Novel Polysaccharide-Decorated Poly(Isobutyl Cyanoacrylate) Nanoparticles

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Purpose. The aim of this work was to synthesize new surface-modified nanoparticles using a radical emulsion polymerization of an alkyl cyanoacrylate.

Methods. Isobutyl cyanoacrylate was polymerized in nitric acid 0.2 M containing a polysaccharide (0.1375 g) and cerium (8×10^{-2} M). After 1 h, the pH was adjusted to 7.0, and the nanoparticles were purified by dialysis. Nanoparticle characterization included scanning electron microscopy, quasi-elastic light scattering, zeta potential determination, measurements of the complement activation induced by different polysaccharide-coated nanoparticles and of the antithrombic activity of heparin.

Results. Dispersions of spherical particles were obtained using various polysaccharides. The particle diameter varied from 90 nm to several micrometers, and the zeta potential depended on the molecular weight and the nature and charge of the polysaccharide. Surface analysis performed by ESCA confirmed the presence of polysaccharides at the nanoparticle surface. The nanoparticles were very stable, and the biologic activity of the polysaccharide was preserved. Complement activation was influenced by the polysaccharide characteristics.

Conclusions. A new method based on radical emulsion polymerization of isobutyl cyanoacrylate initiated by polysaccharides and cerium was developed to prepare nanoparticles. It leads, in a single step, to nanoparticles with surface properties defined by the polysaccharide. This method is a new concept for the development of biomimetic drug carriers with multiple functions.

KEY WORDS: poly(isobutyl cyanoacrylate); polysaccharides; nanoparticles; surface properties.

INTRODUCTION

When given intravenously, drugs are distributed throughout the body as a function of their physicochemical properties. A pharmacologically active concentration is reached in the targeted tissue at the expense of massive contamination on the rest of the body. For highly toxic substances such as cytostatic agents, this poor specificity raises a toxicologic problem that represents a serious obstacle to effective therapy. Thus, one of the main interests of using polymer nanoparticles as drug carrier systems is to control both the spatial and temporal delivery of a biologically active substance into the body.

During the last decade, it has been demonstrated that surface properties of nanoparticles were the key factor that determined their *in vivo* fate (1–6). Colloidal drug carriers with protein-repulsive surface properties were designed in order to reduce the opsonization by serum proteins and complement activation because these phenomena play a major role in the rapid clearance of the particles from the bloodstream and in their recognition by macrophages of the mononuclear phagocytes system (MPS). The most popular approach consisted in the formation of a hydrodynamic cloud around the particles by grafting poly(ethylene glycol) (PEG) chains at the carrier surface (2,4,7). The result is that the opsonization of the nanoparticles through their adsorption of serum protein is reduced, and consequently the nanoparticles are less recognized by macrophages. However, in the view of targeting, PEG-coated nanoparticles are passive systems because their modification of tissue distribution is basically a result of the difference in microvascular permeability between healthy and altered tissue and of their long circulating properties. To achieve active targeting with molecular recognition, more specific properties should be added to the nanoparticle surface, which has not yet been successfully achieved. This is the reason why we propose here the design of new nanoparticles obtained from copolymers containing oligo- or polysaccharides. Indeed, oligo- and polysaccharides are universally exposed at the cell surfaces as well as at the surface of virus and bacteria. Moreover, these components are involved in cell surface properties including tissue addressing and transport mechanisms (8). Other polysaccharides display biologic activity such as antithrombic properties, antiviral and antibacteria activity or can be involved in cell growth regulation phenomena (9). Thus, the grafting of polysaccharides on the nanoparticle surface would not only open new features in the design of biomimetic drug delivery systems but will also propose a new concept for the obtaining of self active drug delivery devices.

The first polysaccharide-coated nanoparticles proposed in the literature were made of non-biodegradable polymer which is not acceptable for the development of a drug delivery system for systemic administration to humans (10). Therefore, the preparation of biodegradable nanoparticles such as poly(alkyl cyanoacrylate) (PACA) nanoparticles was found to be very challenging also because, from a technical point of view, the linkage of polysaccharides requires a radical emulsion polymerization of the corresponding monomers (11). Indeed, alkyl cyanoacrylates (ACA) are among the most reactive compounds polymerizing spontaneously according to a well established anionic mechanism (12,13). Thus, this paper describes the synthesis and characterization of polysaccharide-coated PACA nanoparticles by a new emulsion polymerization of an ACA (e.i. isobutyl cyanoacrylate) performed in the presence of different polysaccharides and initiated by a redox system including cerium ions and the polysaccharide itself.

