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Influence of the initiator system, cerium–polysaccharide, on the surface properties of poly(isobutylcyanoacrylate) nanoparticles

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Abstract

Isobutylcyanoacrylate has been used to form nanoparticles in presence of polysaccharides by two methods of polymerization. To have a better understanding of the relation between the structure of the resulting copolymer and the surface properties of the nanoparticles, the redox radical emulsion polymerization (RREP) was investigated and compared to anionic emulsion polymerization developed by Couvreur et al. (AEP Couvreur). The kinetic study showed that at pH1 the nanoparticles were formed in 5 min by RREP against 30 min in anionic emulsion polymerization (AEP pH1). The diameter of the nanoparticles was mainly affected by the concentrations of cerium and dextran and by the molecular weight of the dextran. The zeta potential was controlled by the characteristics of the polysaccharides (molecular weight and charge). The different methods of polymerization of isobutylcyanoacrylate led to nanoparticles with different surface properties.

Keywords: Alkylcyanoacrylate; Polysaccharide; Nanoparticle

1. Introduction

An exciting application of colloidal particles made of biodegradable polymers was their development as carrier for the in vivo delivery of drugs. The main goal of this research was to modify the surface of nanoparticles to interfere on the distribution of the drugs inside the body. Indeed, it was already proved for nanoparticles that their surface properties controlled their interactions with seric proteins [1,2] and, therefore, their biodistribution after administration in the body by intravenous injection [3–9].

Polyalkylcyanoacrylates have been investigated extensively for 20 years to be used as drug carrier for their low toxicity and biodegradability. Anionic emulsion polymerization (AEP Couvreur) was developed by Couvreur et al. [10] to prepare nanoparticles of polyalkylcyanoacrylate covered by dextran. This reaction was spontaneously and quickly initiated by small amount of weak base including hydroxyl groups of water or of dextran. The emulsion polymerization was an interesting way because it allowed to prepare nanoparticles in one step and to avoid the use of organic solvent.

The nanoparticles prepared by this method accumulated only in the liver and the spleen after intravenous administration to animals. These nanoparticles were strongly opsonized by seric proteins and activated the complement system. To modify their surface properties, Peracchia et al. [11] replaced the dextran by poly(ethylene oxide), which was known to have a protein repulsive effect when grafted onto the surface of a polymer device [6,7]. By this way poly(ethylene oxide)—coated nanoparticles could be prepared due to the initiation of the anionic emulsion polymerization of alkylcyanoacrylate by the terminal hydroxyl group of the poly(ethylene oxide) chain. The poly(ethylene oxide)—coated nanoparticles showed a clear reduction of their capacity to induced complement activation [12] suggesting that their in vivo distribution would be dramatically modified compare to the distribution shown by the nanoparticles prepared by anionic emulsion polymerization in the presence of dextran.

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More recently, another method of polymerization of alkylcyanoacrylate was developed by Chauvierre et al., and introduced nanoparticles coated with polysaccharides [13]. By electron microscopy, they appeared spherical with a regular size [13,14]. Polysaccharides are encountered on the surface of many living structure including membrane cells and viruses, where they are used as mask of antigenic structures or are involved in signaling or specific recognition phenomena. One of the main advantages of polysaccharide compared to poly(ethylene oxide) is that they contain chemical groups on which it is possible to graft other ligand for recognition of the target cell for example. Moreover, there are many different polysaccharides with some having biological activity. Thus, it was believed that the coating of biodegradable nanoparticles by polysaccharide could open new perspectives in the development of biomimetic drug delivery systems with versatile surface properties. Passirani et al. [15] developed a redox radical emulsion polymerization, allowing the preparation of non-biodegradable poly(methyl methacrylate) nanoparticles, which could be coated with either dextran or heparin. In this reaction, the polymerization was initiated by the redox system composed of the polysaccharide (i.e. dextran or heparin) and Ce^{4+}. Applied to alkylcyanoacrylates this reaction produced very stable nanoparticles and was successfully used with different polysaccharides having a molecular weight ranging from 10.000 to 500.000 g/mol [14]. They showed different properties, in interaction with seric proteins, than the properties of the nanoparticles made by anionic emulsion polymerization carried out with dextran. The aim of the present work was to study in details this new method of redox radical emulsion polymerization of alkylcyanoacrylate (RREP) to have a better knowledge of the characteristics of the nanoparticles produced, depending their conditions of preparation. In this work, we investigated the rate of the formation of the nanoparticles and the influence of the composition of the polymerization system on the characteristics of the nanoparticles produced in term of their size and surface properties.

2. Experimental

2.1. Materials

Isobutylcyanoacrylate (IBCA) was used as monomer and was kindly provided as a gift by Loctite (Dublin, Ireland). Dextran 66,900 g/mol, dextran 10,000 g/mol and dextran sulphate 10,000 g/mol were purchased from Sigma (Saint-Quentin Fallavier, France). Dextran 40,000 g/mol and chitosan medium molecular weight were purchased from Fluka (Saint-Quentin Fallavier, France), and dextran sulphate 36,000–50,000 g/mol was supplied from ICN Biomedicals (Orsay, France). All chemicals were reagent grade and used as purchased.

