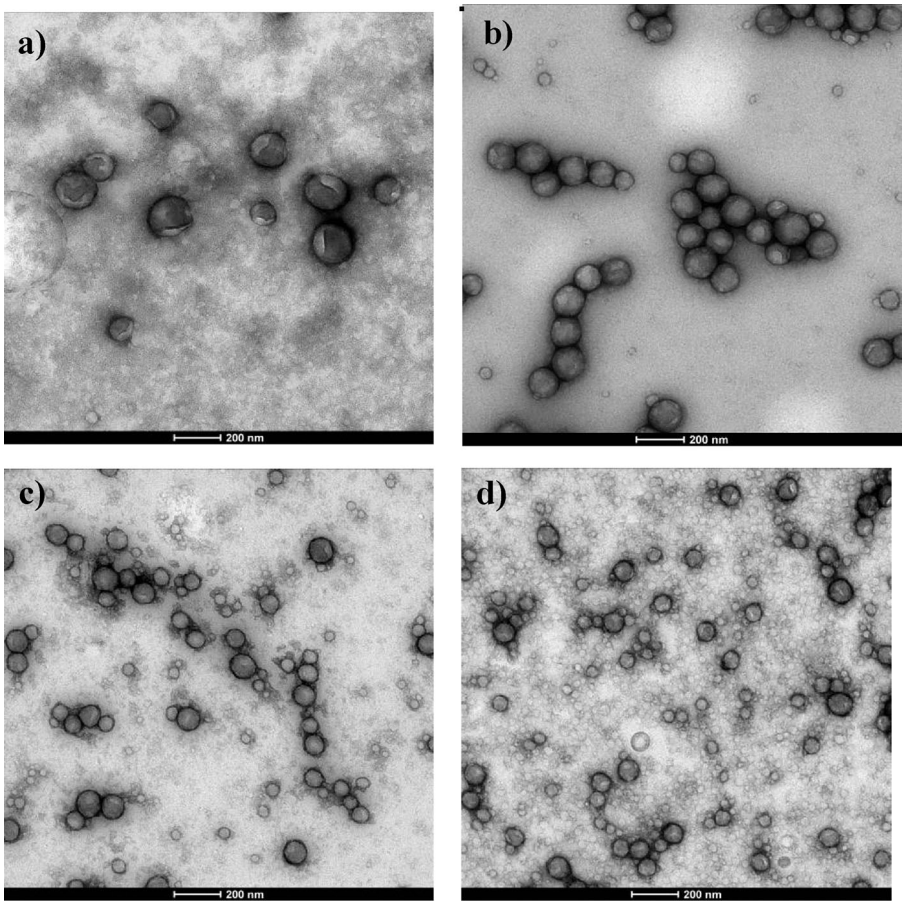

Figure 4.
Morphology of particles obtained by MMA polymerization with varied casein concentration: a) 3 pphm; b) 6 pphm; c) 25 pphm and d) 40 pphm.

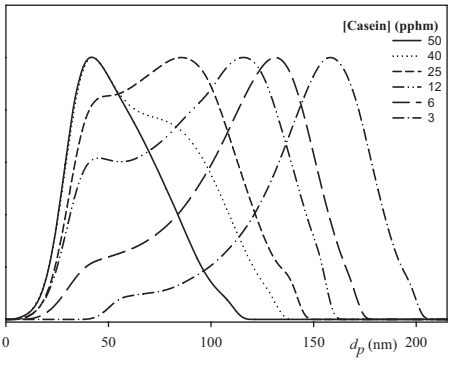

Figure 5.
Mass PSDs for the MMA polymerization in the presence of varied casein concentrations. (PSDs were normalized to the same maximum).

concentration (3 pphm), latexes exhibited almost complete grafted fractions of both casein and PMMA, and the PSD was mainly constituted by a population of high d_p with a small shoulder at low d_p. As the casein concentration was increased from 3 up to 25 pphm, an increasing contribution of the population of low d_p on the PSD was observed, due to the increment of the ungrafted fraction of casein and hence of the second way of nucleation. On the extreme of high casein concentration (40–50 pphm), the ungrafted fraction of both casein and PMMA is too high, and the population of low d_p represented the main contribution on the PSD, indicating that the

Table 3.
Effect of the TBHP concentration on the MMA polymerization in the presence of high casein concentrations.

	Cas 25	Cas 25*	Cas 50	Cas 50*
Casein (pphm)	25	25	50	50
TBHP (pphm)	0.20	0.34	0.20	0.34
x (%)	91	92	89	91
d_p (nm)	120	114	113	106
AGE (%)	68	57	53	47
CGE (%)	26	25	5	6
g-casein (%wbm)	6.5	6.2	2.5	3.0
N_p (#/L)	6.2×10^{16}	7.2×10^{16}	6.2×10^{16}	8.1×10^{16}

principal source of particle formation was the second way of nucleation.

