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Pegylated Nanoparticles from a Novel Methoxypolyethylene Glycol Cyanoacrylate-Hexadecyl Cyanoacrylate Amphiphilic Copolymer

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Purpose. The aim of this work was to develop PEGylated poly(alkylcyanoacrylate) nanoparticles from a novel methoxypolyethyleneglycol cyanoacrylate-co-hexadecyl cyanoacrylate copolymer.

Methods. PEGylated and non-PEGylated nanoparticles were formed by nanoprecipitation or by emulsion/solvent evaporation. Nanoparticles size, zeta potential and surface hydrophobicity were investigated. Surface chemical composition was determined by X-ray photoelectron spectroscopy. Nanoparticle morphology was investigated by transmission electron microscopy after freeze-fracture. Nanoparticles cytotoxicity was assayed *in vitro*, onto mouse peritoneal macrophages. Cell viability was determined through cell mitochondrial activity, by a tetrazolium-based colorimetric method (MTT test). Finally, the degradation of PEGylated and non-PEGylated poly(hexadecyl cyanoacrylate) nanoparticles was followed spectrophotometrically during incubation of nanoparticles in fetal calf serum.

Results. Monodisperse nanoparticles with a mean diameter ranging between 100 and 200 nm were obtained using nanoprecipitation or emulsion/solvent evaporation as preparation procedures. A complete physico-chemical characterization, including surface chemical analysis, allowed to confirm the formation of PEG-coated nanoparticles. The PEGylation of the cyanoacrylate polymer showed reduced cytotoxicity towards mouse peritoneal macrophages. Furthermore, the presence of the PEG segment increased the degradability of the poly(hexadecyl cyanoacrylate) polymer in presence of calf serum.

Conclusions. We succeeded to prepare PEGylated nanoparticles from a novel poly(methoxypolyethyleneglycol cyanoacrylate-co-hexadecyl cyanoacrylate) by two different techniques. Physico-chemical characterization showed the formation of a PEG coating layer. Low cytoxicity and enhanced degradation were also shown.

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ABBREVIATIONS: poly(MePEGCA-co-HDCA), poly(methoxypolyethylenglycol cyanoacrylate-co-hexadecyl cyanoacrylate); MePEG, methoxypolyethylenglycol; MePEGCA, methoxypolyethylen glycol cyanoacetate; HDCA, *n*-hexadecyl cyanoacetate; poly(MePEGCA), poly(methoxypolyethylenglycol cyanoacrylate); PHDCA, poly(hexadecyl cyanoacrylate); MTT, tetrazolium-based colorimetric assay.

KEY WORDS: poly(ethylene glycol); poly(alkyl cyanoacrylate); nanoparticles; amphiphilic copolymers.

INTRODUCTION

The therapeutic potentialities of injectable particulate drug carriers (polymeric nanoparticles and liposomes) may be compromised by particle recognition by the macrophages of the mononuclear phagocyte system (MPS) and their rapid elimination from the bloodstream after intravenous injection (1). After adsorption of blood proteins (opsonins) onto their surface, colloidal systems accumulate in MPS organs, such as liver and spleen (1): thus, the therapeutic activity of compounds entrapped within these carriers is limited to the MPS.

Long-circulating nanoparticles or liposomes can be obtained by modifying their surface with hydrophilic, flexible and non-ionic polymers, such as poly(ethylene glycol) (PEG) (2–4). A PEG coating covalently bound to the carrier is preferable, particularly in the case of biodegradable particles, where an adsorbed coating layer is likely to be unstable *in vivo* (5). In the case of liposomes, long-circulating vesicles are generally prepared directly from PEGylated lipids (4). In the case of polymeric nanoparticles, the synthesis of amphiphilic PEG-R copolymers (where PEG is the hydrophilic segment and R represents a general hydrophobic unit) allows the preparation of PEGylated nanoparticles by classical procedures (6,7), ensuring the stability of the bound PEG. In the literature, various PEG-R amphiphilic copolymers have been described (8,9), but in nanoparticle technology, biodegradable diblock or multiblock PEG-R copolymers have been developed only from polyesters (6,7,10) and polyanhydrides (10).