2.2. Preparation of chitosan of various molecular weights

2.2.1. Depolymerization of chitosan

Chitosan was selectively depolymerized by reaction with sodium nitrite at various concentrations [16]. Typically, 100 ml of a solution of chitosan (2%) in acetic acid (6%) was depolymerized during 1 h with 10 ml of sodium nitrite, which concentration was either 0.04 or 0.08 mol/l, at room temperature and under magnetic stirring. Chitosan was precipitated by raising the pH to 9 with sodium hydroxide. The precipitate was recovered by filtration and washed extensively with acetone before being dried. Chitosan dissolved in acetic acid (0.1 M) was further purified by dialysis against water during 24 h. The resulted solutions were freeze dried before storage.

2.2.2. Measurement of molecular weight

The molecular weight of chitosan was determined from capillary viscosity measurements. Briefly, the reduced viscosity of solutions of chitosan of various concentrations (0.1–2.5 g/l) in acetic acid 0.1 M, NaCl 0.2 M was measured in a Ubbelohde tube (53710/1 Schott Geräte) at 25 °C (Bath CT1450 Schott Geräte and cooling system CK100 Schott Geräte) using a viscometer AVS400 (Schott Geräte). The intrinsic viscosity $[\eta]$ was then deduced from the reduced viscosity measured for each solution of chitosan by extrapolation at zero concentration. The molecular weight was determined by using the Mark Houwink Sakurada equation: $[\eta]=K\times M^{a}$, with $K=1.81\times10^{-3}$ and $a=0.93$ [17].

2.3. Polymerization procedure

To highlight the differences between the anionic and the radical reactions, the polymerization of isobutylcyanoacrylate in presence of dextran was made in three different conditions named: redox radical emulsion polymerization, anionic emulsion polymerization Couvreur and anionic emulsion polymerization at pH1.

2.3.1. Redox radical emulsion polymerization (RREP)

The radical polymerization was carried out according to the method described by Chauvierre et al. [13]. A polysaccharide (dextran, dextran sulphate or chitosan) (0.1375 g) was dissolved in 8 ml of nitric acid (0.2 M) in a glass tube at 40 °C under gentle stirring and argon bubbling. After 10 min, 2 ml of a solution of cerium(IV) ammonium nitrate (8×10^{-2} M) in nitric acid (0.2 M) and 0.5 ml of IBCA were successively added under vigorous agitation, enough to create a vortex. Argon bubbling was maintained during 10 min. The reaction was left to continue under gentle stirring for 50 min. After cooling to room temperature, 1.25 ml of an aqueous solution of trisodium citrate dihydrate (1.02 M) was added to the polymerization medium corresponding to the preparation performed with dextran or with dextran sulphate. The pH of all the

preparations was adjusted to 7.0 with NaOH 1 N. The suspension obtained appeared as a milky dispersion of polymer particles.

2.3.2. Anionic emulsion polymerization Couvreur (AEP Couvreur)

The anionic emulsion polymerization was performed according to the procedure described by Couvreur et al. [10]: dextran (0.05 g) was dissolved in 10 ml of hydrochloric acid (pH 2.5) at room temperature. Then, 100 μl of IBCA were added dropwise under vigorous stirring. After the reaction was completed (generally 3 h), the pH was adjusted to 7.0 with NaOH 1 N. The resulted suspension was less turbid than the suspension obtained by RREP.

2.3.3. Anionic emulsion polymerization at pH1 (AEP pH1)

The polymerization media was prepared by dissolving dextran (0.4139 g) in 30 ml of nitric acid (0.2 M), whose pH was lower than 1. IBCA (1.5 ml) was added under strong magnetic stirring and left to polymerize at room temperature under magnetic stirring during 24 h. The pH was lower than in AEP Couvreur to slow down the anionic polymerization. The concentrations in dextran and in IBCA were the same than in RREP but the ceric ions were removed to avoid the radical polymerization.

2.4. Purification of nanoparticles

All the polymer suspensions resulted from the different polymerizations were purified by dialysis (Spectra/Por® membrane 100,000 g/mol molecular weight cut off (MWCO), Biovalley, Marne la Vallée, France) two times against 1L of distilled water for 90 min and once overnight. The purified suspensions were stored at 4 °C until use or freeze dried. For freeze-drying the suspensions were frozen at −18 °C and freeze-dried during 48 h (Christ Alpha 1–4 freeze dryer, bioblock Scientific, Illkrich, France) without using cryo-protecting agent.

