Radical Emulsion Polymerization of Alkylcyanoacrylates Initiated by the Redox System Dextran−Cerium(IV) under Acidic Aqueous Conditions

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ABSTRACT: A radical emulsion polymerization of alkylcyanoacrylates (ACA) has been investigated to produce core−shell nanoparticles coated with polysaccharides. It was initiated by dextran on which a radical was created by reaction with cerium(IV) ions in an aqueous acidic medium. The redox radical polymerization developed in this study occurred much faster at pH 1 than the spontaneous anionic polymerization of ACA. It was applied to several ACA and has led to the formation of very stable suspensions of nanoparticles. The mean diameter of the nanoparticles depended on the monomer. Different analysis showed that the polymer forming the nanoparticles was an amphiphilic dextran based copolymer of poly(alkylcyanoacrylate). This copolymer exhibited surfactant properties. The size, morphology, and ζ potential of the nanoparticles have been determined as well as the mobility of the polysaccharide chains at the nanoparticle surface, which was investigated by electron paramagnetic resonance.

Introduction

Over the last 20 years, numerous efforts have been made to develop polymer nanoparticles with surface modified properties for controlling in vivo delivery of drugs. Most of the approaches were based on the synthesis of amphiphilic copolymers which were used to prepare nanoparticles by different suitable techniques.[1,2] Emulsion polymerization is another well-known way to produce polymeric nanoparticles.[3] It has been developed to carry out the radical polymerization of numerous monomers leading to high molecular weight polymers and to latexes of very well-defined characteristics. Radical emulsion polymerization was not so much used to prepare polymer drug carriers up to now.[4] In addition, this polymerization led to non biodegradable polymeric nanoparticles which were not suitable for applications in humans.

Among the different monomers able to polymerize through a radical polymerization mechanism, only alkylcyanoacrylates (ACA) and their derivatives lead to the formation of biodegradable polymers.[5,6] However, the radical polymerization of such monomers has hardly ever been described even in bulk.[7] These monomers are among the most reactive ones and their anionic polymerization is usually spontaneously and quickly initiated by small amounts of a weak base including the hydroxyl ions of water.[8−11] This is the reason, in contrast with the other monomers which are easily polymerized in aqueous emulsion by radical polymerization, such a polymerization was obviously not applicable to ACA. Indeed, poly(alkylcyanoacrylate) (PACA) nanoparticles, used as drug carriers, were designed on the basis of an anionic emulsion polymerization which was spontaneously initiated in the aqueous polymerization medium in the presence of acids.[12] The initiating species inducing the anionic polymerization were either the hydroxyl group of water or any weak base including some types of drugs or stabilizing agents dissolved in the aqueous polymerization medium.[13−15]

The aim of the present study was to develop a redox radical polymerization of ACA initiated by a redox mechanism in order to design a new method of synthesis of biodegradable drug carrier system consisting of polysaccharide decorated PACA nanoparticles. This polymerization was designed using the couple polysaccharide and cerium(IV) ions as the redox radical polymerization initiator according to Casinos[16] and to Passirani et al.[17] The main interest of developing a new method for the synthesis of PACA is to obtain new properties regarding the in vivo fate of the nanoparticles. PACA nanoparticles obtained by the anionic polymerization according to the method of Couvreur et al.[12] are rapidly cleared from the bloodstream by macrophage uptake.[18] In contrast, the nonbiodegradable dextran-poly(methyl methacrylate) nanoparticles prepared according to the redox radical polymerization showed a prolonged circulation time in the blood leading to a different in vivo fate.[19] Thus, the obtaining of biodegradable nanoparticles by this polymerization method would open new perspectives for the delivery of drugs in vivo. However, because of the high reactivity of ACA, such a redox radical emulsion polymerization required a sufficient control of the spontaneous anionic polymerization. Thus, the emulsion polymerization of ACA was investigated at very low pH in the presence and in the absence of the redox radical polymerization initiator. The formation of the polymer particles resulting from the emulsion polymerization performed under both conditions was followed in situ by the continuous monitoring of the optical density of the polymerization medium. The suspensions of the polymer particles and the polymer produced were characterized by using different methods. New approaches were also developed in order to investigate, on one hand, the mobility of the polysaccharide chains at the nanoparticle surface and,

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on the other hand, the surfactant properties of the polymers forming the nanoparticles.

Experimental Section

Materials. Isohexylcyanoacrylate (IHCA), isobutylcyanoacrylate (IBCA), *n*-butylcyanoacrylate (NBCA), *n*-propylcyanoacrylate (NPCA), ethylcyanoacrylate (ECA) and 2-methoxyethylcyanoacrylate (2MECA) monomers were kindly provided as a gift by Loctite (Dublin, Ireland). Dextran M_w 71 kDa and dextran M_w 15−20 kDa were respectively purchased from Sigma and Fluka (Saint-Quentin Fallavier, France). All chemicals were reagent grade and used as purchased.

Emulsion Polymerization Experiments. Emulsion polymerization of ACA was performed in various aqueous media which differed in their pH and composition in dextran and cerium(IV) ions. All the other conditions were kept constant within the different experiments. The pH of the polymerization medium was fixed at 1, 2, and 3 with, respectively, 0.2 , 10^{-2}, and 10^{-3} M aqueous solutions of nitric acid.

The redox radical emulsion polymerization (REP) was performed as follows:

Dextran (0.1375 g) was dissolved in 8 mL of nitric acid 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 and 0.5 mL of IBCA were successively added under vigorous agitation. Argon bubbling was maintained for 10 min. The reaction was left to continue under gentle stirring for 40 min. After cooling to room temperature, 1.25 mL of an aqueous solution of 1.02 M trisodium citrate dihydrate was added to the polymerization medium and the pH was adjusted to 7.0 with NaOH 1 N. Experiments were named respectively [REP-pH1], [REP-pH2], or [REP-pH3], depending on the pH of the polymerization medium.

Other polymerization conditions were used to evaluate the spontaneous emulsion polymerization (SEP) of ACA resulting from anionic initiation. For the polymerization [SEP+Ce^{4+}-pH1], the medium was composed of 8 mL of 0.2 M nitric acid without dextran. Then, 2 mL of cerium(IV) ions solution were added before 0.5 mL of IBCA. For the polymerizations [SEP+dextran-pH1], [SEP+dextran-pH2], and [SEP+dextran-pH3], the media were prepared with dextran (0.1375 g) solubilized in 10 mL of 0.2 , 10^{-2}, or 10^{-3} M nitric acid before addition of 0.5 mL of IBCA. The same protocol as described for REP was then followed.

In general, the monomer used was IBCA, except in some experiments in which IBCA was replaced by IHCA, NBCA, NPCA, ECA, or 2MECA.

The polymerization medium (12 mL) was purified by dialysis (Spectra/Por membranes, 100 000 Da molecular weight cut off (MWCO), Biovalley, Marne la Vallée, France) three times against 1 L 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 purified suspensions were frozen at −18 °C and freeze-dried during 48 h (Christ Alpha 1−4 freeze-dryer, Bioblock Scientific, Illkirch, France) without using cryo-protecting agent.

