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# The controlled intravenous delivery of drugs using PEG-coated sterically stabilized nanospheres☆

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## Abstract

Injectable blood persistent particulate carriers have important therapeutic application in site-specific drug delivery or medical imaging. However, injected particles are generally eliminated by the reticuloendothelial system within minutes after administration and accumulate in the liver and spleen. To obtain a coating that might prevent opsonization and subsequent recognition by the macrophages, sterically stabilized nanospheres were developed using amphiphilic diblock or multiblock copolymers. The nanospheres are composed of a hydrophilic polyethylene glycol coating and a biodegradable core in which various drugs were encapsulated. Hydrophobic drugs, such as lidocaine, were entrapped up to 45 wt% and the release kinetics were governed by the polymer physico-chemical characteristics. Plasma protein adsorption was drastically reduced on PEG-coated particles compared to non-coated ones. Relative protein amounts were time-dependent. The nanospheres exhibited increased blood circulation times and reduced liver accumulation, depending on the coating polyethylene glycol molecular weight and surface density. They could be freeze-dried and redispersed in aqueous solutions and possess good shelf stability. It may be possible to tailor "optimal" polymers for given therapeutic applications.

### Keywords

Long-circulating nanoparticles; Biodegradable polymers; Polyethylene glycol; Hydrophilic coating; Reduced liver accumulation; Intravenous drug administration

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## 1. Introduction

There has been a growing interest in the development of a colloidal drug carrier which is small enough for intravenous administration and possesses an adequate circulation half-life in order to enable drug release into the vascular compartment in a continuous and controlled manner.

There are numerous potential applications for such a system: the protection of sensitive therapeutically active molecules against in vivo degradation, the reduction of toxic side effects which can occur when some highly active drugs like those used for cancer treatment are administered in the form of a solution, the increase of patient comfort by avoiding repetitive bolus injection or the use of perfusion pumps, and the achievement of more favorable drug pharmacokinetics.

The successful delivery of macromolecular drugs is often problematic [1]. Protein drugs generally cannot be administered orally, since they are hydrolyzed or denatured in the gastrointestinal tract. Parenteral administration also poses some problems, since proteins are usually quickly metabolized and eliminated. The typical pharmacokinetic half-lives of proteins range from 2 to 30 min [1]. The pharmacokinetics of these compounds could be improved by encapsulating them into long-circulating drug delivery devices.

The effective use of pharmacologically active substances in the chemotherapy of cancer, viral infections, and many other diseases suffers from non-specific toxicity and poor tissue specificity of drugs. Polymeric nanoparticles are possible carriers for targeting these compounds by the intravenous route in order to increase the compound's effectiveness in the diseased tissue and reduce general toxicity [2,3].

Various types of systems (liposomes, emulsions, micelles, and nanoparticles) were developed over the last decade in order to achieve controlled intravenous drug delivery or targeting to specific tissues. However, most of these systems, via recognition by the phagocytic cells (mainly the cells of the mononuclear phagocyte system and the polymorphonuclear leukocytes), are detected as foreign products and quickly removed from blood circulation. Essentially, macrophages located in the reticuloendothelial system (RES) (of which the Kupfer cells in the liver comprise 85–95% of the total intravascular phagocytic capacity [4]) play a crucial role to phagocytize injected particles. For example, polystyrene (PS) particles as small as 60 nm disappear from blood within minutes [5]. Similar short half-lives were observed whatever the chemical composition of the injected particles: albumin [6], poly(lactic acid) (PLA) [7,8], poly(lactic acid-co-glycolic acid) (PLGA) [9], polycyanoacrylate [10] or polyacryl starch [11]. Therefore, using these types of systems, it is only possible to target drugs to RES organs, mainly the liver.

It is generally assumed that the rapid particle phagocytosis is mediated by the adsorption of certain blood components (opsonins) onto the surface of the particles; for example, in vitro experiments with liposomes [12] showed that serum complement, one of the most important components of the opsonin system of the body, and in particular C3, strongly activates phagocytosis by macrophages. Other complements, such as C4, also seem to be involved in the phagocytic process [13].

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The main challenge for administering particulate drug carriers into the vascular compartment is site-selective drug delivery. Such a carrier would avoid indiscriminate interactions with the RES, selectively reach the desired tissue, release the active compound with an optimal rate and finally degrade into non-toxic elements, which can be eliminated from the body and which do not cause side-effects.

Various attempts were made to achieve long blood circulation times by avoiding RES recognition, mainly by chemically attaching or adsorbing appropriate polymers or molecules at the particle surface [5,14–21], which would reduce or minimize the interaction with opsonins.

After reviewing some of the long-circulating systems thus obtained (focused on nanoparticles and liposomes), this article will discuss a newly developed type of particle with an increased blood half-life: poly(ethylene glycol) (PEG)coated biodegradable nanospheres [22–33]. Nanospheres (or nanoparticles) are defined as macromolecular solid colloidal particles of less than 1 $\mu$m, in which drugs or other biologically active molecules are dissolved, entrapped or encapsulated, chemically attached to the polymers or adsorbed to the particle surface [34].

