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Poly(alkylcyanoacrylates) as biodegradable materials for biomedical applications

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Abstract

This review considers the use of poly(alkylcyanoacrylates) (PACAs) as biomedical materials. We first present the different aspects of the polymerization of alkylcyanoacrylate monomers and briefly discuss their applications as skin adhesives, surgical glues and embolitic materials. An extensive review of the developments and applications of PACAs as nanoparticles for the delivery of drugs is then given. The methods of preparation of the nanoparticles are presented and considerations concerning the degradation, in vivo distribution, toxicity and cytotoxicity of the nanoparticles are discussed. The different therapeutic applications are presented according to the route of administration of the nanoparticles and include the most recent developments in the field.

Keywords: Poly(alkylcyanoacrylates); Polymerization; Nanoparticles; Degradation; Therapeutic applications

Contents

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1. Introduction

Poly(alkylcyanoacrylates) (PACAs) were not employed as polymers until the early 1980s [1]. However, the corresponding monomers, alkylcyanoacrylates, have been used since at least 1966 because of their excellent adhesive properties resulting from the bonds of high strength they are able to form with most polar substrates, including living tissues and skin [2]. Therefore, the monomers have been used extensively as tissue adhesives for the closure of skin wounds [3–7], as surgical glue, and as embolitic material for endovascular surgery [6,8]. More recently, one application of the polymers consists of the use of PACAs as drug nanoparticulate carriers [9–15]. This very exciting area of research, which emerged in the 1980s [1,16,17], has gained increasing interest in therapeutics, especially for cancer treatments, which generally involve highly toxic molecules in contact with healthy tissue. Other molecules of interest, including poorly stable compounds such as peptides and nucleic acids, have been combined with PACA nanoparticles for targeting purposes [12,13,18]. Today, PACA nanoparticles are considered the most promising polymer colloidal drug delivery system and are already in clinical development for cancer therapy [19–23].

This review thus considers, in detail, PACAs as materials for biomedical applications. The different aspects presented include a short summary of the polymerization and biomedical applications of the monomers and an extensive review of the more recent developments and applications of PACAs applied as nanoparticles for drug delivery.

2. Polymerization of alkylcyanoacrylates and biomedical applications of the monomers

2.1. Polymerization of alkylcyanoacrylates

The monomers generally occur as clear and colorless liquids with a low viscosity and are highly reactive compounds, extremely difficult to handle in their pure form [2,4,23–25]. They display a remarkable tendency to polymerize because of their strong reactivity. Inhibitors are essential to maintain their stability. Indeed, alkylcyanoacrylates are able to polymerize extremely rapidly in the presence of moisture or traces of basic components.

The polymerization of alkylcyanoacrylates can theoretically occur according to three different mechanisms, namely free-radical, anionic and zwitterionic polymerization. In practice, the anionic and zwitterionic routes are strongly favored because they are rapidly initiated at ambient temperature (Fig. 1). Classical initiators of anionic polymerization are anions (i.e. I^-, CH_3COO^-, Br^-, OH^-, etc.), weak bases such as alcohols and water and amino acids encountered in living tissues [2]. Initiation of polymerization by the amino acids of proteins is responsible for the strong binding power of these components to the skin, as will be explained below.

2.2. Biomedical applications

Alkylcyanoacrylates have been used for decades as adhesives, mainly for consumer applications where speed of cure is needed. These monomers have also been applied in the biomedical area as

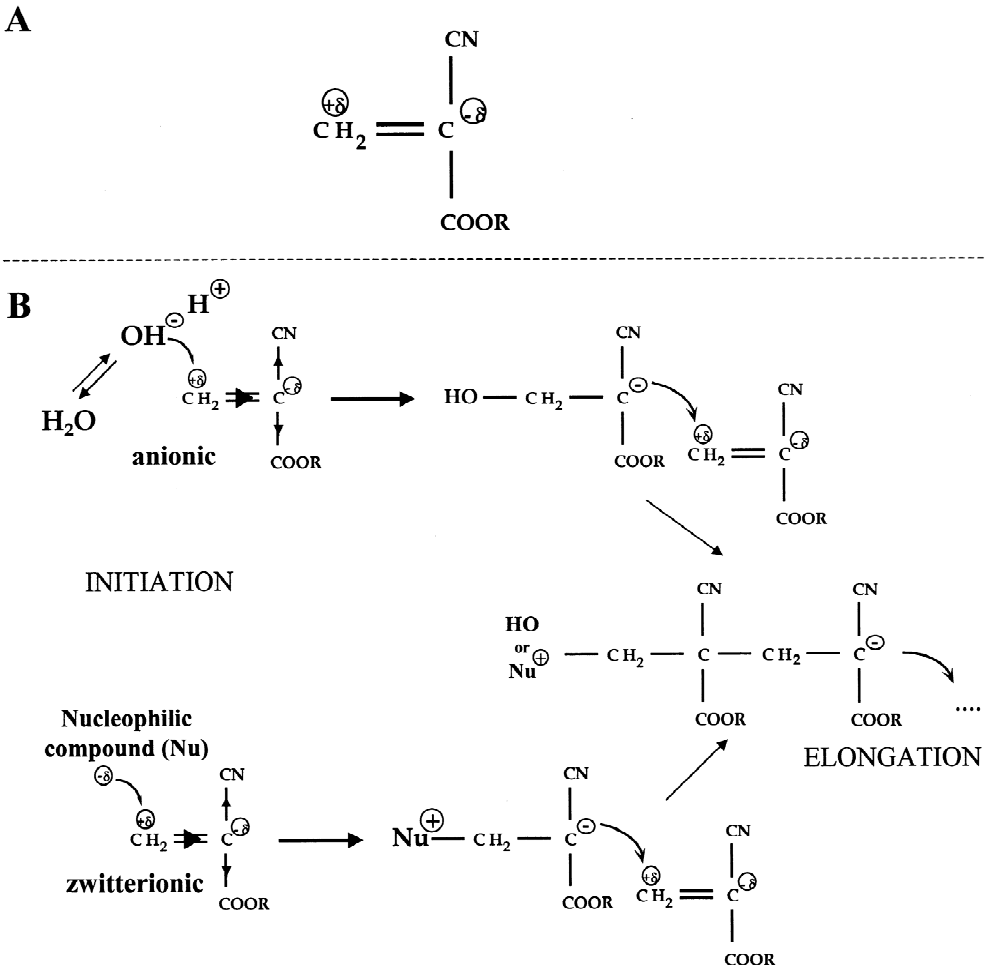

Fig. 1. Chemical structure of alkylcyanoacrylates (A) and scheme of their anionic and zwitterionic polymerization (B).

tissue adhesives since 1964, when methylcyanoacrylate was first tested for the closure of 3-cm-long cystotomies in dogs. At present, use as a tissue adhesive is an improved and popular method of wound closure, being faster, less painful and more economical than suturing. It is mainly applied to the closure of simple lacerations and surgical wounds [5,7,26,27]. Today, methylcyanoacrylate (Biobond) is still in use in Japan [28], whereas early cyanoacrylates were removed from distribution in other countries because of rapid in vivo degradation, resulting in significant tissue toxicity and inflammation [27]. They were replaced by longer-chain alkylcyanoacrylates such as *N*-butylcyanoacrylate (Indermil®, liquiband®), which is used clinically in Europe, Canada and the USA, and octylcyanoacrylate (Dermabond®), which received Food and Drug Administration approval in 1998 and is now marketed in the USA for skin wound closure after lacerations or incisions [27]. Upon application, the liquid monomer formulation polymerizes instantaneously into a thin polymer film that adheres tenaciously to the mucosal tissue. The polymer film also creates a mechanical barrier which maintains a natural healing environment for the area to heal [29]. Octylcyanoacrylate forms strong bonds across opposed wound edges and provides a flexible water-resistant coating, inhibiting microbial growth, there-

fore avoiding the occurrence of infections [30]. This new adhesive presents advantages over *n*-butylcyanoacrylate, including a higher breaking strength, flexibility and resistance to splintering after drying [30]. Octylcyanoacrylate also appears to be a stronger tissue adhesive than its corresponding fibrin sealant counterpart [7]. Some authors claim that complications are virtually non-existent [26]. No evidence of histotoxicity has been reported with this monomer, which is considered a promising alternative to the standard wound closure method, providing a faster repair of traumatic lacerations and surgical incisions with similar cosmetic outcomes [29]. Furthermore, this method of wound closure is readily accepted by patients, especially by children. One disadvantage of the current octylcyanoacrylate formulation is its slow rate of biodegradation. It has also been stressed that these adhesives are only for external use.

The internal use of octylcyanoacrylate has not yet been approved and applications beyond the skin are considered by certain authors as unwise and potentially dangerous to patients [7]. Despite this, alkylcyanoacrylates are under investigation for many other applications [28]. They are even the main liquid adhesives under clinical investigation for use in the vascular system as embolitic material and are being considered to play an important role in managing abnormalities, especially in arteriovenous malformations [6,8]. For instance, Trufill n-BCA®, a combination of *n*-butylcyanoacrylate and tantalum powder, is delivered through the arterial system to stop bleeding atrioventricular malformations and is then removed during repair. Another active area of research is the treatment of gastric varices, for which cyanoacrylate glues are still first choice [31].

3. Poly(alkylcyanoacrylate) nanoparticles

Nanoparticles is a general word used to designate small-sized polymer particles with a diameter ranging from several tens to several hundred nanometers. It includes colloidal particles of different structures: nanospheres, nanocapsules, core-shell nanospheres and core-shell nanocapsules. The PACA nanoparticle family comprises nanospheres, oil- and water-containing nanocapsules and core-shell nanospheres

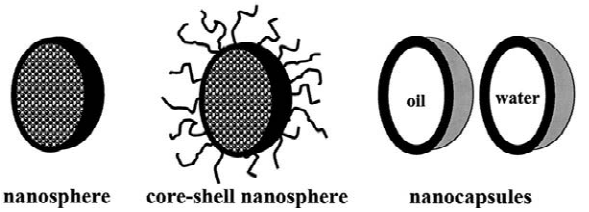

Fig. 2. Schematic representation of the different types of PACA nanoparticles produced.

(Figs. 2 and 3). PACA nanoparticles can be prepared either by polymerization of alkylcyanoacrylate monomers or directly from the polymers (see Section 3.1, Fig. 4). These nanoparticles were developed in order to design biodegradable drug carrier systems for targeting a drug to tissues, cells or subcellular compartments, as explained in Sections 3.2–3.5.