Monitoring of Polymerization. A Teflon ring with two holes, one at 0° and the other one at 180°, was mounted around a glass tube to serve as support for two optical fibers. The first optical fiber (100 μm core) was used to carry the incident light from the light source HL-2000-Cal (Ocean Optics Europe, Lannion, France), to the glass tube and the second optical fiber (200 μm core) was used to carry the transmitted light form the glass tube to the plug-in spectrometer PC 2000 (Ocean Optics Europe, Lannion, France). During the experiment, a PC computer connected to the plug-in spectrometer and equipped with a SpectraWin 4.1 software recorded optical density spectra of the polymerization medium in a wavelength range comprised between 400 and 800 nm. The sample time was fixed at 120 ms. The dark spectrum was recorded before the addition of the cerium(IV) ions solution (REP and SEP + Ce^{4+}) or of nitric acid (SEP + dextran) and when the incident light was still on the "off" position. The reference spectrum was recorded immediately after adding this solution and when the incident light was "on". Recording started just after the addition of the monomer, and spectra were recorded every 30 s for 50 min.

The optical density of the polymerization medium measured at 650 nm was taken from the different recorded spectra. A mean value from three different determinations was calculated for each time point to follow the evolution of the optical density of the polymerization medium with time.

Nanoparticle Characterizations. The morphology of the nanoparticles was investigated by Scanning Electron Microscopy using a Philips XL 30 scanning electron microscope (Philips, Limeil Brevannes, France). Diluted nanoparticle suspensions (1/10 to 1/500 (v/v) in distilled water) were spread on an aluminum disk and left to dry at room temperature before being coated with gold using an Edwards Sputter Coater S150 (Edwards, Gennevilliers, France).

The size 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°. The samples were diluted to the 1/100 (v/v) in MilliQ water. The results corresponded to the average of three determinations and were the mean hydrodynamic diameter of the dispersed particles, the standard deviation of the size distribution and the polydispersity index. The polydispersity index given by the apparatus is equivalent to the variance of the log-normal distribution.

The ζ potential of the polymer particles was measured using a Zetasizer 4 (Malvern Instruments Ltd., Orsay, France). Dilutions of the suspensions (1/200 (v/v)) were performed in KCl 1 mM.

Polymer Characterizations. Solubility. A 5 mg sample of polymer (dextran, poly(isobutylcyanoacrylate (PIBCA), or polymer prepared with IBCA in experiment [REP-pH1]) was weighed into a glass tube, and 500 μL of a solvent chosen within acetone, ethyl acetate, ethanol, dimethyl sulfoxide (DMSO), tetrahydrofuran (THF) and MilliQ water was added. The samples were homogenized and left at 19 °C for 72 h. After this period of time, the samples in which an undissolved polymer was still present were considered as insoluble whereas polymers which appeared totally dissolved in the solvent were considered as soluble.

Elemental Analysis. The composition in dextran and PIBCA of the polymer obtained after emulsion polymerization of IBCA in experiment [REP-pH1] was determined by elemental analysis based on their content of carbon, nitrogen, oxygen and hydrogen (Service Central d'Analyses du CNRS, Vernaison, France). The percentage in dextran (X) and in PIBCA (Y) of the polymer obtained in experiment [REP-pH1] was deduced considering the composition of the respective homopolymers (i.e., dextran and PIBCA) and using the following system of equations giving the composition of carbon C, nitrogen N, oxygen O, and hydrogen H:

$$C_{[\mathrm{REP-pH\ 1}]} = XC_{\mathrm{dextran}} + YC_{\mathrm{PIBCA}} \quad (1)$$

$$N_{[\mathrm{REP-pH\ 1}]} = XN_{\mathrm{dextran}} + YN_{\mathrm{PIBCA}} \quad (2)$$

$$O_{[\mathrm{REP-pH\ 1}]} = XO_{\mathrm{dextran}} + YO_{\mathrm{PIBCA}} \quad (3)$$

$$H_{[\mathrm{REP-pH\ 1}]} = XH_{\mathrm{dextran}} + YH_{\mathrm{PIBCA}} \quad (4)$$

$$C_{[\mathrm{REP-pH\ 1}]} + N_{[\mathrm{REP-pH\ 1}]} + O_{[\mathrm{REP-pH\ 1}]} + H_{[\mathrm{REP-pH\ 1}]} = 100 \quad (5)$$

$$X + Y = 100 \quad (6)$$

Since dextran has no nitrogen ($N_{\mathrm{dextran}} = 0\%$), the content in PIBCA of the polymer obtained in [REP-pH1] can be deduced from eq 2 and therefore X can be determined using eqs 1, 3, 4, or 6.

Infrared Spectroscopy. The Fourier transform infrared (FTIR) spectra of dextran, PIBCA, and polymer from experiment [REP-pH1] performed with IBCA, were obtained using a Bruker IFS 66 spectrometer (Bruker Instrument Inc., Wissembourg, France) from a KBr tablet compressed at 10

tons with an hydraulic press (Eurolabo, Meaux, France) containing 1% (w/w) of the respective polymers.

Raman Spectroscopy. The Raman spectra of the same polymers in powder were obtained using a Labram spectrometer (Dilor, Longjumeau, France) equipped with a 5 mW helium−neon Laser ($\lambda = 633$ nm).

Nuclear Magnetic Resonance (NMR). The solid-state ^{13}C CP/MAS NMR spectrum was recorded with a Bruker MSL-400 spectrometer (Bruker Instrument Inc., Wissembourg, France) equipped with a Bruker double resonance magic angle spinning (MAS) probe head. The sample was rotated at a frequency of 10.5 kHz. ^{13}C CP/MAS NMR experiments at 100.6 MHz were performed using cross-polarization with a contact time of 2 ms and with TPPM decoupling during acquisition. The numbers of scans were 1024 for dextran, 29 696 for PIBCA, and around 28 000 for the polymer obtained from the experiment [REP-pH1] performed with IBCA.

Determination of the Polymer Surface Active Properties. The study of the surface active properties of the polymers was performed by measuring the stability of emulsions prepared with 3 mL of ethyl acetate, 3 mL of MilliQ water and 12 mg of a polymer (see below), with the help of a Turbiscan MA 2000 (Formulaction, Toulouse, France). The Turbiscan made it possible to follow phase separation occurring in unstable emulsions and provided us with a quantitative analysis of the phenomenon. The emulsions were formed according to a defined procedure of agitation (vortex 30 s, 2 handle turnings, vortex 30 s, 20 handle turnings, vortex 30 s). After the mixtures were stirred, 5 mL samples of the emulsions were immediately introduced into the measurement cell of the Turbiscan. The light transmission ($\lambda = 850$ nm) across the sample and the retro-diffusion of the incident beam were simultaneously recorded every minute during a period of 30 min by scanning the vertical height of the sample with a resolution of 40 μm. The polymers employed to prepare the different emulsions were dextran, PIBCA, and the polymers obtained from the polymerization of IBCA respectively under conditions [REP-pH1] and [SEP+dextran-pH1]. A mixture of PIBCA (6 mg) and dextran (6 mg) and an emulsion without polymer were also used as controls.