2.5. Nanoparticles characterization

2.5.1. Particle size

The diameter of the nanoparticles was measured at 20 °C by quasi-elastic light scattering using a Nanosizer N4 PLUS (Beckman-Coulter, Villepinte, France) operating at the angle of 90 °C. The samples were diluted in Milli Q water by 1/300 (v/v) for nanoparticles made by RREP and by 1/150 (v/v) for nanoparticles formed by AEP Couvreur and AEP pH1. To evaluate the stability of the dispersion in saline conditions, the samples were diluted in aqueous solution of NaCl (0.16 mol/l). The size was measured 30 min after the dilution was performed.

The results were expressed as the average of the mean hydrodynamic diameter of the dispersed particles obtained from three determinations. The standard deviation of the size distribution and the polydispersity index were also given. The polydispersity index given by the apparatus is equivalent to the variance of the log-normal distribution. The dispersion was considered as monodisperse if the polydispersity index was lower than 0.1.

2.5.2. Zeta potential

The electrostatic surface charge of the polymer particles was deduced from the electrophoretic mobility using a Zetasizer 4 (Malvern Instruments Ltd, Orsay, France). Dilution of the suspensions (1/200 (v/v)) was performed in KCl 1 mM.

2.5.3. Turbidity of the suspension

The turbidity of the latex was evaluated by measuring the absorbance of diluted suspension at 400 nm using a UV–Vis Spectrophotometer DU70 (Beckman, Villepinte, France). All the samples were analyzed at the same dilution factor 1/100 in Milli Q® water.

2.6. Monitoring of polymerization reaction

2.6.1. Rate of particle formation

To determine the rate of formation of nanoparticles in polymerization at pH1, we measured, at different times, the diameter of the particles in two different processes: redox radical emulsion polymerization and anionic emulsion polymerization at pH1. The beginning of the reaction was defined by the addition of the monomer.

For the two procedures, the diameter was measured by sampling 100 or 200 μl of the media and diluting it in 2 ml of Milli Q® water. The unreacted monomer remaining in the sample immediately precipitated upon dilution due to a fast uncontrolled polymerization initiated by the hydroxyl group of the water used for the dilution. The nanoparticles already formed under controlled polymerization conditions remained in suspension whereas the part of polymer, which appeared as a consequence of the dilution of the sample, precipitated and settled down rapidly at the bottom of the sample. The diameter of the particles was measured every minute during the first 5 min and then every 10 min for 50 min.

2.6.2. Yield of the reaction

The yield of the polymerization was evaluated by gravimetric determination. A known amount of the dispersion was freeze dried at different times of the polymerization reaction. The mass of the dry residue was compared to the amount of the polymer, which was theoretically expected if 100% of monomer was converted into the polymer. To avoid the recapture of water, the products were weighted right after the completion of the freeze drying procedure.

2.6.3. Evaluation of the amount of unreacted dextran

The amount of unreacted dextran ($Dex_{unreacted}$) was evaluated by the measure of the difference (ΔD_R) between

the dry residue obtained before and after the dialysis. This difference was due to the elimination during the dialysis of the cerium (n_{ce4+}), the citrate (n_{ci}) and the unreacted dextran. The amounts of citrate and cerium involved in the polymerization were known. Thus the unreacted dextran was deduced from the following relationship:

$$\mathrm{Dex}_{\mathrm{unreacted}} = \Delta D_{\mathrm{R}} - (n_{ce4+} + n_{ci}) \quad (1)$$

2.7. Study of the influence of the composition of the polymerization medium on the nanoparticle characteristics

The RREP was performed in various conditions of concentrations of dextran (5–17 g/l), cerium (0.0008–0.02 mol/l) and IBCA (20–63 g/l). For each polymerization, the diameter was measured to investigate the influence of the parameter. For the variation of dextran concentration (5–17 g/l), the amount of unreacted dextran was also evaluated. In this series of experiment the ratio between the concentration of dextran chains and the number of radicals was constant. It was achieved by the use of a constant ratio between the concentration of cerium and the concentration of dextran. This ratio was equal at the value used in the process of RREP described above: 0.0012 (mol/g). For other experiments, only the concentration of the component was modified. In the case of the study of the influence of the concentration of cerium, the concentration of dextran was maintained at 13.7 g/l and the concentration of IBCA at 48 g/l. For the experiments designed to study the influence of the concentration of IBCA, the concentration of dextran was 13.7 g/l and the concentration of cerium was 0.016 mol/l. All the other experiment procedures were the same as these described in Section 2.3.1.

3. Results and discussion

3.1. Rate of particle formation

The rate of formation of the nanoparticles was investigated in the case of the RREP and of the AEP pH1. Both polymerizations performed at pH1, lead to the formation of colloidal suspension.

The evolution of the diameter and of the polydispersity index at different times after the addition of the monomer was shown in Figs. 1(A) and (B) and 2. To measure the size of the dispersed particles of polymer, which were formed in the reaction medium during the polymerization, the samples were diluted in water at neutral pH. The increase of the pH caused by the dilution induced the immediate precipitation of the polymer, which resulted from fast and uncontrolled polymerization of the remaining IBCA not converted into the polymer during the controlled RREP or AEP pH1. These precipitates were easy to separate from the polymer formed under controlled polymerization conditions because they were sedimented in a few minutes whereas the previously formed polymer remained in suspension.