MATERIALS AND METHODS

Materials

Isobutyl cyanoacrylate (IBCA) was kindly provided by Loctite (Dublin, Ireland). Dextran (Mw 71,000 Da), heparin (Mw 19,000 Da), dextran sulfate (Mw 50,000 and 10,000 Da), and hyaluronic acid (Mw 80,000 to 8,000,000 Da) were purchased from Sigma (Saint-Quentin Fallavier, France). Dex-

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tran (Mw 15,000 Da), dextran sulfate (Mw 500,000 Da), and chitosan (Mw 400,000 Da) were obtained from Fluka (Saint-Quentin Fallavier, France). Dextran sulfate (Mw 40,000 and 7,000 Da) was supplied by ICN Pharmaceuticals (Costa Mesa, CA, USA). Pectin (Mw 70,000 to 100,000 Da) was purchased from Citrus colloids (Hereford, UK). Cerium IV ammonium nitrate, nitric acid, sodium hydroxide and tri-sodium citrate dihydrate were obtained from Fluka (Saint-Quentin Fallavier, France). All chemicals were reagent grade and used as purchased.

PC2000 plug-in spectrometer, optic fibers (100 and 200 μm) and HL-2000-Cal calibrated light source from Ocean Optics Europe (Lannion, France) were used to monitor the emulsion polymerization of isobutyl cyanoacrylate. The configuration of the spectrometer should be follows: first coefficient (0.389298), second coefficient (-2.1235^{-005}), and intercept (185.073).

Preparation of Poly(Isobutyl Cyanoacrylate) (PIBCA) Nanoparticles Coated with Polysaccharides

Polysaccharides were dissolved in nitric acid (8 ml, 0.2 M) at 40°C, under gentle stirring and argon bubbling. After 10 min, 2 ml of a solution of cerium ammonium nitrate (8×10^{-2} M in 0.2 M nitric acid), and 0.5 ml of IBCA were added under vigorous stirring. Argon bubbling was maintained for 10 min. The reaction was allowed to continue at 40°C under gentle stirring for 40 min. After cooling to room temperature, tri-sodium citrate (1.25 ml, 1.02 M) was added to the polymerization medium and the pH was adjusted to 7.0 with NaOH.

Polysaccharides consisted of 0.1375 g of either pure dextran 71 kDa, dextran 15 kDa, dextran sulfate of different molecular weights, heparin or of a mixture of dextran 71 kDa (0.0688 g) and heparin (0.0688 g). For chitosan, pectin and hyaluronic acid, 0.0230 g of one of these polysaccharides was solubilized in 8 ml of nitric acid (0.2 M) between 40 to 50°C under strong agitation overnight. Remaining undissolved material was eliminated by filtration (1.2 μm).

For further analysis, the nanoparticle suspensions (12 ml) obtained with polysaccharides of molecular weight lower than 100 kDa were purified by dialysis against 1 liter of distilled water (dialyzing membrane with a molecular weight cut-off of 100 kDa from Spectra/Por®, Biovalley, Marne la vallée, France). The dialysis has been performed three times at room temperature for 90 min and one additional time overnight. Particles obtained with polysaccharides of molecular weight over 100 kDa can be purified by ultracentrifugation.

Purified nanoparticles were either stored at +4°C or frozen at –18°C and freeze-dried during 48 h in a Christ alpha 1–4 freeze dryer (Bioblock Scientific, Illkirch, France) without cryo-protecting agent.

Monitoring of the Polymerization

A Teflon® ring with two holes, one at 0°, the other one at 180° was mounted around the glass tube in order to adapt the optic fibers for the *in situ* monitoring of the optical density of the polymerization medium resulting from polymer formation. An optic fiber (100 μm) was used to carry the light from the light source to the glass tube and another (200 μm) was used to collect the transmitted light to be analyzed by the plug-in spectrometer. A PC computer recorded one hundred optical density spectra of the polymerization medium from 400 to 800 nm. The acquisition time of one spectrum was automatically fixed at 120 ms. The dark spectrum was recorded before adding 2 ml of cerium ammonium nitrate ($8x10^{-2}$ M in 0.2 M nitric acid) and when the incident light was still ′off′. The reference spectrum was recorded immediately after adding 2 ml of cerium ammonium nitrate and when the incident light was ′on′. The kinetic 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 given by the plug-in spectrometer at 650 nm (pixel 1284) was determined from the recorded spectra to deduce the particle-forming rates which resulted from the mean optical density variation of the polymerization medium vs. time.

Scanning Electron Microscopy

Nanoparticle suspensions were successively diluted in MilliQ® water to 1/100 (v/v). The dilutions were spread on an aluminium disc and dried at room temperature. The dried nanoparticles were then coated with gold metal using an Edwards sputter coater S150 prior to be observed with the scanning electron microscope (Philips XL 30, Philips, France).

Particle Size Analysis

The mean particle diameter and the size distribution were determined at 20°C by quasi-elastic light scattering using a Nanosizer® N4 PLUS (Beckman-Coulter, Villepinte, France). The scattered angle was fixed at 90°. Nanoparticle samples were diluted in MilliQ® water. The apparatus provides with the mean hydrodynamic diameter (peak), the standard deviation of the size distribution (width) and the polydispersity index. Particles with a diameter bigger than 1 μm were evaluated using a Laser Granulometer® LS 230 (Coulter, France) according to the Fraunhofer's theory.

