



Synthesis of poly(alkyl cyanoacrylate)-based colloidal nanomedicines

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Nanoparticles developed from poly(alkyl cyanoacrylate) (PACA) biodegradable polymers have opened new and exciting perspectives in the field of drug delivery due to their nearly ideal characteristics as drug carriers in connection with biomedical applications. Thanks to the direct implication of organic chemistry, polymer science and physicochemistry, multiple PACA nanoparticles with different features can be obtained: nanospheres and nanocapsules (either oil- or water-containing) as well as long-circulating and ligand-decorated nanoparticles. This review aims at emphasizing the synthetic standpoint of all these nanoparticles by describing the important aspects of alkyl cyanoacrylate chemistry as well as the experimental procedures and the different techniques involved for the preparation of the corresponding colloidal devices.

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Nanotechnology has emerged as a promising area of research in which scientists from both academia and industry put a lot of effort, hoping for the best with regard to life in future. It is a highly multidisciplinary field which consists of engineering functional systems at the molecular scale and covers applied physics, materials science, interface and colloid science, supramolecular chemistry as well as chemical, mechanical, and electrical engineering. One of the direct applications of nanotechnology is devoted to the medical and pharmacology areas, also called nanomedicine, the most famous example being nanoparticle drug delivery.

Indeed, a crucial impulse was given to nanomedicine with the development of various types of drug-carrier nanodevices, made possible by means of multidisciplinary approaches—organic and polymer chemistry, physicochemistry, pharmacology, etc. Among suitable nanodevices for drug delivery, nanoparticles on the basis of biodegradable poly(alkyl cyanoacrylate) (PACA) polymers have appeared as an established technology for colloidal nanomedicine.

Introduced more than 25 years ago in the field of pharmacology, PACA drug carriers have demonstrated significant results in numerous pathologies such as cancer, severe infections (viral, bacteriologic, parasite) as well as several metabolic and autoimmune diseases, well-reviewed in the recent literature.^{1–6} As a complementary work, the objective of the present review is to emphasize the synthetic aspect of these colloidal carriers by describing, as precisely as possible, the chemistry of the cyanoacrylate monomers, their polymerization as well as the different structures and morphologies of the corresponding nanoparticles. In particular, description of this PACA-based nanotechnology will start from the simplest nanocarriers to more sophisticated and ‘smart’ drug delivery devices. The reader who would like a more exhaustive point of view about the biologic and pharmaceutical aspects of PACA nanoparticles as well as the drugs successfully incorporated in such colloidal devices is referred to the above-mentioned references. ■

ALKYL CYANOACRYLATE MONOMERS AND RELATED POLYMERS

General Features of Alkyl Cyanoacrylate Monomers

Alkyl cyanoacrylates are widely known monomers, extremely appreciated for their very high reactivity

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DOI: 10.1002/wnan.015

and the excellent adhesive properties of the resulting polymers. On one hand, the famous Superglue (manufactured by Henkel), which contains short alkyl chain cyanoacrylates, is commonly employed by the general public for repairing and do-it-yourself activities, whereas longer alkyl chain cyanoacrylates have been developed for biomedical purposes such as surgical glue for the closure of skin wounds^{7–13} and embolitic material for endovascular surgery.^{10,11,14} Indeed, several commercial products have emerged from the use of cyanoacrylates in the biomedical area, mainly devoted to tissue adhesion. For instance, methyl cyanoacrylate (MCA, Figure 1) is the main component of the Biobond tissue adhesive and longer alkyl ester chain cyanoacrylates, such as *n*-butyl cyanoacrylate (*n*BCA, Figure 1) or octyl cyanoacrylate (OCA, Figure 1), were commercialized under the trademarks of Indermil, Liquidband, and Dermabond, respectively.

The synthesis of alkyl cyanoacrylate monomers has been described in the patent literature since 1949.^{15–18} Basically, the main strategy to achieve α -cyanoacrylates comprises two steps. First, the corresponding alkyl cyanoacetate is reacted with formaldehyde in the presence of a basic catalyst, to form PACA oligomers (by the so-called Knoevenagel condensation reaction). The catalyst is a base, either inorganic (e.g., sodium or potassium hydroxide, ammonia) or organic (e.g., quiloline, piperidine, dialkyl amines). Then, pure alkyl cyanoacrylate monomer is recovered by a thermal depolymerization reaction of the previously obtained oligomers, using suitable stabilizers such as protonic or Lewis acids

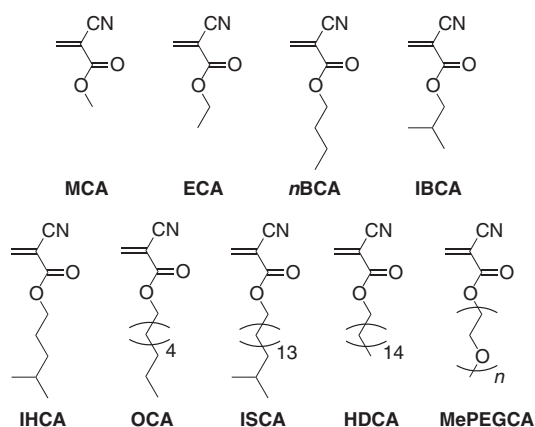


FIGURE 1 | Structure of alkyl cyanoacrylates described in the literature: methyl cyanoacrylate (MCA), ethyl cyanoacrylate (ECA), *n*-butyl cyanoacrylate (*n*BCA), isobutyl cyanoacrylate (IBCA), isohexyl cyanoacrylate (IHCA), octyl cyanoacrylate (OCA), isostearyl cyanoacrylate (ISCA), hexadecyl cyanoacrylate (HDCA), and methoxypoly(ethylene glycol) cyanoacrylate (MePEGCA).

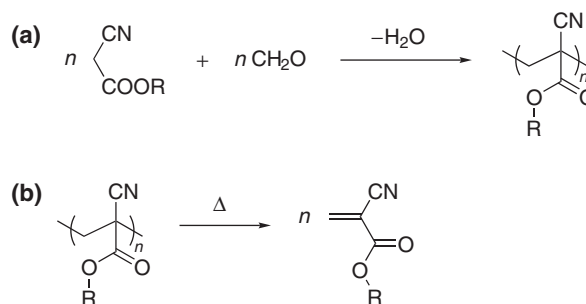


FIGURE 2 | Synthesis of alkyl cyanoacrylate monomer via Knoevenagel condensation reaction (a) and subsequent thermal depolymerization (b).

with small amounts of a free-radical inhibitors to prevent repolymerization (Figure 2).

