Book Chapter

Effect of pH and Monomer Dosing Rate in the Anionic Polymerization of Ethyl Cyanoacrylate in Semicontinuous Operation

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Published **November 25, 2019**

This Book Chapter is a republication of an article published by Hened Saade, et al. at International Journal of Polymer Science in March 2015. (Hened Saade, Suleidi Torres, Cynthia Barrera, Julieta Sánchez, Yolanda Garza, and Raúl G. López, “Effect of pH and Monomer Dosing Rate in the Anionic Polymerization of Ethyl Cyanoacrylate in Semicontinuous Operation,”

International Journal of Polymer Science, vol. 2015, Article ID 827059, 9 pages, 2015. <https://doi.org/10.1155/2015/827059>.)

How to cite this book chapter: Hened Saade, Suleidi Torres, Cynthia Barrera, Julieta Sánchez, Yolanda Garza. Effect of pH and Monomer Dosing Rate in the Anionic Polymerization of Ethyl Cyanoacrylate in Semicontinuous Operation. In: Prime Archives in Polymer Technology. Hyderabad, India: Vide Leaf. 2019.

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Acknowledgments: National Council of Science and Technology (CONACyT) supported this research through grants 2014-223227 and 232753 (Laboratorio Nacional de Materiales Grafénicos). One of the authors (C. Barrera) acknowledges the scholarship from CONACyT. The authors are grateful to Marcelina Sánchez for her technical assistance in characterization work.

Conflicts of Interest: The authors declare no conflict of interest

Abstract

Nanoparticles of poly(ethyl cyanocrylate) with more than 10 % solids content were prepared by semicontinuous heterophase polymerization at monomer starved conditions varying the initial pH in the interval 1-1.75 and at two monomer dosing rates. Measurements by scanning-transmission electron microscopy allowed to identify an inverse dependence of particle size on pH. Furthermore, all the polymerizations conducted at the slower monomer dosing rate rendered two particle populations, with the larger one formed from the aggregation of a fraction of the smaller particles. It was believed that the so slow addition of the monomer caused the formation of very small but instable particles, thereby a fraction of which aggregated to reduce the total interface particles-aqueous phase, increasing the latex stability. An increase in the monomer dosing rate led to larger and more stable particles in such way that only one population of nanoparticles with around 40 nm in average diameter was obtained.

Keywords

Poly(Ethyl Cyano Acrylate) Latexes; High Solid Content; Poly(Ethyl Cyano Acrylate) Nanoparticles sub-50 nm

Introduction

Poly(alkyl cyanoacrylate), PACA, nanoparticles are a very interesting material for the design of drug delivery nanosystems [1,2] due to their well-known biocompatibility and biodegradability [3,4]. Since Couvreur *et al.* reported the anionic polymerization of methyl cyanoacrylate (MCA) and ethyl cyanoacrylate (ECA) in emulsion [5], a number of research groups have documented studies on nanoparticles preparation and their loaded with different drugs [3,4]. In accordance with the reports on the subject, polymerization in an aqueous media stabilized with an amphiphile is the most widely used technique for preparing PACA nanoparticles [3,4].

In agreement with the specialized literature, the type and surfactant concentration [6-11] and the initial pH [7-9,12-15] are the most studied variables in the PACA nanoparticles preparation. All of these works emphasize the influence of these variables on particle size, mainly due to the possible use of drug-loaded PACA nanoparticles in the development of drug delivery systems, in which ultrafine nanoparticles ranging 10-50 nm are very attractive due to the increase in their efficacy [16,17]. Reports in the literature indicate that different types of dextrans [6,10,12-14] and Tweens [5,6,11,12] are the most evaluated surfactants in the emulsion polymerization for PACA obtaining and that in general the smaller sizes are obtained when Tweens were used. A revision of the quoted reports indicates that average diameters as large as 400 nm were obtained by Behamet *al.* [13] who used Dextran 70 in emulsion polymerization of butyl cyanoacrylate (BCA) at different pH's between 2 and 3. In contrast, Douglas *et al.* reported the obtaining of poly (butyl cyanoacrylate) (PBCA) nanoparticles with around 50 nm in average diameter when using Tween 60 in an emulsion polymerization at pH 2.25 [6]; in fact, they obtained larger particles when tested Tweens with lower molecular weight (TW20 and TW40), which suggested an inverse relationship between particle size and Tween molecular weight. On the other hand, the reports on pH influence have demonstrated that polymerizations carried out at pH's lower than 1 and higher than 3.5 produce very large particles and that smaller particles are