All determinations were performed in triplicate.

ESCA

A Surface Science Instrument (SSI) spectrometer (based at ITODYS Laboratory, University Paris VII) equipped with a monochromatic Al Kα X-ray source (1486.6 eV) was used at a spot size of 1000 μm. The take-off angle relative to the sample holder surface was 35°. The pressure in the analysis chamber was *ca.* 5×10^{-9} mbar. The pass energy was set at 150 and 100 eV for the survey and the narrow scans, respectively. The step size was 1.12 eV for the survey spectra and 0.078 for the narrow scans. Charge compensation was achieved with a flood gun of 4 eV electrons. Data processing was achieved with a Winspec software, kindly supplied by the Laboratoire Interdisciplinaire de Spectroscopie d'Electrons (LISE, Namur, Belgium). The spectra were calibrated against the N1s peak PIBCA centered at 399.6 eV, a binding energy value reported by Beamson and Briggs (14) for poly(acrylonitrile) and poly(methacrylonitrile). The surface composition was determined using the manufacturer's sensitivity factors. The fractional concentration of a particular element A (% A) was computed using:

$$\%A = \frac{(I_A/s_A)}{\sum(I_n/s_n)} \times 100\% \quad (1)$$

where I_n and s_n are the integrated peak areas and the sensitivity factors, respectively.

Zeta Potential

Zeta potential of nanoparticles diluted in NaCl 1 mM was measured on a Zetasizer 4 (Malvern Instruments Ltd., Malvern, UK).

Determination of the Nanoparticle Concentration in the Suspension and Evaluation of the Nanoparticle Composition

The nanoparticle concentration in the suspension was evaluated by gravimetry. 1 g of the purified nanoparticle suspension was freeze-dried and the dry residue corresponding to the nanoparticles was weighted to deduce the percentage of nanoparticles contained in 1 g of the suspension.

Composition in polysaccharide and PIBCA of the copolymers forming the nanoparticles were determined by elemental analysis (CNRS, Vernaison, France) in comparison to pure polysaccharide and PIBCA homopolymers. The results were expressed as percent (w/w) of the dry nanoparticles.

Evaluation of Complement Activation by 2D Immunoelectrophoresis of C3

The specific activation of C3 complement component induced by different nanoparticles in human serum was evaluated using human serum and by comparative measurements of C3 cleavage, as previously described (15–17). Practically, human serum was obtained after calcifing plasma from healthy donors and stored at −80°C until use. Nanoparticles were incubated for 1 h at 37°C with human serum diluted to 1:4 in veronal buffer containing 0.015 mM Ca^{2+} and 0.05 mM Mg^{2+} ions (VBS^{2+}) under gentle agitation. To ensure a valid comparison of the different nanoparticles, sample volumes with equal surface area of hydrated particles (1,000 cm^2) were incubated with 100 μl of the diluted human serum. A nanoparticle relative surface area was calculated by the following equation:

$$S = 3V/r = 3m/rd \quad (2)$$

where S is the relative surface area developed by the nanoparticles in cm^2, V the volume (in cm^3) of n spherical particles of average hydrodynamic radius r (in cm), m the weight (in g) of dry residue contained in 1 cm^{-3} of nanoparticle suspension and d the copolymer density (taken to a value of 1 for all polymer particles).

Each sample (5 μl) was subjected to a first electrofocusing electrophoresis on 1% agarose gel. The second-dimension electrophoresis was carried out on Gelbond® films in agarose gel plates containing a polyclonal antibody to human C3 (Complement C3 antiserum raised in goat, Sigma, France), recognizing both C3 and C3b. The films were finally stained with Coomassie blue (Sigma). Serum diluted in VBS^{2+} and serum in which calcium and magnesium ions have been chelated by ethylenediaminetetraacetic acid (EDTA) were both used as negative controls of complement activation. Sephadex G 25 superfin (Pharmacia, Orsay, France) was used as a positive control.

Analysis of Heparin Activity of Nanoparticles Coated with Heparin

Antithrombic activity of nanoparticles coated with heparin was evaluated by activated cephalin time measurements using a coagulometer ST4 (Diagnostica stago, Gennevilliers, France). The results were expressed in U.I per ml of nanoparticle suspension. The apparatus was previously calibrated with heparin standards.

(The calibration curve equation was: $Y = 0.0042\ X - 0.1895$ ($r^2 = 0.999$)).

Antithrombic activity of nanoparticles was also evaluated by the routine anti-Xa factor activity measurements using a coagulometer ST1 (Diagnostica stago, Gennevilliers, France).

Finally, the results were expressed in USP taking into account the concentration of heparin in 1 ml of the heparin-PIBCA nanoparticle suspension.