From that moment on, the synthetic protocol remained almost unchanged. It was only slightly modified and improved essentially by playing with the nature of the solvent mixture,^{19,20} by applying a transesterification approach for making cyanoacrylates bearing longer alkyl ester chains,²¹ or by using a more efficient catalyst (namely pyrrolidine) for the condensation step.²²

Polymerization of Alkyl Cyanoacrylates in Homogeneous Media

On the fringe of typical vinyl monomers [styrenics, (meth)acrylates, etc.] is the alkyl cyanoacrylate family, which seems to be an exotic class of polymerizable compounds. Indeed, due to the presence of two powerful electro-withdrawing groups in the α -carbon of the double bond, namely ester (COOR) and cyano (CN), alkyl cyanoacrylate monomers exhibit a remarkable reactivity toward nucleophiles such as anions (hydroxide, iodide, alcoholate, etc.) or weak bases (alcohol, amine, etc.), resulting in a very high polymerization rate. Even traces of one of the above-mentioned compounds in the reaction medium are sufficient to initiate such a fast polymerization. This explains why alkyl cyanoacrylates are extremely difficult to handle under their pure form and that batches of these monomers are usually maintain stable with a small amount of acidic stabilizers (e.g., SO₂, sulfonic acid, etc.).

PACA can be synthesized according to three distinct types of polymerization: (1) anionic, (2) zwitterionic, and (3) radical (Figure 3). In practice, because of the exceptional reactivity of alkyl cyanoacrylate derivatives, anionic and zwitterionic polymerization mechanisms are by far predominant under conventional experimental conditions with

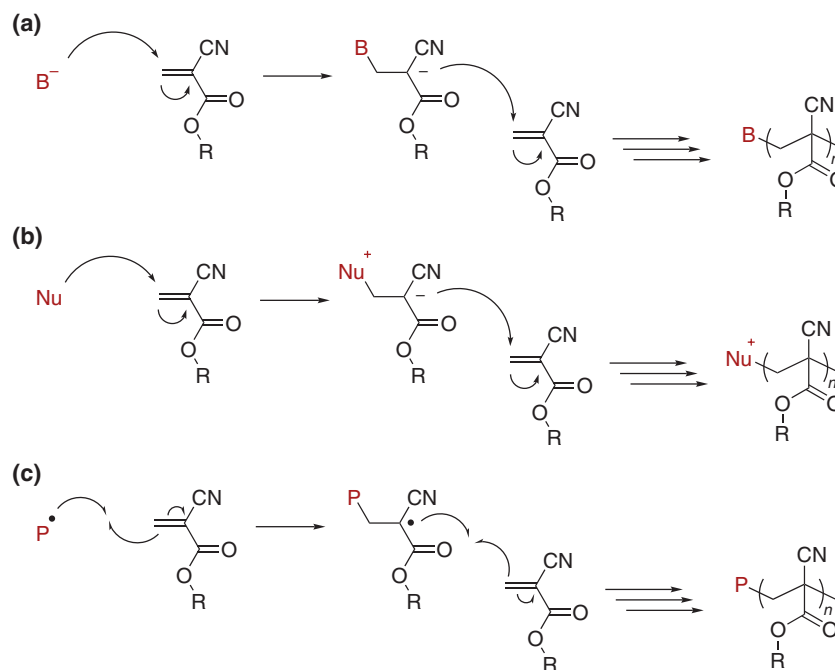


FIGURE 3 | Initiation and propagation steps involved during anionic (a), zwitterionic (b), and radical (c) polymerizations of alkyl cyanoacrylate monomer initiated by a base (B⁻), a nucleophile (Nu), and a radical (P[•]), respectively.

respect to a pure radical process. This explains why studies on alkyl cyanoacrylates polymerization in both homogeneous (i.e., bulk or solution) and heterogeneous (i.e., emulsion, microemulsion) media were mainly devoted to anionic and zwitterionic processes.

Synthesis of Homopolymers

In this field, an extensive work has been accomplished by Pepper and coworkers to get a better understanding of the involved polymerization mechanisms depending on the experimental conditions.^{23–26} Indeed, the homopolymerization in solution of ethyl cyanoacrylate (ECA, Figure 1) and *n*BCA were initiated either by simple anions (CH₃COO⁻, CN⁻, I⁻, etc.) or by covalent organic bases (Et₃N, pyridine, etc.), leading to anionic or zwitterionic polymerizations, respectively.²³ For zwitterionic polymerization of *n*BCA, the influence of the nature of the initiator as well as other experimental conditions (inhibiting species, presence of water, etc.) on both the main characteristics of the obtained polymer (number-average molecular weight, molecular weight distribution) and polymerization kinetics (monomer conversion, polymerization rate, etc.) were investigated through a small library of covalent organic bases such as phosphine,^{26–29} pyridine^{24,27} and amine^{25,27,30} derivatives. Considering anionic polymerization, the same research group used tetrabutyl ammonium salts (hydroxide, bromide, acetate, and substituted

acetates) as the initiating species for the polymerization of *n*BCA at 20–40°C in tetrahydrofuran (THF) and reported a nearly ideal living polymerization in the case of the hydroxide-based initiator.^{31–33}

Even though anionic and zwitterionic mechanisms are more likely to occur for the polymerization of alkyl cyanoacrylates, free-radical polymerization was believed to be the main chain-extension process during homopolymerization^{34–37} and copolymerization^{37,38} carried out in bulk when a suitable inhibitor is introduced in the reaction medium. However, even under these specific inhibition conditions, anionic polymerization is not totally suppressed but is made negligible regarding the timescale of the polymerization reaction. In particular, Canale et al.³⁴ used in 1960, boron trifluoride–acetic acid complex while conducting free-radical bulk polymerization of MCA at 60°C initiated by azobisisobutyronitrile (AIBN), whereas Bevington et al.³⁶ used propane-1,3-sultone as an efficient inhibitor against anionic polymerization for the free-radical polymerization of MCA in bulk or in 1,4-dioxane at 60°C, initiated by AIBN or benzoylperoxide (BPO). In 1983, Yamada et al.³⁷ polymerized ECA in bulk at 30°C with a small amount of acetic acid or propane-1,3-sultone and from their results, they extracted very high propagation rate constants: $k_p = 1622 \text{ l mol}^{-1} \text{ s}^{-1}$ in the presence of acetic acid and $k_p = 1610 \text{ l mol}^{-1} \text{ s}^{-1}$ in the presence of propane-1,3-sultone. As a

comparison, methyl methacrylate (MMA) which is considered as a highly reactive monomer gave $k_p = 450 \text{ l mol}^{-1} \text{ s}^{-1}$ at 30°C .³⁹

Synthesis of Copolymers

Alkyl cyanoacrylates were also copolymerized with more 'common' vinyl monomers through a free-radical process (using trifluoride–acetic acid complex as an efficient inhibitor against anionic polymerization) to give different kinds of copolymers, depending on the nature of the comonomer.³⁸ Random copolymers with MMA were achieved in bulk, whereas alternating copolymers with styrene were reported in benzene solution at 60°C under AIBN initiation. Considering bulk properties, random copolymers with 10% MMA exhibit physical properties similar to the PMCA homopolymer, whereas alternating copolymers with styrene had an enhanced thermal stability compared with random copolymers. Hall et al., who previously investigated the reactions of electron-rich olefins with electron-poor olefins,^{40–42} confirmed the alternating copolymer starting from a 1:1 styrene : MCA mixture, either initiated by AIBN under UV light at 40°C in benzene solution or produced spontaneously at room temperature.⁴³ However, when using other comonomers such as isobutyl vinyl ether, *p*-methoxystyrene or β -bromostyrene, copolymerizations with MCA led to mixtures of (co)polymers and/or small adducts.⁴³

In 1978, a comprehensive synthetic approach of bis(alkyl cyanoacrylate)s was proposed by Buck starting from anthracene adducts.⁴⁴ These difunctional alkyl monomers derived from cyanoacrylates were copolymerized with monofunctional alkyl cyanoacrylates such as MCA and isobutyl cyanoacrylate (IBCA, Figure 1), resulting in crosslinked macromolecular adhesive compositions exhibiting superior mechanical properties under both dry and wet environments than the noncrosslinked counterparts, which could be advantageously employed as pit and fissure sealant in dentistry.