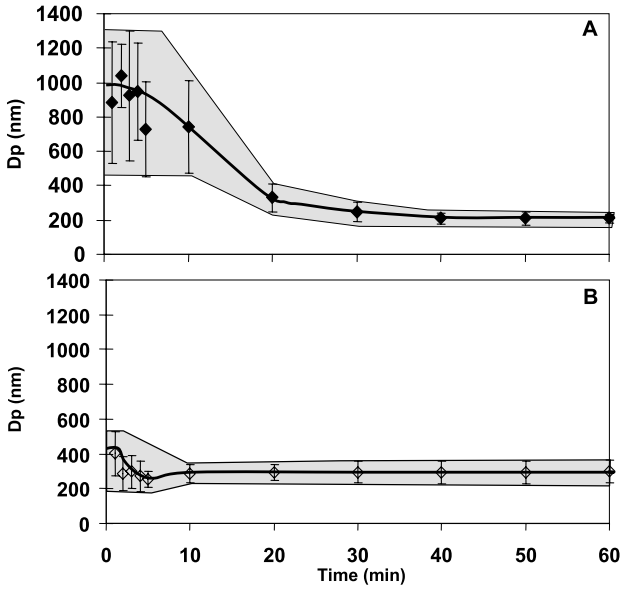

Fig. 1. Evolution of the hydrodynamic diameter of the particles during the polymerization of isobutylcyanoacrylate in presence of dextran. (A) AEP pH1, (B) RREP, ■ area of size distribution.

In the case of the AEP pH1, the size of the particle was around 1 μm and the polydispersity index was high during the first 5 min (Fig. 1(A), 2). It can be due to the presence of some aggregates in the suspension formed by the uncontrolled polymerization of the remaining IBCA and too small to be eliminated by sedimentation. These aggregates were the sign that the polymerization was not finished and explained the high value of the polydispersity index. After 5 min of polymerization, the size distribution of the particles remained large but the diameter decreased to reach a value close to the final value. The decrease of the diameter was due to the decrease of the amount of the remaining IBCA and to the increase of the number of polymer particles resulting from the controlled polymerization performed at pH 1. After 20 min of polymerization, the polydispersity index was equal to 0.1, showing that the

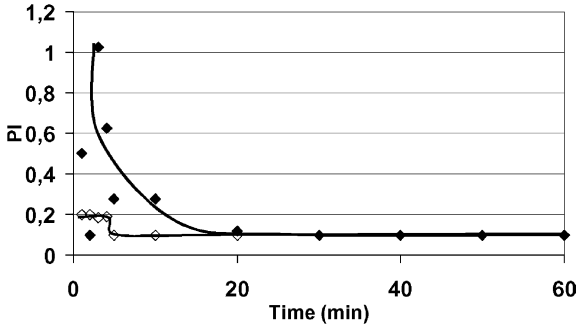

Fig. 2. Evolution of the polydispersity index (PI) of the suspension during the polymerization of isobutylcyanoacrylate in presence of dextran. ◇ RREP/AEP, ♦ pH1.

size distribution was unimodal. Thus, the formation of the nanoparticles by the AEP pH1 appeared to be a rather long process. In contrast, the samples obtained from the RREP showed particles with much defined diameter after 1 min of reaction and the particle size reached the final value at 5 min after the beginning of the polymerization. In this case the polydispersity index was equal to 0.1 after 5 min of polymerization (Fig. 1(B), 2).

These results suggested that the polymerization initiated by the redox system dextran-Ce^{4+} occurred much faster than the AEP pH1 initiated by the hydroxyl groups of dextran. They are in agreement with the data obtained by Chauvierre et al. [18], who monitored the polymerization by turbidity measurements. In the present work, the results showed that the size of the nanoparticles reached the final diameter only 5 min after the beginning of the RREP. This also suggested that the radicals responsible for the initiation of the polymerization were probably created at the beginning of the reaction and were very active. This was also observed by Wallace et al. [19], who showed that the ceric ions were very reactive with the *cis*-glycol groups at the chain end of a dextran molecule.

In contrast, the AEP pH1 occurred much slowly. The delay observed before the diameter of the particle reached a stable value with a narrow size distribution was much longer than the RREP. Indeed it took 30 min to reach a constant value of diameter in the case of the AEP pH1 compared to the 5 min required in the case of the RREP. As suggested by Douglas et al. [20] in the case of the AEP pH1, the initiation of the polymerization is believed to be initiated by the hydroxyl groups of the dextran chains leading to an anionic polymerization of the alkylcyanoacrylates. Such groups are weak acids. In the experimental conditions used in the present work, the very low pH was unfavorable for their ionization. Thus, it can be expected that the initiation of the polymerization induced by the polysaccharide was strongly slowed down.