We have chosen the more rapidly biodegradable poly(alkyl cyanoacrylate) (PACA) polymer as a suitable material for the development of polymeric injectable carriers. Previously, the only attempt at developing "stealth" PACA nanoparticles, by adsorption of Poloxamer/Poloxamines, was not successful *in vivo* (5). In a first approach, we succeeded in obtaining PEG-poly(isobutyl cyanoacrylate) (PIBCA) nanoparticles by emulsion/polymerization of the isobutyl cyanoacrylate monomer in an aqueous acidic medium in presence of PEG (11). PEG initiated the polymerization and was chemically coupled to the growing PIBCA. Other authors reported a zwitterionic synthesis of PEG-triphenylphosphine-PIBCA block copolymers(12) and the preparation of nanoparticles from this preformed material. Indeed, polymerization of IBCA initiated by phosphines showed near ideal living polymerization (13), whereas anionic polymerization initiated by PEG alkoxide salts, due to the well known high reactivity of the cyanoacrylate monomers and the high propagation rate during ionic polymerization (14), did not allow copolymers of defined structure to be obtained (12). However, the presence of the phosphine group in the polymer structure could lead to toxicity, making this copolymer unsuitable for intravascular administration.

More recently, we have synthesized a novel poly(methoxypolyethyleneglycol cyanoacrylate-co-hexadecyl cyanoacrylate) amphiphilic material for the preparation of PEG-coated PACA nanoparticles (15). We performed a single step condensation of methoxypolyethyleneglycol (MePEG) cyanoacetate with formaldehyde via the formation of the aminoderivate. A fatty alkyl chain was inserted into the polymer structure during the

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condensation reaction in order to modulate the hydrophobicity of the final compound.

The aim of this work was to develop PEG-coated PACA nanoparticles from this novel poly(methoxypolyethyleneglycol cyanoacrylate-co-hexadecyl cyanoacrylate) copolymer. A complete physico-chemical characterization, including surface chemical analysis, was carried out in order to confirm the formation of PEG-coated nanospheres prepared by nanoprecipitation or emulsion/solvent evaporation. We investigated whether the PEGylation of the cyanoacrylate polymer was able to reduce its cytotoxicity towards mouse peritoneal macrophages. Furthermore, we studied the effect of the presence of the PEG segment on the degradability of the poly(hexadecyl cyanoacrylate) polymer.

MATERIALS AND METHODS

Materials

The poly(methoxypolyethyleneglycol cyanoacrylate-co-hexadecyl cyanoacrylate) (poly(MePEGCA-co-HDCA)) copolymer (Fig. 1) was synthesized by condensation of methoxypolyethylene glycol (MePEG) 2K cyanoacetate with *n*-hexadecyl cyanoacetate in ethanol, in presence of formalin and dimethylamine. Details have been given elsewhere (15). MePEGCA and HDCA were also condensed separately in order to obtain respectively poly(methoxypolyethyleneglycol cyanoacrylate) (poly-(MePEGCA)) and poly(hexadecyl cyanoacrylate) (PHDCA), to use as controls.

Poly(vinyl alcohol) (PVA) (MW 15,000, 86–89% hydrolyzed, Fluka) was employed as colloidal stabilizer in the aqueous phase for the preparation of control non-PEGylated nanoparticles. All other reagents were of analytical grade.

Preparation of the Nanoparticles

The solubility at room temperature of 50 mg of the poly-(MePEGCA-co-HDCA) copolymer was tested in common organic solvents (0.5–1% w/v) used in nanoparticle technology, such as ethanol, acetone, methylene chloride and chloroform. The copolymer was considered very soluble (++) in the case of immediate dissolution, poorly soluble (+) in the case of a slower but complete dissolution under mechanical stirring, and insoluble (−) in the case of only partial dissolution.