RESULTS

According to several authors (18–20), the mechanism of the redox radical polymerization initiated by cerium ions and dextran would lead to the formation of a copolymer between the polysaccharide and the poly(isobutyl cyanoacrylate) (18,19). Practically, after addition of the cerium ammonium nitrate, an immediate and rapid increase of the optical density of the polymerization media was noted (Fig. 1). The initial slope of the curves at time zero varied from 45 to 61% per min indicating that the polymerization was quite fast. Since the different curves were rather superimposed, no influence

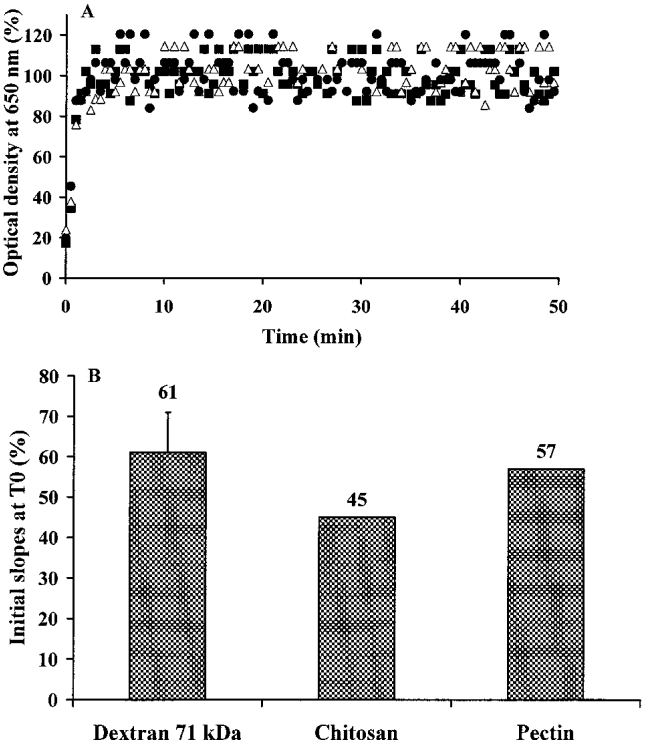

Fig. 1. (A) Radical emulsion polymerization of IBCA initiated by dextran 71 kDa (■), chitosan (△), and pectin (●) as followed by the increase of turbidity of the polymerization medium. (B) Initial slopes of the curves at time zero (Below). A standard deviation of 10% has been determined with dextran 71 kDa (n = 10).

of the polysaccharide on the polymerization rate could be highlighted (Fig. 1).

Scanning electron microscopy performed on the suspension showed the presence of spherical particles of regular shape and size (Fig. 2). The particle diameters evaluated by photon correlation spectroscopy or by laser granulometry was ranging from 93 nm to 59 μm depending on the polysaccharide used for the synthesis (Table I). It seems that chitosan, pectin and hyaluronic acid are the polysaccharides with the higher molecular weight, produced particles of bigger size (micrometer range). On the contrary, the low molecular weight dextran sulfate (7 kDa) led to nanoparticles with a large diameter, a wide size distribution and a high polydispersity index. For the other dextran sulfate with higher molecular weights (from 10 kDa to 500 kDa), the diameter of the nanoparticles increased with the molecular weight and the size dispersion was much narrow (Table I). This tendency was also observed with dextran. Interestingly, nanoparticles obtained with a blend of dextran 71 kDa and heparin showed an intermediate diameter compared to the nanoparticles obtained with the single corresponding polysaccharide (Table I). The polymerization performed at a larger scale (5 times the volume of the classic experiments) lead to nanoparticle suspensions with the same characteristics than those produced in smaller batches (data not shown).

The theoretical value of the oxygene to carbon atomic ratio expected for PIBCA and calculated from its chemical formulae is 0.25. The experimental value found from the ESCA analysis of PIBCA was 0.28 in agreement with the theoretical value given above. This ratio was increased to 0.43, 0.49 and 0.40 for PIBCA nanoparticles prepared with dextran, the blend of dextran and heparin and heparin respectively. The nitrogen to carbon atomic ratio decreased from a value of 0.12 for PIBCA to 0.09 for the nanoparticles with polysaccharides. When nanoparticles were prepared with heparin or with a blend of heparin and dextran they showed the presence of about 1% of sulfur and of its counter ion sodium which concentration differed whether the nanoparticles were prepared with heparin alone 2.43% or with the blend of heparin and dextran 0.31%.

Zeta potential of the nanoparticles are given in Fig. 3. Negatively charged polysaccharides (heparin and dextran sulfate) lead to nanoparticles with a highly negative zeta potential (around – 45 mV). No influence of the molecular weight of these polysaccharides on the surface charge could be noted. Nanoparticles prepared with dextran (neutral polysaccharide) 15 kDa or 71 kDa showed a less negative zeta potential (of –19 mV and –11 mV respectively).