More sophisticated macromolecular architectures such as diblock and triblock copolymers comprising poly(ethylene glycol) (PEG) and PACA blocks were also synthesized in homogeneous media via zwitterionic polymerization.⁴⁵ The synthesis involved the preparation of triphenylphosphine end-capped monohydroxyl and dihydroxyl PEGs, giving the corresponding monofunctional and difunctional macrozwitterionic initiator. The polymerization of IBCA was then initiated with each one of the macroinitiators in THF at ambient temperature to afford PIBCA-*b*-PEG

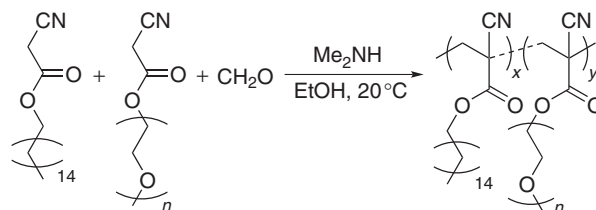


FIGURE 4 | Synthesis of random, comb-like poly[(hexadecyl cyanoacrylate)-*co*-methoxypoly(ethylene glycol) cyanoacrylate] [P(HDCA-*co*-MePEGCA)] copolymer via Knoevenagel condensation reaction.

diblock and PIBCA-*b*-PEG-*b*-PIBCA triblock copolymers with tuneable compositions in good match with the initial stoichiometry.

Synthesis of poly[(hexadecyl cyanoacrylate)-*co*-methoxypoly(ethylene glycol) cyanoacrylate] [P(HDCA-*co*-MePEGCA)] comb-like copolymers exhibiting amphiphilic properties was reported by Peracchia et al.⁴⁶ This original approach derived from Knoevenagel condensation reaction where corresponding cyanoacetates, namely hexadecyl cyanoacetate and PEG monomethyl ether cyanoacetate, were reacted with formaldehyde in the presence of dimethylamine as the catalyst (Figure 4). Thanks to the slow, *in situ* formation of the cyanoacrylate monomers, it allowed the polymerization process to be better controlled compared with a direct anionic polymerization. Besides, the composition of the copolymer (and thus its hydrophilicity/hydrophobicity) can be adjusted simply by varying the initial cyanoacetates feed ratio.

POLY(ALKYL CYANOACRYLATE)-BASED NANOPARTICLES

General Consideration on the Synthesis of Poly(alkyl cyanoacrylate) Nanoparticles

Nanoparticle is a collective name for two different types of colloidal objects, namely nanospheres (NS) and nanocapsules (NC), which can be separately obtained depending on the preparation process. Basically, nanospheres are matrix systems constituted by the polymer in which the drug is physically and uniformly dispersed, whereas nanocapsules are vesicular systems in which the drug is solubilized in a liquid core, either water (w-NC) or oil (o-NC), surrounded by a thin polymer layer (Figure 5).

During the last 25 years, an important breakthrough in this field has been witnessed with the development of PACA nanoparticles as colloidal drug carriers. Polymerizations in heterogeneous media (i.e., emulsion, dispersion, miniemulsion,

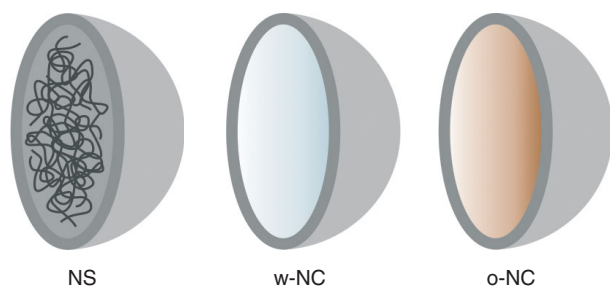


FIGURE 5 | Schematic representation of nanospheres (NS), water-containing nanocapsules (w-NC), and oil-containing nanocapsules (o-NC).

microemulsion)^{47,48} and spontaneous emulsification techniques^{49–51} are two well-known approaches for the preparation of polymeric particles, which have also been intensively used for the confection of PACA nanoparticles as colloidal drug carriers for *in vivo* administration.

Synthesis of Nanospheres

In 1979, Couvreur et al. first developed a simple process to directly generate stable MCA or ECA nanospheres, consisting of a dropwise addition of the monomer into a vortexed HCl solution ($2 < \text{pH} < 3$) containing a nonionic or a macromolecular surfactant.⁵² Since then, numerous studies aiming at establishing relevant parameters governing the polymerization kinetics as well as the characteristics of the macromolecules and the nanospheres have been reported. It has been shown that the nature and the concentration of the surfactant played a significant role on the particle size,^{53–61} whereas the type of both the monomer and the surfactant strongly influenced the molar mass of the obtained polymer.^{55–58} Besides the monomer concentration,^{53,59,60,62} the pH of the reaction medium^{53,55–58,60–64} and the concentration of sulfur dioxide (acting as a polymerization inhibitor)⁵⁷ were also crucial parameters which strongly affected the macromolecular and/or colloidal properties of the nanospheres. The size of the colloidal objects which can be obtained usually ranged from 50 to 300 nm,^{54,59,60} which is a well-adapted window for colloidal drug delivery devices, especially by intravenous administration.