3.1. Preparation of poly(alkylcyanoacrylate) nanoparticles

3.1.1. Preparation of nanospheres by emulsion polymerization

Emulsion polymerization is a very popular approach used to synthesize polymer colloids with a matrix structure (nanospheres, Fig. 2a). The emulsion polymerization of alkylcyanoacrylates was first introduced by Couvreur et al. in 1979 [1] to design nanoparticles with biodegradable polymers for the in vivo delivery of drugs. The polymerization media used for the polymerization of alkylcyanoacrylates for this purpose are usually very complex. In these systems, the polymerization is initiated by the hydroxyl ions of water, and elongation of the polymer chains occurs according to an anionic polymerization mechanism (Fig. 1). It should be pointed out that the anionic polymerization of such a reactive monomer can be controlled in an aqueous medium. The main parameters involved are the adjustment of the pH with a strong mineral acid such as hydrochloric acid and the concentration of the anionic polymerization inhibitor (SO_2) in the monomer [32]. The size of the nanospheres produced can vary from 50 to 300 nm [33–35]. As an example of this procedure, the monomer (100 μl) is dispersed in acidified water containing a surfactant or a stabilizing agent (10 ml

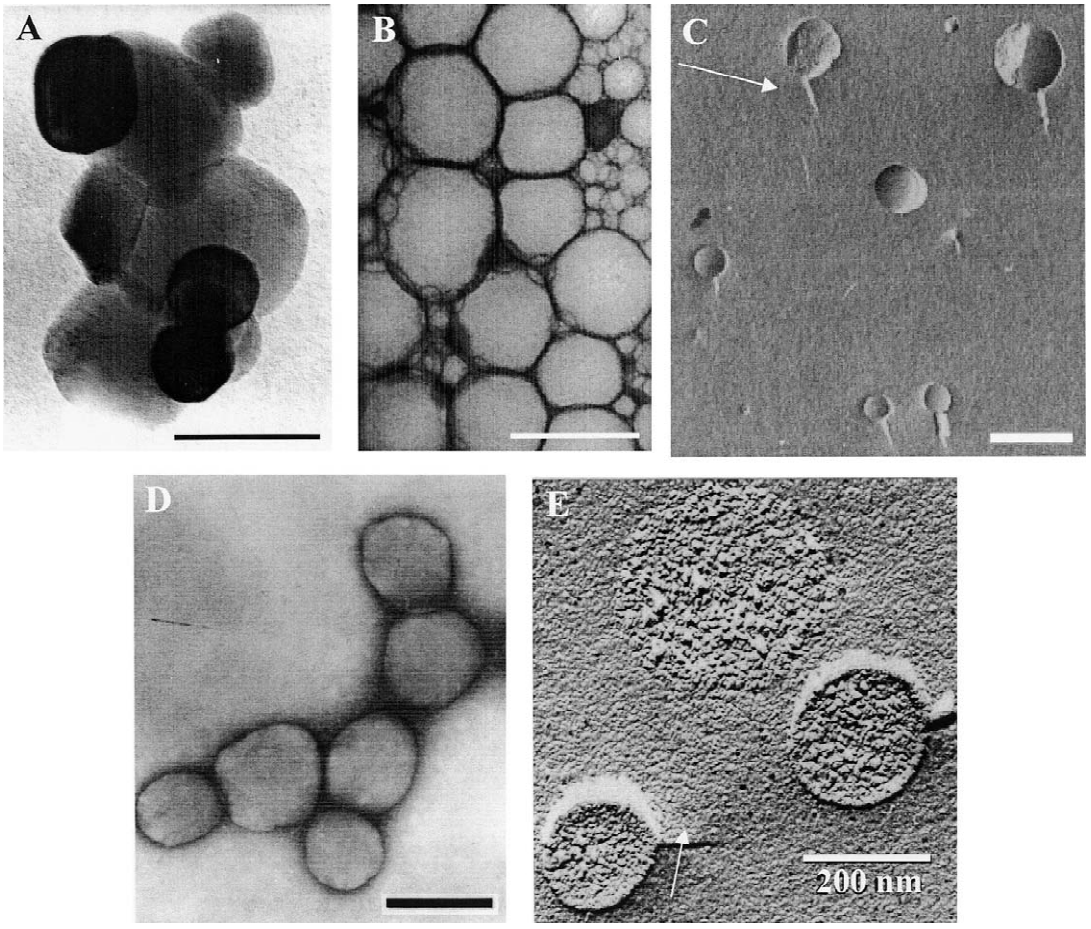

Fig. 3. Example electron micrographs of PACA nanoparticles obtained under different conditions. (A) Oil-containing nanocapsules after staining with uranyl acetate. (B) Oil-containing nanocapsules after staining with phosphotungstate acid. (C) Water-containing nanocapsules after cryofracture and shadowing with platinum. (D) Nanospheres after staining with uranyl acetate. (E) Nanospheres after cryofracture and shadowing with platinum (bars 200 nm; the arrow in cryofractures (C) and (D) indicates shadowing orientation).

of a 0.5–1% solution of Pluronic F68 or dextran 70 at pH 2.5 adjusted with HCl) and allowed to polymerize spontaneously for a few hours (3–4 h) under strong magnetic stirring.

Many drugs can be entrapped in PACA nanospheres [14,33]. The addition of cyclodextrins to the polymerization medium can promote the association of poorly water-soluble drugs with the PACA nanospheres [36].

It should be pointed out that certain drugs are reported to be able to initiate the polymerization of alkylcyanoacrylates, leading to loss of their biological activity [37–39]. However, such side reactions could also be used advantageously to promote the covalent binding of certain compounds to the nanospheres when stable association is required. Naphthalocyanines [40], a series of photosensitizers used in the phototherapy of tumors, and a series of molecules containing diethyltriaminepentacetic acid (DTPA) capable of complexing radioactive metals for radiolabelling of nanoparticles in medical imaging [41], have been associated with nanospheres using this approach. These reactions have also been used to produce nanospheres with modified surface properties, allowing the covalent coupling of macromolecules onto the nanosphere surface [33,42–45].

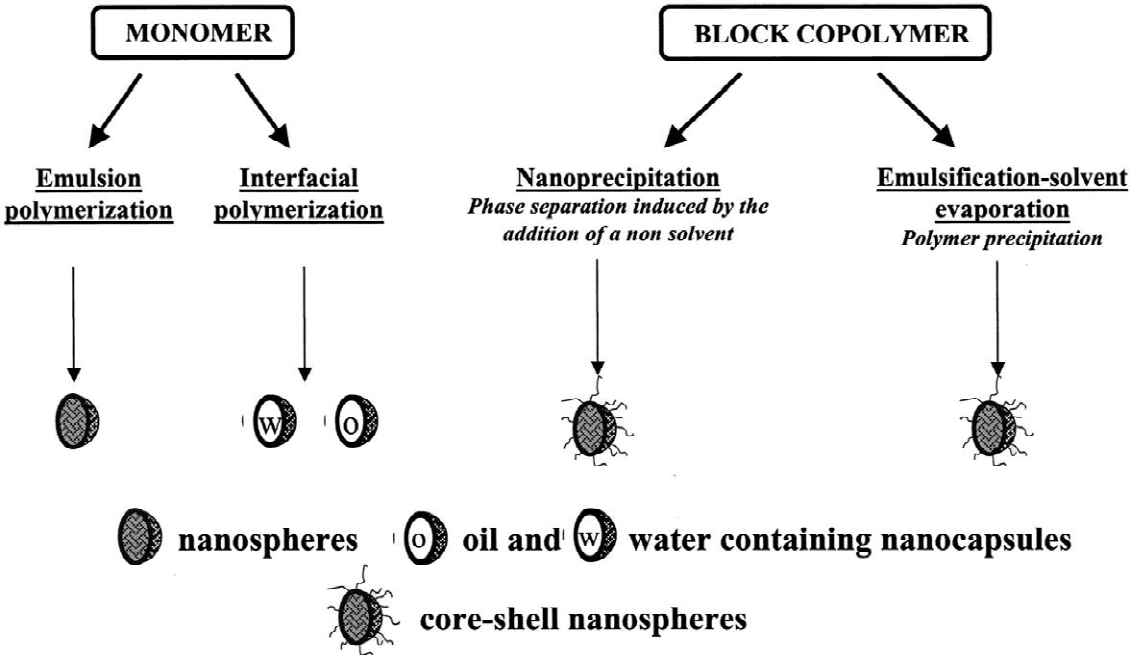

Fig. 4. Summary of the different methods developed for the preparation of PACA nanoparticles with the corresponding types of nanoparticles produced.

3.1.2. Preparation of nanocapsules by interfacial polymerization

Methods based on interfacial polymerization have been developed to prepare nanocapsules consisting of a liquid core surrounded by a thin polymer envelope [46–50]. The reactions are performed either in water-in-oil or in oil-in-water emulsion systems or in microemulsions, leading to the production of water-containing or oil-containing nanocapsules, respectively.

Oil-containing nanocapsules are obtained by the polymerization of alkylcyanoacrylates at the oil/water interface of a very fine oil-in-water emulsion [46]. An organic phase (oil, 1 ml; isobutyl-2-cyanoacrylate, 0.125 ml; drug dissolved in ethanol or acetone, 25 ml) is injected into the aqueous phase (50 ml) containing a hydrophilic surfactant (Pluronic® F68, 0.25%) under strong magnetic stirring. The nanocapsules form immediately to give a milky suspension. The organic solvent is then evaporated. In such a system, the organic solvent, acetone or ethanol, which is totally miscible with water, serves as a monomer vehicle and the interfacial polymerization of alkylcyanoacrylate is believed to occur at the surface of the oil droplets that form during emulsification [12,51–53]. To promote nanocapsule formation, an ideal oil/ethanol ratio of 2% in the organic phase has been suggested [51] and the use of aprotic solvents such as acetone and acetonitrile has been recommended [54]. Protic solvents, such as ethanol, *n*-butanol and iso-propanol, were found to induce the formation of nanospheres in addition to nanocapsules.

Nanocapsules prepared by this method contain more than 90% oil by weight [53], allowing the efficient encapsulation of soluble oily substances [55]. Because of the extremely fast formation of the polymer shell around the oil droplets, highly water-soluble molecules such as insulin can also be encapsulated with high encapsulation yields (up to 97%) if these compounds are suspended in the oily phase [52,56–58].

Water-containing nanocapsules may be obtained by the interfacial polymerization of alkylcyanoacrylate in water-in-oil microemulsions. In these systems, water-swollen micelles of surfac-

tants of small and uniform size are dispersed in an organic phase. The monomer is added to the microemulsion and polymerizes at the surface of the micelles. The polymer forms locally at the water–oil interface and precipitates to produce the nanocapsule shell [47,49,50]. Nanocapsules obtained by this method are of special interest for the encapsulation of water-soluble molecules such as peptides [50] and nucleic acids, including antisense oligonucleotides [49]. For intravenous administration, aqueous core-containing nanocapsules can be transferred into an aqueous continuous phase by ultracentrifugation of the oily suspension over a layer of pure water containing Span® 80 [49].