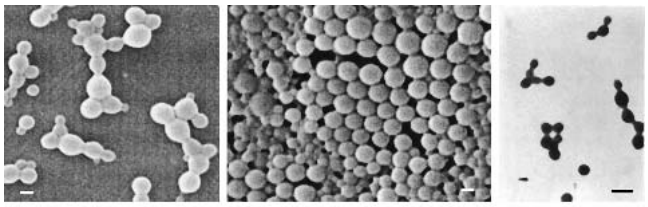

Fig. 2. Electron microscopy of PIBCA nanoparticles coated with dextran 71 kDa (scanning electron microscopy) (A, B) and coated with heparin (transmission electron microscopy) (C). *Bars* represent 200 nm.

Table I. Hydrodynamic Diameters of PIBCA Nanoparticles and Microspheres Prepared with Different Polysaccharides and Obtained after Purification

PIBCA nanoparticles prepared with	Hydrodynamic diameters		
	Peak (nm)	Width (nm)	Polydispersity index
Dextran 71 kDa	297	80	0.105
Dextran 15 kDa	200	47	0.074
Dextran 71 kDa and heparin	186	67	0.267
Heparin	93	33	0.259
Dextran sulfate 500 kDa	408	107	0.114
Dextran sulfate 50 kDa	333	102	0.158
Dextran sulfate 40 kDa	274	64	0.072
Dextran sulfate 10 kDa	192	47	0.081
Dextran sulfate 7 kDa	805	286	0.270
Chitosan	59 μm	3 μm	—
Pectin	30 μm	2 μm	—
Hyaluronic acid	32 μm	2 μm	—

Gravimetry measurements indicated that the polymer content of the suspensions obtained using dextran 71 kDa and heparin were respectively of 3.5 ± 0.5% (w/w) and 2.7 ± 0.7% (w/w). The yield of the nanoparticle formation was comprised between 95 and 100% based on the monomer conversion. The polysaccharide content of the copolymer forming the nanoparticles was 22 ± 1% (w/w) for those prepared with dextran 71 kDa and 11 ± 4% (w/w) for those obtained with heparin. The heparin concentration of the suspension of heparin-PIBCA nanoparticles was 2.97 mg/ml.

Figure 4 shows the variation of the diameter and of the size distribution of nanoparticles prepared in the presence of either dextran 71 kDa or heparin after synthesis, purification and during storage. No change in these parameters could be detected over the period of 36 months.

The capacity of different polysaccharide-coated nanoparticles to activate the complement has been evaluated by measuring the cleavage of C3 by 2D-immunoelectrophoresis after incubation with serum. In this experiment, a polyclonal anti-C3 antibody was used in order to detect both C3 (non activated complement) and C3b (activated complement). The negative and positive controls revealed that the serum was not auto-activated (Fig. 5 A,B) and that the antibody could detect both C3 and C3b when a strong complement activator was added to the serum (Fig. 5C). The electrophoregram given by the nanoparticles prepared with dextran 15 kDa (Fig. 5D) showed a high peak for the activated complement whereas almost no non activated complement could be detected. On the contrary, nanoparticles prepared with dextran 71 kDa (Fig. 5E) showed almost the same pattern than the electrophoregram obtained with the negative control (Fig. 5B). The nanoparticles obtained with heparin and with the mixture of dextran 71 kDa and heparin gave the same electrophoregrams showing no trace of C3b (Fig. 5F,G). Finally, nanoparticles prepared with dextran sulfate showed none of the C3 peaks (Fig. 5H). The results of a semi quantitative analysis of the electrophoregrams taking into account the ratio between the peak height (in mm) measured for C3b and for C3 showed that nanoparticles prepared with dextran 71 kDa presented only a limited increase of this ratio (0.20) (Fig. 5E) compared to the negative control (0.13) (Fig. 5B)