For a more fundamental standpoint, several tentative mechanisms have been postulated.^{65,66} It has been reported that the emulsion/dispersion polymerization in acidic medium is not that trivial and proceeds via a stepwise, anionic mechanism comprising reversible propagation and reversible termination steps^{63,64} (Figure 6). Basically, PACA oligomers are formed in the monomer droplets and are reversibly

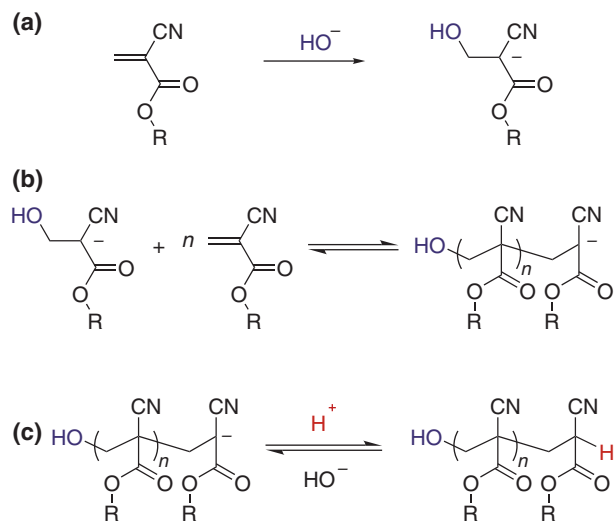


FIGURE 6 | Schematic representation of poly(alkyl cyanoacrylate) formation via the stepwise anionic polymerization mechanism in emulsion/dispersion. Initiation step (a), reversible propagation step (b), and reversible termination step (c).

terminated by the acid-inhibiting agents present in the monomer. This step is followed by a re-initiation reaction of terminated species by still living chains, leading to further polymerization until a molecular weight balancing is reached, similar to depolymerization/repolymerization events.⁶⁶ One should be aware that in all these mechanisms, the polymerization is postulated to be initiated by the hydroxyl ions from the aqueous phase independently of other reactants existing in the polymerization medium.

On the basis on an interfacial polymerization mechanism,^{67,68} Limouzin et al. polymerized *n*BCA in emulsion and miniemulsion in the presence of dodecylbenzenesulfonic acid (DBSA) acting as both surfactant and terminating agent (also termed tersurf).⁶⁹ By releasing protons at the water/oil interface, DBSA allowed the interfacial, anionic polymerization to be drastically slowed down through a (reversible) termination reaction and to proceed under a fairly controlled fashion leading to stable high solids content ($\sim 20\%$) *Pn*BCA nanospheres. The miniemulsion technique was also used by Weiss et al. for the preparation of *Pn*BCA nanospheres. By varying the concentration of the surfactant (SDS), and by adding sodium hydroxide as the initiating species, high solids content dispersions up to 10% with average diameters ranging from 110 to 360 nm were obtained.⁷⁰

Synthesis of Nanocapsules

Nanocapsules are reservoir-type nanoparticles in which drugs can be encapsulated according to their

intrinsic solubility. In other words, oil-containing nanocapsules will be able to encapsulate hydrophobic drugs, whereas hydrophilic ones will be efficiently encapsulated into water-containing nanocapsules.⁷¹ The nature of the nanocapsules (i.e., water-containing or oil-containing) is determined by the nature of the dispersed phase involved in a heterogeneous polymerization process, usually emulsion or microemulsion. Basically, the macromolecular shell is formed by the spontaneous anionic polymerization of alkyl cyanoacrylate occurring at the interface between the dispersed and the continuous phase. Historically, oil-containing nanocapsules were first developed by Fallouh et al.⁴⁹ through a simple protocol: a solution of monomer and oil in a water-miscible solvent (usually ethanol) is poured into an aqueous solution of surfactant (usually Poloxamer 188) under vigorous stirring, leading to small oil/monomer droplets at the interface of which the polymerization is initiated by hydroxide ions present in water. Gallardo et al.⁶⁵ reported that the crucial parameters for achieving nanocapsules lies: (1) in the diffusion behavior of the organic solvent (acting as a monomer support) within the aqueous phase, which ultimately governs the reservoir nature of the nanoparticles, and (2) in the simultaneous precipitation of the polymer at the water/oil interface (i.e., the polymer should be insoluble in both the aqueous and the organic phase). Usually, nanocapsules exhibit average diameter ranging from 200 to 350 nm, the latter being governed by several physicochemical parameters such as the nature and the concentration of the monomer and encapsulated drug, the amount of surfactant and oil as well as the speed of diffusion of the organic phase within the aqueous phase. However, Altinbas et al. have demonstrated that when a miniemulsion is applied instead of an emulsion, nanocapsules of an average diameter below 100 nm can be obtained.⁷²

The main drawback often encountered in this approach is the contamination of the nanocapsule population by a substantial amount of nanospheres, resulting from a partial polymerization in the organic phase.⁶⁵ However, it has been shown that an optimized ethanol/oil ratio,^{65,73} the acidification of the organic phase,⁷⁴ and the inhibition of the polymerization in the organic phase by aprotic solvents⁷⁵ (acetonitrile, acetone) each avoided the formation of matrix-type nanoparticles.

Water-containing nanocapsules have been developed more recently than were the oil-containing counterparts. They are usually prepared by water in oil (w/o) (micro)emulsion, also called an inverse (micro)emulsion, using polysorbate, sorbit monoleate or poly(ethylene oxide) lauryl ester (Brij 35)

as surfactants. Basically, the alkyl cyanoacrylate monomer is added to the preformed (micro)emulsion and, in a similar way to that of oil-containing nanocapsules, spontaneous anionic polymerization occurred at the water/oil interface to form a thin PACA layer surrounding an aqueous core. Depending on the nature of the surfactant and the starting system (emulsion or microemulsion), which are parameters governing the surface properties of these colloidal objects, this process led to 50–350 nm diameter, stable nanocapsules.^{76–79}

However, because the inverse (micro)emulsion processes conduct to water-containing nanocapsules dispersed in oil (which are suitable for oral route administration), intravenous injection cannot be directly performed with a nonaqueous dispersing medium. To circumvent this limitation, a recent method aiming at transferring the nanocapsules from an oil-dispersing medium to a water-dispersing medium was recently suggested by Couvreur and coworkers and consisted in a centrifugation step of the nanocapsules onto an aqueous layer.^{77,79}

To synthesize nanocapsules with preformed polymers, homopolymer of alkyl cyanoacrylate are required and synthesized separately, for instance by dripping the monomer in pure water, the polymer being subsequently recovered by lyophilization. The nanocapsules preparation method, also called interfacial deposition, consists of the addition of a solution of the homopolymer and a small amount of oil, for instance Miglyol (which will constitute the oily core of the nanocapsules), into an aqueous phase. The oil-containing nanocapsules form instantaneously by deposition of the homopolymer at the oil/water interface, which precipitate as a macromolecular shell.^{71,80,81} In general, a surfactant is added in the aqueous phase to ensure colloidal stability of these nanocapsules.

Synthesis of Poly(alkyl cyanoacrylate) Nanoparticles with Controlled Surface Properties

In this topic, the major breakthrough is undoubtedly the grafting of PEG, a nonionic, flexible, and hydrophilic polymer, onto nanoparticles (which also applies for other colloidal drug carriers such as liposomes). This approach, termed 'PEGylation', represented a milestone in the drug delivery area.^{82,83} Indeed, non-'PEGylated' nanoparticles are quickly eliminated from the bloodstream because of the adsorption of blood proteins (opsonins) onto their surface, which triggers the recognition of the mononuclear phagocyte system (MPS) by the macrophages.