Size Exclusion Chromatography. Size exclusion chromatography (SEC) was used to determine the molecular weight of dextran after treatment with cerium(IV) ions. The SEC apparatus consisted of a TSK-G4000PW column (Toyo Soda, Tokyo, Japan), an Hitachi pump (model L-6000), an injection valve (Rheodyne) and two sample detectors. The first one was a Hitachi multichannel detector (model L-3000) operating between 200 and 300 nm, and the second one was a differential refractive index detector module (Waters R401). The apparatus was connected to a PC and operated using a software developed by Lesieur et al.[20] The mobile phase consisted of Hepes buffer (10 mM, pH 7.35, NaCl 145 mM) and was used at a flow rate of 1 mL/min. All the samples were prepared at a concentration of 1 mg/mL in Hepes buffer, and the volume injected on the column was 150 μL.

Calibration of the column was performed using dextran standards (Polymer Laboratories, Marseille, France) with a molecular weight ranging from 180 to 1 660 000 g/mol. The total volume of the column, V_t, was evaluated from the peak corresponded from the elution of the small ions contained in the sample. The effective void volume (V_0) of the column was determined using the excluded standard dextran (M_w 1 660 000 Da) by taking the elution volume corresponding to the intercept between the baseline and the left-side half-height tangent of the elution peak. The elution volumes (V_e) of the samples were taken at the intercept of the half-height tangents of the elution peak when it was symmetrical. In the other cases, V_e was evaluated at the position of the apparent maximum of asymmetrical peaks. The column parameter, K_d, was calculated using eq 1 and used to determined the molecular weight of the dextran prepared as described below.[21,22]

$$K_d = (V_e - V_0)/(V_t - V_0) \quad (7)$$

The samples of dextran to be analyzed were prepared according to a procedure similar to that one used to perform polymerization under [REP-pH1] conditions, but without adding the monomer. The protocol was applied using 0.5 mL of a hydroquinone solution (5% (w/w) in MilliQ water) or 0.5 mL of 0.2 M nitric acid instead of the monomer. The samples were then dialyzed as described above by using a membrane of 500 Da MWCO (Spectra/Por) and freeze-dried.

The parent dextrans (dextran not treated with cerium(IV) ions) were analyzed according to the same procedure to provide a reference in order to evaluate the action of the cerium(IV) ions.

Determination of Dextran Chain Mobility. A suspension of polymer particles was prepared by polymerization of IBCA under conditions [REP-pH1] with dextran 15 kDa. A part of the obtained suspension (3 mL) was filtered through 1.2 μm membranes (Sartorius, Palaiseau, France) and dialyzed twice against 1 L of distilled water for 2 h (dialysis membrane 100 000 Da MWCO, Spectra/Por). A final dialysis was performed against 0.1 M phosphate buffer, pH 7.4 (Sigma, Saint Louis, MO). The suspension of nanoparticles was then labeled with a nitroxide free radical containing probe, 4-amino-TEMPO, using a specific method for the labeling of dextran. Carbodiimidazole (27 mg, 0.166 mmol) and 4-amino-TEMPO (11 mg, 0.064 mmol) were dissolved in 0.5 mL of phosphate buffer and added to the 3 mL of the nanoparticles suspension previously prepared. The system was left to react for 48 h at room temperature under gentle magnetic stirring. The labeled nanoparticles were then purified by dialysis three times against 1 L of phosphate buffer for 2 h (Dialysis membrane 100 000 Da MWCO, Spectra/Por). In one experiment, the suspension was freeze-dried and rehydrated with a small amount of MilliQ water.

Electron paramagnetic resonance (EPR) spectra were recorded using a Varian E-4 spectrometer (Varian, Paolo Alto, CA) operating in the X band at 9.15 GHz and working in the TEM_{102} mode. The oscillating magnetic field was maximal at the center of the cavity whereas the electric field was minimal. Flat cells were used as the sample holder. The shape of the EPR spectra of nitroxide free radicals as those of the 4-amino-TEMPO is very sensitive to the Brownian motion of the probe. The absorption results from transitions between the energy levels of the Hamiltonian spin as described elsewhere.[23] The spectra were analyzed according to the Kivelson model[24] in order to determine the rotational correlation time of the probe and to obtain information about the mobility of the dextran chains on which the label has been grafted.

Results

Polymerization. In the absence of dextran, i.e., in the experiment [SEP+Ce^{4+}-pH1], no polymer was formed even after 24 h. In all the other experiments, polymerization occurred after the addition of the monomer and led to the formation of turbid suspensions of colloidal particles. Thus, the increase of the optical density in the medium during polymerization of IBCA performed under the conditions [REP-pH1], [REP-pH2], [REP-pH3], [SEP+dextran-pH1], [SEP+dextran-pH2], and [SEP+dextran-pH3] could be measured (Figure 1). At pH 1, the optical density under the polymerization conditions [REP-pH1] increased rapidly whereas it augmented much slowly after a lag time of a few minutes in the case of the polymerization performed under conditions [SEP+dextran-pH1] (Figure 1A). At pH 2 and 3, the curves showing the variation of the optical density with time for the different emulsion polymerization conditions were almost superimposed (Figure 1, parts B and C).

Similar observations were done with other monomers (IHCA, NBCA, NPCA and ECA): optical density always rises faster with redox radical emulsion polymerization (Figure 2A) than with anionic emulsion polymerization,

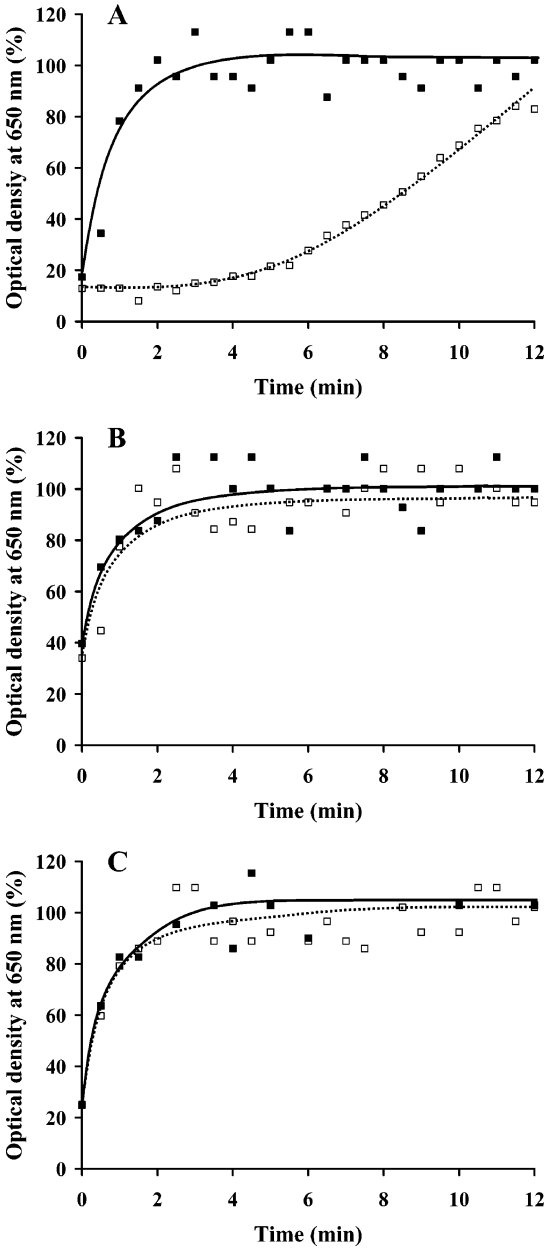


Figure 1. Optical density of the polymerization medium during the polymerization of IBCA with dextran 71 kDa under different conditions. Closed square: [REP-pH1] (A), [REP-pH2] (B), [REP-pH3] (**C**). Open square: [SEP+dextran-pH1] (A), [SEP+dextran-pH2] (B), [SEP+dextran-pH3) (C).

which was dramatically influenced by the nature of the monomer (Figure 2B).