3.1.3. Synthesis of block copolymers and preparation of nanoparticles by nanoprecipitation and emulsification–solvent evaporation

A major problem with colloidal drug carriers after their intravenous administration is their non-specific uptake by the macrophages of the mononuclear phagocyte system. In an attempt to reduce particle interactions with blood opsonins, which facilitate phagocytosis, the development of sterically stabilized nanoparticles with amphiphilic block copolymers has been introduced, leading to a new generation of drug carrier systems. These particles remain in the blood circulation for a longer period of time and their accumulation in the mononuclear phagocyte system organs is reduced. To obtain such long-circulating (Stealth®) nanoparticles, (PACA)–poly(ethylene glycol) (PEG) copolymers have been synthesized using two methods. With the first method, block copolymers are obtained by initiating the polymerization of alkylcyanoacrylates either by monomethoxy (MeO)-PEG-triphenylphosphine or by triphenylphosphine-PEG-triphenylphosphine, leading to diblock MeO-PEG–PACA or triblock MeO-PACA–PEG–PACA-OMe linear copolymers, respectively (Fig. 5A and B) [59]. The second route of synthesis is based on the Knoevenagel reaction of block copolymers, involving the condensation of a cyanoacetate derivative with formaldehyde [60]. This route leads to branched copolymers in which the PEG part constitutes the branches of the copolymer (Fig. 5C). This second method is preferred since it allows the production of very stable nanospheres and avoids the use of the triphenylphosphine group, which may be

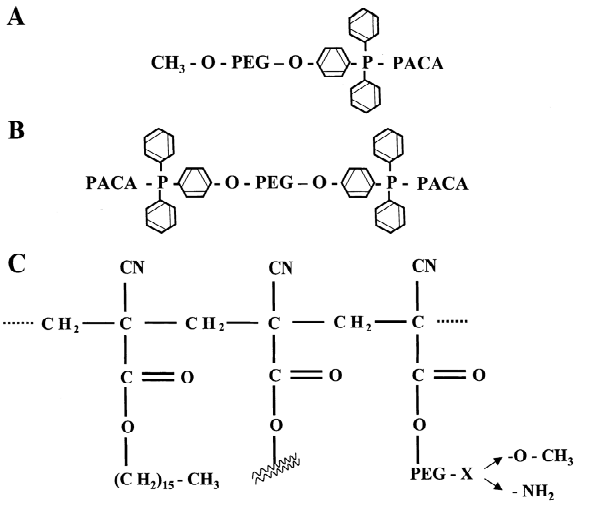

Fig. 5. Structure of amphiphilic PACA-containing block copolymers: PEG-triphenylphosphate–PACA diblock (A) and triblock (B) copolymers [59], and monomethoxy or monoamino poly-(PEGCA-co-HDCA) copolymer (C) [20,60].

toxic [61–63]. In addition, Stella et al. [20] reported the preparation of PEG-coated nanospheres with an amino-terminated PEG-containing copolymer, which were found to be excellent supports for coupling ligands at the nanoparticle surface to develop nanoparticles for cell-selective targeting of drugs. This approach has been used with folic acid for the targeting of KB cancer cells.

Nanospheres are prepared from these copolymers by nanoprecipitation and emulsification–solvent evaporation. Compared with the methods based on polymerization, these approaches have the major advantage that the polymers entering the composition of the nanoparticles are well characterized and their intrinsic physico-chemical characteristics do not depend on the conditions used during the preparation of the nanospheres. Because of the amphiphilic nature of these block copolymers, they self-organize to produce core-shell nanospheres with a hydrophilic surface (Fig. 2).

Nanoprecipitation is a method based on the formation of colloidal polymer particles during phase separation induced by the addition of a non-solvent of the polymer to a rather dilute polymer solution. At that stage, the particles form spontaneously and quasi-instantaneously. The polymer solvent is then

removed from the suspension by rotoevaporation. To facilitate the formation of colloidal polymer particles during the first step of the procedure, phase separation is performed with a totally miscible solvent which is also a non-solvent of the polymer.

The second method, called emulsification–solvent evaporation, also includes two steps. The first consists of the emulsification of the polymer solution in an aqueous phase with the aid of a high-pressure homogenizer or microfluidizer to produce emulsion droplets of very small size. During the second step, the polymer solvent is evaporated, inducing polymer precipitation as nanospheres.

These methods for the preparation of nanoparticles have been applied to poly(poly(ethylene glycol) cyanoacrylate-co-hexadecylcyanoacrylate) (poly-(PEGCA-co-PHDCA)) to prepare PEG-coated nanospheres [20–23,60,64,65].

3.2. Degradation of poly(alkylcyanoacrylate) nanoparticles

The degradation and toxicity of PACA are often discussed in the literature, especially for in vivo applications of PACA nanoparticles as drug delivery systems. Indeed, the suitability of a polymer designed for use as a drug carrier system for humans requires that the material has to be biocompatible, possibly biodegradable, or at least should be able to be excreted (e.g., by the kidneys). These aspects are discussed below.

PACA are bioerodible polymers for which complete excretion of the polymer material will only occur if the nanoparticles were designed using low-molecular-weight polymers. Indeed, even if different pathways for PACA degradation have been described in the literature, the predominant mechanism greatly depends on the surrounding conditions. One of the degradation mechanisms described in the literature consists of the hydrolysis of the ester bond of the alkyl side chain of the polymer (Fig. 6A) [66–68]. Degradation products consist of an alkylalcohol and poly(cyanoacrylic acid), which are soluble in water and be eliminated in vivo via kidney filtration. This degradation has been shown to be catalyzed by esterases from serum, lysosomes and pancreatic juice [69,70] and is believed to occur as the major degradation pathway in vivo. According to this mechanism, nanoparticles are usually degraded within a couple of hours depending on the alkyl side chain length of the PACA forming the nanospheres [2,67,71].

Another mechanism which may also theoretically occur in biological systems consists of an unzipping depolymerization of the parent polymer with immediate repolymerization to give a new polymer with a much smaller molecular weight. The whole phenomenon occurs within a few seconds and is generally induced by a base (Fig. 6B) [25]. However, this mechanism, which may theoretically be induced in vivo by the amino acids of proteins, has never been described. Due to its very rapid occurrence, it is expected that it will be extremely difficult to observe, especially in complex systems such as biological media.

The well-known inverse Knoevenagel reaction, resulting in the production of formaldehyde and cyanoacetic ester, has also been reported, but is limited in water and at physiological pH, yielding only 5% degradation after 24 h. This mechanism has been claimed to occur in vivo in the early stage of the development of PACA for biomedical applications; it was even believed to be responsible for the toxic effects of the nanoparticles [2,72–75]. However, this degradation pathway is too slow to compete with the other, much more rapid, mechanisms occurring in vivo catalyzed by enzymes [73,76,77].

3.3. In vivo distribution of nanoparticles after intravenous administration

The main attraction of PACA nanoparticles is their ability to achieve tissue targeting and enhance the intracellular penetration of drugs. After intravenous administration, PACA nanoparticles are taken up by the liver, by the spleen and, to a smaller extent, by bone marrow [78]. Within tissues, nanoparticles are mainly taken up by macrophages of the mononuclear phagocyte system [79,80]. However, the architecture of the organs has been shown to play a role in the localization of the nanospheres. For instance, in the spleen of mice, uptake was mainly observed in metallophilic macrophages of the marginal zone, whereas, in the rat, which has a sinusoidal spleen similar to that of humans, particles were found in the red pulp macrophages [81]. The uptake of nanoparti-

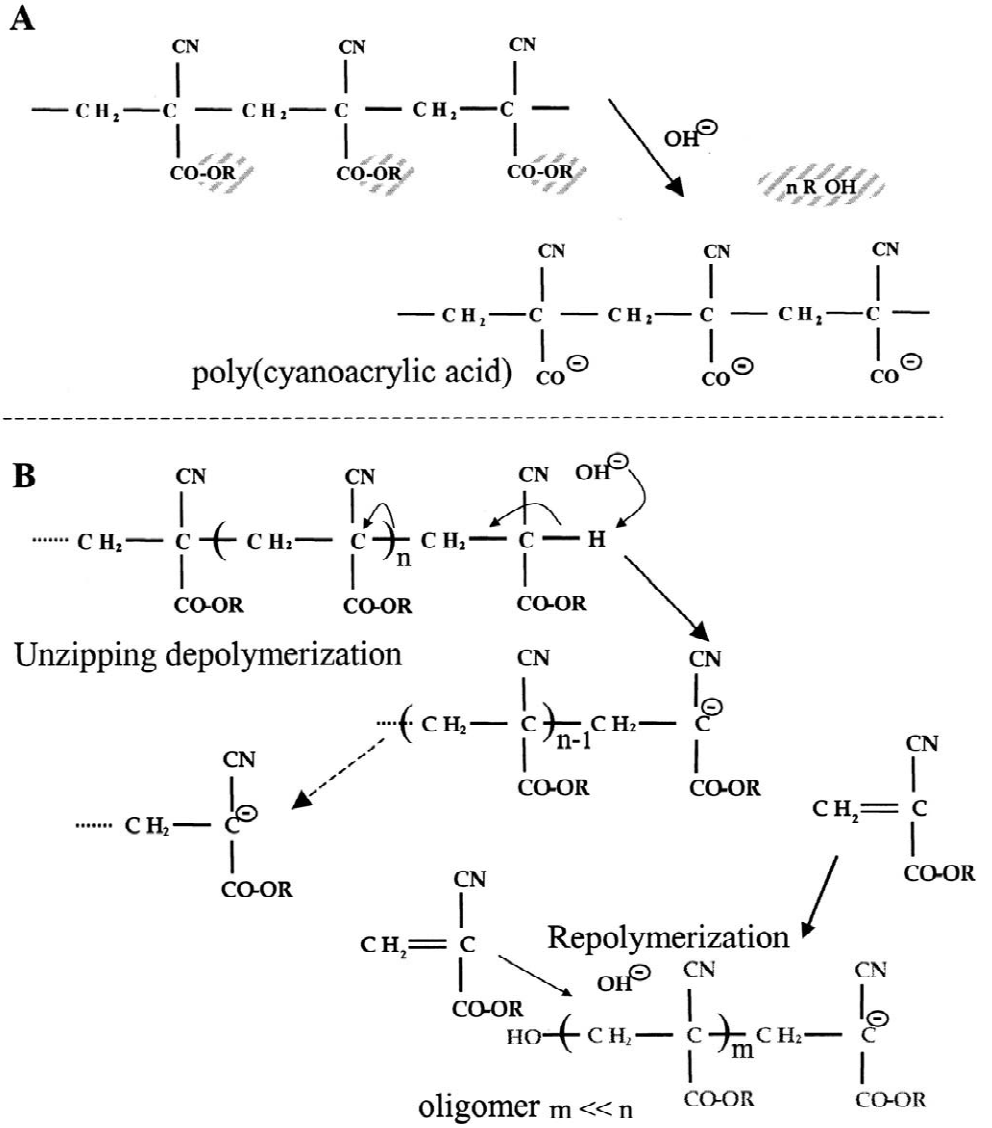

Fig. 6. Degradation pathways of poly(alkylcyanoacrylate). (A) Enzymatic degradation catalyzed by esterases and leading to the production of alkylalcohol and poly(cyanoacrylic acids) [66–69]. (B) Unzipping depolymerization–repolymerization mechanism producing oligomers of PACA [25].

cles by macrophages occurs via an endocytosis process (Fig. 7), after which the particles end up in the lysosomal compartment [79] where they are degraded, producing soluble, low-molecular-weight compounds that are then eliminated from the body by renal excretion [66]. Due to their strong lysosomal localization, one can imagine that nanoparticles are not suitable for the targeting of drugs to the cytoplasm of cells. To avoid their entrapment within the lysosomal compartment, several compounds able to destabilize the lysosomal membrane are added to the nanoparticulate system (e.g. cationic surfactants) [82], allowing some drugs to be delivered to the cell cytoplasm.