obtained at pH's close to 2 [12,13]. It is noticeable that practically all works reporting particle sizes are based on measurements by quasi-elastic light scattering (QLS), while only a few include electron microscope determinations [5,9]; however, the latter do not show particle size distributions from micrographs, but they use these results as complement of QLS measurements. Nevertheless, the report of Yang et al. [9] calls attention due to the very small particle sizes of PBCA they said have obtained. These authors documented the results of emulsion polymerization of BCA, showing micrographs with two particle populations, one displaying diameters smaller than 50 nm and another with much larger sizes. It would have been interesting to know about the fraction of the particle populations in the latexes, however taking into account the turbidity of the dispersions mentioned by the authors, surely larger particles constituted the largest fraction.

The aim of the study presented herein was to elucidate the effect of the reaction medium pH and the rate at which the monomer is dosed on the particle size during the ECA polymerization in an aqueous dispersion containing Tween 80 (TW80). To our best knowledge, the use of this surfactant, polyoxyethylene (20) sorbitanmonooleate (1310 g/mol in molecular weight), has not been reported in the PACA obtaining in aqueous dispersion. Moreover, in contrast with that reported in the specialized literature for other alkyl cyanoacrylates polymerizations using other surfactants, the effect on the particle size documented here was determined with basis on electron microscope measurements. The polymerizations were conducted at different pH's between 1 and 1.75 in a semicontinuous manner, which means that ECA was dosed over a micellar solution. This monomer adding policy has been used successfully by our group under the name of *semicontinuous heterophase polymerization at monomer starved conditions* to obtain polymeric nanoparticles with average diameters substantially less than 50 nm [18-20]. Despite those polymerizations proceeded via free radical mechanism, it was expected that this technique also would lead to very small particles, taking into account that the ECA anionic polymerization also would take place in surfactant stabilized

compartments. Such small nanoparticles would be an excellent candidate for preparing high-efficacy drug delivery nanosystems.

Experimental Section

Reagents

ECA (99%) was purchased from Sigma-Aldrich (Toluca, México) and stored at 4°C; TW80 was obtained from Oxiteno (Guadalajara, México); both of them were used as received. De-ionized and triple-distilled water was drawn from a T S Barnstead E-Pure 4-Holder Water Purification Sytem.

Polymerizations

Reactions were conducted in a 25 mL jacketed glass reactor equipped with a reflux condenser and mechanical agitation following the procedure described below. Twenty g water and 0.35 g TW80 were charged into the reactor, after which the mixture was subjected to 450 rpm agitation and the temperature stabilized in 35°C; the reaction was started by the beginning of the monomer addition. In all polymerizations 2 g ECA were added by employing two different addition total times, 4 and 2 hours, which equals to 0.0083 and 0.0166 g/min dosing rates, respectively. After the end of the monomer addition the reaction was allowed to proceed for a further period of 3 hours. The water pH in the polymerizations was initially adjusted to 1.0, 1.5 and 1.75 for the ECA addition period of 4 h, while for the period of 2 h, only the adjustment to pH 1.75 was carried out. The monomer conversion at sampling time was determined by gravimetry.