The initial slopes of the curves as presented in Figures 1 and 2 are given in Figure 3. This still shows more clearly that the initial increase of the optical density under the polymerization conditions [REP-pH1] was much faster than under conditions [SEP+dextran-pH1], except for the monomer 2MECA (Figure 3B). As suggested in Figure 1, no difference appeared between the polymerizations performed at pH 2 and 3 whatever the polymerization conditions (REP or SEP + dextran) (Figure 3A).

Nanoparticle Characterizations. The suspension obtained after polymerization of IBCA under conditions [REP-pH1] consisted of rather monodispersed spherical nanoparticles as observed by scanning electron microscopy (Figure 4). The mean hydrodynamic diameter of

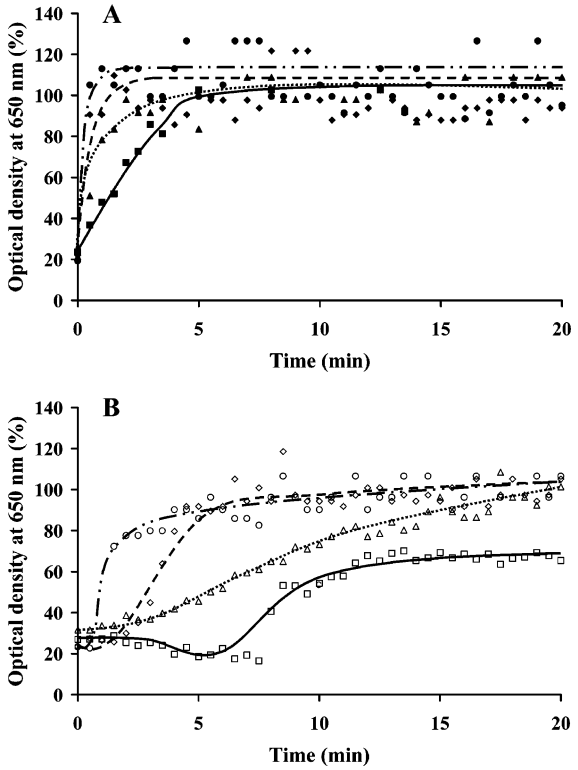


Figure 2. Optical density of the polymerization medium during the emulsion polymerization of IHCA (square), NBCA (triangle), NPCA (diamond), and ECA (cercle). The conditions of polymerization were either [REP-pH1] (A) (closed symbols) or [SEP+dextran-pH1] (B) (open symbols).

these nanoparticles as measured by quasi-elastic light scattering was around 300 nm and consistent with the scanning electron microscopy (Table 1A). After redispersion of the freeze-dried PIBCA nanoparticles, the mean hydrodynamic diameter, the size distribution, and the polydispersity index of the suspension were almost not modified. These values were kept constant over time too, even after 36 months of storage (Table 1A). The size of nanoparticles prepared with the different ACA monomers under conditions [REP-pH1] were between 227 and 443 nm (Table 1B). The mean hydrodynamic diameter of the nanoparticles generally decreased with alkyl chain length of the monomer (Table 1B). Nanoparticles prepared under conditions [REP-pH1] with a lower molecular weight dextran, i.e., 15 kDa instead of 71 kDa, were found to have a mean hydrodynamic diameter of 200 nm, a standard deviation of size distribution of 47 nm, and a polydispersity index of 0.074 when IBCA was used as starting monomer.

The ζ potentials of the nanoparticles prepared by polymerization of IBCA under [REP-pH1] conditions using dextran 15 kDa and dextran 71 kDa were, respectively, −19 and −11 mV.

Polymer Characterizations. When the polymer was prepared under [REP-pH1] conditions using IBCA as a monomer, it was found to be insoluble in all solvents tested whereas dextran was soluble in MilliQ water or DMSO and PIBCA (homopolymer) was soluble in acetone, ethyl acetate, and THF. Thus, further characterizations of this polymer were performed with methods making solid-state analysis possible. The FTIR, Raman and NMR (^{13}C CP/MAS) spectra are shown in Figures 5A,B and 6, respectively. Spectra were compared to those obtained from pure dextran and PIBCA (data not shown for FITR and Raman). The FTIR

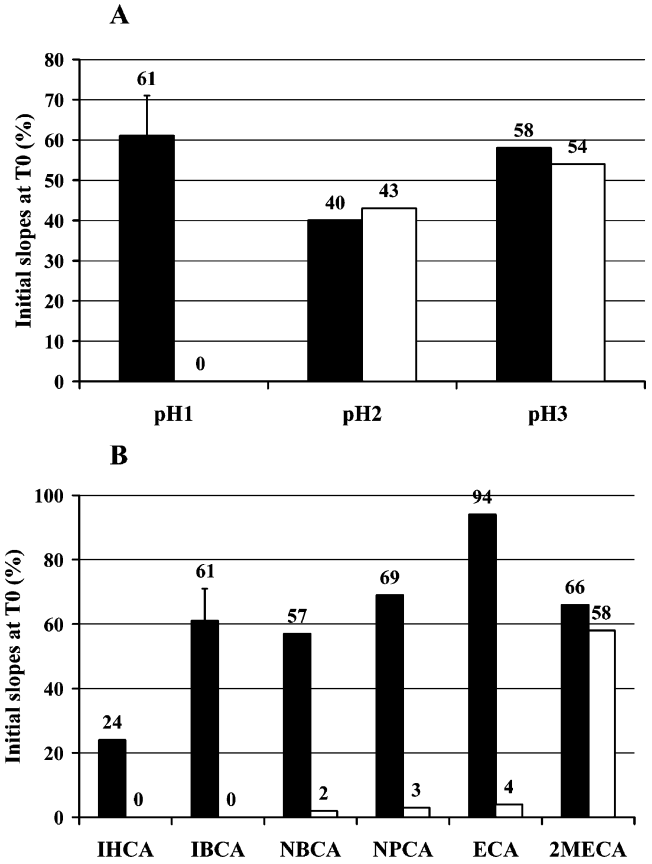


Figure 3. Initial slopes of the curves calculated from Figures 1 and 2. (A) Polymerization of IBCA performed at different pH according to the redox radical emulsion polymerization (REP) (black columns) and to the spontaneous emulsion polymerization with dextran 71 kDa (SEP + dextran) (white columns). (B) Polymerization of the different monomers performed under conditions [REP-pH1] (black columns) and [SEP+dextran-pH1] (white columns) with dextran 71 kDa. The standard deviation was determined with IBCA and dextran 71 kDa ($n = 10$).