In the case of RREP, it was checked that the yield of polymerization as well as the diameter of the particles remained constant after one hour. As summarized in Table 1, the yield of polymerization determined gravimetrically reached a stable value around 90% after 1 h of polymerization. In the same time the size of the nanoparticles remained perfectly constant. These results indicated that the RREP of the alkylcyanoacrylate was totally finished after 1 h.

Table 1
Evolution of the yield and the hydrodynamic diameter in redox radical emulsion polymerization as a function of time of polymerization

Time (h)	1	3	5
Yield (%)	92	91	90
Hydrodynamic diameter (nm)	270±13	277±14	278±14

3.2. Influence of the composition of the polymerization medium on the characteristics of the nanoparticles produced by RREP

The RREP was further investigated in order to study the influence of the concentrations of dextran, cerium and monomer on the characteristics of the nanoparticle produced. All measurements were performed after 1 h of polymerization to insure that the polymerization was completed as suggested by the results of the evaluation of the yield of polymerization. The results are presented in Figs. 3 and 4.

3.2.1. Influence of the concentration of dextran

In the present series of experiments, the ratio between the concentration of cerium and the concentration of dextran was constant to keep the same number of radicals per dextran chain. Thus, by increasing the concentration of dextran in the polymerization medium, the difference between these experiments was the number of dextran

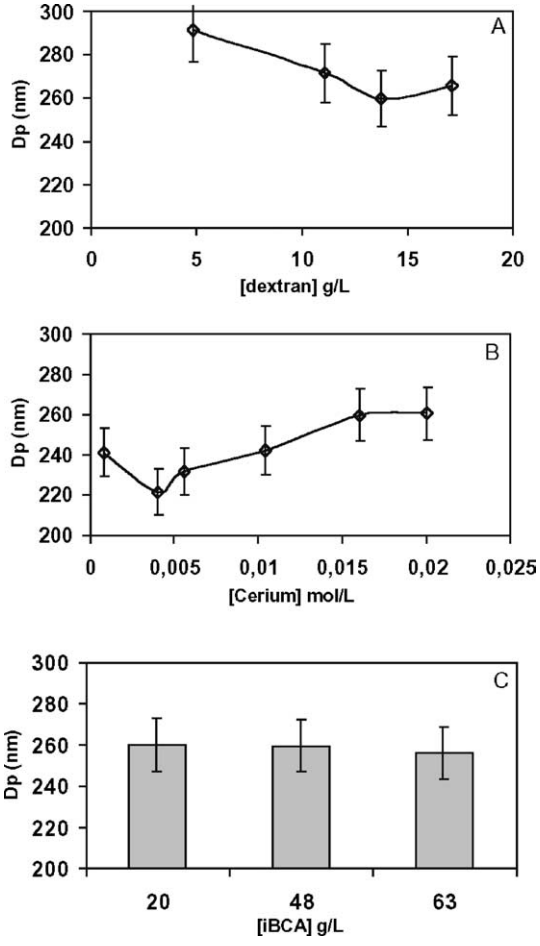

Fig. 3. Variation of nanoparticles diameter in RREP as a function of concentration of the components. (A) Dextran with [Ce]/[Dex]= 0.0012 mol/g, [IBCA]=48 g/l, (B) cerium with [Dex]=13.7 g/l, [IBCA]=48 g/l, (C) Isobutylcyanoacrylate with [Dex]=13.7 g/l, [Ce]= 0.016 mol/l.

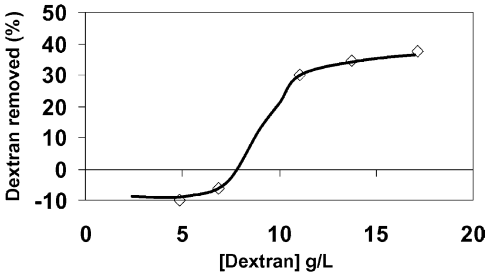

Fig. 4. Amount of unreacted dextran as a function the concentration of dextran added in the RREP with [Ce]/[Dex] = 0.0012 mol/g, [IBCA] = 48 g/l.

chains which were allowed to initiate the polymerization reaction, whereas the size and the structure of these chains were expected to remain constant.

By increasing the concentration of dextran from 5 to 14 g/l the diameter of the nanoparticles formed slightly decreased from 290 to 260 nm (Fig. 3(A)). Over a concentration of 14 g/l the diameter of the nanoparticles formed remained constant at 260 nm. The decrease of the diameter observed up to 14 g/l of dextran in the polymerization medium can be explained by a better stabilization of the nanoparticles due to the increase of the concentration of the dextran. To explain why the diameter of the nanoparticles remained constant over a concentration of dextran of 14 g/l, the amount of unreacted dextran was evaluated. The results presented in Figs. 4 showed that all the dextran introduced in the reaction medium was involved in the polymerization reaction when the concentration of dextran were lower than 7 g/l. It increased up to 40% of dextran introduced in the polymerization for concentrations of dextran higher than 7 g/l.