Characterization

Particle Size

Determinations of particle size distributions for latexes samples were carried out by using a JEOL JSM-7401F scanning-transmission electron microscope (STEM). For the measurements a dilution containing about 2.5 g solids per liter was prepared, depositing one drop of it on a copper grid and allowing to dry. After that the samples were stained with a drop of a 2 wt.% aqueous solution of phosphotungstic acid. The

diameters of a variable number of particles were manually measured one by one from the set of micrographs by using the image analysis program ImageJ 1.37c. From these data, D_w , D_n and PDI (D_w/D_n), being D_w and D_n the weight- and number-average diameters and PDI the polydispersity index, were calculated using the following equations:

$$D_n = \frac{\sum_i n_i D_i}{\sum_i D_i} = \frac{\sum_i n_i D_i}{n} \quad (1)$$

$$D_w = \frac{\sum_i n_i D_i^4}{\sum_i n_i D_i^3} \quad (2)$$

Results and Discussion

Polymerizations carried out at pH 1, 1.5 and 1.75 attained final conversions of 99.4, 99.5 and 98.6 %, respectively, which corresponds to 10.4, 10.6 and 10.3 % solids content, respectively. This looks interesting due to in accordance with the specialized literature solid contents no higher than 5-6% are common in the PACA latexes obtained by emulsion polymerization [6-12,15]. It is pertinent to make clear that practically none of the quoted reports mentions monomer conversion values, so the corresponding solid contents were estimated from the reported formulations and assuming total monomer conversion.

Figures 1to 3 include representative micrographs of the final latexes samples prepared at pH 1, 1.5 and 1.75, respectively, where is evident the existence of two particle populations. The contrast in the particles appearance in the micrographs would arise from a no homogeneous sample staining. These figures also include the histograms of particle diameters for each one of the populations, which were constructed from the size manual measurement of a great number of particles in the sets of micrographs. The formation of two particle population in the alkyl cyanoacrylates polymerization has been reported by Yang

et al. [9] who obtained one population showing 10-30 nm in size and another one where particles were larger than 50 nm as determined by electronic microscopy, when they polymerized BCA using chitosan as stabilizer. On the other hand, Table 1 shows the values for D_n , polydispersity index and the number of particles measured to obtain those values, corresponding to each one of the populations in the prepared latexes.

Table 1: Results of STEM measurements for samples from polymerizations at different pH's.

pH	Population 1			Population 2		
	Measured particles	D_n (nm)	D_w/D_n	Measured particles	D_n (nm)	D_w/D_n
1.0	536	22.0	1.4	157	119.9	1.2
1.5	774	14.4	1.4	264	67.3	1.3
1.75	1833	11.9	1.3	599	58.9	1.2

Figure 1: Micrograph of a sample taken at the end of the polymerization at pH 1.0. The histograms of smaller (a) and larger nanoparticles populations (b) are also included.

Figure 2: Micrograph of a sample taken at the end of the polymerization at pH 1.5. The histograms of smaller (a) and larger nanoparticles populations (b) are also included.

Figure 3: Micrograph of a sample taken at the end of the polymerization at pH 1.75. The histograms of smaller (a) and larger nanoparticles populations (b) are also included.

From the micrographs in Figures 1 to 3 is evident that the large particles are formed by the aggregation of a fraction of the smaller ones and that the individuality of the latter is preserved, that is to say, that they do not coalesce. Additionally, an inspection of data in Table 1 indicates that the average size of both population decreases as pH values increases and that the populations of small particles are formed of very small particles, 22.0 and 11.9 nm in D_n for polymerizations at pH 1 and 1.75, respectively. Values as small than these, determined by electron microscopy have not been previously reported in the literature.

In the following possible explanations on the formation of such small particles, the obtaining of smaller ones at the higher pH and why a fraction of the smaller particles aggregates to give rise larger ones will be provided.