As a consequence, these nanoparticles are ineluctably accumulated in MPS organs such as the liver and the spleen, restricting the therapeutic activity of the entrapped compounds to liver diseases (i.e., hepatic primary hepatocarcinoma or metastasis as well as liver intracellular infections). In contrast, when covered by PEG chains, the obtained nanoparticles are able to efficiently escape this recognition system, resulting in long-circulating, colloidal devices, also called 'stealth' nanoparticles.^{82,83}

After it has been demonstrated that PACA nanoparticles can be seen as very promising biodegradable drug carriers (the BioAlliance Pharma spin-off company is now producing doxorubicin-loaded PACA nanoparticles for clinical uses in phase II/III trials with resistant liver hepatocarcinoma as main indication), their complexity was further increased by performing appropriate tuning of their surface properties in order to control their *in vivo* fate.

Surface Modification of Nanospheres

First attempts concerning surface modification of PACA nanospheres logically concerned the 'PEGylation' concept, either via a simple adsorption of PEG chains onto the nanoparticles or by a covalent linkage of PEG chains with PACA polymers. However, the adsorption approach does not fit the covalent linkage criteria and is not really suitable as long as it has been demonstrated that these kinds of assemblies (PACA nanoparticles on which poloxamer 388 or poloxamine 908 was adsorbed) are not stable during *in vivo* administration, resulting in a loss of coating and no significant influence on the biodistribution pattern.⁸⁴ Thus the covalent bond of the PEG chains at the surface of the nanoparticles is a prerequisite for this kind of application.

Basically, different types of hydrophilic molecules have been anchored, on purpose, to the surface of PACA nanoparticles (Figure 7). Efficient surface modification of nanospheres can be achieved either *in situ* during the polymerization in aqueous dispersed media or from preformed amphiphilic copolymers during emulsification processes.

Concerning previous studies about anionic/zwitterionic emulsion polymerization of alkyl cyanoacrylate, the hydrophilic molecules introduced in the recipes (SDS, dextran, poloxamer, Tweens, cyclodextrins, etc.) were solely used as stabilizing agents for investigating their effect on the stability, the average diameter, and the particle size distribution. However, it was not fully understood at this time that some of them, especially those containing nucleophilic functional groups, might take part in the initiation of the polymerization, leading to a partial formation

of surface-active macromolecules. This point is of great importance since nanoparticles with covalently anchored stabilizing moieties at their surface would behave differently in a biologic medium than those with adsorbed surfactants. As a consequence, this is only later on that researches have been strictly devoted to surface engineering of PACA nanoparticles in order to investigate any subtle change of the surface properties of the nanoparticles on their *in vivo* fate.

However, almost unmarked, early works by Douglas et al. postulated that dextran or β -cyclodextrin may also initiate the polymerization of butyl cyanoacrylate (BCA) resulting in the formation of amphiphilic copolymers, helping to stabilize the nascent nanoparticles.⁵⁴ This approach was revisited by Peracchia et al. using different linear PEGs acting as stabilizers and initiators for the emulsion polymerization of IBCA^{85,86} (Figure 7(a) and (b)). It was demonstrated that PEG chains exhibited different conformations at the surface of the nanospheres: (1) hairy nanospheres with PEG monomethyl ether due to a single initiation site (Figure 7(a)) or (2) long loops using PEG due to the divergent chain growth (two initiating sites) during the polymerization of IBCA⁸⁷ (Figure 7(b)). In the same spirit, the use of polysaccharides, such as dextran, dextran sulfate, chitosan, and thiolated chitosan, as stabilizing/initiating agents under similar experimental conditions also led to stable nanospheres in the 100–500 nm range, exhibiting different surface properties; for instance, positively charged with chitosan^{61,88,89} and from rather neutral to negatively charged with dextran derivatives.^{88,90,91}

So far, anionic (mini)emulsion polymerization was the most widespread and straightforward technique to synthesize PACA nanospheres. Even though, in that case, the mechanism is on the basis of anionic propagating species,^{63,64} Chauvierre et al. recently adapted Couvreur's original protocol to a free-radical emulsion polymerization process, thanks to the polysaccharide/cerium IV (Ce^{4+}) ions redox couple as the initiator⁹² (Figure 8). Because of the fast radical initiation rate, anionic polymerization is negligible regarding the timescale of the experiment which makes way for a free-radical chain growth process. This technique was also employed for the emulsion polymerization of alkyl cyanoacrylate using different kinds of polysaccharides,^{89,90,93,94} allowing a direct comparison with nanospheres obtained from anionic emulsion polymerization. The first difference is the conformation of polysaccharide chains at the surface of the nanospheres in direct relation with the structure of the copolymer. Indeed, anionic emulsion polymerization led to grafted copolymers, whereas linear block

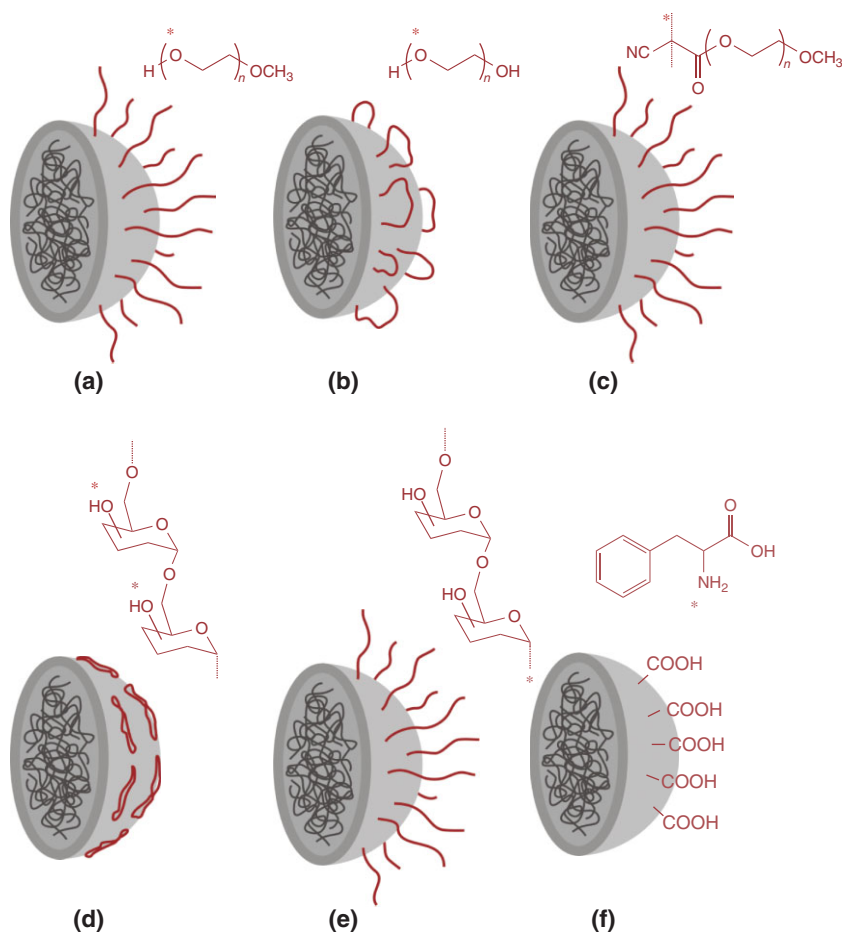


FIGURE 7 | Schematic representation of poly(alkyl cyanoacrylate)-based nanospheres with controlled surface properties using poly(ethylene glycol) monomethyl ether (a), poly(ethylene glycol) (b), poly[(hexadecyl cyanoacrylate)-*co*-methoxypoly(ethylene glycol) cyanoacrylate] copolymer (c), polysaccharide chains under anionic initiation (d), polysaccharide chains under redox initiation (e), and amino acids (f). The moiety anchored at the surface of the nanoparticles is marked by single asterisk.