The stagnation of the diameter observed for the higher concentrations of dextran (over 14 g/l) can be related to the fact that a part of dextran was in excess and was not involved in the initiation step of the polymerization. This part of dextran remained free in the dispersing medium of the nanoparticles and can be removed from the nanoparticle dispersion by dialysis using the membrane with a high molecular weight cut off (100,000 g/mol).

The stagnation of the diameter of the nanoparticles and the increase of the percentage of dextran in excess seemed to indicate that over the critical concentration of dextran, keeping the ratio $[Ce^{4+}]$/[dextran] constant, a saturation of the surface of the nanoparticles was reached leading to nanoparticles with constant characteristics.

3.2.2. Influence of the concentration of cerium

The cerium is a part of the redox system involved in the initiation of the polymerization by the formation of the radical by reaction with dextran. By increasing the concentration of cerium keeping the concentration of dextran constant (13.7 g/l), it was expected that more radicals can be created. In addition, because the reaction was carried out at very acidic pH, it leads to a simultaneous cleavage of the dextran chain [21]. The number of cleavage should be increased with the concentration of cerium, leading to smaller fragments of dextran chain. As shown in Fig. 3(B), the size of the nanoparticles was influenced by the concentration of Ce^{4+} introduced in the polymerization medium.

At a very low concentration, below 0.004 mol/l, the diameter of the nanoparticles decreased with the concentration of cerium up to 220 nm. Then the diameter of the nanoparticles increased up to a concentration of cerium of 0.015 mol/l. A further increase of the concentration of cerium did not modify the diameter of the nanoparticles, which remained constant at 260 nm.

This result was surprising because it was expected that a low concentration of cerium would induce a low concentration of radicals and consequently a low rate of cleavage of the dextran chain leading to a low reduction of their molecular weight. Thus, it would be expected that the diameter of the nanoparticles would decrease with the increasing concentration of cerium. This was actually the case at very low concentration of cerium. However, over a concentration of cerium of 0.004 mol/l the contrary was observed. To explain the increase of the nanoparticle diameter with the concentration of cerium, a variation of the stabilization properties of the copolymer can be consider. Indeed, the copolymer formed by the RREP was made of two different parts, which had different properties. The dextran part was hydrophilic, whereas the poly(isobutylcyanoacrylate) part was hydrophobic. A variation of the balance between these hydrophobic and hydrophilic parts can explain the variation of the size of the nanoparticles. As explained before, because the reaction of dextran with cerium ions leads to a cleavage of dextran chains in our experimental conditions, different concentrations of cerium can actually lead to copolymers with different hydrophobic/hydrophilic balance resulting in the nanoparticles of different size.

3.2.3. Influence of the concentration of isobutylcyanoacrylate

The monomer concentration showed no apparent effect on the nanoparticle diameter (Fig. 3(C)). This result can be explained by the structure of the nanoparticles produced. Indeed, during the polymerization, the copolymer, which consisted of dextran and poly(alkylcyanoacrylate), self associated in core shell nanoparticles. In these nanoparticles, the core was composed of the poly(isobutylcyanoacrylate) part of the copolymer and the shell was constituted by the dextran chains. The poly(isobutylcyanoacrylate) is very hydrophobic. Then it can be suggested that this polymer was collapsed in the core of the nanoparticle. On the other side, the dextran, which is very hydrophilic was much expanded and swollen by the water at the surface of the nanoparticles forming the shell. Following this hypothesis, it can be expected that the size of the nanoparticles is less affected by a modification of the molecular weight of

the poly(alkylcyanoacrylate) part of the copolymer due to a variation of the concentration of the monomer compare to any modifications of the compositions of the polymerization system affecting the characteristics of the dextran chains as shown previously (Fig. 3(A) and (B)).

Increase of the amount of IBCA in the polymerization medium did not influence the diameter of the nanoparticles formed. To investigate if the increase of the amount of monomer would influence or not the number of particles formed, the turbidity of the suspensions were measured after dilution of the suspension in water at the same dilution factor. Since the nanoparticles had the same diameter, it can be expected that this measurement would provide a direct measurement of the concentration in particles. As indicated in Table 3, the absorbance increased with the concentration of IBCA. This indicated that the number of particles increased with the concentration of IBCA. It can be expected that the increasing number of particles was stabilized by dextran, which is present in excess in the polymerization medium.

Thus, these series of experiments showed that the diameter of the nanoparticles was much influenced by the components which induced modification of the dextran shell of the nanoparticle. The variation of the cerium and dextran concentrations allowed to control the size of the nanoparticles. These two components formed the polymerization initiation system, which can be considered as a critical step for the formation of the nanoparticles and which governed their characteristics. Another interesting point, the number of particles in the colloidal suspension can be controlled by the concentration of the monomer, IBCA.