Respecting to the cause of the smallness of PECA particles obtained in this study, it is believed that the slow rate at which the monomer was added to the miscellar solution, is the reason behind this finding. This arises from the operation at semicontinuous heterophase polymerization, technique developed by our group [18-20] that allows to obtain polymeric nanoparticles with diameters less than 50 nm, solid contents up to 25-30% and all surfactant in the latex stabilizing the particles. However, this technique requires to operate at the so-called *monomer-starved conditions* during the monomer addition period [21]. Krackeler and Naidus [21] coined this term when they explained the smaller particle sizes obtained in the emulsion polymerization of styrene carried out in semicontinuous mode compared to the batch process. These authors resorted to the correlation developed for emulsion polymerization by Smith and Ewart [22] for predicting the number of particles (N_p) for the case-II kinetics, in which N_p is inversely proportional to the volume growth rate of the polymer particles during nucleation period. When the particles are saturated with monomer, they grow at their maximum rate; as a consequence, the particle nucleation is minimum. In semicontinuous emulsion polymerization particle monomer saturation is attained by operating at the so-called *monomer-flooded conditions*. In contrast the operation under monomer-starved conditions,

meaning that the monomer in the particles is below the saturation concentration, slows down the particle growth rate resulting in a larger number of smaller particles. These conditions are achieved by adding the monomer at very slow dosing rates.

The inverse dependence between pH and particle size could be explained as follows. As is well-known [13] the mechanism of anionic polymerization of alkyl cyanoacrylates in aqueous dispersions containing micelles includes an initiation via hydroxyl ions in the aqueous phase, which react with the monomer near the swollen micelles surface, giving rise to a particle. In our case, it should be considered that TW80 contains OH groups into its structure, which also can initiate the polymerization, giving more soluble water oligomers. The consequence is a delay in the entry of this oligomer kind to the surfactant stabilized compartments whether to form a particle or added to the polymer chains in an already existent particle. Then, these anionic radicals propagate inside the particles until the growing chains terminate by reacting with protons present in the monomer, which come from the aqueous phase and monomers stabilizer. Given that the surfactant concentration is the same in all polymerizations, the concentration of hydroxyl ions in the aqueous phase would be determinant in the initiation rate and, as consequence, in the particle formation; this means that the higher hydroxyl ions concentration, the more particles are formed. Thus, in view that hydroxyl ions concentration increases as pH increases, an increase in the particle number and consequently smaller particle sizes in the latexes should be expected.

Table 2 shows additional data concerning to the particle aggregation in the polymerizations. Here, the particle surface area covered by one surfactant molecule was estimated by first calculating N_p in the latex using the following equation:

$$N_p = \frac{6C_p}{\pi\rho_p D_n^3} \quad (3)$$

Where C_p is the polymer concentration in the latex in g/mL of water, D_n , the number-average diameter in nm, and, ρ_p , the polymer density in g/mL, taken as 1.1 g/mL for PECA. The

number of surfactant molecules stabilizing the particles was calculated from the molecular weight of the surfactant (1310g/mol), its amount in the formulation and assuming that all surfactant is placed on the particles. The values of particle surface area that one surfactant molecule covers shown in Table 2 range from 3.1 to 5.7 nm² and they increase as pH increases. In general, these values are very high when compared with, for example, the value of 0.5 for the ionic surfactant sodium dodecyl sulfate (SDS) [23], which suggests that TW80 molecules provide only a partial stabilization to PECA particles. Data in Table 2 show indirectly that the number of surfactant molecules per surface area decreases as pH increases, which suggests that the increasing number of hydroxyl ions in the aqueous phase contributes to latex stabilization. However, due to the partial stabilization provided by the surfactant molecules, a fraction of the particles aggregates to reduce the total interfacial area seeking for more stable latex. Table 2 also includes the estimated number of average small particles that aggregates to form a large one, which were obtained from the volume ratio of large to small particles for each polymerization. Then, using these numbers and simple geometric calculations, it was obtained an estimation of the reduction on the total interfacial area given by the sum of each one of the areas of the average small particles that aggregates to form a large one. The results shown in Table 2 indicate a similar reduction for all polymerizations of around 83-85%, that is to say, that the surface area of one average large particle represents around 15-17% of the area of all average small particles that had to aggregate to form it.