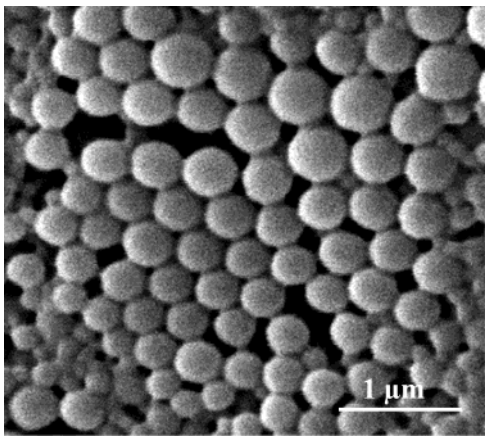


Figure 4. Scanning electron microscopy of nanoparticles prepared by the polymerization of IBCA under [REP-pH1] conditions using dextran 71 kDa.

spectrum (Figure 5A) showed absorption bands at 3200−3600 cm^{-1} corresponding to the OH group of dextran. The bands at 2250 and 1750 cm^{-1} were respectively attributed to the CN stretching vibration and to the ester carbonyl of the PIBCA. The Raman spectrum (Figure 5B) also showed bands of the OH groups of dextran and of the ester carbonyl and CN groups of PIBCA. Finally, the ^{13}C CP/MAS NMR

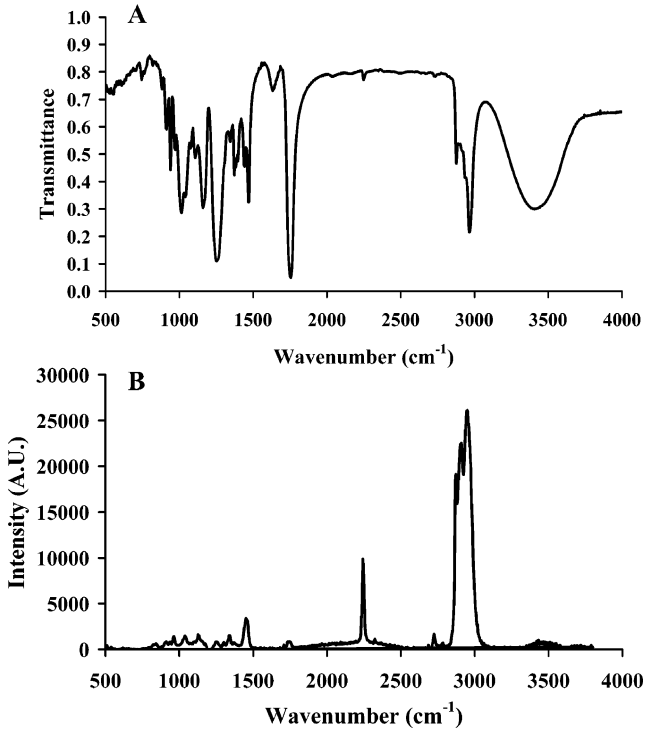


Figure 5. FTIR spectrum (A), Raman spectrum (B) of the polymer obtained by polymerization of IBCA under [REP-pH1] conditions using dextran 71 kDa.

Table 1. (A) Hydrodynamic Diameters of the Nanoparticles Obtained after Polymerization of IBCA under the Conditions [REP-pH1] Using Dextran 71 kDa and (B) Hydrodynamic Diameters of the Nanoparticles Prepared by Polymerization of Various ACA Monomers under [REP-pH1] Conditions

	hydrodynamic diameters (nm)	std dev of size distribn (nm)	polydispersity index
A. Polymerization of IBCA under the Conditions [REP-pH1]			
after synthesis	290	73	0.089
after dialysis	297	80	0.105
storage 2 months	282	52	0.044
storage 8 months	290	66	0.068
storage 30 months	286	78	0.114
storage 36 months	294	76	0.090
after redispersion	312	53	0.042
B. Polymerization of Various ACA Monomers under [REP-pH1] Conditions			
IHCA	236	34	0.025
NBCA	227	63	0.117
NPCA	275	97	0.256
ECA	443	175	0.421
2MECA	375	127	0.242

spectrum (Figure 6) was consistent with the other spectra. The signals at 19, 27, 43, 73, 115, and 166 ppm could be attributed to the contribution of carbon atoms of PIBCA (Figure 6) whereas the peak at 98 ppm could be specifically attributed to dextran. Table 2 presents the results from the elemental analysis of the homopolymers of dextran and of PIBCA as well as the composition in carbon, hydrogen, oxygen, and nitrogen of the polymer obtained in experiment [REP-pH1]. From these results, it can be determined that the polymer obtained in experiment [REP-pH1] showed a composition of 22% (w/w) dextran and 78% (w/w) PIBCA.

Finally, the ability of the polymer to stabilize an emulsion was investigated with a Turbiscan. Figures 7A, 6B, 7C, and 7D show the vertical profiles of light transmission across reference emulsions prepared respectively either without polymer, or with PIBCA, or