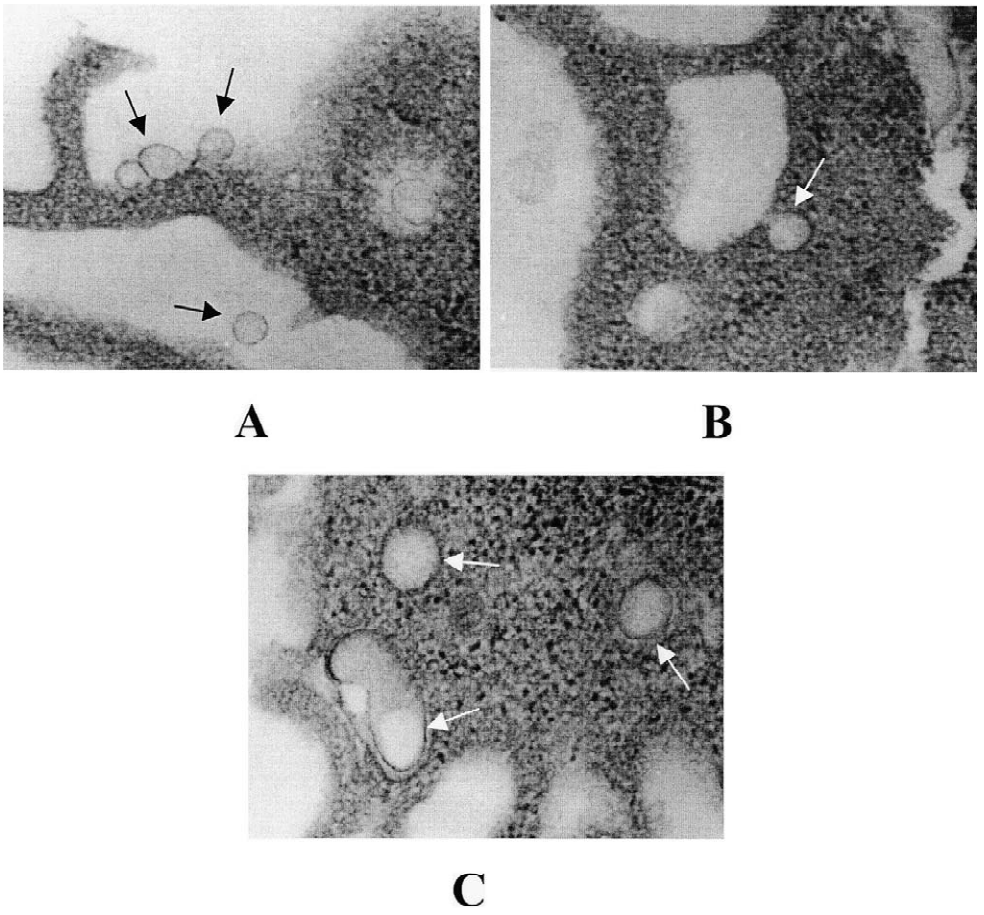

Fig. 7. Endocytosis of PACA nanospheres by J774 macrophages in culture cells as observed by transmission electron microscopy. (A) Interactions of nanospheres with the cell membrane (arrows). (B) Endocytosis of a nanosphere. (C) Nanospheres in phagosomes (arrows).

Coating PACA nanospheres with PEG results in a lower uptake of nanoparticles by the mononuclear phagocyte system and a longer circulation time in the blood [63,83,84]. As a consequence, these so-called 'stealth®' nanoparticles would be able to extravasate across the endothelium, which becomes permeable due to the presence of solid tumors. However, from the point of view of targeting, these 'stealth®' nanoparticles are simple and passive systems with no specific targeting ligands. They basically exploit both the differences in microvascular permeability between healthy and altered tissues and their long-circulating properties.

3.4. Toxicity and cytotoxicity of poly(alkylcyanoacrylate) nanoparticles

Nanoparticles are generally captured by the macrophages of the mononuclear phagocyte system after intravenous administration. However, depending on their surface characteristics, nanoparticles may be more or less opsonized, so that coating nanoparticles with hydrophilic polymers results in a significant modification of the body distribution profile. It is evident that the particle distribution profile in the body may influence the toxicity of the polymer–drug entity. Indeed, the altered phar-

macokinetics and disposition of drugs when associated with nanoparticles should induce novel modalities of cells, tissues or receptor exposures, as well as novel drug metabolism and drug interactions. For instance, in some cases, alteration of the drug distribution profile by linkage to nanospheres could considerably reduce the toxicity of a drug because of the decrease in drug accumulation in organs where the most acute toxic effects are exerted. This concept was illustrated with doxorubicin, which displays severe acute and chronic cardiomyopathy. After intravenous administration to mice, plasma levels of doxorubicin were higher when the drug was adsorbed onto nanospheres and, at the same time, the cardiac concentration of the drug was dramatically reduced [85]. In accordance with the observed distribution profile, doxorubicin associated with nanospheres was found to be less toxic than free doxorubicin [86].

On the contrary, if the cardiac toxicity of doxorubicin is clearly reduced after linkage with PACA nanoparticles [86,87], increased bone marrow toxicity may be observed [80]. Thus, the evaluation of potential novel toxicities of polymeric site-specific drug delivery systems includes the search for a depression or activation of the mononuclear phagocyte system. As far as the mononuclear phagocyte system is concerned, it was observed that PACA nanoparticles did not induce a physical blockade of this tissue after repeated administration, although a temporary depletion of blood opsonins was observed [88]. For this route of administration, hemocompatibility also needs to be evaluated in terms of embolies due to particle aggregation or hemolysis due to erythrocyte damage by nanoparticles or their degradation products. For other means of administration (transmucosal, oral, regional administration, etc.), it is especially important to determine the absence of local tissue irritation (histocompatibility). In this case, the test of lesion reversibility is very important to evaluate the acceptability of the nanoparticle system. On the other hand, as reported by Ammoury et al. [89], the encapsulation of drugs such as indomethacin into PACA nanocapsules may also protect mucosae against the ulcerative properties of the drug.

Results from the toxicological evaluation of PACA nanoparticles motivated the beginning of clinical trials for human cancer [19,90]. A phase I trial confirmed the good tolerance of the drug carrier, since only secondary effects due to the associated drug were observed.

Cytotoxicity is another important parameter to consider with nanoparticulate drug delivery systems since they can be phagocytosed by cells. As reported by Maassen et al. [91], the cytotoxicity of nanospheres may be due to many factors, such as (1) the release of degradation products, (2) the stimulation of cells and subsequent release of inflammatory mediators, and (3) membrane adhesion. The first factor, cytotoxicity attributed to the presence of degradation products, has been reported by several authors. Kante et al. [92] incubated PACA nanospheres with macrophages, and morphological cell changes were characterized by electronic microscopy. Only in the presence of poly(methylcyanoacrylate) was cell membrane perforation observed. By contrast, no morphological changes were observed with poly(butylcyanoacrylate). Another study [93] was carried out by incubating nanoparticles with hepatocytes. Cell toxicity was assessed using a dye-exclusion test and by measuring lactate dehydrogenase leakage. Poly(butylcyanoacrylate) nanospheres did not induce any cytotoxicity at a concentration of 75 μg/ml. Nevertheless, membrane damage appeared at a polymer concentration of 150 μg/ml. These data were confirmed by incubating poly(isobutylcyanoacrylate) nanospheres with L929 fibroblasts, where no cell mortality was induced after 3 and 7 h incubation at a polymer concentration of 100 μg/ml. However, after 24 and 48 h, an increased mortality rate was observed [71]. Kubiak et al. [94] have shown that poly(isobutylcyanoacrylate) nanospheres are more cytotoxic than poly(isohexylcyanoacrylate) by measuring [^{3}H]leucine incorporation in resistant and sensitive cancer cells (DC3F). Gipps et al. [95] studied the interaction of PACA nanoparticles with mesenchymal malignant and normal cells. Using nanospheres made with PACA of different side-chain length (poly(methylcyanoacrylate), poly(ethylcyanoacrylate), poly(butylcyanoacrylate), poly(isobutylcyanoacrylate), poly(isohexylcyanoacrylate)), the authors showed that the cell viability, as

determined by the uptake of neutral red, was influenced dramatically by the nature of the polymer. Polymers with short alkyl side chains appeared more toxic than polymers with longer alkyl groups, whereas cytotoxicity was found to be independent of the cell type used. Morphological changes in the cell membrane were also characterized by transmission electron microscopy. With poly(isobutylcyanoacrylate), a loss of adhesion followed by dilation of the rough endoplasmic reticulum of the cells was observed. Perforation of the cell membrane occurred later in the damage sequence. The toxicity of PACA nanospheres was also investigated by measuring cell growth inhibition of Swiss 3T3 fibroblasts. Poly(isobutylcyanoacrylate) and poly(ethylcyanoacrylate) were found to inhibit cell proliferation to a lower extent than poly(ethoxy-ethyl-2-cyanoacrylate) and poly(methylcyanoacrylate) nanospheres. The extent of cell growth inhibition decreased with increasing molecular weight of the polymer [75]. In addition, Lherm et al. [71] have shown that, after incubating PACA nanoparticles with L929 fibroblasts, only nanoparticles with slow degradation kinetics (i.e. long alkyl side chain) were non-toxic. From these studies, it can be concluded that the cytotoxicity of alkylcyanoacrylate polymers is clearly dependent upon the length of the alkyl side chain, with a very low toxicity for the longer alkyl side chains. This observation may be related to the burst release of degradation products during the incubation of poly(methylcyanoacrylate) or poly(ethylcyanoacrylate) nanoparticles, since the hydrolytic degradation of poly(alkylcyanoacrylates) increases with decreasing alkyl chain length [2,69].

A second mechanism due to membrane adhesion was also found to be involved in the cytotoxicity of PACA nanoparticles. Even though polymers with longer alkyl chains are less toxic, poly(ethylcyanoacrylate) nanoparticles are more cytotoxic to L929 fibroblasts than poly(methylcyanoacrylate) nanoparticles. The reason for this is that poly(methylcyanoacrylate) is very rapidly degraded in the culture medium and does not adhere to the cell membrane. On the contrary, poly(ethylcyanoacrylate) is more cytotoxic because the particles probably first adhere to the cell membrane and then release their biodegradation products locally [71].

Finally, the release of inflammatory mediators induced by the contact of PACA nanoparticles with cells was studied by Gaspar et al. [96], showing that PACA nanoparticles could stimulate macrophages. The release of hydrogen peroxide from the cells was observed, whereas no release of cytokines could be detected.

It should be stressed that one has to be very careful with toxicity studies performed in vitro because the nanoparticles-to-cell ratio involved under such conditions is dramatically higher than it would be after in vivo administration. In addition, if degradation products are partially or totally responsible for the cytotoxicity of PACA polymers, under in vivo conditions these products are generally eliminated from their site of degradation, thus the contact time with the cells would be considerably lower than in vitro.

In vitro cell culture experiments are clearly useful to clarify the polymer–cell interaction, but they are of poor toxicological relevance without being complemented by ex vivo and in vivo assays. This was the reason why ex vivo models were used by Fernandez-Urussuno et al. [97,98] to study the possible alteration of hepatocytes after repeated administration of PACA nanoparticles to rats. After treatment, rat hepatocytes were isolated and both secretion of inflammation proteins (α-1 acid glycoprotein) and the oxidative response were observed. However, these effects were reversible after treatment with PACA nanospheres was discontinued. Such an hepatocyte reaction was assumed to be due to the release of mediators from Küpffer cells, in which the nanoparticles concentrated after intravenous administration. This hypothesis was confirmed by showing that particles coated with a hydrophilic block copolymer (therefore escaping Küpffer cell uptake) do not induce any inflammatory response [97].

Finally, from a toxicological point of view, it is noteworthy that the association of a drug with nanoparticles can also induce a higher intracellular concentration of the drug and even allows non-intracellularly diffusible drugs to concentrate in certain intracellular compartments (lysosomes) [99]. This approach, which has been used to improve the efficacy of certain drugs, may induce unexpected toxicity in the case of compounds with an apparent low cytotoxicity because they usually diffuse poorly

intracellularly. This was demonstrated with propidium iodide, free or associated with polymeric nanoparticles [100]. In addition, the pathway and velocity of cellular absorption can influence the intracellular distribution of the drug–polymer association, with, as a consequence, altered cytotoxicity.