copolymers were achieved under redox radical initiation (Figure 8), leading respectively to compact loops (Figure 7(d)) and hairy polysaccharide chains (Figure 7(e)) at the surface of the nanospheres.^{88,93}

The size of the polysaccharide-decorated nanospheres was in the 80–800 nm range and depended on: (1) the molecular weight of the polysaccharide, where a minimum value of about 6000 g mol⁻¹ was required for ensuring an efficient colloidal stability^{88,89} and (2) on the nature of the polysaccharide: dextran-decorated nanospheres exhibited an average diameter below 300 nm, dextran sulfate and chitosan led to a larger average diameter of about 350–600 nm,⁸⁸ whereas the use of heparin conducted to 90-nm nanospheres.^{95,96}

Another crucial difference resulting from the surface conformation of the hydrophilic chains, for either PEG derivatives or polysaccharides, concerns the measure of the complement activation,^{87,88} which

is known to play a significant role in the non-specific recognition events of the immune system. Indeed, according to Peracchia et al., nanospheres bearing big loops because of α,ω -dihydroxyl PEG (Figure 7(b)) were shown to better prevent complement consumption than do the hairy nanoparticles obtained from PEG monomethyl ether⁸⁷ (Figure 7(a)). Besides, Bertholon et al. demonstrated that, for both dextran and chitosan, an increase of the length of the compact loops (Figure 7(d)) resulted in an increase of complement activation, whereas the opposite effect was obtained by increasing the length of the hairy polysaccharide chains⁸⁸ (Figure 7(e)), which clearly demonstrated that complement activation is highly sensitive to any change of the surface chain conformation. In a recent work, it was also suggested that the conformation of the coating material also affects the cytotoxicity profile of PACA nanoparticles.⁹⁷

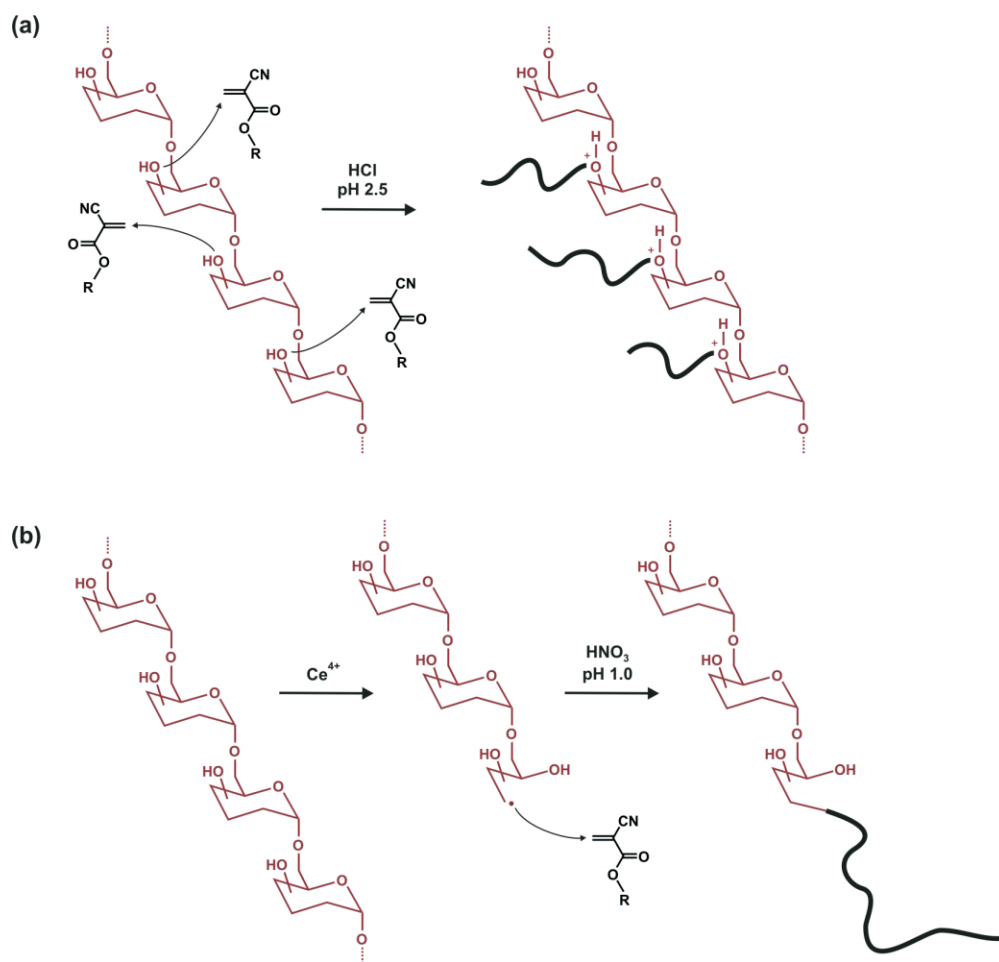


FIGURE 8 | Anionic emulsion polymerization of alkyl cyanoacrylates initiated by hydroxyl groups of dextran (a) and redox radical emulsion polymerization (RREP) of alkyl cyanoacrylates initiated by dextran/cerium IV (Ce^{4+}) ions redox couple (b).

Recently, an interesting synthetic pathway to functionalize PACA nanospheres using amino acids was proposed by Weiss et al.⁷⁰ The authors used a miniemulsion process to prepare a stable pH 1 dispersion of *n*BCA nanoparticles stabilized by SDS as the surfactant. Polymerization was then triggered by the addition of nucleophilic compounds such as amino acids (for instance, glycine), leading to functionalized, stable nanospheres (as already discussed earlier, the similar miniemulsion process has been applied to nonfunctionalized nanospheres when sodium hydroxide was added as the initiator). This method allowed: (1) the solids content to be increased up to 10 wt% with average diameter ranging from 80 to 350 nm, depending on the amount of surfactant as well as the nature of the amino acid and (2) a convenient surface functionalization by amino acid moieties (Figure 7(f)).