### 1.1. Long-circulating drug delivery systems: PEG-based coatings

Particle size and shape greatly influence their organ distribution. To circulate through the smallest capillaries, the particle size should be less than 5 $\mu$m. Moreover, a diameter of less than 200 nm is required to avoid spleen filtering effects [35]. In addition, the surface of the particles should possess RES-avoiding properties (be stealthy with regard to phagocytic cells). Allen [19] wrote “if you want to be invisible, look like water”; indeed, particles with hydrophilic surfaces, upon which water molecules can readily adsorb, have longer blood half-lives [5,14–20].

Particles with neutral surfaces seem the most appropriate with regard to blood persistence [18,36]. Various attempts were directed towards altering the carriers’ surface properties to reduce their RES clearance. This alteration was performed by adsorbing or chemically attaching appropriate hydrophilic and neutral polymers to their surface.

### 1.2. Coatings obtained by polymer adsorption

In the case of PS nanoparticles, the rapid uptake by RES may be partially overcome by coating the particles with artificial surfactants such as Poloxamine 908 [5] or 1508 [14]. Poloxamer and Poloxamine surfactants contain blocks of PEG and blocks of poly(propylene glycol) (PPG). The more hydrophobic PPG blocks adsorb on the PS surface and the more hydrophilic PEG blocks stick out of the surface and form a protective coating [37]. PEG was adsorbed into carboxylated PS and poly(styrene-butadiene)latexes from water [38].

The adsorption was kinetically controlled and decreased with an increase in surface roughness and polarity. However, non-degradable PS particles are not realistic therapeutic systems. Similarly to PS particles, poly(methyl-methacrylate) colloidal carriers coated by Poloxamer [39] circulate longer in blood.

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The polymer adsorption approach was adapted from model PS particles to degradable PLGA particles [40] coated by adsorption of amphiphilic diblock PEG-PLA diblock copolymers, which advantageously replaced the non-degradable surfactants previously used (Poloxamer and Poloxamine) [5,14]. However, a comparison of the in vivo results obtained with the two systems, PLGA/PEG-PLA and PS/Poloxamer 908, suggested that the adsorbed layers of PEG-PLA might have a lower stability or resistance to protein adsorption than Poloxamer 908 ones.

The adsorbed polymers might desorb in vivo, due to replacement by blood compounds with a higher affinity for the particle surface [18], and this would pose problems for their intravenous administration. To increase the coating stability, polymer chemical attachment is an alternative to polymer adsorption.

### 1.3. Coatings obtained by polymer chemical attachment

Model spherical PS particles with covalently bound PEG2K (molecular weight 2000 Da) chains on their surface were less sequestered by the liver than PS ones [41]. Moreover, a correlation was found between the PEG surface density and the blood half-life. At the highest PEG surface density, the increase in blood circulation time was higher than that obtained with particles coated by Poloxamine 908 adsorption [41].

PEG 350 was attached to the surface of PS latex particles [42]. By assuming that all the PEG present in the system was located at the surface, it was calculated that one PEG molecule covers about 1 $nm^2$.

PEG was covalently attached to the surface of poly(alkyl cyanoacrylate) particles [43] or to the surface of chemically cross-linked albumin nanospheres [20]. In the latter case the particles' uptake by cell culture macrophages was significantly reduced. Poly(aspartic acid) polymers with pendant PEG chains and adriamycin (a hydrophobic drug) chemically attached to them formed long-circulating micelles [44]. Covalently bound to proteins, PEG forms macromolecular conjugates with longer blood circulation time than native proteins and with reduced immunogenicity and antigenicity [45]. Attached to surfaces, PEG was shown to increase their biocompatibility [46] and reduce the adsorption of proteins and blood components [47–51].

When appropriate polymers are attached to their surface, liposomes are an important class of long-circulating particles, because they are generally composed of substances naturally occurring in the body. Although liposomes were hypothesized to make good drug delivery systems more than 20 years ago [52], in some cases they have circulation half-lives as short as a few minutes, as a result of their rapid uptake into the cells of the RES. Thus, many of their therapeutic applications have been limited to the delivery of drugs to this system; liposomes were used to treat leishmaniasis [53] or to manipulate macrophage function, for example by delivering immune modulators [54], among other applications. To enable additional applications, such as the delivery of drugs to tumor cells, liposomes with increased blood half-lives were designed (termed "Stealth" or sterically stabilized), by attempting to mimic the surface structure of blood cells. For example, carbohydrates [55] and polysaccharides such as monosialoganglioside GM1 [56] were attached to the

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liposomes' surface and a significant increase in blood circulation times was achieved. A coating with PEG not only dramatically increased the liposomes' blood half-life [15,16], but also solved some problems related to the use of GM1, such as its costliness and its difficulty in purification. So far, a PEG coating appears to be superior to other polymers in conveying significant advantages to liposomes. A few of these advantages are: decreased RES uptake, increased blood residence time, increased stability to contents leakage, increased flexibility in lipid composition, and dose-independent pharmacokinetics [19].

However, the amount of PEG which can be included in the liposome lipid bilayers decreases with increasing PEG molecular weight [57]; from 15 mol% in the case of PEG 120 to 5–7 mol% in the case of PEG2K and PEG5K. Above 7.5 mol% of PEG 1900, a liposome dissolution was observed [58].

The bilayer rigidity is an essential factor to prolong the liposome circulatory half-life, for example by decreasing the interactions with plasma proteins. A low bilayer rigidity can lead to destabilization of the liposome membrane and release of encapsulated drug, thus aborting the function of the microparticle delivery system [59,60].