3.5. Therapeutic applications of poly(alkylcyanoacrylate) nanoparticles

Polymer nanoparticles are very promising drug delivery systems for a wide range of applications. Among the different biodegradable nanoparticles developed over the last 25 years, an important part of the published literature has been devoted to PACA nanoparticles, which have been tested for different therapeutic purposes and routes of administration.

3.5.1. Intravenous administration

3.5.1.1. Application to the treatment of intracellular infections

Intracellular infections have been established as a field of interest for drug delivery by means of nanospheres. Indeed, infected cells may constitute a 'reservoir' for microorganisms which are protected from antibiotics inside lysosomes. The resistance of intracellular infections to chemotherapy is often related to the low uptake of commonly used antibiotics or to their reduced activity at the acidic pH of lysosomes. To overcome these effects, the use of ampicillin, a β-lactam antibiotic, bound to PACA nanospheres was proposed as an endocytozable formulation [101]. The effectiveness of poly(isohexylcyanoacrylate) nanospheres was tested in the treatment of two experimental intracellular infections.

First, ampicillin-loaded nanospheres were tested in the treatment of experimental *Listeria monocytogenes* infection in congenitally athymic nude mice, a model involving chronic infection of both liver and spleen macrophages [102]. After adsorption of ampicillin onto nanospheres, the therapeutic activity of the ampicillin was found to increase dramatically over that of the free drug. Bacterial counts in the liver were reduced at least 20-fold after linkage of ampicillin to poly(isohexylcyanoacrylate) nanospheres. In addition, ampicillin-loaded nanoparticles were capable of ensuring liver sterilization after two injections of 0.8 mg of nanosphere-bound drug, whereas no such sterilization was observed with any of the other regimens tested. The reappearance of living bacteria in the liver after cessation of treatment was probably due to a secondary infection derived from other organs such as the spleen, which was not completely sterilized by the treatment [102].

Secondly, nanosphere-bound ampicillin was tested in the treatment of experimental salmonellosis in C57/BL6 mice, a model involving an acute fatal infection [101]. All mice treated with a single injection of nanoparticle-bound ampicillin (dose 0.8 mg) survived, whereas all control mice and all those treated with unloaded nanospheres died within 10 days post-infection. With free ampicillin, an effective cure required three doses of 32 mg each. Lower doses (3×0.8 mg and 3×16 mg) delayed, but did not reduce, mortality. Thus, the therapeutic index of ampicillin, calculated on the basis of mice mortality, was increased by 120-fold when the drug was bound to nanospheres.

In order to clarify the mechanism by which PACA nanospheres improve the antimicrobial efficacy of ampicillin, Forestier et al. [103] compared the efficacy of ampicillin bound to poly(isobutylcyanoacrylate) nanospheres with that of free ampicillin in terms of survival of *L. monocytogenes* in mouse peritoneal macrophages cultivated in vitro. After 30 h incubation, nanosphere-bound ampicillin reduced the number of viable bacteria by 99% as compared to the controls, whereas with free ampicillin the number of bacteria was slightly lower than in the controls. Nanoparticle–ampicillin thus appears to be much more effective than free ampicillin for inhibiting the intracellular growth of *L. monocytogenes*.

With in vitro *Salmonella typhimurium*-infected macrophages, the situation was slightly more complicated since the bactericidal effect of ampicillin-bound poly(isohexylcyanoacrylate) nanospheres was poor, although the intracellular capture of ampicillin was dramatically increased and its efflux in the extracellular medium reduced [104]. In another study, confocal microscopy and transmission electron microscopy were used to establish the intracellular trafficking of ampicillin-bound poly(isohexylcyanoacrylate) nanospheres and its relation to bacteria within subcellular compartments (Fig. 8)

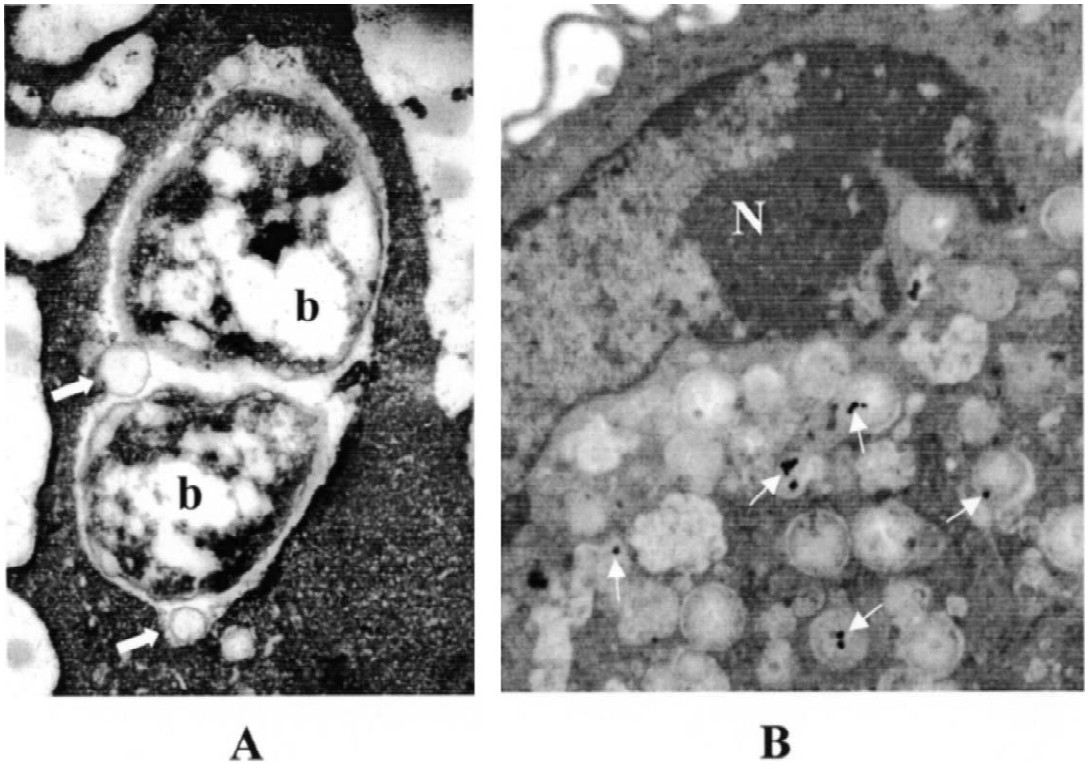

Fig. 8. Transmission electron microscopy of J774 macrophages infected with *Salmonella typhymurium* incubated with ampicillin-loaded PACA nanospheres. (A) Colocalization of nanospheres (arrows) and a dividing bacterium (b) in the same vaccuole inside the macrophage. (B) Ultrastructural autoradiograph of a macrophage treated with [^{3}H]ampicillin-loaded PACA nanospheres. Silver grains (arrows) due to the presence of radioactive material ([^{3}H]ampicillin) were located inside the cells on spherical bodies that may correspond to bacteria in the form of spheroplasts, because of the action of the antibiotic, a β-lactam, on the outer membrane, resulting in a loss of rigidity.

[105]. Data obtained from this study clearly demonstrated the active uptake by phagocytosis of ampicillin-bound poly(isohexylcyanoacrylate) nanospheres by murine macrophages and their localization in the same vacuoles as the infecting bacteria, but in a restrictive way [105]. Furthermore, using [^{3}H]ampicillin-loaded nanospheres, the radioactivity due to ampicillin could clearly be localized as being associated with the membrane of intracellular bacteria under lysis due to the presence of the antibiotic (Fig. 8B). It is difficult to understand the limited bactericidal effect of ampicillin-bound nanospheres measured in vitro in this model. The most probable explanation may be found in one of the known antibiotic resistance mechanisms of *S. typhimurium* involving the inhibition of phagosome–lysosome fusion required for the nanospheres (in phagosomes) to reach the bacteria (in lysosomes) [106]. If this hypothesis is correct, the high efficiency observed in vivo would be due to the specific targeting of the infected tissues (rich in macrophages), rather than to efficient intracellular targeting, as was assumed.

In order to eliminate both dividing and non-dividing bacteria, a fluoroquinolone antibiotic, ciprofloxacin, has been associated with poly(isobutylcyanoacrylate) and poly(isohexylcyanoacrylate) nanospheres. In an animal model of persisting Salmonella infection, although an effect on the early phase of the infection was observed, neither free nor nanosphere-bound ciprofloxacin was able to eradicate truly persisting bacteria [107].

Since they accumulate in the mononuclear phagocyte system, nanospheres also hold promise as drug carriers for the treatment of visceral leis-

hmaniosis [108]. Thus, poly(isohexylcyanoacrylate) nanospheres were used as a carrier of primaquine, the activity of which was increased 21-fold against intracellular *Leishmania donovani* when associated with nanospheres [96]. A part of the activity was attributed to the fact that the phagocytosis of nanospheres lead to the induction of a respiratory burst which was more pronounced in infected than in non-infected macrophages [96]. Dehydroemetine is another drug candidate for this treatment. The heart toxicity of this drug can be reduced after linkage with nanospheres [109].

3.5.1.2. Application to the treatment of non-resistant cancers

When given intravenously, anticancer drugs are distributed throughout the body as a function of the physico-chemical properties of the molecule. A pharmacologically active concentration is reached in the tumor tissue at the expense of massive contamination of the rest of the body. For cytostatic compounds, this poor specificity raises a toxicological problem which represents a serious obstacle to effective therapy. The use of colloidal drug carriers could represent a more rational approach to specific cancer therapy. In addition, the possibility of overcoming multi-drug resistance might be achieved by using cytostatic-loaded nanospheres.

The anti-tumor efficacy of doxorubicin-loaded PACA nanospheres was first tested using the lymphoid leukemia L1210 as a tumor model. In this study, one intravenous injection of doxorubicin-loaded poly(isobutylcyanoacrylate) nanospheres was found to be more effective against L1210 leukemia than the drug administered in its free form following the same dosing schedule [87]. However, although the increased life-span of mice injected with doxorubicin-loaded poly(isobutylcyanoacrylate) nanospheres was twice as long as the increased life-span for free doxorubicin, there were no long-term survivors. The effectiveness of doxorubicin-loaded poly(isohexylcyanoacrylate) nanospheres against L1210 leukemia was even more pronounced than that of doxorubicin loaded onto poly-(isobutylcyanoacrylate) nanospheres. The drug toxicity was markedly reduced, so that impressive results were obtained at doses for which the therapeutic efficiency of free doxorubicin was completely masked by the overpowering toxicity of the drug [87]. Preliminary experiments suggested that one intravenous bolus injection of doxorubicin-loaded nanospheres was more active, in L1210-bearing mice, than perfusion of the free drug for 24 h.