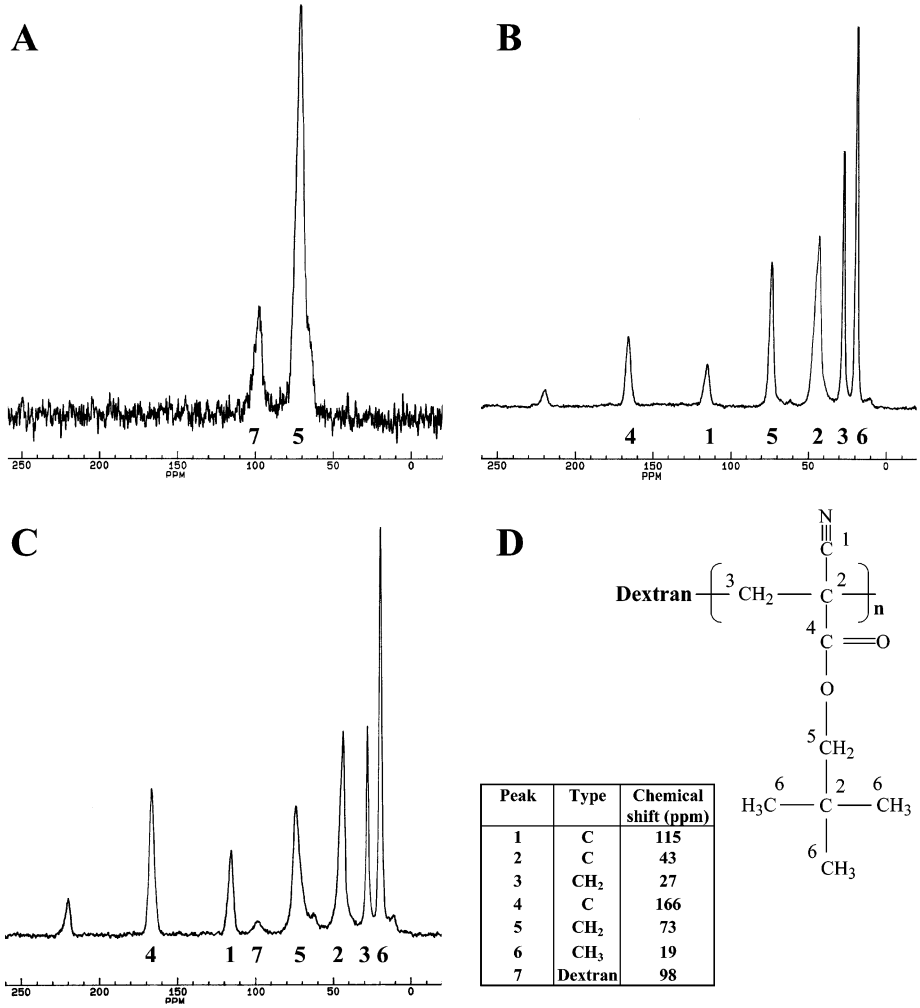


Figure 6. ^{13}C CP/MAS NMR spectrum of dextran (A), of PIBCA (B) and of the polymer obtained by polymerization of IBCA under [REP-pH1] conditions using dextran 71 kDa (C). (D) Analysis of the NMR spectrum given in part C and suggestion of the chemical structure of the polymer.

Table 2. Elemental Analysis of Dextran, PIBCA, and the Polymer Prepared by the Polymerization of IBCA under [REP-pH1] Conditions

component	dextran (%)	PIBCA (%)	polymer [REP-pH1] (%)
C	39.26	61.81	56.25
N	0	9.12	7.05
O	53.82	21.54	28.22
H	6.58	7.28	7.08

Table 3. Results of Elution Parameters and Molecular Weights of Parent Dextrans 71 and 15 kDa, after Treatment with Cerium(IV) or with Cerium(IV) and Hydroquinone

	K_d		M_w (kDa)	
peak	1	2	1	2
dextran 71 kDa parent	0.497		45.6	
dextran 71 kDa cerium	0.573	0.850	29.5	1.86
dextran 71 kDa cerium + hydroquinone	0.495	0.882	46.1	1.33
dextran 15 kDa parent	0.679		13.8	
dextran 15 kDa cerium	0.682	0.869	13.5	1.61
dextran 15 kDa cerium + hydroquinone	0.681	0.885	13.6	1.25

with dextran or with a blend of dextran and PIBCA. The vertical profiles of the light transmission across the emulsion prepared with a polymer prepared by polymerization of IBCA under [SEP+dextran-pH1] conditions is given in Figure 7E. The results obtained for the emulsion prepared with the polymer prepared by polymerization of IBCA under [REP-pH1] conditions is given in Figure 7F. For all the reference emulsions, a high level of transmission was recorded at both ends of the samples at time 0. The thickness of these domains was further increased after 2 min, and only a thin turbid (transmission = 0) band remained at the half-height of the samples. The profiles recorded at time 0 presented in Figure 7, parts E and F, were slightly different than those presented by the reference emulsions. The turbid domain detected at the middle of the sample was wider in the emulsion prepared with the polymer obtained under the conditions [SEP+dextran-pH1]. The turbid zone (transmission = 0) was almost complete in the emulsion prepared with the polymer obtained under [REP-pH1] conditions since only a small peak of transmission was recorded at the top of the sample. After 2 min, the profile of Figure 7E (polymer under [SEP+dextran-pH1] conditions) became very similar than those given by the reference emulsions. On the contrary, the transmission profile of the emulsion prepared with the polymer obtained under conditions [REP-pH1] remained closed to zero in a large part of the sample. The peak of transmission located at the top of the sample increased slightly but remained rather narrow.

The molecular weight of dextran 15 and 71 kDa after treatment by the cerium(IV) ions was evaluated by SEC. The chromatograms obtained for the parent dextrans, and for the dextrans treated with cerium(IV) ions alone or together with hydroquinone are presented in

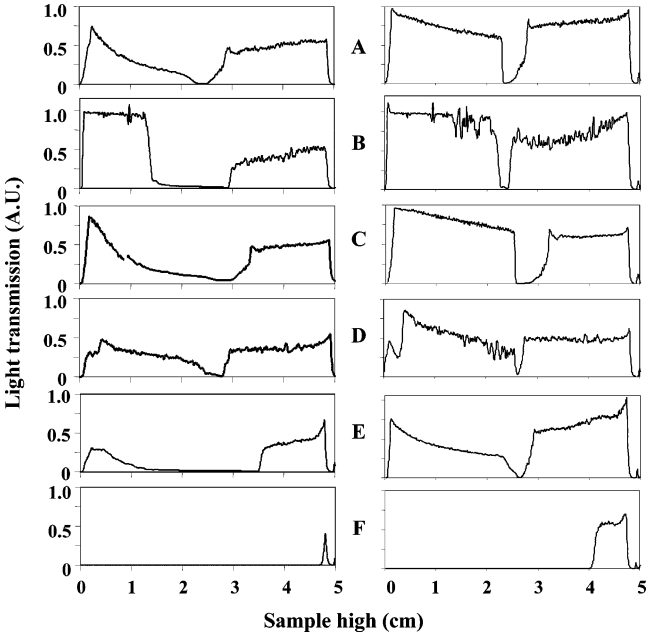


Figure 7. Vertical profile of the light transmission through the emulsions prepared in order to investigate the surface-active properties of the polymers as recorded by the Turbiscan at time zero (left column) and after 2 min (right column). Emulsion prepared without polymer (A), with PIBCA (B), with dextran 71 kDa (C), with a mixture of dextran 71 kDa and PIBCA (D), with polymer obtained under [SEP+dextran-pH1] conditions (E), and with polymer obtained under [REP-pH1] conditions (F).

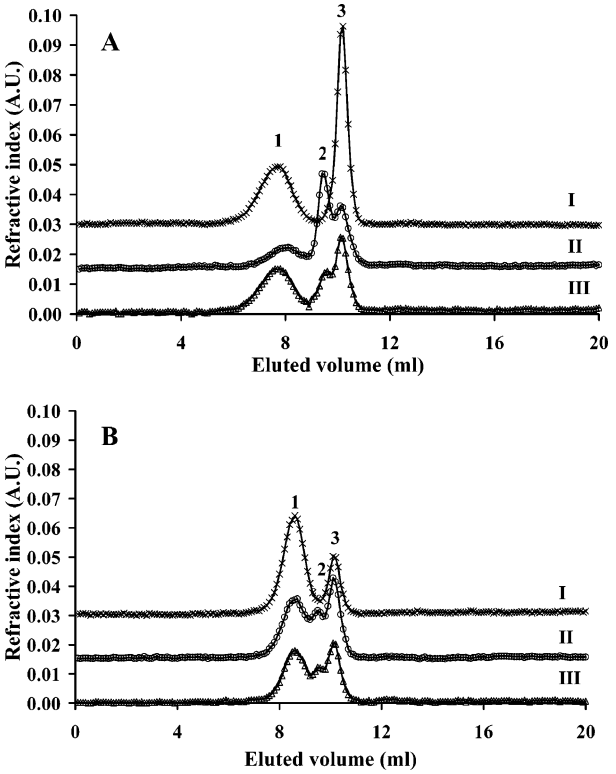


Figure 8. Size exclusion chromatography of dextran treated by cerium(IV) ions with or without hydroquinone. (A) The parent was dextran 71 kDa. (B) The parent was dextran 15 kDa. Chromatogram I: parent dextran. Chromatogram II: parent dextran treated with cerium(IV) ions. Chromatogram III: parent dextran treated with cerium(IV) ions and hydroquinone.