Effect of the TBHP Concentration

It is observed that the amount of casein and acrylic grafted (i.e, the particle compatibilization) is importantly restricted when a high concentration of casein is used. One can speculate that, at high protein contents, the TBHP concentration is limiting the initiation and the particle formation via casein grafting. Table 3 compares the influence of increasing the TBHP concentration from 0.20 up to 0.34 pphm in the MMA polymerization with 25 pphm and 50 pphm of casein. The increase of the TBHP concentration favored the formation of more polymer particles (N_p was increased). However, contrary to what was expected, new particles were mostly formed via the second way of nucleation over the mechanism of compatibilized particles nucleation, because the fraction of grafted PMMA (i.e., the AGE) was reduced without practically modifying the MMA conversion and the amount of grafted casein. These results indicate that the TBHP is also responsible, together with the ungrafted casein concentration, of the formation of particles by the second way of nucleation.

Proposed Mechanism for Particle Nucleation

Based on the previous results, we propose that the particle formation and growth could follow the mechanism represented in Figure 6. Before starting the polymerization, the reaction system is composed by: i) casein (either dissolved in aqueous phase or present in the form of micelles); ii) MMA (partially solubilized in aqueous phase and contributing to swell the hydrophobic inner part of the casein micelles, and the rest remaining as monomer droplets); and iii) TBHP (partitioned between the aqueous and hydrophobic phases). Step 0 represents the redox initiation between the amino groups of the casein and TBHP, forming amino and terbutoxy radicals.[12] In step 1a, casein grafting is produced in aqueous phase at the beginning of the reaction through graft polymerization of MMA from casein radicals. Also, the propagation of terbutoxy radicals produces MMA homopolymer radicals (step 1b). In step 2a, the graft-copolymer radicals become water insoluble and self-assemble to form micelle-like microdomains.[13] These microdomains have a hydrophobic interior to drive TBHP molecules, tert-butoxy propagating radicals (step 2ba) and MMA monomers into the self-assembled micellar microdomain. Step 3a involves the formation of compatibilized particles as the result of the MMA polymerization therein via both graft polymerization and homopolymerization pathways. Thus, the formation of compatibilized particles by the abovementioned initial nucleation mainly involves steps 1a, 2a and 3a. Note that the TBHP dissolved inside the compatibilized polymer particles initiates the polymerization by reacting with the amine groups of the grafted casein in the casein-PMMA interface, increasing the particle size and the fraction of grafted acrylic,

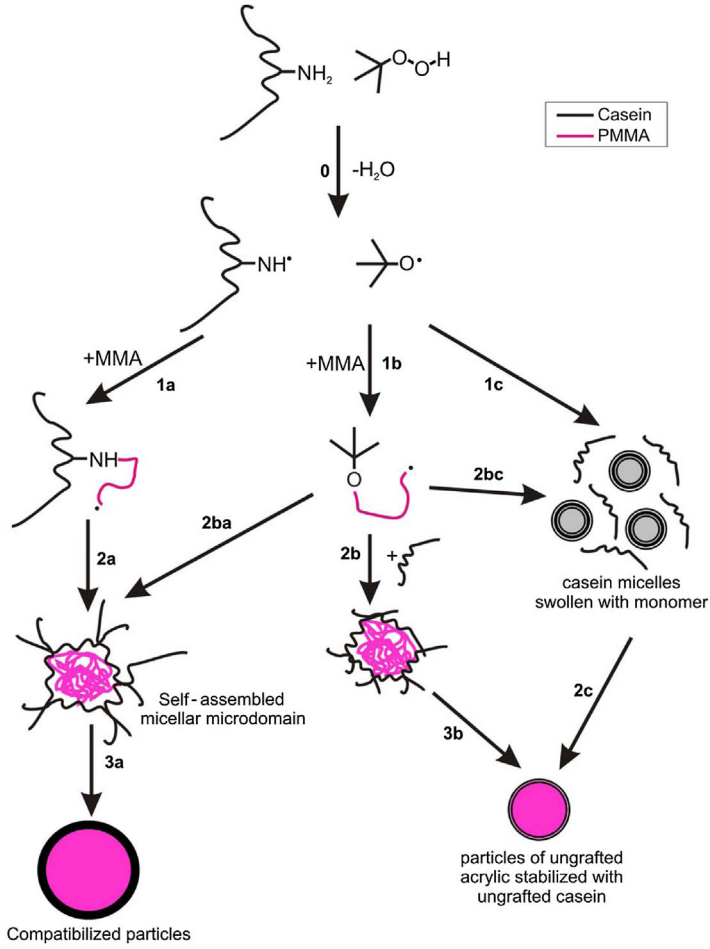

Figure 6.
Proposed mechanism for the hybrid particles generation in the MMA polymerization initiated with TBHP and varied casein concentrations.

without changing the amount of grafted casein.