The superiority of doxorubicin targeted with the aid of PACA nanospheres was later confirmed in a murine hepatic metastases model (M5076 reticulosarcoma) [110]. Irrespective of the dose and the administration schedule, the reduction in the number of metastases was much greater with doxorubicin-loaded nanospheres than with free doxorubicin, particularly if treatment was given when the metastases were well established. The improved efficacy of the targeted drug was clearly confirmed by histological examinations showing that both the number and the size of the tumor nodules were smaller when doxorubicin was administered in its nanoparticulate form [110]. Furthermore, liver biopsies of animals treated with the nanosphere-targeted drug showed a lower cancer cell density inside tumor tissue. Necrosis was often less widespread with the nanosphere-associated drug than in the control group and the group treated with free doxorubicin. Studies performed on total homogenates of livers from both healthy and metastases-bearing mice showed extensive capture of nanoparticulate doxorubicin by the liver; no difference in hepatic concentrations was observed between healthy and tumor-bearing animals [110]. In order to elucidate the mechanism behind the enhanced efficiency of doxorubicin-loaded nanospheres, doxorubicin measurements were made in both metastatic nodules and neighboring healthy hepatic tissue. This provided quantitative information concerning the drug distribution within these tissues [86]. During the first 6 h after administration, exposure of the liver to doxorubicin was 18 times greater for nanosphere-associated doxorubicin. However, no special affinity for the tumor tissue was detected and the nanospheres were observed by electron microscopy to be located within Küpffer cells (macrophages). However, at later time points, the amount of drug in the tumor tissue increased in nanosphere-treated animals to 2.5 times the level found in animals given free doxorubicin. Since uptake of nanospheres by neoplastic tissue appeared to be unlikely, this increase in the doxorubicin concentration in tumor tissue probably resulted from

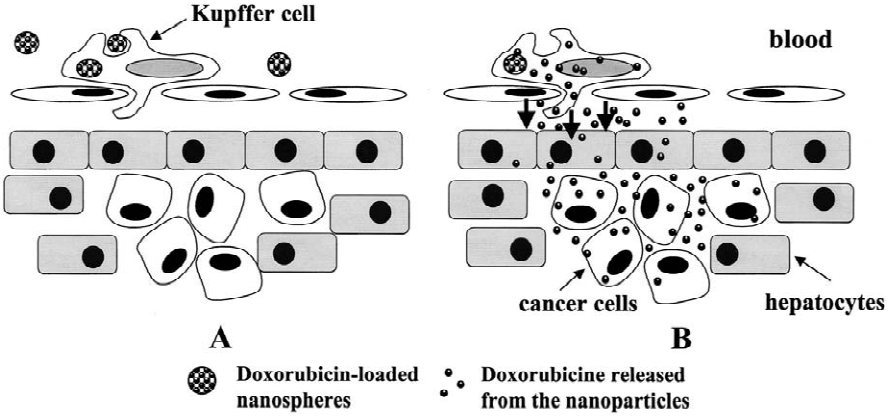

Fig. 9. Mechanism proposed to explain the action of doxorubicin-loaded PACA nanospheres in the treatment of liver metastasis. (A) Uptake of the nanospheres by Küpffer cells. (B) Degradation of the doxorubicin-loaded nanospheres in the intralysosomal compartments of the Küpffer cells, inducing the release of the drug and diffusion into the surrounding tissue.

doxorubicin released from healthy tissue, in particular Küpffer cells (Fig. 9). Hepatic tissue could play the role of a drug reservoir, from which prolonged diffusion of the free drug (from nanospheres entrapped in Küpffer cell lysosomes) towards neighboring malignant cells occurs (Fig. 9).

This hypothesis raises the question of the long-term effect of an 18-fold increase in the doxorubicin concentration in the liver. Although toxicological data have shown that doxorubicin-loaded nanospheres are not significantly or unexpectedly toxic to the liver in terms of survival rate at high doses, body weight loss and histological appearance [111], this possibility should be borne in mind, especially since a temporary depletion in the number of Küpffer cells, and hence the ability to clear bacteria, was observed in rats treated with doxorubicin-loaded liposomes [112]. Nanoparticle-associated doxorubicin also accumulates in bone marrow, leading to a myelosuppressive effect [80]. This tropism of carriers might be useful for the delivery of myelostimulating compounds such as granulocyte colony stimulating factor to reverse the suppressive effects of intense chemotherapy [113].

Another approach to cancer therapy considered PEG-coated PACA nanoparticles as a long-circulating carrier for the targeting of recombinant tumor necrosis factor-α (rHuTNF-α) to tumor tissue [23,64]. rHuTNF-α was successfully associated with PEG-coated nanospheres consisting of poly(PEGCA-co-PHDCA) [23,64]. The pharmacokinetics and antitumor effect were evaluated in vivo in mice with sarcoma-180 cells implanted intradermally. As expected because of the difference in microvascular permeability between healthy and tumoral tissue, greater accumulation of rHuTNF-α in the tumor and increased antitumoral activity were found when this compound was injected intravenously as PEG-coated nanospheres compared to the free drug [23].

To develop cell-selective targeting, folic acid has been conjugated to PEG-coated nanospheres consisting of poly(PEGCA-co-PHDCA) [20], the rational behind this construction being that folic acid-binding protein is frequently overexpressed on the surface of human cancer cells. The nanospheres were prepared using a copolymer containing PEG with a terminal amino group. This amino group was exposed at the particle surface and subsequent coupling with activated folic acid was successful. Based on plasmon resonance assays, it was found that the folate-conjugated nanospheres interacted much more with folate-binding protein than nanospheres coated with PEG. Folate-decorated nanospheres additionally showed a greater affinity for the receptor compared to a single molecule of folic acid. This can be explained by the cooperative interactions obtained with the nanos-

pheres [20]. Folic acid-decorated nanospheres offer interesting perspectives for the selective targeting of anticancer compounds to tumoral cells and tissues.

3.5.1.3. Application to the treatment of resistant cancers

The ability of tumor cells to develop simultaneous resistance to multiple lipophilic compounds is a major problem in cancer chemotherapy. Cellular resistance to anthracyclines has been attributed to an active drug efflux from resistant cells linked to the presence of transmembrane P-glycoprotein, which is not detectable in the parental drug-sensitive cell line. Drugs such as doxorubicin appear to enter the cell by passive diffusion through the lipid bilayer. Upon entering the cell, these drugs bind to P-glycoprotein, which forms transmembrane channels and uses energy from ATP hydrolysis to pump these compounds out of the cell [114]. To solve this problem, many authors have proposed the use of competitive P-glycoprotein inhibitors, such as the calcium channel blocker verapamil, which are able to bind to P-glycoprotein and overcome pleiotropic resistance. However, since verapamil exhibits serious adverse effects, its clinical use to overcome multidrug resistance is limited. This is why the effect of nanospheres loaded with doxorubicin, the resistance to which is known to be related to the presence of P-glycoprotein, was evaluated. The cytotoxicity of free doxorubicin, doxorubicin-loaded poly(isohexylcyanoacrylate) nanospheres (mean diameter 300 nm) and nanospheres without drug against sensitive (MCF7) and multi-drug resistant (doxorubicin R MCF7) human breast cancer cell lines was compared [115]. MCF7 cells were more sensitive to free doxorubicin than doxorubicin R MCF7 cells with a 150-fold difference in the IC_{50} values. No significant difference was observed in the survival rate of MCF7 cells treated with free doxorubicin or doxorubicin-loaded nanospheres. In contrast, for doxorubicin R MCF7, the IC_{50} for doxorubicin was 130-fold lower when doxorubicin-loaded nanospheres were used instead of free doxorubicin [115]. These results indicate that PACA nanospheres provide an effective carrier for introducing a cytotoxic dose of doxorubicin into the pleiotropic resistant human cancer cell line Dox R MCF7.

Complementary experiments, conducted with other sensitive and resistant cell lines, have confirmed this efficacy of nanospheres [116,117]. Doxorubicin resistance was circumvented in the majority of cell lines tested, and encouraging results were obtained in vivo in a P388 model growing as ascites [116]. Further studies were undertaken to elucidate the mechanism of action of PACA nanospheres. The incubation time and number of particles per cell were important factors [118] and, when poly(isobutylcyanoacrylate) nanospheres were used, doxorubicin accumulation within P388/ADR-resistant leukemic cells increased compared with the free drug, although no endocytosis of nanospheres occurred [119]. On the other hand, when the less rapidly degradable poly(isohexylcyanoacrylate) nanospheres were used, reversion was observed in the absence of increased intracellular drug concentration [120]. The degradation products of PACA nanospheres (mainly poly(cyanoacrylic acid)) were also able to increase both the accumulation and cytotoxicity of doxorubicin, although they were soluble in the culture medium. Hence, reversion of resistance seems to be due both to the adsorption of nanospheres on the cell surface and to the formation of a doxorubicin–poly(cyanoacrylic acid) complex (an ion pair) which facilitates the transport of the drug across the cell membrane (Fig. 10) [120,121].

In the light of the results obtained with doxorubicin-loaded PACA nanospheres in the liver metastases model described above [110], the role of macrophages as a reservoir for doxorubicin was tested in a two-compartment, co-culture system in vitro with both resistant and sensitive P388 cells [122]. Even after prior uptake by macrophages, doxorubicin-loaded poly(isobutylcyanoacrylate) nanospheres were able to overcome resistance. However, this reversion was only partial. It was decided to take advantage of the particulate drug carrier effect to associate an anti-cancer drug and a compound capable of inhibiting P-glycoprotein. This approach was tested with doxorubicin and cyclosporin A bound to the same nanospheres, and was found to be extremely effective in reversing P388 resistance [123]. The association of cyclosporin A with nanospheres would ensure that it reaches the same sites as the anti-cancer drug at the same time and would also reduce its toxic side-effects.

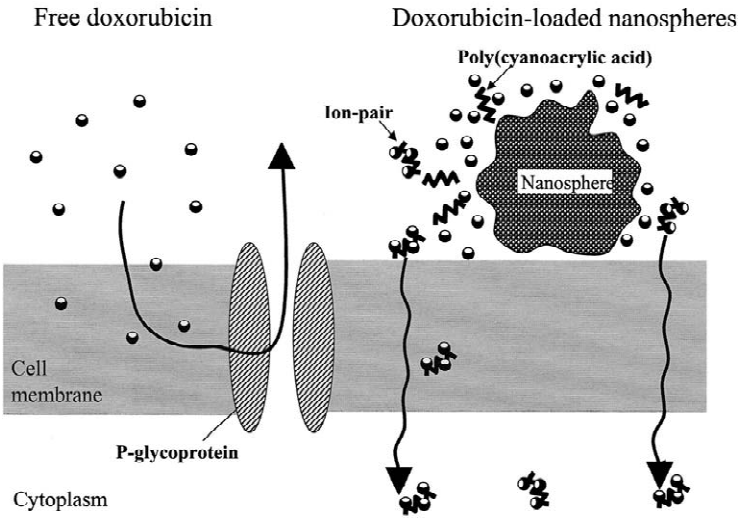

Fig. 10. Proposed mechanism for the reversion of multidrug resistance by means of doxorubicin-loaded PACA nanospheres. Given as free drug, doxorubicin is pumped out of the cell by P-glycoprotein (A). When given as PACA nanospheres, doxorubicin and degradation products such as poly(cyanoacrylic acid) are released locally from the nanospheres adsorbed on the cell membrane. Together they can form ion pairs, which facilitate the penetration of doxorubicin into resistant cells (B) [120].

3.5.1.4. Application to the delivery of oligonucleotides

Oligodeoxynucleotides are potentially powerful new drugs because of their selectivity for particular gene products in both sense and anti-sense strategies. However, using oligonucleotides in therapeutics is a challenge to pharmaceutical technology because of their susceptibility to enzymatic degradation and their poor penetration across biological membranes. Thus, nanoparticle preparations might be an interesting alternative for protecting oligonucleotides from degradation in biological fluids. In the case of PACA nanospheres, since oligonucleotides have no affinity for the polymeric matrix, association has been achieved by ion pairing using a cationic surfactant, cetyltrimethylammonium bromide (CTAB) or diethylaminoethyl-dextran (DEAE), adsorbed onto the nanosphere surface [82,124].