Figure 8, parts A and B, respectively, for dextran 71 and 15 kDa**.** All the chromatograms showed a third peak

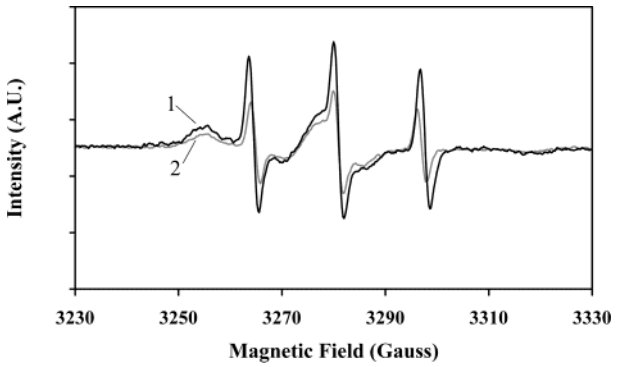


Figure 9. EPR spectrum of PIBCA nanoparticles prepared under conditions [REP-pH1] using dextran 15 kDa and labeled with 4-amino-TEMPO before (1) and after freeze-drying and redispersion in MilliQ water (2).

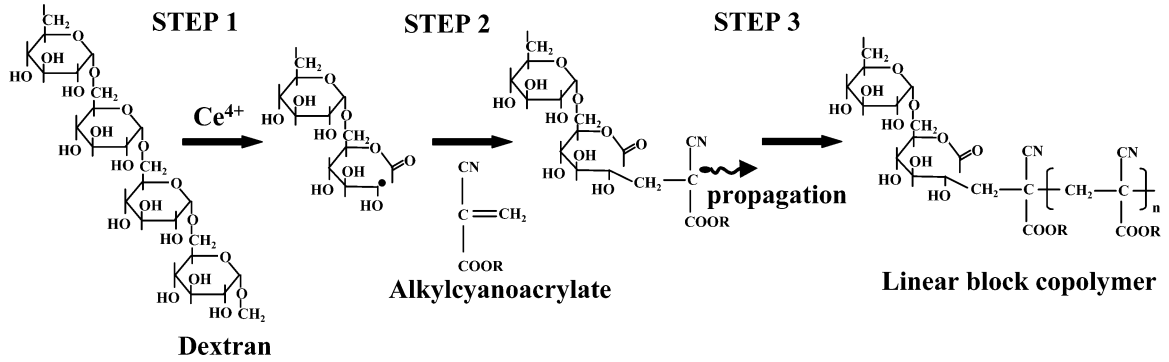
(peak 3) at an elution volume of 10.15 mL which corresponded to the signal produced by the small ions containing in the sample including NaCl. This very reproducible peak was used as an internal standard to calculate the total volume of the column. The chromatograms of the parent dextrans showed another symmetric peak (peak 1) at a lower retention time which depended on the molecular weight of the analyzed dextran (either 15 or 71 kDa). Chromatograms of dextrans treated by cerium(IV) ions with or without hydroquinone showed a third peak (peak 2) located between peaks 1 and 3. The values of K_d determined for peaks 1 and 2 are reported in Table 3 together with the values of the corresponding molecular weight as determined from the calibration curve. Peak 2 corresponded to dextran with a molecular weight ranging from 1250 to 1860 Da. The molecular weight determined for the peak 1 depended on the molecular weight of the parent dextran and whether hydroquinone was added after the cerium(IV) ions in the preparation media.

Evaluation of Dextran Chain Mobility by EPR. Figure 9 shows EPR spectra of the labeled nanoparticles obtained by the polymerization of IBCA under conditions [REP-pH1] before and after lyophilization and rehydration. The two spectra were very similar and showed three rather narrow Lorentzian peaks. The analysis of the spectra according to the Kivelson model[24] led to the determination of a correlation time of 1.4×10^{-10} s and to a proportion of 80% slow movements and 20% fast movements of the labeled dextran chains located at the nanoparticle surface.

Discussion

The purpose of this study was to design new PACA nanoparticles decorated with polysaccharides. This aim has been achieved according to a new synthetic method based on the redox radical polymerization of alkylcyanoacrylate monomers performed in emulsion in the absence of surfactant. Such a reaction was successfully initiated using dextran as a polysaccharide model on which a radical was created at the end of the chain oxidized by reaction with cerium(IV) ions and cleaved in acidic medium. Since ACAs are highly reactive monomers polymerizing spontaneously in emulsion after anionic initiation,[12] the main challenge of this study was to promote the redox radical polymerization by inhibiting the anionic spontaneous polymerization. Thus, conditions under which the redox radical polymerization initiators were not added, i.e., [SEP+Ce^{4+}-pH1], [SEP+dextran-pH1], [SEP+dextran-pH2], and

Scheme 1. Scheme of the Redox Radical Initiation of the Polymerization of ACA Using the Couple Dextran and Cerium(IV) Ions at pH 1[a]



[a] Step 1: formation of the radical at a chain end of dextran due to cerium(IV) ions, inducing simultaneous splitting of the parent dextran chain in acidic medium. Step 2: initiation of the redox radical emulsion polymerization of ACA by dextran radical. Step 3: propagation of the polymerization leading to a linear block copolymer (dextran-*co*-PACA).

[SEP+dextran-pH3], were investigated. In the absence of dextran, i.e., under conditions [SEP+Ce^{4+}-pH1], no polymerization could occur at pH 1. On the contrary, spontaneous polymerization could be initiated when the polymerization medium contained dextran even at pH 1, i.e., under conditions [SEP+dextran-pH1]. This result which was in contradiction with some data of the literature[25−27] could be explained by an initiation of the polymerization by the OH groups of dextran leading to the formation of a graft copolymer with a dextran backbone and grafted PIBCA chains in agreement with a previous study by Douglas et al.[27] Formation of polymer particles could be delayed for a few minutes only at pH 1 as evidenced by monitoring the optical density of the polymerization medium. On the contrary, at pH 2 and 3, the polymerization started much faster without lag time. This is explainable by the fact that anionic polymerization occurred spontaneously due to the rapid initiation by the OH^- of water.

When the polymerization was performed in the presence of the redox radical initiators consisting of the combination of dextran and cerium(IV) ions, it started immediately even at pH 1 (Figure 1A) as evidenced by the rapid increase of the optical density of the polymerization medium. This result demonstrates that a redox radical polymerization of ACA could be initiated at pH 1. Indeed, at this pH polymer particles appeared much faster than when the polymerization was performed in the system [SEP+dextran-pH1] in the absence of cerium(IV).