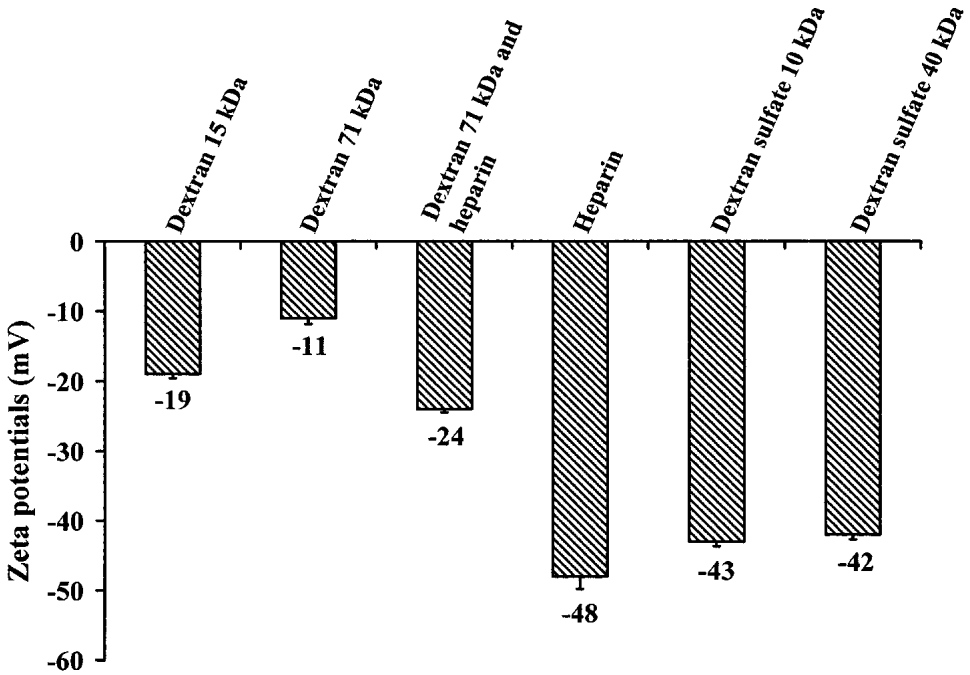

Fig. 3. Zeta potentials of PIBCA nanoparticles coated with different polysaccharides. *Bars* represent mean ± SD (n = 10).

whereas a dramatic increase of C3b to C3 ratio (7.6) was observed with the nanoparticles prepared with dextran 15 kDa (Fig. 5D).

Finally, the antithrombic activity was found to be 112 USP (333 ± 30 U.I per ml) and 137 USP (408 ± 50 U.I per ml) for nanoparticles coated with heparin as measured by the activated cephalin time and by the anti-Xa factor activity respectively. The activity of the heparin used for the preparation of the nanoparticles was 175 USP as measured in the same conditions.

DISCUSSION

This study describes the design of new poly(isobutyl cyanoacrylate) nanoparticles with modified surface properties. Their preparation is based on a original emulsion polymer-

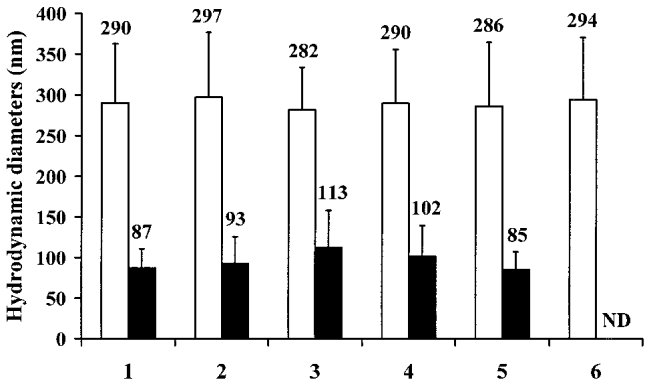

Fig. 4. Hydrodynamic diameters of PIBCA nanoparticles coated with dextran 71 kDa or heparin after synthesis (1), dialysis (2), and storage at 4°C without preservative agents for 2 months (3), 8 months (4), 30 months (5), and 36 months (6). Dextran 71 kDa-PIBCA (*white columns*). Heparin-PIBCA (*black columns*). *Bars* represent mean ± width (n = 3).

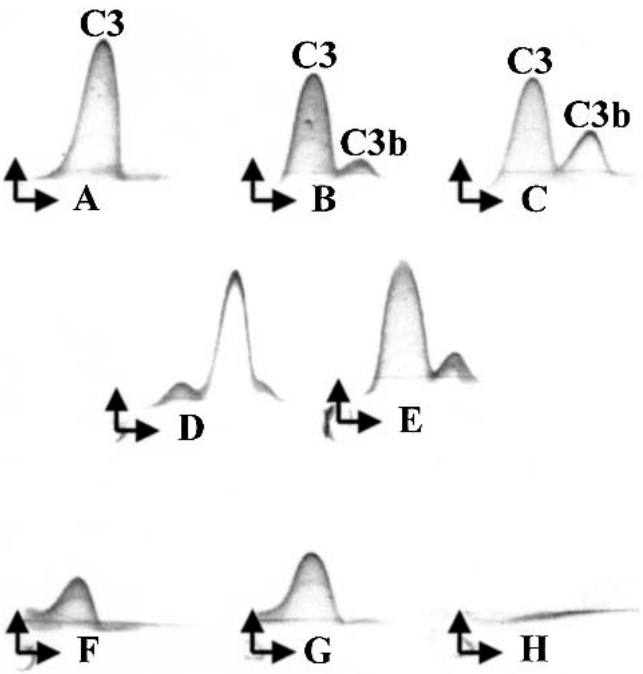

Fig. 5. Crossed immunoelectrophoresis of C3 antigens in human serum diluted 1:4 in VBS^{2+} after 1 h incubation with nanoparticle suspension at constant surface area (1,000 cm^2). (A) Serum/VBS^{2+}/EDTA 10 mM 1:3 (v/v); (B) serum/$VBS2^{+}$; (C) serum/VBS^{2+}/Sephadex G25; (D) serum/VBS^{2+}/dextran 15 kDa-coated nanoparticles; (E) serum/VBS^{2+}/dextran 71 kDa-coated nanoparticles; (F) serum/VBS^{2+}/heparin-coated nanoparticles; (G) serum/VBS^{2+}/dextran 71 kDa and heparin-coated nanoparticles; (H) serum/VBS^{2+}/dextran sulfate 40 kDa-coated nanoparticles.