The oligonucleotides bound to the nanospheres were protected from nuclease degradation in vitro [82] and their intracellular uptake was increased [125]. In addition, PACA nanospheres were able to concentrate intact oligonucleotides in the liver and spleen [126]. Antisense oligonucleotides formulated in this way are also able to specifically inhibit mutated Ha-*ras*-mediated cell proliferation and tumorigenicity in nude mice [127].

However, these nanospheres have two drawbacks: (i) their toxicity, mainly due to the presence of CTAB [128]; and (ii) the rapid desorption of the oligonucleotides in the presence of serum, which results from PACA nanosphere surface erosion by serum esterases [126]. This is why PACA nanocapsules with an aqueous core containing the oligonucleotides were developed recently [49,128]. Stability studies demonstrated that the PACA nanocapsules were able to protect the oligonucleotides from degradation by serum nucleases and that this protection was more efficient than that obtained with CTAB-coated PACA nanospheres [82,129].

Phosphorothioate oligonucleotides directed against EWS Fli-1 chimeric RNA were encapsulated within PACA nanocapsules and tested for their efficacy in vivo against experimental Ewing sarcoma in mice after intratumoral administration [130]. Only intratumoral injection of antisense-loaded nanocapsules lead to a significant inhibition of tumor growth at a cumulative dose of 14.4 nmol. No antisense effect could be detected with the free oligonucleotide. In a previous study, using the same antisense sequence as

the free drug, Tanaka et al. [131] demonstrated inhibition of tumor growth in a similar model, but a cumulative dose of 500 nmol oligonucleotide was needed. With PACA nanocapsules it was possible to obtain a comparable effect with a 35-fold lower dose. Therefore, nanocapsule technology allows smaller phosphorothioate doses to be used and thus avoid the toxicity and loss of specificity resulting from phosphorothioates at higher doses [132].

The mechanism by which oligonucleotides in nanocapsules lead to a significant effect on tumor growth may be explained by the protection of the oligonucleotide afforded by the nanocapsules which, in addition, may act as a controlled release system for the oligonucleotide within the tumor. Thus, the use of phosphorothioates at low doses combined with nanocapsules may represent a new and safe option for the administration of antisense oligonucleotides in vivo.

3.5.1.5. Application to the passage of the blood–brain barrier

The blood–brain barrier is an insuperable obstacle for a large number of drugs, such as antibiotics, antineoplastic agents, and a variety of central nervous system-active drugs, especially neuropeptides. PACA nanoparticles coated with polysorbate 80 have been proposed to overcome this barrier and to deliver drugs to the brain [133]. Drugs that have been transported successfully into the brain using this carrier include the hexapeptide dalargin [134], the dipeptide kytorphin [133], loperamide [135], tubocurarine [136], the NMDA receptor antagonist MRZ 2/576 [137,138], and doxorubicin [139,140]. PACA nanoparticles may be especially helpful for the treatment of disseminated and very aggressive brain tumors. Intravenously injected doxorubicin-loaded, polysorbate 80-coated nanoparticles lead to a 40% cure in rats with intracranially transplanted glioblastomas 101/8 [139,140]. The mechanism of nanoparticle-mediated transport of drugs across the blood–brain barrier has not been fully elucidated. The most likely mechanism is endocytosis followed by transcytosis by the endothelial cells lining the blood capillaries of the brain, as shown by fluorescence labelling and confocal laser scanning microscopy observations [141,142]. Nanoparticle-mediated drug transport to the brain depends on coating the particles with polysorbates, especially polysorbate 80 [143]. Polysorbate 80-coated PACA nanospheres were found to adsorb apolipoprotein E from blood plasma and therefore may mimic low density lipoprotein, having specific receptors at the surface of endothelial cells of the blood–brain barrier. After such recognition, the drug may be released into these cells from the nanospheres and diffuse into the brain interior, or the particles may be transcytosed. Other processes such as tight junction modulation or P-glycoprotein inhibition may also occur (Fig. 11). Moreover, these mechanisms may run in parallel or may be cooperative, thus enabling efficient drug delivery to the brain. However, Olivier et al. [144] have shown that non-specific permeabilization of the blood–brain barrier, probably related to the toxicity of the carrier, may account for the central nervous system drug penetration when associated with poly-(butylcyanoacrylate) nanoparticles and polysorbate 80.

Calvo et al. [22,145] evaluated the ability of long-circulating, PEG-coated PACA nanoparticles consisting of the amphiphilic copolymer poly(PEGCA-co-HDCA) to diffuse into brain tissue after intravenous administration to mice and rats. Based on their long-circulating characteristics, PEG-coated nanoparticles penetrated into the brain to a greater extent than all the other nanoparticle formulations tested, including polysorbate 80-coated nanospheres. Particles were localized in the ependymal cells of the choroid plexus, in the epithelial cells of the pia mater and ventricles, and to a smaller extent in the capillary endothelial cells of the blood–brain barrier. These phenomena occurred without any modification of blood–brain barrier permeability, whereas polysorbate 80-coated nanoparticles owe their efficacy, in part, to blood–brain barrier permeabilization induced by the surfactant. Poloxamine 908-coated nanoparticles, which also exhibit long-circulating properties, failed to increase the brain concentration, probably because of their inability to interact with cells [22]. The concentration of PEG-coated nanoparticles in the central nervous system, especially in white matter, was shown to be greatly increased in comparison to conventional non-PEG-coated nanoparticles. In addition, this increase was significantly higher in pathological situations where blood–brain barrier permeability is augmented and/or macro-

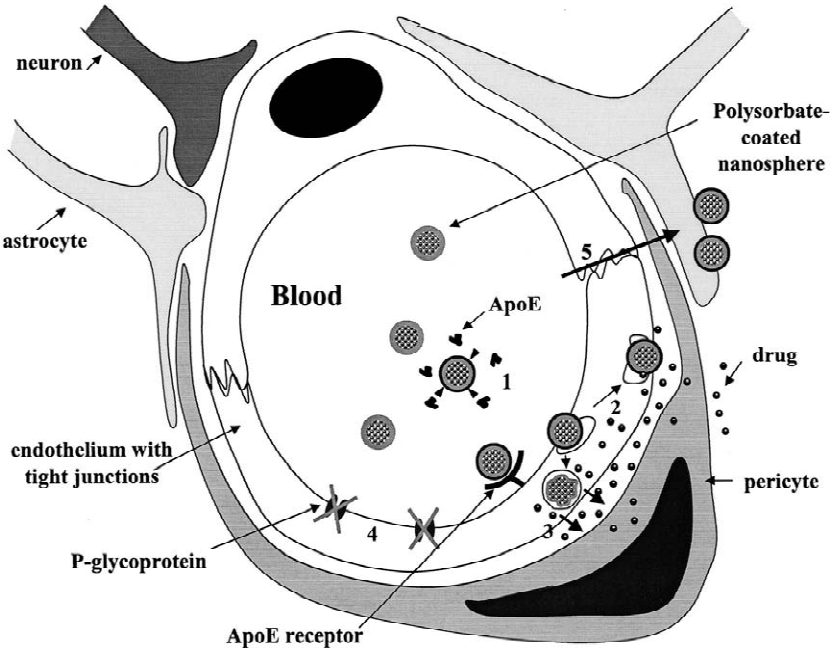

Fig. 11. Hypothetical mechanism of drug delivery to the brain by means of polysorbate 80-coated PACA nanospheres. (1) Adsorption of apolipoprotein E (ApoE) onto the nanospheres; (2) transcytosis of the nanospheres; (3) endocytosis followed by intracellular degradation of the nanospheres, resulting in release of the drug and diffusion towards the interior of the brain; (4) inhibition of P-glycoprotein; (5) modulation of tight junction opening.

phages have infiltrated. Passive diffusion and macrophage uptake in inflammatory lesions seems to be the mechanism underlying such brain penetration. This was clearly documented after administration of PEG-coated nanospheres to rats bearing an experimental allergic encephalomyelitis [145]. In addition, these PEG-coated nanospheres showed, in comparison with conventional non-PEG-coated nanoparticles, a higher uptake by the brain of scrapie-infected animals, which may be useful for targetting drugs for the treatment of prion diseases [65].

3.5.2. Oral route

There are numerous reports showing that uptake and translocation of nanoparticles and microparticles takes place after oral administration to animals [146–148]. Different mechanisms have been proposed to explain the translocation of particulate material across the intestine: (i) uptake via Peyer's patches or isolated lymphoid follicles; (ii) intracellular uptake; and (iii) intercellular/paracellular passage [149]. The mechanism of nanoparticle uptake depends on the nature of the nanoparticle. The uptake of PACA nanocapsules by Peyer's patches has been shown by Damgé et al. [148]. When administered in the lumen of an isolated ileal segment of the rat, PACA nanocapsules were found preferentially over Peyer's patches, through which they passed massively and rapidly [148]. Nanocapsules were clearly visible in M-cells and in intercellular spaces around the lymph cells. Intracellular uptake of nanospheres has been proposed by Kreuter et al. [150] based on electron-microscopic autoradiographic investigations showing radioactivity in epithelial and goblet cells after oral administration of poly(hexylcyanoacrylate) nanospheres labeled with ^{14}C. The translocation of particles by a paracellular pathway was evidenced in a study performed by Aprahamian et al. [151] using poly(isobutylcyanoacrylate) nanocapsules. The nanocapsules were filled with an iodinized oil (lipiodol) in order to render them detectable using a scanning electron microscope equipped with an energy-dispersive X-ray spectrometer. When administered in an isolated segment of dog jejunum,

they appeared as vesicles associated with intraluminal mucus. Subsequently, they were observed in intravillus capillaries in close contact with red blood cells or adsorbed to the inner wall of endothelial cells. Among these three mechanisms, and according to many studies involving nanoparticles consisting of other biodegradable and non-degradable polymers, translocation via uptake in Peyer's patches seems to be the major pathway for rapid and substantial passage after oral administration of nanoparticles. Although it might occur in certain situations, the passage of particles between absorptive cells is rather less likely if the barrier of tight junctions has not been disrupted. Although there are abundant reports from various independent workers showing evidence of the absorption of particulate systems by the gastrointestinal tract, the oral absorption of nanoparticles remains a controversial issue. However, even if a more exact estimation of the quantity of absorbed particles is needed, as well as a better understanding of the factors affecting particle uptake, it must be concluded that translocation of small-sized particles such as PACA nanoparticles is possible. The question remains if the extent of particle translocation is compatible with a strategy of drug administration with therapeutic perspectives. This is discussed below.

3.5.2.1. Oral delivery of peptides, proteins and vaccines

Poly(isobutylcyanoacrylate) nanocapsules were shown 15 years ago to be able to encapsulate insulin and to increase its activity as assessed by a reduction of glycemia after oral feeding [56]. Several aspects of this phenomenon are surprising: encapsulation of a hydrophilic drug in the oily core of nanocapsules; reduction of glycemia was only obtained with diabetic animals; and hypoglycemia appeared 2 days after a single oral administration and was maintained for up to 20 days depending on the insulin dose, although the amplitude of the pharmacological effect (minimum level of blood glucose) did not depend on the insulin dose. Damgé et al. [148] and Lowe and Temple [58] suggested that nanocapsules could protect insulin from proteolytic degradation in intestinal fluids as observed in the presence of different proteolytic enzymes in vitro. Furthermore, later studies showed that insulin did not react with the alkylcyanoacrylate monomer during nanocapsule formation and was located within the oily core rather than adsorbed on the surface [52].