The growing MMA radicals obtained by propagation of terbutoxy radicals (step 1b) could coagulate producing new microdomains of uncompatibilized PMMA stabilized by adsorption of free casein (step 2b). These new microdomains, with a hydrophobic interior, drive tert-butoxy radicals and MMA monomer into their core. On the other hand, the rather hydrophobic tert-butoxy radicals produced in aqueous phase by step 0 or the growing radicals obtained from step 1b, could enter into the casein micelles swollen with monomer (steps 1c and 2bc). These steps become more important at high casein concentration, where the availability of micelles is greater. The TEM image of Fig. 7 confirms the existence of casein micelles for a solution of 20 mg/mL of protein, equivalent to the casein concentration of latex Cas 25. Propagation of alcoxy radicals with the MMA dissolved inside both the microdomains produced after step 2b and the nucleated casein micelles generated after steps 1c and 2bc give place to new polymer particles constituted by ungrafted PMMA and ungrafted casein, recognizing this phenomenon as the second way of

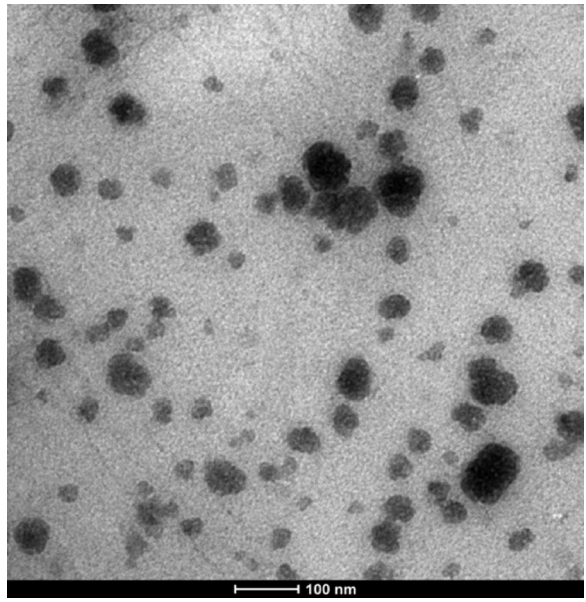

Figure 7.
TEM images of casein micelles of a 20 mg/ml casein solution containing 0.4% of Na_2CO_3.

nucleation (steps 3b and 2c, respectively). It is worth to remark that the so called second way of nucleation, where both free casein and tert-butoxy radicals participate, could be present from the beginning of the polymerization.

In the cases where the casein concentration is low, most of the protein is grafted at the beginning of the polymerization (Figure 2), limiting the casein concentration in aqueous phase and the micelles formation. Consequently, the later occurrence of steps 0 in aqueous phase is strongly restricted; and then the predominant way of progress of the polymerization is the initiation inside the compatibilized particles. Also, the availability of amine groups in the casein-PMMA particle-interface is restricted, and hence it could be the responsible of the observed limiting conversion of MMA at low casein concentration. In contrast, when the casein concentration is high, particle formation mainly occurs through a second way of nucleation, following the possible b and c paths of Figure 6. Under these conditions, the fractions of both grafted casein and grafted acrylic result reduced (Table 2) and a large fraction of the produced particles are constituted by ungrafted acrylic stabilized with ungrafted casein.

In particular note that the increment of casein concentration from 3 up to 12 pphm favors the grafting reaction in the aqueous phase (steps 0 and 1a), improving the weight of grafted-casein. However, a further addition of casein increases the availability of ungrafted casein (dissolved and in the form of micelles) which promotes the second way of nucleation, thus producing a reduction of the effective weight of grafted protein. Similarly, the increment in the TBHP concentration enhances the availability of tert-butoxy radicals and promotes the second way nucleation.

Conclusion

The MMA emulsion polymerization in the presence of casein was investigated by employing different concentrations of protein, with the aim of producing hybrid acrylic-casein latexes. The characterization

of the molecular microstructure of hybrid casein/PMMA composites allowed us to determine the fraction of grafted casein and grafted acrylic polymer, which helped to understand the involved particles nucleation mechanism. Two main competitive mechanisms were identified along the batch polymerization of MMA in the presence of casein and with TBHP as initiator. The casein/TBHP initiation promoted the formation of compatibilized particles at the beginning of the polymerization, while a second way of nucleation (probably present along the whole process) by hydrophobic radicals coagulation or absorption into the protein micelles, generated uncompatibilized particles.

The employed casein concentration could favor one nucleation mechanism over the other. Polymerization with low casein concentration mainly produced compatibilized acrylic/casein particles (i.e., with a high fraction of each component chemically compatibilized). However, as the casein concentration increased, the nucleation of uncompatibilized particles (i.e., composed by ungrafted acrylic and casein) became more important, and at a casein concentration of 50 pphm the second way of nucleation was predominant.

Finally, the present results suggest that the employed process should be modified to improve the nanoparticles compatibilization when a high casein concentration was required.

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