ization reaction of isobutyl cyanoacrylate which is initiated by a polysaccharide on which a radical has been created by reaction with cerium ions (pH 1) (18,20). Results of the monitoring of the optical density of the polymerization medium (Fig. 1) demonstrated that the polymerization reaction started readily after the addition of the monomer to give a very stable suspension of spherical colloidal polymer particles as shown by scanning electron microscopy (Fig. 2) and by light scattering (Fig. 4). The rate of increase of the optical density as evaluated by the calculation of the initial slope of the curves at time zero showed no difference between the polymerization carried out with polysaccharides of different nature. This also suggested, that the initiation of the polymerization was extremely fast since 50% of the maximum of optical density was reached already after one minute. The data obtained here are completely different from those reported in the literature when ACA polymerization is performed at pH 1 in a water medium but in the absence of cerium ions. In this case, the anionic polymerization either never occurs or occurs according to a slower rate (12,13,21–23). Moreover, the polymer particles resulting from the spontaneous anionic polymerization of isobutyl cyanoacrylate at pH 1 were quite unstable. Thus, our results strongly suggest that radical polymerization occurs with a fastest rate than the anionic polymerization. This is explainable by the high reactivity of the initiation step of the polymerization in which all the radicals are produced at the same time by the action of cerium ions on the polysaccharides which are ready to initiate the polymerization when as soon as the monomer is introduced in the medium. The resulting dispersion is composed by a polysaccharide-poly(isobutyl cyanoacrylate) copolymer which is able to spontaneously auto-associates to form the nanoparticles (19). The polymer particles are likely stabilized by the hydrophilic polysaccharide moiety as already suggested by Passirani *et al.* (10). It is noteworthy that this polymerization reaction could be reproduced with various polysaccharides having different molecular weights and charges which allows to obtain polymer particles with various sizes and charges. The polysaccharide content of the nanoparticles prepared with dextran 71kDa and heparin was found to be comprised between 10 to 20%. This fraction of polysaccharide which is strongly associated with the nanoparticles can be involved in the nanoparticle properties whereas the unreacted excess of polysaccharide introduced in the polymerization medium was eliminated during the purification performed by dialysis.

The size of the polymer particles was shown to mainly depends on the molecular weight of the polysaccharide used considering the series of experiments performed with dextran sulfate. In can be observed that the lower the molecular weight, the smaller the particles were. However, a minimum molecular weight for the polysaccharide seemed to be required to insure the stability of polymer particles formed as suggested by the experiment performed with dextran sulfate 7 kDa. This can be explained by the fact that the nanoparticles which formed are stabilized by the newly synthesized copolymer. Thus, the size of the final nanoparticles that formed will be gouverned by the characteristics of the stabilizing copolymer which may depends on the respective length of the hydrophilic (polysaccharide) and hydrophobic [poly(isobutyl cyanoacrylate)] parts. Nevertheless, stable polymer particles with a wide range of size and a narrow size distribution could be synthesized by this new method using a set of different polysaccharides. The further characterizations were performed on the nanoparticles mainly obtained with dextran and heparin and for some experiments with dextran sulfate which represent relevant systems for the development of drug carriers for the intravenous route of administration.