Nanoparticles were formed by nanoprecipitation (16) or by emulsion/solvent evaporation (17). For the first procedure, the polymer (50, 100, 150 mg) was dissolved in 10 ml of tetrahydrofuran (THF), and the polymer solution was added, under magnetic stirring, to 20 ml of water. Particle precipitation occurred immediately. After solvent evaporation by Rotavapor®, an aqueous suspension of nanoparticles (2.5, 5, 7.5 mg/ml, respectively) was obtained. In the second procedure, the polymer (10, 20, 30, 40 mg) was dissolved in 2 ml CH_2Cl_2. In preliminary studies, we tested other organic solvents (methylene chloride, chloroform and their mixtures with acetone and ethyl acetate) in the preparation. The organic phase was then emulsified with 30 ml water, and the o/w emulsion formed was sonicated for 1 minute (Vibracell, Sonics & Materials Inc., Danbury, CT, USA). After solvent evaporation by magnetic stirring at room temperature, an aqueous suspension of nanoparticles was obtained. Nanoparticles were centrifuged and redispersed in water to obtain the desired final concentration (5, 10, 15 mg/ml). In both procedures, after evaporation of the organic solvent, the nanoparticle suspensions were filtered (prefilters Millex®, AP20, Millipore), and stored at 4°C or lyophilized (Christ Lyophilizer, D) over 24 hours (−30°C/+30°C) at 0.001 mbar pressure.

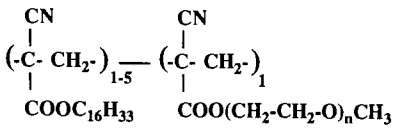

Fig. 1. Structure of the poly(MePEGCA-co-HDCA) copolymer.

Physico-chemical Characterization of the Nanoparticles

Particle Size and Stability

The size of nanoparticles was determined at 20°C by Quasi-Elastic Light Scattering (QELS), with a nanosizer (Coulter® N4MD, Coulter Electronics, Inc., Hialeah, FL, USA). The influence of the organic solvent, of the polymer concentration in the organic phase and of the preparation method was studied. Nanoparticle stability was evaluated by measuring nanoparticle size during 4 weeks of storage at 4°C.

Particle Surface Charge and Hydrophobicity of PEGylated and Non-PEGylated Nanoparticles

The surface charge of the nanoparticles, prepared by both nanoprecipitation and emulsion/solvent evaporation procedures, was investigated through zeta potential measurements in water (Zetasizer 4, with a multi-8 correlator 7032, Malvern Instr.).

Surface hydrophobicity of the nanoparticles was investigated by hydrophobic interaction chromatography (HIC). The nanoparticle suspension (1 ml) was injected into a column (bed volume 10 ml) (Pharmacia Biotechnology, Uppsala, S) filled with propyl agarose and connected to a pump (Minipuls II Gilson, Prosciences). Elution was performed using phosphate buffer saline (PBS). Particles which interacted with the gel were removed by washing with PBS containing Triton® X-100 (0.1% w/v) after elution of the first 40 ml. The flow rate was 0.8 ml/min. The optical density of the eluted samples was determined at 350 nm (Beckman 25 spectrophotometer).

Particle Surface Chemical Composition

XPS (X-ray photoelectron spectroscopy) was used to determine the composition of the nanoparticle surface (18). XPS signals were recorded using a VG Scientific ESCALAB MK2 system operated in the constant analyzer energy mode. An Al Kα X-ray source was used at a power of 200 W, and the pass energy was set at 20 eV. The pressure in the analysis chamber was about 5×10^{-8} mbar. Wide scans and high resolution C(1S) peaks were analyzed.

Particle Morphology

PEGylated nanoparticles prepared by nanoprecipitation were observed by transmission electron microscopy after freeze-fracture. A small drop of an aqueous particle suspension

was deposited on a copper planchet and rapidly frozen in liquid propane. Fracturing, etching and shadowing, using Pt-C, were performed in a Balzers freeze-etch unit. The replicas were observed in a Phillips 301 electron microscope.

Particle Cytotoxicity

The cytoxicity of the copolymer was evaluated by determining the viability of the mouse macrophage line J774 after incubation with different concentrations of PEGylated and non-PEGylated nanoparticles (5-1000 μg/ml) for 3 hours. The number of viable cells was determined by the estimation of their mitochondrial reductase activity using the tetrazolium-based colorimetric method (MTT) (19).