### 1.4. Mechanism of action of the PEG coating

The research work summarized in the previous paragraphs shows that hydrophilic PEG-based coatings significantly increase the nanoparticle or liposome blood circulation time. Among hydrophilic polymers, PEG has the advantage to be considered non-toxic and was approved by the Food and Drug Administration (FDA) for internal use in humans [61]. The blood half-life of PEG extended from 18 min to 1 day as the PEG molecular weight increased from 6 K to 190 K [62].

Unraveling the mechanistic action of hydrophilic and in particular PEG-based coatings would be a step forward in the design of particles with optimized surface properties. A vast amount of research work has been devoted to a better understanding of the mechanism of the PEG coating utilized to extend particle blood circulation times. A generally assumed mechanism is based upon the formation of a sterically hindered, hydrophilic coating which avoids opsonization by plasma proteins [63–65]. The hydrophilicity was considered a main requirement, but it turned out not to be a sufficient one. Indeed, liposomes were coated with series of hydrophilic polymers, among which maltopentaose, estimated to be more hydrophilic than PEG5K, but they were still removed from blood circulation very rapidly in mice [65]. Dextran-coated liposomes circulate shorter than PEG-coated ones [66], in spite of the more hydrophilic nature of dextran compared to PEG.

It has been proposed [63,65] that besides hydrophilicity, chain fiexibility is another major feature necessary for the coating polymers to provide long-circulating particles. Due to the transient, flexible and rapidly changing structure of PEG, the immune system would have difficulties in modeling an antibody around it [67]. The protective layer of PEG is considered as a "cloud" of possible chain conformations, with a density high enough to prevent the interactions of opsonins with the surface of the particles; only if a polymer chain posesses both hydrophilicity and flexibility properties (to enable a high number of possible chain conformations), can it serve as an effective coating protector for particles against

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opsonization [63]. Besides PEG, other possible candidates were considered, such as poly(acrylamide) or poly(vinylpyrrolidone). Preferably, the protective polymers should not contain hydroxyl groups (like polysaccharides), which are targets for complement C3, or amine groups (like polylysine), which are targets for C4 [63].

The conditions that lead to protein repulsion from hydrophobic plane surfaces to which PEG chains were attached to one chain end in a "brush" configuration were recently studied [68,69]. These authors elaborated a mathematical model taking into account the four types of interactions between a protein and a hydrophobic substrate (Fig. 1). The best conditions for protein repulsion were found to be long PEG chain length and high surface density [68]. Let $D$ be the distance between the anchorage to the substrate of two terminally attached PEG chains (cf. Fig. 1). In the case of small proteins (approximated as spheres with a radius of 2 nm), $D$ should be around 1 nm, whereas for larger proteins (6–8 nm), $D$ should be around 1.5 nm.

It has been suggested that both the reduction in the adsorption of opsonins and the selective adsorption of certain plasma components (dysopsonins) prevent the recognition and uptake of nanoparticles by macrophages; the competition between these two mechanisms is believed to be the key in controlling the particle uptake by macrophages, and hence their biodistribution [70]. Muir et al. [71] suggested that two serum components (one with a molecular weight below 30 000 Da and the other with a molecular weight higher than 100 000 Da) are the principal factors which result in a dysopsonic action.

The interactions between injected particles and blood components are complex. These compounds might reversibly or irreversibly adsorb on the surface of the particles, and might be replaced by others. Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) will probably be a helpful tool to gain insight into these phenomena [72].

## 2. PEG-coated nanospheres

Even if the exact mechanism which leads to an increase in the blood half-life of PEG-coated surfaces has not been entirely elucidated, the sum of the research work presented in the previous paragraphs clearly shows that a PEGbased coating (especially if obtained by chemical attachment) is a key in the design of long-circulating particles, and it has inspired recent approaches to design PEG-coated nanoparticles [22–33].

As model PS particles, these nanospheres have compact polymeric cores (in order to ensure good stability) but the cores are degradable. Composed of poly(hydroxy acids) such as PLA or PLGA, the cores should degrade by hydrolysis into harmless elements which could be excreted (such as lactic and glycolic acids), in order to avoid particle accumulation in the body. Investigations of the potential use of PLA for medical purposes date from 1966 [73]. PLA, PLGA and polycaprolactone (PCL) showed a good ability to encapsulate various drugs. By adjusting the physico-chemical characteristics of these polymers, different encapsulation properties and release kinetics could be obtained [3].

As with PEG-coated liposomes, the PEGcoated nanospheres have PEG chains attached to their surface at one chain end, in a brush configuration (as in Fig. 1), which should avoid or

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reduce the interactions with blood proteins and therefore impart RES-avoiding properties. To achieve the core-shell structure described above, block amphiphilic polymers of the type PEG-R were synthesized. "R" is chosen among the bioerodible polymers cited above (PLA, PLGA, PCL). The two blocks have a tendency to easily phase-separate in the presence of water [74] and have different solubilities in water and organic solvents. This property was used to obtain the core-shell structure by an o/w emulsification procedure (Fig. 2). For this, PEG-R polymers were dissolved in an organic solvent immiscible with water (such as ethyl acetate or methylene chloride). The o/w emulsion was formed in an aqueous phase, and the organic solvent was allowed to slowly evaporate. This led to a progressive increase in polymer concentration inside the droplets. R is insoluble in water, but highly soluble in the organic solvent; conversely, PEG is highly water-soluble, soluble in methylene chloride, and practically insoluble in ethyl acetate. This leads to a tendency of PEG chains to migrate towards the water phase to form sterically stabilized particles (Fig. 2), with a core presumably mostly composed of R chains. After complete solvent evaporation, the nanosphere core solidifies, thus entrapping the hydrophobic biologically active molecules.