Table 2: Data concerning to the particle aggregation in the polymerizations conducted at Different pH's.

pH	Particle surface area covered by a surfactant molecule (nm ²)	Number of average small particles that aggregates to form a large particle	Reduction in total area of small particles after aggregation in one larger particle (%)
1.0	3.1	162	84.5
1.5	4.7	102	82.4
1.75	5.7	121	83.2

Another feature that deserves to be treated is the difference in the polydispersity index values between the two populations observed in Table 1; in all cases the smaller nanoparticles populations show a broad distribution, while those corresponding to the larger particles are consistently less broad. The broad distribution are usually related to continuous particle nucleation along the reactions, due to the great difference between the sizes of the particles formed early in the polymerization, who grow along all the time and those nucleated at the final stages, with a reduced growth period. Continuous nucleation is common in semicontinuous heterophase polymerization at monomer starved conditions [18-20] under which the polymerizations in this study were conducted, and it is consequence of the very low monomer concentration in the particles that prevails along the reactions. On the other hand, the less broad distributions of larger particles populations suggest that another mechanism to form particles is acting, in this case, the already mentioned smaller particles aggregation. Furthermore, an heterogeneous aggregation, that is to say, small with large particles both belonging to the smaller particles population should occur to give rise a less width particle distribution.

To acquire some understanding about how the particle size evolves along the polymerizations another reaction at pH 1.75 and 4 hours of monomer addition was conducted, taking a sample after 35 min from the beginning of monomer dosing and another one at the end of the polymerization. The sample taken early in the reaction showed a global conversion of 11.1%, while that taken at the end of the polymerization gave a value of 99.0%. Figures 4 and 5 include representative micrographs of the 35 min and final samples, respectively, in which can be seen two particles populations. As in the samples obtained at the end of the polymerizations at different pH's the larger particles are formed by the aggregation of a fraction of the smaller ones, which preserve their individuality. Additionally, the corresponding histograms of particle diameters for each population were included in those figures. Table 3 shows D_n , polydispersity index and the number of particles measured to obtain these values for the same samples. The average sizes of the smaller particles population in Table 3 indicate that the

particles formed at early stages in the polymerizations are smaller than those obtained at the final stage. This means that the smaller particles grow as the polymerization evolves probably through the recruiting of anionic radicals formed near their surface by the reaction of the hydroxyl ions and the terminal OH groups of the surfactant with the monomer molecules which finally results in a new polymer chain inside the particle. Following the same procedure used in the analysis of the data obtained in the polymerizations conducted at different pH's, it was found that around 661 average smaller particles should aggregate to form one large particle at 35 min from the reaction beginning. In this case, the surface area of the average large particle represents around 11% of the sum of the areas of all the average small particles that had to aggregate to generate it. At the end of the polymerization, the number of average small particles that aggregates to give rise one average large particle is only around 108 and the surface area of the large particle is now equivalent to approximately 17% of the sum of the areas of all the small particles before aggregation. It is notable the good reproducibility of the D_n values obtained at the end of both polymerizations carried out at pH 1.75: 11.9 and 12.1 nm (mean value, 12.0 ± 0.1 nm) for the smaller particles population and 58.9 and 57.7 nm (mean value, 58.3 ± 0.8 nm) for the larger one. This result contributes to discard any artifact from electron microscopy technique as causing the two particle populations formation. The differences between the results at 35 min from the reaction beginning and those at the end, could be due to the very small size of the particles at the early stage of the polymerization that makes them more unstable, promoting their aggregation in a number enough to more reduce their total area thereby increasing the latex stability.

Table 3: Results of STEM measurements for samples taken from polymerization at pH 1.75.