The surface characteristics of the nanoparticles were investigated using ESCA and by measuring the zeta potential. The analysis of lyophilized samples of nanoparticles prepared with heparin and dextran was performed using ESCA and compared to the results obtained in the same analytical conditions with the homopolymer of PIBCA. The data obtained showed that dextran and heparin dramatically affected the oxygene to carbon atomic ratio considering the corresponding value obtained for PIBCA. In parallel, the nitrogen to carbon atomic ratio was decreased as it could be expected for coated PIBCA nanoparticles with the polysaccharides considered in this work. It is also interesting to point out that only the nanoparticles prepared with heparin showed the presence of sulfur under the form of sulfonate because of the simultaneous detection of sodium which is the counter ion. The dramatic difference in sodium concentration evaluated from the ESCA analysis as well as the difference in the oxygen to carbon atomic ratio found between the nanoparticles prepared with heparin alone and with the blend of heparin and dextran indicated that the nanoparticles were respectively coated with heparin and with a blend of heparin and dextran. Measurements of zeta potential were achieved on dispersed samples. This parameter is sensitive to changes in the surface composition of colloids especially if charged molecules are present on the nanoparticle surface (24–26). It gives different information than the surface composition obtained by ESCA which is a technique applied on highly dessicated samples placed under high vacuum conditions during the analysis. Results showed that the zeta potential of the nanoparticles prepared with different polysaccharides clearly depended on the charge of the polysaccharides used. The results confirm that the polysaccharide was more likely located at the nanoparticle surface. Interestingly, nanoparticles obtained with a mixture of dextran 71 kDa and heparin showed intermediate value of zeta potential, in between the values obtained with the nanoparticles prepared with one of these polysaccharides alone. This result also suggests that dextran 71 kDa and heparin were present together on the nanoparticle surface in agreement with the analysis of the nanoparticles by ESCA. This clearly demonstrates that it is possible to modulate the charge of the obtained nanoparticles by using a combination of polysaccharides during their synthesis. The difference in zeta potential observed with the two types of nanoparticles prepared with dextran of different molecular weight (dextran 15 kDa and dextran 71 kDa) could be attributed to a slight contribution of the negatively charged poly(isobutyl cyanocrylate) core of the particles. It also suggests that neutral polysaccharides required a minimum molecular weight to hide the negative charge of the nanoparticle core.

Another useful technique for nanoparticle surface characterization is the evaluation of the complement activation. This was found to give a good predictive result of the *in vivo* fate of the nanoparticles after intravenous administration, especially regarding their uptake by the macrophages of the MPS (3,10,17). In the view of the development of new drug delivery systems with surface properties modulated by the presence of different polysaccharides it appeared interesting

to investigate if the nanoparticle capacity to activate the complement also differ with the type of polysaccharide coating. The analysis of both the non-activated and activated complement component C3 is achieved by 2D-immunoelectrophoresis using an anti-C3 antibody. A typical immunoelectrophoretic pattern of a serum in which the complement has been activated by particles was given by the positive control obtained with Sephadex beads. The negative control showed only a very low level of self activation of the serum indicating that suitable experimental conditions were fulfill to carry the analysis of the nanoparticles (Fig. 5B). Nanoparticles prepared with dextran 15 kDa and 71 kDa did not produce the same level of complement activation. While dextran 15 kDa-PIBCA nanoparticles induced a strong activation of the complement, the activation produced by dextran 71 kDa-PIBCA nanoparticles were only slightly higher than the one of the negative control. The fact that dextran 15 kDa-PIBCA nanoparticles produced a high level of complement activation could be attributed to the low molecular weight of the polysaccharide covering the nanoparticle surface. As with the zeta potential, it seems that dextran 15 kDa is too small to achieve the complete coating of the nanoparticle surface, which explains that the PIBCA core of the nanoparticle still contributes to the surface properties. According to the immunoelectrophoregrams, the nanoparticles coated with heparin did not produce any activated C3 component. Interestingly, the same immunoelectrophoretic profile was obtained with nanoparticles prepared with a mixture of dextran 71 kDa and heparin suggesting that in both cases complement activation was abolished because of the presence of heparin at the nanoparticle surface. This is explainable by the fact that heparin is a specific inhibitor of complement activation (27). The immunoelectrophoresis performed on serum incubated with nanoparticles prepared with dextran sulfate did not reveal the presence of any of the C3 components. It is supposed that complement either non activated or activated was so strongly adsorbed onto the nanoparticle surface that its release was prevented under the electrophoretic conditions used for this analysis. These nanoparticles showed a very unusual behavior considering their interaction with complement that have never been described before with other types of nanoparticles. In front of this very unusual case, the 2D-immunoelectrophoresis technique did not allow to conclude whether the dextran sulfate-coated nanoparticles activated or did not activated complement. Nevertheless, complement activation data show that the surface properties of the nanoparticles were controlled by their polysaccharidic surface decoration.

Finally, measurements of the antithrombic activity of nanoparticles coated with heparin were performed in order to verify whether the activity of a biologically active polysaccharide could be preserved after employing the radical emulsion polymerization process developed in this study. The data obtained showed that the biologic activity of heparin was preserved during the synthesis of the nanoparticles by this method. This opens interesting prospects for the formulation of nanoparticles carrying their own biologic activity.

In conclusion, this study shows that radical emulsion polymerization of isobutyl cyanoacrylate leads to new polysaccharide-coated nanoparticles within a single step of synthesis. The method was shown to be very flexible and could be applied with different polysaccharides allowing to modulate the surface properties of the nanoparticles by choosing the type of polysaccharide. Thus, nanoparticles with well define surface characteristics may be produced as well as nanoparticles displaying interesting biologic activity. The fact that nanoparticles with mixed polysaccharide surface composition could also be produced opens very interesting feature for the design of colloids with surface properties combining the characteristics of several polysaccharides. This is a step forward toward the development of new biomimetic systems.

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