Particle Degradability

The degradation of PEGylated and non-PEGylated PHDCA nanoparticles was studied during incubation of nanoparticles in fetal calf serum (0.5 mg/ml) at room temperature, under mechanical stirring. Degradation was followed by spectrophotometric measurements using a UV/VIS spectrometer (Lambda 11, Perkin Elmer), according to a previously published procedure (20). The reduction in light transmission at 450 nm was determined. Light transmission measurements provide information about the time required for the degradation of the particles. The method can be employed when polymer degradation occurs by surface erosion, as in the case of PACA nanoparticles (21), rather than by bulk hydrolysis. PACA can be degraded by hydrolysis of the lateral alkyl chain, mainly by enzymatic degradation, with a decrease of the degradation rate with increasing length of the alkyl chain (21).

RESULTS AND DISCUSSION

Synthesis of the Copolymer

In order to obtain a novel poly(MePEGCA-co-HDCA) polymer), we used a base-catalyzed condensation of MePEGCA and HDCA with formaldehyde. The poly(MePEGCA-co-HDCA) copolymer, with MePEG cyanoacetate/hexadecyl cyanoacetate ratios ranging between 1:1 and 1:5, was synthesized successfully (15). Table I reports all the data related to the composition (obtained by NMR spectra) and weight average MW of the copolymers obtained (calculated by GPC chromatogram), depending on the initial PEG/hexadecyl ratio. The theoretical MW was obtained by considering the MW of the constitutional repeating units, that was 2085 for the MePEG cyanoacetate, 327 for the hexadecyl cyanoacetate and 30 for the formaldehyde.

The hydrophobicity of the polymer was easily modulated by adjusting the MePEGCA/HDCA ratio. In this respect, to produce nanoparticles with "stealth" properties, it would be desirable to use the copolymer with the highest MePEGCA/HDCA ratio for nanoparticle preparation, in order to ensure a high PEG surface density. Indeed, it is expected that, due to the amphiphilic properties of the copolymer, the PEG chains would migrate to the interface with the aqueous phase during nanoparticle preparation, and localize at the surface of the formed particles. In contrast, a lower MePEGCA/HDCA ratio would assure a better yield of production of insoluble nanoparticles. For the present study, a copolymer with a 1:5 PEG/hexadecyl ratio was chosen (Fig. 1).

The separate condensation of MePEGCA and HDCA led to the formation of poly(MePEGCA) and PHDCA polymers respectively, to be used as controls.

Table I. Composition and MW of the Copolymers Obtained by Condensation/Polymerisation of MePEG Cyanoacetate with Increasing Amounts of Hexadecylcyanoacetate (1:1/1:5)

Initial ratio MePEGCA/HDCA	Polym. comp.[a]	Weight-avg. MW	
		Theoretical[b]	Observed[c]
1:5	1:5.6	3719	3780
1:4	1:4	3506	3441
1:3	1:2.5	3410	3102
1:2	1:2	2962	2763
1:1	1:0.6	2373	2424

[a] Calculated from 1H-NMR spectra.
[b] Calculated by considering the constitutional repeating units according to the initial ratio.
[c] Calculated from GPC chromatograms.

Solubility of the Copolymer

First, copolymer solubility was determined in the organic solvents employed in nanoparticle technology. As illustrated in Table II, the copolymer (1:5 PEG/hexadecyl ratio) was found to be insoluble in most of the organic solvents miscible with water, such as ethanol and acetone, with the exception of THF. This can be explained by the presence of the highly hydrophobic fatty alkyl chains in the copolymer structure. On the other hand, the copolymer was found to be soluble in all chlorinated solvents, as well as in their 1:1 mixtures with acetone or ethyl acetate.