Sampling time	Population 1			Population 2		
	Measured particles	D_n (nm)	D_w/D_n	Measured particles	D_n (nm)	D_w/D_n
35 min	4637	7.3	2.63	103	63.6	1.6
Final	823	12.1	1.6	193	57.7	1.4

Figure 4: Micrograph of a sample taken at 35 min from the beginning of the replica of the polymerization at pH 1.75. The histograms of smaller (a) and larger nanoparticles populations (b) are also included.

Figure 5: Micrograph of a sample taken at the end of the replica of the polymerization at pH 1.75. The histograms of smaller (a) and larger nanoparticles populations (b) are also included.

The results obtained in the second polymerization conducted at pH 1.75 allowed to know that two particle populations are formed since the beginning of the polymerization and that the aggregation of a fraction of small particles would occur along the polymerization. This suggests that probably the monomer dosing rate is so low that the system is forced to generate very small but unstable particles. To test this hypothesis a polymerization with a faster ECA dosing rate (0.0166 g/min) was conducted, since in accordance with the findings in semi continuous hetero phase polymerization at monomer starved conditions [19-21] larger and more stable particles should be obtained, avoiding this way the particle aggregation. This polymerization gave a final conversion of 91.2 %, which corresponds to 9.7 % in solids content.

The micrograph and the corresponding histogram in Figure 6 show that indeed only one particle population is observed when the polymerization was carried out at a faster monomer dosing rate. From the measurement of around one thousand particles a D_n value of 42.3 nm and a polydispersity index of 1.4 were determined. Using the same calculation procedure previously employed, it was found that in this case one molecule of surfactant covers approximately 1.6nm^2 of particle surface area, which is much smaller value than those obtained in the polymerizations carried out at the slower monomer dosing rate, indicating higher particle stability. Only one population of larger and more stable particles obtained as result of an increase in the monomer dosing rate confirms the hypothesis given above. This finding, along the good reproducibility observed in the average size of smaller and large particle population from two polymerizations carried out at pH 1.75 and 4 h of monomer addition, also allows to discard that the two particle populations observed in all other polymerizations to be caused by any artifact of electronic microscopy technique. Moreover, as mentioned earlier, OH groups of TW80 can act as polymerization initiators, which could result in particle nucleation. The possibility that nucleation arising from these groups could contribute to formation of two particle population was also discarded in the light of this mentioned finding.

Figure 6: Micrograph and histogram of a sample taken at the end of the polymerization at pH 1.75 and 0.017 g/min in monomer dosing rate.

Conclusions

Semicontinuous heterophase polymerization at monomer starved conditions conducted at different initial pH's in the range 1 to 1.75 allowed to obtain latexes containing very small particles of PECA and solid contents around 10.5%, which are substantially higher than those reported in the literature on preparing PACA latexes. Irrespective of the polymerization pH, two particle populations are formed since the early stages of those polymerizations conducted at 4 h of monomer addition; the smaller particle sizes at the end of the reaction were obtained in the polymerizations carried out at pH 1.75. STEM measurements indicate that larger particles are formed by the aggregation of a fraction of the smaller ones, which preserve their individuality. A polymerization conducted at a pH 1.75 and faster monomer dosing rate than that used in the rest of the reactions allowed to obtain particles with a D_n value near to 40 nm, distributed in only one population. This finding indicates that while it is possible to obtain smaller particles when the monomer is dosed at a very low rate, they are instable thereby a fraction of them aggregates

to form a population of larger particles, increasing in this way the latex stability. Taken into account the potential use of biocompatible and biodegradable particles with diameters smaller than 50 nm, the results reported herein could be employed as a basis to investigate on pH's and monomer dosing rate using TW80 or more effective surfactants, that allow to prepare latexes with stable particles less than 40 nm in average diameter distributed in only one population, preserving the relatively high solids content attained in this study.

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