This result obtained with IBCA could also be achieved with all the other monomers tested, indicating that the redox radical polymerization could be applied to different ACA. Interestingly, differences concerning the polymerization kinetics were observed depending on the alkyl chain length of the ester group of the ACA (Figures 2 and 3). Thus, anionic spontaneous polymerization initiated by the OH groups of dextran was more rapid in the case of the shortest alkyl chain length. As a consequence, it was expected that redox radical polymerization would be easier to promote with ACA possessing a long alkyl chain. In addition, it was observed that the redox radical polymerization could only be applied to ACA. Indeed, no difference in turbidity increase could be evidenced between the spontaneous and the redox radical emulsion polymerization conditions when using 2-methoxyethylcyanoacrylate.

The suspension of polymer particles prepared by the redox radical polymerization of IBCA under conditions [REP-pH1] consisted of well-defined nanoparticles with a narrow size distribution and a slightly negative ζ potential. The suspension was very stable with time and could be stored for at least 36 months without showing any variation of the size parameters. The nanoparticles could also be freeze-dried and redispersed in water without the need of cryo-protecting agents which is an obvious advantage for further pharmaceutical applications. Regarding the ζ potential, the lower values measured with nanoparticles prepared with dextran 15 kDa (as compared with dextran 71 kDa) may be explained by the fact that negative charge of PACA was less efficiently shielded with low molecular weight dextran.

The data resulting from the analysis of the polymer forming the nanoparticles gave convergent arguments that it was a copolymer composed of dextran and PIBCA. For instance, elemental and spectral analysis have shown that the polymer formed included both dextran and PIBCA in its composition as expected by the mechanism of the redox radical polymerization investigated in this study (Scheme 1, steps 2 and 3). This suggests that the polymer synthesized by the redox radical polymerization was a copolymer. However, the structure of the copolymer could not be evidenced by the FTIR, Raman, and NMR spectra by the appearance of a new signal corresponding to the creation of a covalent bond between dextran and PIBCA. This is however easily explainable considering the mechanism by which the copolymer would form (Scheme 1, steps 2 and 3), since if only one bond would be created per copolymer, the signal produced by this single bond would be too small to appear on the spectra when compared to the signals given by the other repeated groups of the rest of the copolymer.

To demonstrate the effective formation of such a copolymer, two indirect methods were used additionally. First, the newly synthesized polymer was found insoluble in the solvents of both dextran and PIBCA, respectively. Second, according to the reaction scheme (Scheme 1), the obtained copolymer should take a linear structure with blocs of dextran and of PIBCA which will arrange as diblock copolymers or as multiblock copolymers depending of the mechanism of the polymerization termination mechanism. Since PIBCA is hydrophobic and dextran is hydrophilic, such a copolymer should exhibit amphiphilic properties. Then, the capacity of the synthesized polymer to stabilize an emulsion was investigated using an original approach based on the

scanning of the turbidity along the height of the emulsion with a Turbiscan. The results clearly showed that the polymer obtained by redox radical emulsion polymerization could improve significantly the stability of the emulsion whereas the homopolymers and a blend of them were unable to stabilize the same emulsion. This polymer appeared clearly amphiphilic supporting the hypothesis that it was a copolymer composed of dextran and PIBCA. Interestingly, the copolymer prepared by anionic emulsion polymerization under [SEP+dextran-pH1] conditions was found to be less efficient in improving the stability of the model emulsion, suggesting that the polymer formed by the redox radical emulsion polymerization was different in its structure and displayed higher surface-active properties.

Since the polymer obtained by redox radical polymerization was insoluble in the solvents generally used for molecular weight determinations, it was decided to evaluate the molecular weight of the dextran part of the copolymer by simulating the initiation of the polymerization which occurred on dextran by using a system with cerium(IV) ions but in which the monomer was not added. SEC analysis of the resulting dextran showed that the action of cerium(IV) ions on parent dextrans (15 or 71 kDa) led to dextran chains with a bimodal molecular weight distribution. This result confirms that dextran was split by the reaction with cerium ions under our conditions. Taking into account the molecular weight values deduced from the chromatograms, it was concluded that the parent dextran chains were split at a specific point located 7 to 10 glucose units from the chain end because both parent dextrans released the same oligomer. Degradation of the parent dextran chains into the glucose oligomer could be stopped in the presence of hydroquinone, which is known to react with radicals. This result suggests that the radical created on the dextran chains could also initiate the polymerization of a monomer as well. It additionally suggests that once a component reacted on the radical created at the dextran chain end, this dextran chain is then protected from a further attack by the cerium(IV) ions and could not be split again. Thus, in the case of a polymerization reaction, the elongation leading to the formation of polymer chains could only occur from a single point of the dextran chain resulting in the formation of a linear block copolymer. The fact that dextran chains were protected from a further attack by cerium(IV) ions appeared with the experiments performed on the parent dextran 71 kDa in which the total number of dextran chains exposed to a given concentration of cerium(IV) ions was much lower than in the experiments performed with parent dextran 15 kDa. Therefore, the degradation of the parent dextran 71 kDa could be more pronounced in the absence of hydroquinone than in the presence of this compound, and a difference in molecular weight of the longer fragments between the two experimental conditions could be evidenced. In contrast, such a difference was much more difficult to observe for the smallest parent, dextran. Taking into account that the mean hydrodynamic diameter and the ζ potential of the nanoparticles were affected by the molecular weight of the parent dextran, it could be proposed that only dextran chains corresponding to the higher molecular weight fragments produced by the action of the cerium(IV) ions could be incorporated into the copolymer. Indeed, the shortest fragment obtained from both parent dextrans possessing the same molecular weight would have produced nanoparticles of the same hydrodynamic diameter and ζ potential which was not observed.

Finally, to verify that dextran was well located at the surface of the nanoparticles and in order to investigate the mobility of these chains, dextran was labeled with a free radical nitroxide probe (4-amino-TEMPO) and analyzed by EPR. This study revealed that dextran molecules were freely accessible at the surface of the nanoparticles making the coupling reaction possible. The EPR spectra also showed that, after freeze-drying and rehydration of the nanoparticles, the mobility of dextran molecules was the same as before freeze-drying. This result together with those concerning the size determination suggested that the nanoparticles were not affected by the freeze-drying process even at the molecular level on the nanoparticle surface.

In conclusion, this study has shown that very stable suspensions of PACA nanoparticles coated with dextran could be prepared by a single-step process according to a new synthetic method based on a redox radical emulsion polymerization of ACA. At pH 1, the well-known spontaneous anionic emulsion polymerization of ACA could be sufficiently delayed to promote the occurrence of redox radical polymerization, which was rapidly initiated by dextran and cerium(IV) ions. The nanoparticles produced were composed of an amphiphilic dextran-based copolymer of PACA which was shown to be endowed with surface-active properties. Further research is now on the way to apply this new methodology to other polysaccharides with in vivo recognition properties.

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