Particle Size and Stability

Monodisperse nanoparticles with a mean diameter in the size range 100-200 nm were prepared in a single step by nano-

Table II. Solubility of the Synthesized Copolymer (1:5 PEG/Hexadecyl Cyanoacetate Ratio) in Different Organic Solvents Employed for the Preparation of Colloidal Nanoparticles

Solvent	Solubility PEG-PHDCA nanoparticles
acetone	−
ethyl acetate	−
ethanol	−
THF	+
methanol	−
methylene chloride	++
chloroform	++
methylene chloride/acetone	++
methylene chloride/ethyl acetate	+
chloroform/acetone	++
chloroform/ethyle acetate	++

++ Very soluble.
+ Soluble.
− Practically insoluble.

Table III. Size of PEGylated and Non-PEGylated Nanoparticles Obtained by Nanoprecipitation (Organic Solvent: Tetrahydrofuran) and by Emulsion/Solvent Evaporation (Organic Solvent: Methylene Chloride) as a Function of the Polymer Concentration

Polymer conc (mg/ml)	Particle size (nm)				
	nanoprecipitation			emulsion/solvent evaporation	
	PEG-PHDCA		PHDCA	PEG-PHDCA	PHDCA
		after 4 weeks storing			
5	98	101	–	190	–
10	109	114	–	201	–
15	162	154	299	190	–
20	–	–	–	199	400

Note: Index of polydispersity <0.1 for all samples.

precipitation or emulsion/solvent evaporation, the size depending on polymer concentration in the organic phase, preparation procedure (diffusion- or emulsion-based) and the nature of the organic solvent. When the nanoprecipitation technique was used, monodisperse nanoparticles with a mean diameter ranging between 98 and 162 nm were obtained (Table III), depending on the polymer concentration in the organic phase. By decreasing the polymer concentration in the organic phase, a linear decrease in the particle size was observed. The organic solvent used was THF, since, as explained above, the copolymer was insoluble in the other common solvents miscible with water.

In the case of the preparation of nanoparticles by the single emulsion/solvent evaporation technique, several organic solvents were tested. Nanoparticles with a mean diameter between 118 and 199 nm were obtained, with an unimodal size distribution except in the case of mixtures containing ethyl acetate, where the reported nanometric size corresponded to only 20% of the particle population, and the rest of the particles exhibited a diameter of >1 μm (Table IV). Thus, we analyzed the size distribution as a function of the polymer concentration in the organic phase with methylene chloride as the organic solvent. As shown in Table III, decreasing of the polymer concentration in the organic phase did not lead to a decrease of particle size after solvent evaporation. Thus, the nanoprecipitation technique allowed better control of particle size, compared to the emulsion/solvent evaporation technique.

Furthermore, due to the presence of PEG in their structure, no further stabilizer was needed for the preparation of these nanoparticles, which were observed to be very stable: storage during 4 weeks at +4°C storing did not show any significant change in nanoparticle size (Table III).

Table IV. Size of Nanoparticles Obtained by Emulsion/Solvent Evaporation in Different Organic Solvents

Solvent	Particle size (nm)
methylene chloride	199
methylene chloride/acetone	169
methylene chloride/ethyl acetate	154[a]/>1000
chloroform/acetone	166
chloroform/ethyl acetate	118[a]/>1000

[a] Represents 20% of the particle population (% of the signal intensity).

Particle Surface Charge and Hydrophobicity

Surface charge analysis suggested the presence of PEG at the surface of the particles: PEGylated nanoparticles exhibited a zeta potential value close to neutrality, compared with non-PEGylated nanoparticles (−25/−30 mV) (Fig. 2). Indeed, in stabilized suspensions, a zeta potential value close to the neutrality is due to non ionic materials on the particle surface. The preparation method affected slightly the zeta potential of the particles (PEGylated or not). However, in the case of PEGylated nanoparticles, this slight difference (5–10 mV) can be considered as significant, and suggested a better localization of the PEG chains at the particle surface in the case of the emulsion/solvent evaporation procedure. This could be explained by a slower formation of solid particles during the preparation, allowing a reorganization of PEG chains and self assembly of the amphiphilic copolymer. On the contrary, in the nanoprecipitation procedure, the diffusion of the organic solvent (THF in our case) towards water and polymer precipitation under the form of solid nanoparticles are immediate events.