### 2.1. Degradable PEG-Rand $PEG_n$-R copolymers: synthesis and characterization

One research trend is to synthesize new tailored biocompatible polymers. For example, triblock R-PEG-R polymers were synthesized to take both advantage of the softness and flexibility of the PEG blocks and of the rigidity and hydrophobicity of the "R" blocks (PLA or PCL), in order to obtain new materials with desired mechanical and degradation behavior [75–82].

To prepare PEG-coated nanospheres, PEG-R polymers were synthesized by ring opening polymerization of monomers (lactide and/or glycolide, caprolactone) in the presence of monomethoxy PEG (MPEG) [22,24,30]. The catalyst chosen was stannous octoate, widely used to prepare PLA polymers and approved by the FDA as a food stabilizer [83]. Toxicological data are available for this catalyst [84]. Moreover, among different catalysts used, stannous octoate was the most effective to synthesize PLA-PEGPLA polymers [78].

The polymerization reaction was followed by gel permeation chromatography to determine molecular weight and polydispersity [25]. Fig. 3 shows typical gel permeation chromatograms of reaction mixtures after various times (starting material: MPEG, lactide and glycolide). The consumption of monomers (lactide and glycolide) is observed from a decrease of peak "D". Simultaneously, peak "P" (corresponding initially to MPEG) shifts towards lower retention times (higher molecular weights), indicating that an addition reaction takes place at the hydroxyl end group of MPEG. Finally, only one peak is obtained, corresponding to diblock PEG-R polymers. After purification, the exact chemical composition of the polymers was determined by $^{1}$H- and $^{13}$C-NMR spectroscopy [22].

The crystallinity and glass transition temperature (*Tg*) of a series of diblock PEG-PLGA polymers were determined by differential scanning calorimetry (DSC) [25] (Fig. 4). The first heating trace shows an endothermic peak corresponding to the fusion of the crystallites in the sample; the melting enthalpy corresponds to the total amount of PEG in the sample, indicating that a phase separation occurred between PEG and PLGA. An X-ray study conducted with these samples confirmed that only PEG crystallizes in the samples; the

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diffraction chromatograms showed only specific peaks corresponding to PEG crystallites, the PLGA polymers being amorphous [22].

After the first run, the samples were quenched by rapid cooling and presented an amorphous structure. The second heating trace at 10 °C/min did not show a melting endotherm but a single *Tg* (Fig. 4). We suppose that the single *Tg* observed in this case is due to an entanglement effect of PEG and PLGA chains, that kinetically could not phase-separate during the rapid cooling. The calculated *Tg* follows the Fox law for polymer blends:

$$(1/Tg)_{\mathrm{PEG-PLGA}}=(w/Tg)_{\mathrm{PEG}}+(w/Tg)_{\mathrm{PLGA}}$$

where *w* is the weight fraction of each polymer block.

Multiblock $PEG_n$-$R_m$ copolymers were also synthesized [26,29]. First, several monomethoxy monoamine PEG ($MPEG-NH_2$) chains were attached together at one chain end by reaction with citric, mucic, tartaric acid or other polyfunctional molecules. The remaining hydroxyl groups were further used to initiate the ring-opening polymerization of "R". The structure of the polymers thus obtained (when R=PLA) is indicated in Scheme 1.

The morphology, degradation and drug encapsulation behavior of copolymers containing PEG blocks strongly depends on their composition. For example, poly(L-lactic acid)-PEG-poly(L-lactic acid) (PLLA-PEG-PLLA) polymers crystallize forming spherulites with a morphology depending on copolymer composition, hydrolysis time and crystallization temperature [85]. The degradation of poly(D,L-lactic acid)-PEG-poly(D,L-lactic acid) triblock copolymers was studied [74]. The rate of water uptake in films prepared of these polymers was biphasic, suggesting two possible mechanisms. Compared to PLA, PLA-PEG-PLA copolymers showed faster degradation kinetics, but the overall biological response to both types of implants was good and comparable in both cases [86].

The diffusion of dyes through degraded semicrystalline films prepared from PLLA-PEG-PLLA polymers was further studied [87]. The diffusion rate increases as the PEG content in the copolymers increases. The steady state of mass flux was not reached over a diffusion time of 1000 h, because of polymer degradation.

Bovine serum albumin was encapsulated in PEG-PLA and PEG-PLGA [88] and PLA-PEG-PLA or PLGA-PEG-PLGA [89] microspheres. Striking differences were observed with the respective homo- or copolymers with regard to microsphere morphology, degradation behavior, and drug release patterns.

To form PEG-coated nanospheres, diblock PEG-R polymers were used and not triblock R-PEG-R polymers. R-PEG-R would lead to the formation of PEG coils on the surface with two anchoring points to the core. For the same PEG chain length, the maximum coating thus obtained would be half as thick as the one obtained when diblock polymers are used. As a result, the PEG coating on R-PEG-R nanospheres would be less effective than the one on PEG-R particles, in order to prevent opsonization and increase blood circulation times.