The preparation of ‘PEGylated’ nanoparticles from preformed polymers is a well-established

technique which first requires the synthesis of amphiphilic copolymers with PEG segments. PIBCA-*b*-PEG diblock and PIBCA-*b*-PEG-*b*-PIBCA triblock copolymers were synthesized from phosphine end-capped PEG macroinitiators.⁴⁵ With diblock copolymers, unimodal size distribution and stable nanoparticles in the range of 100–700 nm were obtained by nanoprecipitation or emulsification/solvent evaporation, the average diameter being controlled mainly by the amount of organic solvent and by the composition of the polymers. However, the presence of phosphine groups within the synthesized polymers may be a toxicological issue.

The amphiphilic, biodegradable copolymers comprising poly(hexadecyl cyanoacrylate) hydrophobic units and methoxypoly(ethylene glycol) cyanoacrylate hydrophilic units (Figure 4) were used to prepare the corresponding P(HDCA-*co*-MePEGCA) nanospheres exhibiting a biodegradable PACA core and a shell of excretable PEG chains^{46,98,99}

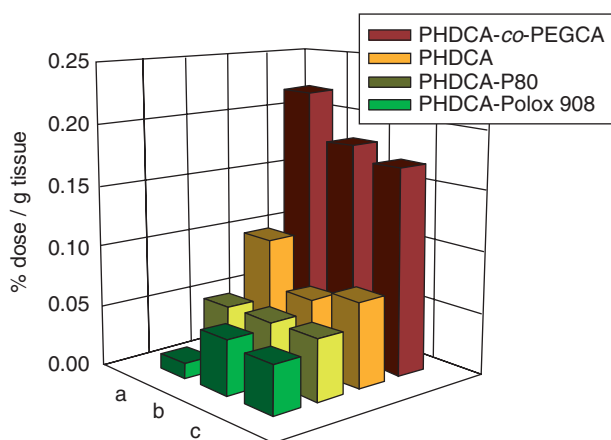


FIGURE 9 | Concentration of radioactivity in right hemisphere (a), left hemisphere (b), and cerebellum (c), after intravenous administration of 60 mg kg⁻¹ of [¹⁴C]-P(HDCA-co-MePEGCA) nanoparticles, poloxamine 908-coated [¹⁴C]-PHDCA nanoparticles, polysorbate 80-coated [¹⁴C]-PHDCA nanoparticles, and uncoated [¹⁴C]-PHDCA nanoparticles (mice at 1 h postinjection).

(Figure 7(c)). Nanoprecipitation or emulsification/solvent evaporation techniques employing P(HDCA-co-MePEGCA) polymers led to very stable 'PEGylated' nanospheres with average diameters in the 100–200 nm range and monomodal size distributions.⁹⁸ These materials showed a reduced cytotoxicity toward mouse peritoneal macrophages, and the presence of the PEG segments was found to increase the degradability of the polymer in the presence of calf serum.⁹⁸ Besides, as a result of the PEG coating, an extended circulation time in the bloodstream was demonstrated.¹⁰⁰

The impressive result deriving from the use of these stealth nanoparticles is their ability to significantly cross the blood–brain barrier (BBB) compared with non-PEGylated counterparts and those with preadsorbed surfactants such as polysorbate 80 or poloxamine 908^{2,101–104} (Figure 9).

This unique feature suggested that P(HDCA-co-MePEGCA) nanospheres exhibited appropriate properties for entering the central nervous system (CNS) via the BBB. Even though a passive diffusion because of an increased permeability of the BBB (when locally disrupted at the tumor site) may not be ruled out, the mechanism by which those nanoparticles preferentially crossed the healthy BBB was assigned to a specific adsorption of apolipoprotein E and B-100 (Apo E and B-100) onto P(HDCA-co-MePEGCA) nanospheres leading to their translocation mediated by low-density lipoprotein receptors (LDLR).^{105–107} The involvement of Apo E on the translocation through the BBB of polysorbate 80-covered PACA nanoparticles was also reported by Kreuter's group

who hypothesized the formation of lipoprotein particle mimics recognized by the LDLR gene family in the brain endothelial cells of the BBB.¹⁰⁸

Synthesis of 'PEGylated' Nanocapsules

To the best of our knowledge, the only examples of 'PEGylated' PACA nanocapsules were reported by Brigger et al.⁸¹ and Li et al.,^{109,110} both using P(HDCA-co-MePEGCA) copolymers.⁴⁶ Although Brigger et al.⁸¹ prepared the corresponding stealth, oil-containing nanocapsules by the interfacial deposition technique, Li et al. used a water-in-oil-in-water (w/o/w) double emulsion process to achieve 'PEGylated', water-containing nanocapsules as tumor necrosis factor- α carriers.^{109,110} This two-step emulsification protocol started by the emulsification of the aqueous phase containing the drug into the organic phase in which the P(HDCA-co-MePEGCA) copolymer was dissolved (w/o), followed by its addition into an aqueous PVA solution (w/o/w). Stable nanocapsules of about 140–150 nm in diameter were then collected by centrifugation.

Addressed Poly(alkyl cyanoacrylate) Biodegradable Nanoparticles

For the forthcoming years, the most exciting challenge in drug delivery, irrespective of the nature of the drug carriers (i.e., liposome, nanoparticles), will be undoubtedly the synthesis of efficient ligands-decorated colloidal devices for achieving specific cells targeting, on the basis of molecular recognition processes. Indeed, the main drawback of previous generation of drug carriers is their non-specific drug release behavior. Nanoparticles are indeed unable to be efficiently addressed to the desired cells and the therapeutic activity of the encapsulated drug may be partly hampered. Even for the remarkable case of brain-targeted P(HDCA-co-MePEGCA) nanospheres,^{2,101–104} the linkage of a judicious ligand at their surface would certainly result in a strongly higher extravasation yield across the BBB.

Thus, if a great deal of effort has been already devoted to this area, a lot of works remain due to be done. The only example of the so-called third-generation PACA nanoparticles involves folate-decorated P(HDCA-co-MePEGCA) nanospheres to target the folate receptor, which is overexpressed at the surface of many tumor cells. For this purpose, the synthetic route for P(HDCA-co-MePEGCA) copolymers⁴⁶ was adapted to the synthesis of a poly[(hexadecyl cyanoacrylate)-co-aminopoly(ethylene glycol) cyanoacrylate]

[P(HDCA-co-H₂NPEGCA)] copolymer, starting from a protected aminopoly(ethylene glycol) cyanoacetate.¹¹¹

Then, the corresponding nanospheres were obtained by nanoprecipitation showing a narrow size distribution for an average diameter of 80 nm. The conjugation with *N*-hydroxysuccinimide–folate (NHS–folate) occurred via an amidation pathway directly at the surface of the nanospheres bearing available amino groups (Figure 10). The specific interaction occurring between the folate-conjugated nanospheres and the folate-binding protein was demonstrated by surface plasmon resonance. The apparent affinity of the folate bound to the nanospheres appeared 10-fold higher than the free folate in solution, because of the multivalency of the folate-decorated nanoparticles.