Hydrophobicity surface studies (Fig. 3) showed that non-PEGylated PHDCA nanoparticles were hydrophobic, and interacted with the propyl-agarose gel phase and were removed only

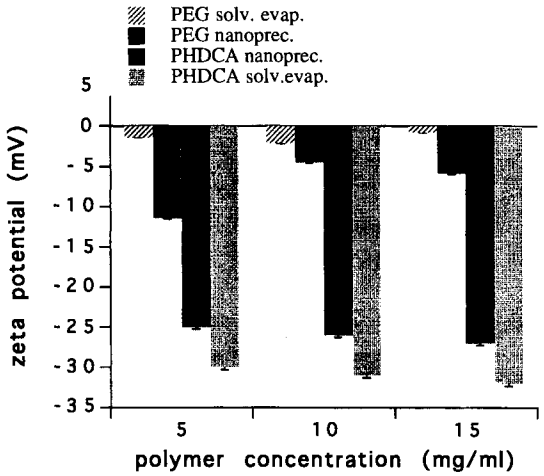

Fig. 2. Zeta potential of PEGylated and non-PEGylated nanoparticles obtained by emulsion/solvent evaporation (organic solvent: methylene chloride) or nanoprecipitation (organic solvent: tetrahydrofuran).

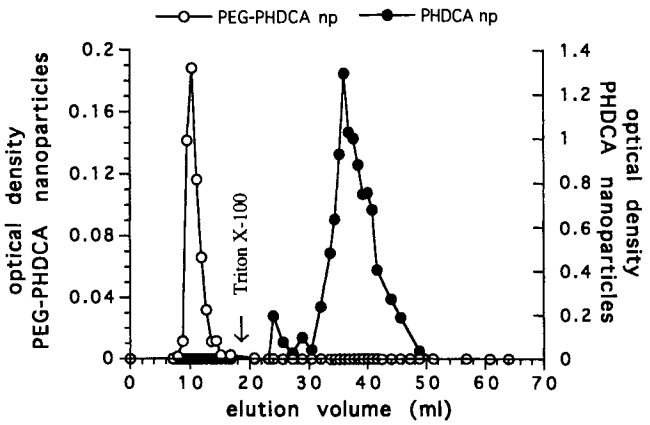

Fig. 3. HIC chromatogram of poly(MePEGCA-co-HDCA) and PHDCA nanoparticles prepared by nanoprecipitation.

by washing with Triton® X-100; on the contrary, as expected, PEGylated PHDCA particles exhibited a more hydrophilic surface, and were eluted easily through the gel phase. Thus, the use of PEGylated material allows the preparation of hydrophilic nanoparticles without any further surface modification.

Particle Surface Chemical Composition

Surface chemical analysis of poly(MePEGCA-co-HDCA) nanoparticles confirmed the presence of a PEG coating layer at their surface. XPS analysis was performed with poly(MePEGCA-co-HDCA) and non-PEGylated PHDCA nanoparticles. The water-soluble poly(MePEGCA) polymer was also used as a control. As depicted in Fig. 4a, the high resolution C(IS) peak from eV callé ou solidaires MePEGCA (A) displayed a major component at 286.1 eV, corresponding to C-O, and a component at 285 eV (C-C and C-H). The same component at 286.1 eV was found for poly(MePEGCA-co-HDCA) nanoparticles (4b), and was less evident for PHDCA (4c), where only a major peak at 285 eV, assigned to C-C and C-H was observed. PEGylated nanoparticles exhibited a PEG layer at their outer surface. Concerning a quantitative evaluation of the amount of PEG at the surface, a study on hydrated samples, where the PEG chains are well extended in the outer layer, is under way.

Particle Morphology

In order to investigate the morphology of PEGylated nanoparticles prepared by nanoprecipitation, and evidence the formation of a PEG coating layer, nanoparticle cross-section was observed by transmission electron microscopy after freeze-fracture. The PEGylated particles (Fig. 5) showed a round shape, but the slightly rough outer surface did not confirm the presence of PEG chains as evidenced by XPS analysis (Fig. 4). Indeed, the PEG layer formed by PEG 2000 is only a few nanometers of length, thus not easy to visualize. The inner core appeared as a porous dense polymeric matrix.