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## 2.2. PEG-coated nanosphere characterization

The PEG-R nanospheres were prepared according to the procedure described in Section 2 and schematized in Fig. 2. Hydrodynamic diameter and size distribution, some of the most prominent features of nanoparticles, were determined by quasi-elastic light scattering, (QELS) [22,23] and compared to those obtained from morphology studies (essentially using electron microscopy). The nanospheres recovered by centrifugation, after preparation, can be lyophilized and redispersed in aqueous solutions and they presented similar size distributions according to QELS studies [22]. Nanosphere powders show good shelf storage properties.

**2.2.1. Morphology—**The spherical shape of PEG-coated nanospheres was validated by scanning electron microscopy (SEM) (Fig. 5) and atomic force microscopy (AFM) [22], a non-destructive technique. SEM needs a previous coating of the sample with gold and/or palladium, whereas AFM allows a higher resolution without the need of coating the nanospheres.

In addition to these techniques, freeze-fracture electron microscopy was used [23] to allow observation of the nanospheres surface ultrastructure (Fig. 6). The samples were frozen, fractured, shadowed by platinum and carbon and the replicas were observed by transmission electron microscopy. Fig. 6 shows typical images of PLGA, PEG20K-PLGA, and lidocaine-loaded PEG20K-PLGA nanospheres. Hemispheric replicas of nanospheres were found in all samples. PLGA nanospheres had a smooth surface, whereas both unloaded and lidocaine-loaded PEG20K-PLGA nanospheres showed typical particles on their surface. These particles with an average greatest diameter of about 12 nm were attributed to the presence of PEG chains at the surface of nanospheres.

PEG20K-coated nanospheres containing 50 wt% lidocaine, a crystalline drug, consistently showed filamentous structures, in addition to the surface particles also found in samples without lidocaine (Fig. 6). These filaments of about 11 nm apparent diameter could be attributed to small lidocaine crystals inside the nanospheres. This hypothesis is in agreement with previous DSC studies performed on nanospheres, which showed lidocaine melting endotherms in these samples [22].

**2.2.2. Size optimization and influence of the surfactant—**The nanosphere hydrodynamic diameter $d$ and size distribution were measured using quasi-elastic light scattering (QELS). This technique was used to follow the nanosphere aggregation and de-aggregation behaviors in aqueous solutions [22].

The influence of different fabrication parameters on $d$ was studied with the aim to obtain particles of less than 200 nm [28]. The size of the PEG5-PLA45 nanospheres was reduced by increasing the concentration of the surfactant used for their preparation, poly(vinyl alcohol) (PVA) or cholic acid, sodium salt (CA). $d$ decreases with reducing the polymer concentration in the organic phase. These results are in agreement with those reported in size optimization studies on PLGA nanospheres [90].

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Several surfactants were tested with regard to their ability to form nanospheres without retaining their composition [28]. Among the anionic biological surfactants tested, CA was the most effective to reduce both hydrodynamic diameter and polydispersity index (Table 1). Only a low amount (less than 6 wt%) of this compound remained associated with the nanospheres after their preparation. In contrast, when PVA was used as a surfactant, a very high amount of it remained in the nanosphere composition, possibly by entanglement with PLA chains in the core. This hypothesis was supported by the fact that, after repetitive washing steps in distilled water, the residual PVA amount still represented as high as 30–50% of PLA or PEG-PLA nanospheres weight.

Some of the surfactants tested were unsuccessful for the preparation of PLA nanospheres, whereas they could be used to prepare PEG-PLA ones. This result was attributed to an additional steric stabilizing effect of PEG chains on the surface of the latter particles.

Whatever the surfactant used, PLA nanospheres have a negative surface charge, as indicated by the negative values of the zeta potential (ZP) (Table 1), ranging from −4 mV (when the non-ionic PVA surfactant was used) to −12 mV (when anionic surfactants were employed). The negative surface charge was attributed to the presence of carboxyl end groups (from PLA), located near the surface and to the presence of adsorbed residual surfactants. In contrast, the ZP of PEG-coated nanospheres was practically zero, regardless of the surfactant used. This result was attributed to a complete screening of the surface charge by the PEG chains covalently attached to it. The PEG coating layer shifts the shear plane of the diffuse layer to a larger distance from the nanospheres, and this results in a decrease of the measured absolute value of ZP.

The ZP studies are in agreement with previous ones dealing with PS [21] or poly($\beta$-malic acidco-benzyl malate) [91] particles coated by Poloxamer or Poloxamine adsorption. The adsorption of these polymers containing PEG blocks resulted in an increase in the surface hydrophilicity and a reduction of the ZP. Similarly, these two characteristics were modified by the covalent attachment of PEG to the surface of albumin nanoparticles [20], with an initial ZP of −37 mV. A coating with MPEG 750 did not modify ZP, but a coating with MPEG2K increased ZP up to −33.5 mV and a coating with MPEG5K up to −23 mV. This gradual modification of ZP was attributed to an increase in the thickness of the PEG coating layer with the chain length of PEG. After a complete screening of the surface charge by additional PEG attachment, the resulting ZP was −5 mV, which was considered sufficient to avoid RES recognition [20].