Biocompatibility and Biodegradation of Poly(alkyl cyanoacrylate) Polymers

The degradation and toxicity of PACA nanoparticles are a crucial point, especially for biomedical applications. Indeed, a drug carrier device is suitable for *in vivo* applications only if it is made of biocompatible, possibly biodegradable, or at least excretable (e.g., by the kidneys) materials. In fact, PACAs are bioerodible polymers for which different degradation pathways have been reported so far (Figure 11).

The predominant mechanism occurs via the hydrolysis of their side chain ester functions,^{55,112,113} producing the corresponding alkyl alcohol and poly(cyanoacrylic acid) as the degradation products, the latter being fully water-soluble and readily eliminated by kidney filtration (Figure 11(a)). This hydrolysis, which is believed to be the main degradation mechanism *in vivo*, proceeds typically in a couple of hours for PACA nanoparticles and is strongly

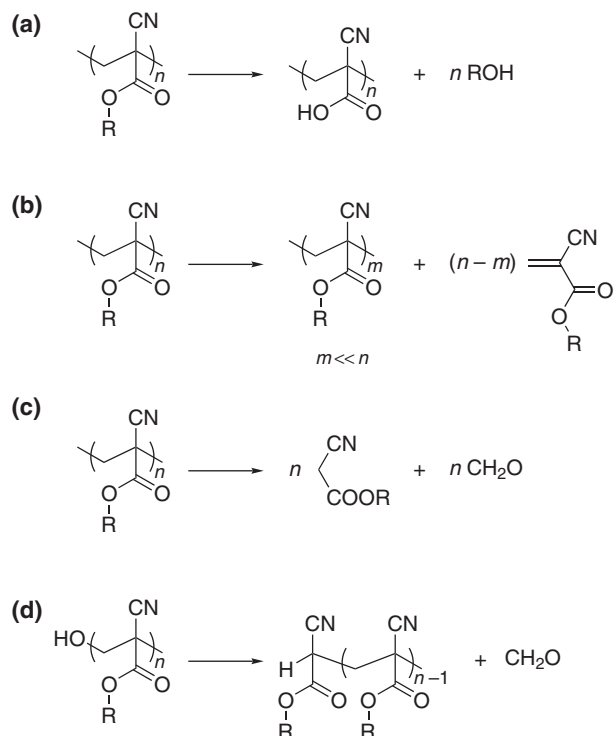


FIGURE 11 | Possible degradation pathways for poly(alkyl cyanoacrylate) (PACA) polymers: hydrolysis of ester functions (a), ‘unzipping’ depolymerization reaction (b), the inverse Knoevenagel condensation reaction (c), and release of formaldehyde from hydrolysis of the α -hydroxyl functions (d).

affected by: (1) the length of the alkyl side chains; the longer the alkyl side chains, the lower the toxicity but the slower the hydrolysis^{55,114,115} and (2) the surrounding environment as it can be strongly catalyzed by esterases from serum, lysosomes, and pancreatic juice.^{116,117} However, a complete excretion of these

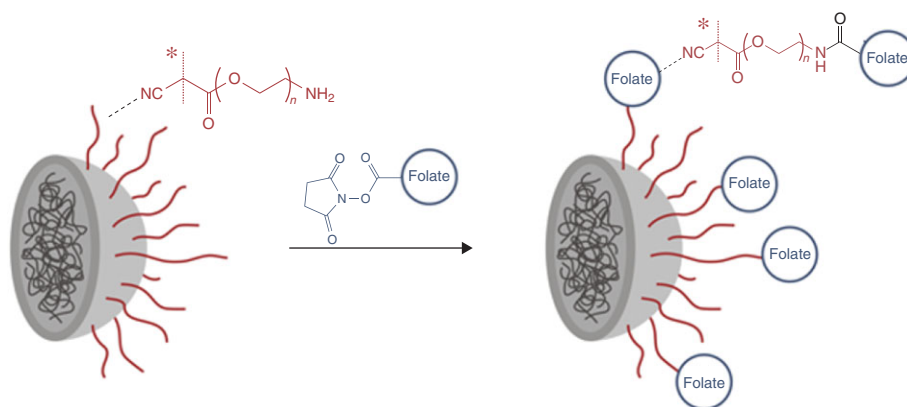


FIGURE 10 | Synthesis of folate-decorated poly[(hexadecyl cyanoacrylate)-co-aminopoly(ethylene glycol) cyanoacrylate] [P(HDCA-co-H₂NPEGCA)] nanospheres. The moiety anchored at the surface of the nanoparticles is marked by single asterisk.

materials would occur only for low-molecular-weight PACA polymers, typically below $10,000 \text{ g mol}^{-1}$.

It has been postulated that the 'unzipping' depolymerization reaction, initiated by a base, could also take part in the biodegradation pathway of PACA,⁶⁶ especially in biologic media where it can be theoretically induced by amino acids of proteins (Figure 11(b)). Following the depolymerization of parent polymers, instant repolymerization to form lower-molecular-weight polymers would occur, even if no clear description of this mechanism has been shown yet, possibly because of its too fast occurrence to be unambiguously observed.

Finally, another suggested mechanism for the degradation of PACA polymers is on the basis of the well-known inverse Knoevenagel condensation reaction, which produces the corresponding alkyl cyanoacetate and formaldehyde (Figure 11(c)), even though the release of formaldehyde might also result from hydrolysis of the α -hydroxyl functions of the polymer chains, provided the hydroxyl ions have been initially used as an initiator⁶⁹ (Figure 11(d)). However, the inverse Knoevenagel condensation reaction has been reported to a lesser extent in aqueous solution at physiological pH and too slow to compete with the above-mentioned enzyme-catalyzed hydrolysis mechanism.^{118–120}

CONCLUSION

Even though the chemistry of alkyl cyanoacrylates is not as straightforward as for other 'common' vinyl monomers, the convergent involvements of organic chemistry, polymer science, and physicochemistry made possible the development of more and more sophisticated, biodegradable PACA-based nanoparticles, successfully used as drug delivery devices. Indeed, from the pharmacology standpoint, PACA nanoparticles fulfill important requirements of ideal drug delivery systems: ease and reproducibility of preparation, ease of storage and administration in a sterile form, satisfying drug-loading capacity, low toxicity, excellent biodegradability, and feasibility for scale-up production. By playing with experimental conditions (nature and amount of reactants, process of preparation, etc.), various types of PACA nanoparticles can be obtained, each of them exhibiting specific features regarding the nature of the drug and/or the way the drug is encapsulated: nanospheres (matrix-type nanoparticles; oil-soluble drug) or nanocapsules (reservoir-type nanoparticles; either oil-soluble or water-soluble drug), nonsurface-modified (mainly devoted to MPS organs) or 'PEGylated' nanoparticles (long-circulating drug carriers) as well as ligand-decorated nanospheres (addressed drug delivery devices). As a result of constant efforts in this field, PACA nanotechnologies have thus opened exciting perspectives for the discovery of novel and more efficient nanomedicines.

NOTES

The authors thank Christine Vauthier, François Ganachaud, and Didier Desmaële for their fruitful discussions. The CNRS and the University Paris-Sud are also acknowledged for financial support.

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