Particle Cytotoxicity

Cell viability studies with mouse macrophages indicated a low cytotoxicity of the nanoparticles composed of poly(MePEG-co-HDCA), even at high concentration. At concentra-

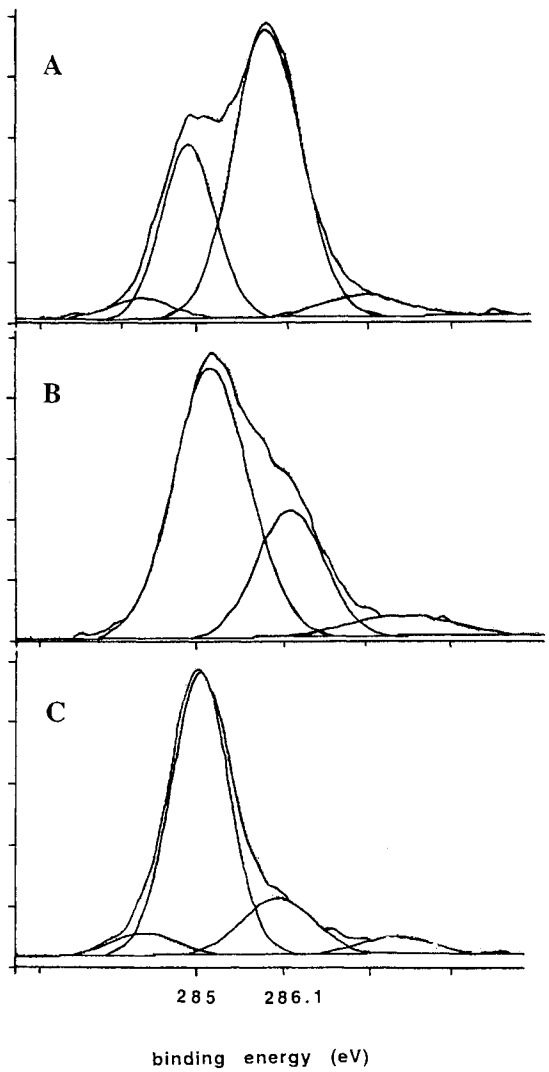

Fig. 4. XPS High Resolution C(1S) spectra of poly(MePEGCA) polymer (A), poly(MePEGCA-co-HDCA) (B) nanoparticles and PHDCA nanoparticles (C).

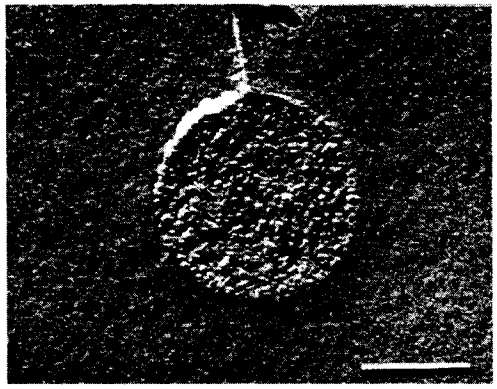

Fig. 5. TEM image after freeze fracture of nanoparticles made of poly(MePEGCA-co-HDCA) (a) and PHDCA (b) bar = 200 nm.

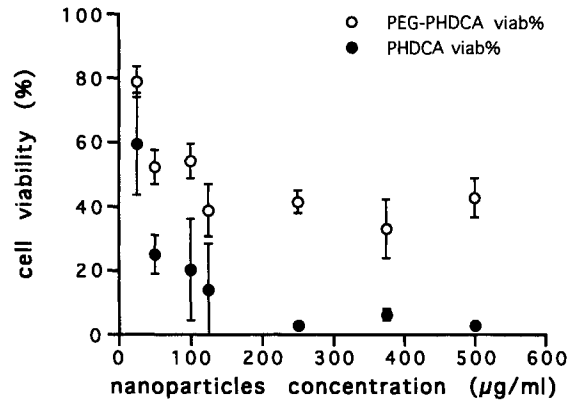

Fig. 6. Effect of poly(MePEG-co-HDCA) (A) and PHDCA (B) nanoparticle concentration on cell viability (3 hours incubation).