**2.2.3. Surface characterization and stability—**X-Ray photoelectron spectroscopy (XPS) was used to determine the relative amount of PEG in the first 5–10 nm surface layers of PEG on the surface of PEG-R and $PEG_3$-PLA nanospheres and to follow its stability during degradation at 37°C in water [22–24,26]. The coating was stable during a 24 h period, in agreement with the dosage of the amount of PEG detached in the supernatant [24]. For example, less than 8% of PEG was detached from PEG5K-PLGA45K nanospheres in the first 60 h of incubation in 0.1 M phosphate buffer solutions (PBS) at 37°C (Fig. 7). In the case of PEG12K-PLGA100K nanospheres, less than 10% PEG was detached in the first 60 h of incubation under the same conditions. Over 3 weeks, the nanosphere mean diameter

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(as determined by QELS) remains pratically constant, then drops down suddenly, presumably when water-soluble oligomers are formed [23].

Intensive studies are underway to develop an exact characterization of the surface of PEG-coated nanoparticles, in particular, to obtain the apparent hydrodynamic layer thickness values. PEG-based coatings modify particle surface composition, charge, and hydrophobicity. Review articles [3,21] summarize some of the most widely used techniques for specific tasks: to determine surface chemical composition (mainly X-ray photoelectron spectroscopy and secondary ion mass spectroscopy (SIMS)), hydrophobicity (hydrophobic interaction chromatography; Rose Bengal binding method; partitioning between two aqueous phases dextran/PEG; contact angle measurement), and surface charge (electrophoresis; laser Doppler anemometry; amplitude weighted phase structuration).

## 3. Drug encapsulation

Various drugs were successfully entrapped inside PEG-coated nanospheres: lidocaine [22,26], prednisolone, carmustine [26], ibuprofen [30], and oligonucleotides [33]. To entrap these active compounds, the incorporation method schematized in Fig. 2 was used; drug and polymer were dissolved together in an organic solvent, and then an o/w emulsion was formed by sonication or microfluidization.

Lidocaine was used as a model hydrophobic drug in an attempt to establish the main factors which determine the encapsulation efficiencies, nanosphere mean diameter and release patterns [22,26]. For these studies, drug-loaded nanospheres were prepared from various diblock PEG-R and multiblock $PEG_n$-R copolymers, where R was chosen among PLA, PLGA, PCL or PSA (the structure of these polymers is given in Scheme 1). Regardless of the polymer used, high loadings and entrapment efficiencies were obtained. The nanosphere mean hydrodynamic diameter and the entrapment efficiency strongly depend upon the polymer physico-chemical characteristics. The hydrodynamic diameter increases with the PEG and R molecular weights and is practically independent of the drug loading. In the case of diblock PEG-PLGA polymers, the entrapment efficiency is practically independent of the PEG molecular weight. Conversely, in the case of $PEG_3$-PLA polymers, a slight decrease of the entrapment efficiency with the PEG molecular weight was observed [26].

Drug release kinetics were also influenced by the nanosphere drug loading (Fig. 8). The possibility of lidocaine crystallization occurring inside the nanospheres at high loadings (above 30 wt%) has been suggested [22]. Part of the drug would homogeneously mix with the chains in the core, and part of it would coexist as crystallites. Freeze-fracture electron microscopy of nanospheres at high lidocaine loadings (Fig. 6) tend to support this hypothesis.

$^{14}$C-Ibuprofen, a drug with a half-life of only a few minutes, was entrapped in PLGA and PEG2K-PLA30K nanospheres. The plasma half-life was increased in the case of PEG2K-PLA30K particles up to 2.5 h [30].

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Prior to their encapsulation into PEG-PLA nanospheres, oligonucleotides were associated by ion pair formation to oligopeptides [33]. The resulting globally neutral complex could be entrapped with high efficiencies into the nanospheres.

## 4. Interactions with blood components and macrophages

The analysis of plasma protein adsorption on nanoparticles has been established using two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) [72]. The nanoparticles are incubated in vitro in human plasma and then separated from the plasma by centrifugation. The adsorbed proteins are washed off the particles and analyzed electrophoretically.

Recent studies with an incubation time of 5 min showed that on PLGA45K nanospheres major proteins were the apolipoproteins J, C-III, E and A-I (Fig. 9). Additionally, for example, considerable amounts of fibrinogen, immunoglobulin G (not shown) and apolipoprotein A-IV were detected. The total amount of adsorbed proteins on the PLGA particles was more than 4 times higher than on all PEG-PLGA nanospheres which were investigated (Fig. 9). The total protein amount was similar on PEG5K-PLGA45K, PEG12K-PLGA100K and PEG20K-PLGA180K.

Especially the amounts of apolipoprotein J and C-III were drastically reduced on all PEG-PLGA particles (Fig. 9). The adsorption of fibrinogen and immunoglobulin G was also decreased by the PEG content in the particles. The latter effect is well in agreement with observations of the protein adsorption on PS particles which were surface-modified with poloxamers [92].

The different PEG-PLGA particles showed similar protein adsorption patterns. Nevertheless, some differences could be detected. For example, PEG5K-PLGA45K particles showed a higher adsorption of apoA-IV than the other PEGPLGA particles. On PEG12K-PLGA100K the highest amount of apoE was detected and on PEG20K-PLGA 180 K the amount of apoC-III was slightly higher than on the other PEG-PLGA particles.