The capacity of insulin nanocapsules to reduce glycemia can also be explained by their translocation through the intestinal barrier, as suggested by Damgé et al. [148]; for example, by the paracellular pathway or via M-cells in Peyer's patches [57]. Recently, the use of Texas Red®-labelled insulin allowed this translocation to be visualized more readily [152]. One hour after oral administration, nanocapsules reached the ileum. The presence of fluorescent areas within the mucosa and even in the lamina propria suggested that insulin-loaded PACA nanocapsules could cross the intestinal epithelium. Although this passage is certainly an important factor, it does not explain the duration of the hypoglycemia. This prolonged action could be due to the retention of a proportion of the colloidal system in the gastrointestinal tract. Interestingly, a prolonged hypoglycemic effect was also observed with insulin entrapped in PACA nanospheres when these nanospheres were dispersed in an oily phase containing a surfactant [153]. This suggests that some components of the nanocapsules can act as absorption promoters. Recently, insulin has also been encapsulated in water-containing nanocapsules [50]. These nanocapsules, dispersed in a biocompatible microemulsion, could facilitate the intestinal absorption of the encapsulated peptide after oral administration, as suggested by the reduced blood glucose level observed in diabetic rats [154].

Peptides other than insulin have been successfully associated with nanocapsules. Among these, octreotide, a somatostatin analogue, has been incorporated in oil-containing PACA nanocapsules, and was found to improve and prolong the therapeutic effect of this peptide after administration by the oral route [155]. Calcitonin has also been encapsulated in both oil-containing and water-containing nanocapsules [48,58,156]. Calcitonin-loaded, oil-containing nanocapsules showed behavior similar to insulin-loaded, oil-containing nanocapsules [57,58]. Using PACA nanocapsules with an aqueous core, the effectiveness of the encapsulated peptide after oral administration to rats was estimated as 45% of the activity obtained after intravenous administration of

the same dose, whereas an absolute bioavailability of 40% was measured [48].

Even if the main limitation to the oral administration of PACA nanoparticles with peptides is that their passage through the intestinal barrier is probably restricted and sometimes erratic, they represent an interesting tool for the oral delivery of antigens. Indeed, M-cells appear to be the main site for the uptake of PACA nanoparticles after oral administration [148] and, furthermore, it is generally accepted that limited doses of antigen are sufficient for mucous immunization. In fact, the oral delivery of antigens may be considered the most convenient means of producing an IgA antibody response. However, this is limited by the enzymatic degradation of antigens in the GI tract and, additionally, by their poor absorption. Thus, it has been postulated that the use of micro- or nanoparticles for the oral delivery of antigens can be efficient if these systems are able to achieve protection of the antigenic molecule. PACA nanoparticles have been shown to enhance the secretory immune response after oral administration in association with ovalbumin [157]. This result was not fully reproduced with poly(acrylamide) nanospheres loaded with the same antigen, leading to the assumption that the antigen was mainly located at the surface of the poly(acrylamide) nanospheres and could have been degraded during its passage through the gut. The relatively high surface concentration of ovalbumin adsorbed onto poly(butylcyanoacrylate) nanospheres may have reduced the ability of the proteolytic enzymes in the gut to gain access to, and to degrade, the antigen, resulting in greater antigen availability.

3.5.2.2. Bioadhesive nanoparticles

Some polymers, either of natural or synthetic origin, have the ability to adhere to wet mucosal surfaces by means of hydrogen bonding or van der Waals forces [158]. With swellable hydrophilic polymers, adhesion is optimal when mucosal contact is made with the dry polymer. Further, progressive hydration of the polymer leads to the formation of a hydrogel, which is responsible for the development of the considerable mucosa adhesion strength [158]. However, in the case of colloidal particles, bioadhesion is achieved with non-swellable polymers such as PACA, and this is mainly due to the inherent tendency of these small particles to develop intimate contact on large mucosal sites [159]. The bioadhesive properties were found to vary with the size and the surface characteristics of the nanoparticles [159–161]. To improve the interactions of nanoparticles with mucosae, lectins have been grafted onto the nanoparticle surface [159], or nanoparticles have been coated with chitosan [45].

Bioadhesion has been tested in vivo. After peroral administration of radiolabelled poly(hexylcyanoacrylate) nanoparticles to mice, whole-body autoradiography showed that, 30 min after administration, the particles were exclusively localized in the stomach [162]. After 4 h, a large quantity of radioactivity was found in the intestine in the form of clusters without macroradiographic evidence of accumulation at specific intestinal sites. On the contrary, a persistent film of nanoparticles adhering to the stomach wall was observed. In this study, very little of the radioactivity was found to be absorbed. In a similar study, microautoradiographs confirmed the presence of radioactivity throughout the gut [150,162]. The amount of radioactivity decreased to 30–40% of the 90-min value within 4–8 h and to 5%, 24 h after dosing. Histological investigation showed radioactivity adjacent to the brush border, incorporated into the underlying cell layers and in goblet cells up to 6 days after administration. However, the exclusive use of a radioactive tracer in these experiments makes the presence of physically intact particles 6 days after administration questionable because of possible degradation of the particles in the gastrointestinal tract.

The pharmacokinetics of several drugs have been improved after oral administration by means of nanoparticles. Most studies were carried out with conventional formulations, which means that the carriers were not specifically designed to improve the bioadhesion performance of the particles. The bioavailability of vincamine was about 25% when administered in an aqueous solution to rabbits. After oral administration of vincamine adsorbed on poly(hexylcyanoacrylate) nanoparticles, the bioavailability reached 40%, probably due to the prolonged period of contact of the drug delivery system with the mucosae [163]. Nanocapsules of poly(isobutylcyanoacrylate) increased the bioavailability of iodine after administration of lipiodol, an iodized

oil, to the jejunum of dogs [164]. With the nanocapsules, the blood level of iodine was prolonged from 75 to over 105 min. This observation was also attributed to the prolonged period of contact between the lipiodol drug and the mucus of the microvilli membrane [164]. Darodipine, a calcium flux inhibitor which causes strong vasodilatation, is characterized by a short half-life time ranging from 2 to 4 h. The use of nanocapsules resulted in a reduction in the intensity of the initial very strong hypotensive effect and prolonged the pharmacological activity of the drug [165].

3.5.2.3. Application to the administration of antiproteases

Saquinavir is a potent HIV-1 and HIV-2 protease inhibitor that has been approved for use in the treatment of patients with acquired immunodeficiency syndrome. However, administering saquinavir by the oral route is a formidable challenge due to its poor absorption pattern. Thus several approaches were investigated to improve its oral bioavailability, among them its association with PACA nanoparticles. Saquinavir-loaded PACA nanospheres could be easily prepared in the presence of a drug–cyclodextrin complex. It was found that large amounts of cyclodextrins remained associated with the particles, resulting in a 20-fold increase in saquinavir loading compared to nanoparticles prepared in the absence of cyclodextrins [36]. This study showed that the loading of saquinavir in PACA nanospheres could be improved dramatically by simultaneously increasing the apparent solubility of the drug in the preparation medium and the amount of cyclodextrin associated with the particles, making these nanospheres an interesting system for oral application. Indeed, Boudad et al. [166] have shown that this system is able to improve significantly the amount and kinetics of saquinavir transported from apical to basolateral sites in the CACO-2 monolayer cell model.

3.5.3. Other routes of administration

PACA nanoparticles have been evaluated as controlled release devices for peptide delivery after subcutaneous administration. Radiolabelled poly(isobutylcyanoacrylate) nanospheres were injected subcutaneously and the autoradiographic pictures revealed a progressive reduction of staining in muscular tissue, suggesting that the nanospheres were progressively biodegraded [167]. These nanospheres were found to release growth releasing factor in a sustained manner and to improve its bioavailability [39]. After association with the nanospheres, the peptide was partially protected from enzymatic degradation, whereas it was very rapidly metabolized at the injection site when administered as the free drug.

Nanoparticle technologies have also been investigated for the administration of drugs to the eye. However, in preliminary experiments, PACA nanoparticles appeared not to be very well tolerated by the ocular mucosae, leading to cell lysis [168]. Thus, the development of ocular therapy by means of nanoparticles has considered the use of other polymers, among which poly(ε-caprolactone) showed good potential for eye administration. Very recently, progress in the area of the research and development of new formulations for ocular therapy encouraged some authors to reconsider PACA nanoparticles in opthalmology. To reduce the observed toxic effect to the eye, PACA nanoparticles were either dispersed in a poly(acrylic acid) or PEG gel [169,170] or coated with PEG chains [171]. These approaches were shown to improve the tolerance of the ocular mucosae to PACA nanoparticles and open new avenues for the further development of such formulations as drug delivery systems for ocular therapy.

4. Conclusion

There are now a significant number of technologies based on cyanoacrylate monomers or polymers for biomedical applications. This is due to the fact that cyanoacrylate monomers are able to form polymeric materials with biodegradable characteristics which may easily be controlled depending on the nature of the cyanoacrylic monomer used. On the other hand, it is unquestionable that the use of cyanoacrylates as surgical glue represents, from a toxicological point of view, a favorable situation. Among the different cyanoacrylate-based technologies, nanoparticles probably offer the most exciting possibilities in terms of medical applications. Indeed, they allow modification of the intracellular

trafficking of drugs, thus opening new ways to reach, in a controlled manner, the intracellular bacteria responsible for opportunistic diseases or to administer anticancer compounds to cells in a manner that is able to bypass the cell P-glycoprotein detoxification process. Also, there are interesting perspectives for the intracellular delivery of molecules such as oligonucleotides, which are able, when protected from nucleases, to modulate gene expression. However, as stated above, many questions remain concerning the intracellular fate of nanoparticles, probably because the answers differ from one cell line to another and from one cyanoacrylic polymer to another. Whatever the answer, there is an urgent need to design nanoparticles able to specifically deliver these molecules, either to the cytoplasm or to the nucleus depending on the target. It is evident that, due to the drawbacks of viral vectors, there is a challenge to deliver genetic material intracellularly by means of nanodispersed synthetic carriers and this represents a new challenge for cyanoacrylate polymers.

Controlling the tissue distribution of nanoparticles after intravascular administration is another challenge, which has been partly solved by the newly designed poly(PEGCA-co-PACA) copolymer that avoids opsonisation, thus reducing liver and spleen uptake and increasing the circulation time in the blood. Due to the leaky vasculature in numerous brain pathologies, very exciting applications are now envisioned in this field. Other, very recent approaches allow the efficient coating of PACA nanoparticles with different polysaccharides, which are also expected to modify the tissue distribution of these particles. However, the exciting potential of this approach has not yet been completely investigated.

The oral administration of peptides and proteins by means of PACA nanoparticles is another interesting perspective. Although the oral absorption of nanoparticles remains a controversial field of research, it would be inconsistent to completely ignore this approach since interesting results have been obtained by various independent research groups. Thus, as discussed above, nanoparticles could also open up very interesting perspectives for the oral delivery of antigens.

Finally, it is surprising that there has been so very little effort expended to develop new molecularly addressed PACA nanoparticles. Indeed, the targeting of these nanotechnologies needs to be urgently improved by adequate decoration of the nanoparticle surface. In this respect, the preliminary results obtained with folic acid for targeting of the folic acid receptor are encouraging, but deserve further development.

The concept of cyanoacrylate polymers for the design of new drug delivery systems emerged from academic pharmaceutical research in Europe. In the last 15 years, cyanoacrylate-based technology has improved and its potential in therapeutics is well documented. Cyanoacrylate nanoparticles have recently entered Phase II clinical trials and results are expected in the treatment of resistant cancers.

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