3.3. Influence of the characteristics of the polysaccharides on the characteristics of the nanoparticles

A series of nanoparticles were prepared by the RREP with different polysaccharides, which differed by their molecular weight and their charge. Dextran (neutral), dextran sulphate (negatively charged), chitosan (positively charged) were used at two molecular weights: 10.000 and 40.000 g/mol. The composition of the polymerization systems was all the same. Only the polysaccharide differed from one experiment to the other. To compare the characteristics of the nanoparticles made by RREP, AEP Couvreur performed in the conditions developed by Couvreur et al. [10], was carried out with dextran 10.000 and dextran 40.000 g/mol. For all the nanoparticles, the diameter was measured and the zeta potential was evaluated from their electrophoretic mobility. It has also been checked that the nanoparticles remained stable in the presence of salt.

Table 2
Absorbance of diluted latex at 400 nm as a function of concentration of IBCA with [dextran] = 13.7 g/l, [ceric ions] = 0.016 mol for nanoparticles made by RREP

[IBCA] g/l	20	48	63
Absorbance	0.3824	0.9100	1.2163

As shown in Table 2, the zeta potential of the nanoparticles obtained by RREP greatly depended on the nature of the polysaccharide. Nanoparticles with negative zeta potential were obtained with dextran sulfate, which is a negatively charged polysaccharide. Nanoparticles with a positively zeta potential were obtained with chitosan, which is a positively charged polysaccharide. Zeta potential of almost neutral value was measured for the nanoparticles prepared with dextran, which is neutral polysaccharide. For these nanoparticles, the zeta potential was slightly negative but the absolute value depended on the molecular weight of the dextran. The less charged nanoparticles were obtained with the dextran of the highest molecular weight. The AEP Couvreur showed more neutral value of zeta potential but the same tendency was observed when compared the nanoparticles prepared with the dextran of different molecular weights.

These results indicated that the zeta potential is defined by the charge status of the polysaccharides. Positive or negative nanoparticles can be obtained using clearly positively or negatively charged polysaccharide. In the case of using neutral polysaccharide, the zeta potential, which was slightly negative, depended on the molecular weight of the polysaccharide. This can be explained by a contribution of the core of the nanoparticle made of poly(alkylcyanoacrylate), which can be more or less hidden by the polysaccharide depending on its molecular weight and on the conformation of the chains at the nanoparticle surface. The difference of the zeta potential measured between the nanoparticles prepared by RREP and the nanoparticles prepared by AEP Couvreur was the sign that the conformation of the chains was important for the properties of the nanoparticles. In the case of the RREP, the reaction was initiated by radicals created on the dextran chains after oxydation of a glycol function by the ceric ions. In strong acidic conditions such as those used in this study the sugar ring of the dextran chains is opened. Radicals are then created at a dextran chain end and the polymerization is initiated at this chain end, followed by a fast propagation [19,21]. As shown elsewhere [18] and confirmed here by the evolution of the nanoparticle diameter (Fig.1(A) and (B)), the radical polymerization occurred much faster than the anionic one and the copolymer formed consisted in a linear diblock copolymer including dextran and PIBCA [13]. In this case, the dextran moieties is expected to adopt an end-on conformation at the nanoparticle surface giving hairy nanoparticles (Fig. 5(A) and (B)). In the case of AEP Couvreur, all the nucleophilic groups and anions present in the aqueous medium are theoretically able to initiate the polymerization of IBCA and the polymerization takes place according to an anionic mechanism [22]. In the acidic conditions used in the AEP Couvreur, the anionic polymerization was rather slow. As shown elsewhere the initiation of the polymerization was mainly induced by the

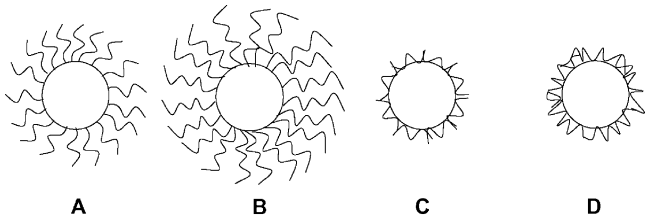

Fig. 5. Representation of the conformation of the polymeric chains at the nanoparticle surface. The polysaccharide was attached at end-on position by RREP with low molecular weight (A) and high molecular weight (B). The polysaccharide was attached at a side-on position by AEP Couvreur with low molecular weight (C) and high molecular weight (D).

hydroxyl groups of dextran since at such a low pH the concentration of the hydroxyl ions resulting from the water dissociation is very low [20]. The PIBCA chains growing on the dextran hydroxyl groups lead to the formation of graft copolymers, with one dextran chain being able to bear several PIBCA chains. In this second case, the dextran moieties is expected to adopt a side-on conformation forming loop and train at the surface of the nanoparticles (Fig. 5(C) and (D)).