Prolonging the incubation time of the particles in the plasma led to considerable changes in the adsorption patterns. After an incubation time of 20 h, apolipoprotein C-III was strongly reduced; it was barely detectable on any of the investigated particles. In contrast to that, the amounts of apoA-IV and apoE were distinctly increased on all the particles, especially on the PLGA nanospheres. The time dependence of adsorption of these proteins was very similar to that on PS particles coated with poloxamers [92].

In general, a distinct reduction of protein adsorption was achieved by employing PEG-PLGA polymers for particle production as compared to pure PLGA, which is in agreement with the generally accepted low protein adsorption of PEG surfaces [93]. The qualitative composition of the adsorbed proteins was changed as well by the introduction of PEG. The differences in the adsorption patterns of the particles with varying PEG contents were probably caused by different PEG surface density rather than different PEG chain length [68,92].

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The relevance of the adsorbed plasma proteins to the in vivo fate of the particles still remains to be uncovered. It has to be kept in mind that not only single proteins but also the composition, i.e., the ratios of the adsorbed proteins and their conformation may be of importance. Special interest has to be directed to the time dependence of adsorption.

PLA70K nanospheres strongly activate the complement system, whereas PEG2K-PLA30K ones hardly do [94]. PLA and PEG2K-PLA30K nanoparticles of identical size (160 nm) were incubated with human platelet-poor plasma [32]. In the case of PLA particles, a diminution of activity of factor V was observed, together with particle aggregation. Conversely, all tests performed on PEG2K-PLA30K particles (concerning global coagulation times and the activity of different plasma coagulation factors) were identical to control samples [32]. The observed absence of PEG2K-PLA30K particle aggregation in plasma is a necessary condition for their intravenous administration.

PEG2K-PLA and PEG5K-PLA nanospheres showed an ability to avoid in vitro macrophage recognition [31]. PEG5K-PLA30K particles were phagocytized 9 times less than PLA ones. PEG5K was more effective in preventing macrophage uptake than PEG2K.

All these results suggest the value of PEG-PLA nanospheres, as opposed to PLA nanospheres, for use as injectable carriers able to escape macrophage recognition. In further studies, the establishment of the correlation of surface properties of the particles with protein adsorption and subsequent in vivo behavior will be approached.

## 5. Biodistribution

PEG-coated and non-coated nanospheres were injected into BALB/c mice [22] to compare their biodistribution. The particles were core-labeled with $^{111}$In, a radioactive γ-emitting compound. For this, diethylene triamine pentaacetic stearyl amide (DTPA-SA) was labeled with $^{111}$In by the transchelation method [95] and encapsulated into the particles during their formation. Due to the hydrophobic character of DTPA-SA, the label was firmly attached to the nanoparticles. It was possible to observe images by γ-scintigraphy of the mouse body after injection of PLGA and PEG20K-PLGA nanospheres [22]. Fifteen minutes after injection, PLGA particles accumulate in the liver and spleen, whereas PEG20K-PLGA nanospheres circulate well and radioactivity in the blood pool (heart and lung) was much higher.

In another series of experiments, nanospheres were injected in mice which were sacrificed and blood and organ samples were counted. Five minutes after injection of uncoated PLGA nanospheres, 66% of nanosphere associated $^{111}$In radioactivity was found in the liver and about 5% in the blood. The results were reversed in the case of PEG20K-coated particles: 17% of injected radioactivity was in the liver and more than 40% in the blood [22]. As expected, non-PEG protected PLGA nanoparticles immediately accumulate in the liver; conversely, 5 h after injection, PEG20K-PLGA nanospheres in the liver did not exceed 30%. In the case of PEG-coated nanospheres, blood circulation times increased with the molecular weight of the protecting PEG chains.

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In addition, the mean diameter and the size distribution have a significant effect on biodistribution; larger particles can be removed by a filtering effect, whereas small particles can be subject to extravasation. For example, in spite of their hydrophilic coating, model PS particles modified with surface adsorbed block copolymers were largely captured by filtration in the red pulp of the spleen when their size was larger than about 250–300 nm [35]. A spleen filtering effect was also observed with PEG-PLGA and $PEG_3$-PLA particles in this size range [96].

## 6. Perspectives and conclusions

PEG-coated nanospheres are a newly developed drug delivery system which has potential applications for intravenous drug administration. Intensive studies are underway to optimize and explore the possibilities offered by this recent system. For example, the biodistribution and the drug encapsulation properties of nanospheres prepared from new diblock and multiblock copolymers are being studied.

The PEG coating provides protection against interaction with blood components and thus prolongs blood circulation time by reducing the particle capture by the RES cells. The degradable core of the nanospheres was formed using a variety of polymers, such as PLA, PLGA, PCL or PSA. Various drugs were encapsulated in the core and released continuously. For a given drug, the release kinetics and the entrapment efficiency were governed by the polymer physico-chemical characteristics, such as chemical structure, molecular weight, and crystallinity. It may therefore be possible to tailor an “optimal” polymer for different therapeutical applications.

Long-circulating PEG-coated nanospheres could function as circulating depots. By slowly releasing the active compound in the blood, the drug plasma concentration profiles could be altered, which results in therapeutic benefits, such as a decreased systemic toxicity. Circulating particles acting as depots might present advantages for the administration of drugs, such as anticoagulants, which might cause bleeding when entrapped in local depots (such as intramuscular inserts). The biodistribution of anti-cancer drugs (e.g., adriamycin) might also be altered, potentially minimizing problems of cardiotoxicity.