tions lower than 200 µg/ml, no significant effect of the PEGylation of the polymer on cell viability was observed. At higher concentrations, cell viability was reduced, and the concentration of PEGylated nanoparticles toxic for the 50% of J774 cells was approximately 500 µg/ml (Fig. 6). However, this value was less than 100 µg/ml for the non-PEGylated PHDCA nanoparticles. Thus, the PEGylation was found to significantly decrease the cytotoxicity of the polymer towards this cell line. Studies are in progress to elucidate the mechanism of this reduced cytotoxicity: a possible explanation could be a reduced nanoparticle internalization by the macrophages, leading to less release of toxic products within the cell, or a reduced contact with the cell membrane.

Particle Degradability

The degradation of the PEGylated PHDCA, as shown in Fig. 7, was faster (30% within 3 hours) than the corresponding non-PEGylated PHDCA polymer, where no degradation occurred in the first 3 hours of incubation. The decrease of the degradation rate with increasing length of the alkyl chain of cyanoacrylic polymers (20) is well established. Therefore, it was not surprising to observe that PHDCA were not degraded during the first 3 hours. In contrast, despite the presence of the high MW PEG segment (2000 Da in a copolymer of total mass of less than 4000 Da), 30% of the PEGylated PHDCA nanoparticles were degraded in the first 3 hours. This increased degradability of the PEG-PHDCA copolymer, compared with the corresponding PHDCA polymer, could be explained by an enhanced penetration of the incubation medium due to the presence of the highly hydrophilic PEG moieties in the copolymer structure. This would facilitate the action of esterases present in the serum, which are known to play a major role in the degradation of polyalkylcyanoacrylates (21). Another explanation could be a more important contribution of the non-enzymatic hydrolysis in the degradation of the PEGylated polymer.

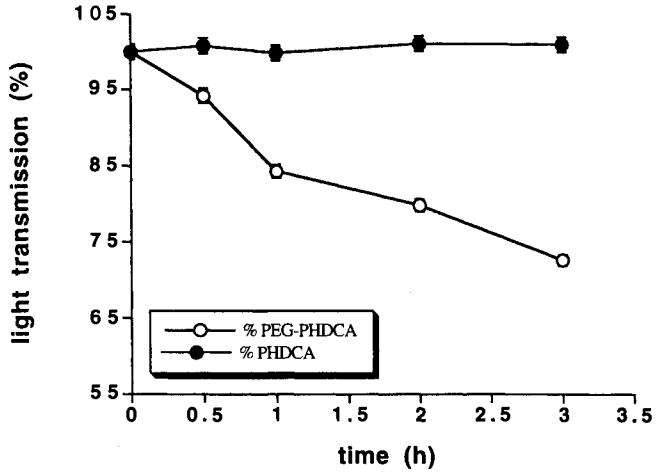

Fig. 7. Degradation profile of poly(MePEG-co-HDCA) and PHDCA nanoparticles in fetal calf serum.

CONCLUSIONS

This work describes the preparation of sterically-stabilized PEG-coated nanoparticles directly from a novel amphiphilic PEG cyanoacrylate copolymer. This amphiphilic copolymer was obtained by condensation/polymerization of PEG- and hexadecyl-cyanoacetate esters with formaldehyde.

Monodisperse PEGylated nanoparticles were easily prepared by nanoprecipitation or emulsion/solvent evaporation. The advantage of preparing PEG-coated injectable nanoparticles from PEG-R copolymers is that a covalent PEG binding would ensure a stable coating, potentially able to prevent any interaction with blood components *in vivo*. A complete physicochemical characterization, including surface chemical analysis, allowed us to confirm the formation of a PEG coating layer at the nanoparticle surface. Preliminary data showed that the PEGylation of the cyanoacrylate polymer was able to reduce its cytotoxicity towards mouse peritoneal macrophages. Furthermore this novel copolymer was shown to be biodegradable, which is extremely important if it is to be administered intravenously.

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