Whereas the molecular weight was shown to have no influence on the zeta potential of the nanoparticles prepared with dextran sulphate and chitosan, it appeared to have a clear influence on the diameter of the nanoparticles (Table 3). Indeed, an increase of the molecular weight of the polysaccharide led to an increase of the nanoparticle size. This was also observed in the case of the RREP carried out with dextran. In contrast for the AEP Couvreur, the effect was the opposite (Table 3). The size of the nanoparticles slightly decreased with the increase of the molecular weight of dextran. The results obtained by the AEP Couvreur were in agreement with those of Douglas et al. [20] and of Yang et al. [23]. The difference of results obtained between the AEP Couvreur and the RREP can be explained once again by a difference in the arrangement of the polysaccharide chains at the nanoparticle surface. Indeed, it can be expected that the size of the nanoparticles can be more affected by the molecular weight of the polysaccharide when it is attached to the nanoparticle surface on the end-on configuration, which was expected to occur in the case of the RREP. In contrast, a side-on attachment of the polysaccharide on the nanoparticle surface, as expected to occur in the case of the AEP Couvreur, would form an outer shell of the nanoparticles that would not depend on the molecular weight of the polysaccharide. Thus, their size in the case of the nanoparticles formed by the AEP Couvreur would remain stable independently of the molecular weight of the polysaccharide (Fig. 5).

Finally, the stability of the nanoparticles was also investigated in saline media. This is an important property for nanoparticles designed for pharmaceutical uses because these particles can be used in complex media such as salting solutions or blood. The stability of the nanoparticles was investigated by measuring the diameter in a solution of NaCl at the physiological concentration (0.16 mol/l or 9% w/v).

For all the type of nanoparticles, there was no aggregation and the polydispersity index was close to 0.1, which showed that the suspension remained stable even in the presence of salt. The stability was due to the steric repulsions between polysaccharide chains, which were not affected by the concentration of salt used in the study. Thus, these nanoparticles can be used in saline media without risk of aggregation.

The nanoparticles obtained by RREP with a charged polysaccharide showed a decrease of the diameter when they were dispersed in the salting solution compared to the diameter measured in the water. The decrease of the diameter of the nanoparticles can be explained by the fact that the electrostatic repulsions, occurring within the polysaccharide shell, were more sensitive to the presence of salt. Indeed, ions of the salt can hide some of the charges present along the charged polysaccharide chains leading to a contraction of the shell thickness and, therefore, a decrease of the hydrodynamic diameter evaluated by quasi-elastic light scattering.

The nanoparticles prepared with dextran by RREP and AEP Couvreur were insensitive to the presence of salt. It

Table 3
Potential zeta and diameter of various nanoparticles made with different polysaccharides and methods

Polysaccharide	Dextran				Dextran sulphate		Chitosan	
Polymerization	RREP		AEP Couvreur		RREP		RREP	
Molecular weight (g/mol)$\times 10^{-3}$	10	40	10	40	10	43	13	36
Zeta potential (mV)	−16.5	−11.52	−2.6	−0.5	−39.9	−44.5	+27.4	+27.9
Diameter (nm)	137	246	214	199	201	320	230	346
Diameter (nm) in NaCl 0.16 mol/l	nd	245	nd	196	nd	299	nd	290

was explained by the fact that these nanoparticles were stabilized by only steric repulsion, which was insensitive to the presence of salt in our conditions. This result seemed to be independent of the conformation of the polysaccharide at the nanoparticle surface.

4. Conclusion

The RREP of alkylcyanoacrylate led to the formation of polymer particles of a few hundred of nanometers in diameter. The size of the nanoparticles was mainly affected by the concentration of the components involved in the polymerization initiation process, i.e. dextran and cerium concentrations, whereas the diameter of the nanoparticles formed was insensitive to the concentration of monomer. This is in agreement with the formation of nanoparticles with a core-shell structure in which the core was formed by the poly(alkylcyanoacrylate) which is highly hydrophobic and the shell was formed by the polysaccharide which is highly hydrophilic and can be swollen by the aqueous surrounding medium. The characteristics of the nanoparticles obtained by redox radical emulsion polymerization can be well adjusted in term of their size and of their zeta potential by the characteristics of the polysaccharide used for the synthesis. Thus, the positively charged, negatively charged and neutral nanoparticles can be obtained according to the nature of the polysaccharide, whereas the diameter of the nanoparticles depended on the molecular weight of the polysaccharide. The redox radical emulsion polymerization was found to allow a higher versatility of the nanoparticles characteristics than the anionic emulsion polymerization previously proposed by Couvreur et al. [10]. It was suggested that the main difference between these two polymerizations resulted in the arrangement of the polysaccharide at the nanoparticle surface. By the redox radical emulsion polymerization, the polysaccharide can be attached to the nanoparticle surface according to an end-on position whereas by the anionic emulsion polymerization the polysaccharide was attached on the nanoparticle surface according to a side-on position.

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