Similarly, a possible use of PEG-coated nanospheres could be the encapsulation of various contrast agents. A number of contrast-enhancing agents have been developed to resolve and contrast tissues for diagnostic imaging (e.g., γ-camera, nuclear magnetic resonance imaging, computed tomography). Gadolinium diethylenetriamine pentaacetic acid encapsulated in liposomes improved the detection of liver metastases in vivo, compared to the free contrast agent [97]. Similarly, PEG-coated nanospheres could be good candidates to encapsulate contrast agents with short blood half-lives in order to alter their biodistribution.

PEG-coated nanospheres represent interesting alternatives as drug delivery systems to PEG-coated liposomes. Their similar size enables similar uses, but the nanospheres may have longer shelf lives and avoid destabilization by interaction with serum components. Moreover, many coating materials such as surfactants might lead to the disintegration of liposomes, but do not affect the nanospheres’ integrity. The nanospheres can also be freeze-dried and reconstituted in aqueous solutions.

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Another advantage that could be taken from the stability of PEG-coated nanospheres is the possibility of attaching antibodies or a fragment of them to the surface of the particles, without destabilizing them, in order to achieve site-specific drug delivery, a major challenge for drug administration. Ideally, these “magic missiles” [98] would accumulate at the diseased tissue and locally liberate the necessary amount of drug.

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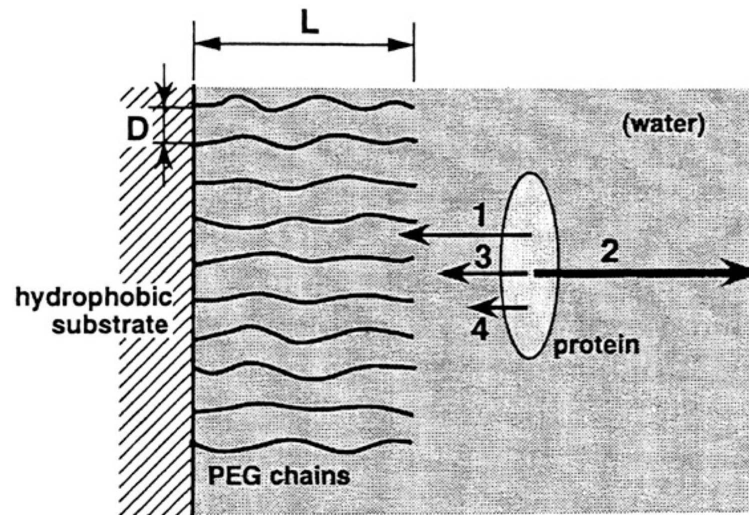


**Fig. 1.**

Interactions between a protein and a hydrophobic substrate with attached PEG chains (adapted from [68]). 1: hydrophobic attraction between the protein and the substrate; 2: steric repulsion resulting from PEG chain constriction; 3: van der Waals attraction between the protein and the substrate; 4: van der Waals attraction between the protein and the PEG chains.

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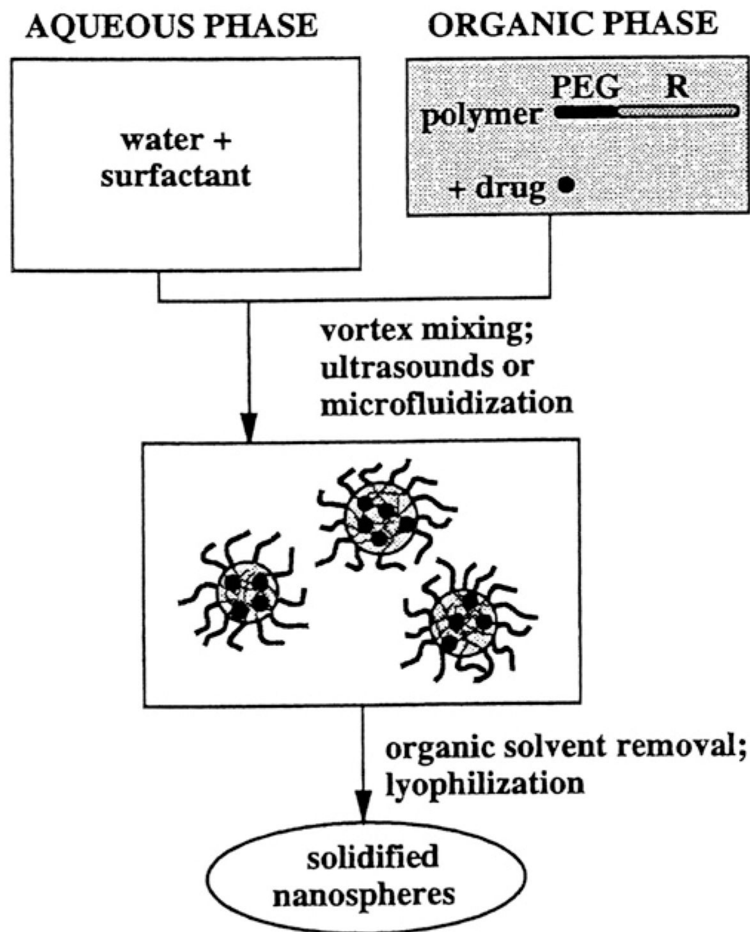


**Fig. 2.**
Schematic representation of the nanosphere fabrication procedure following an emulsion-solvent evaporation procedure.

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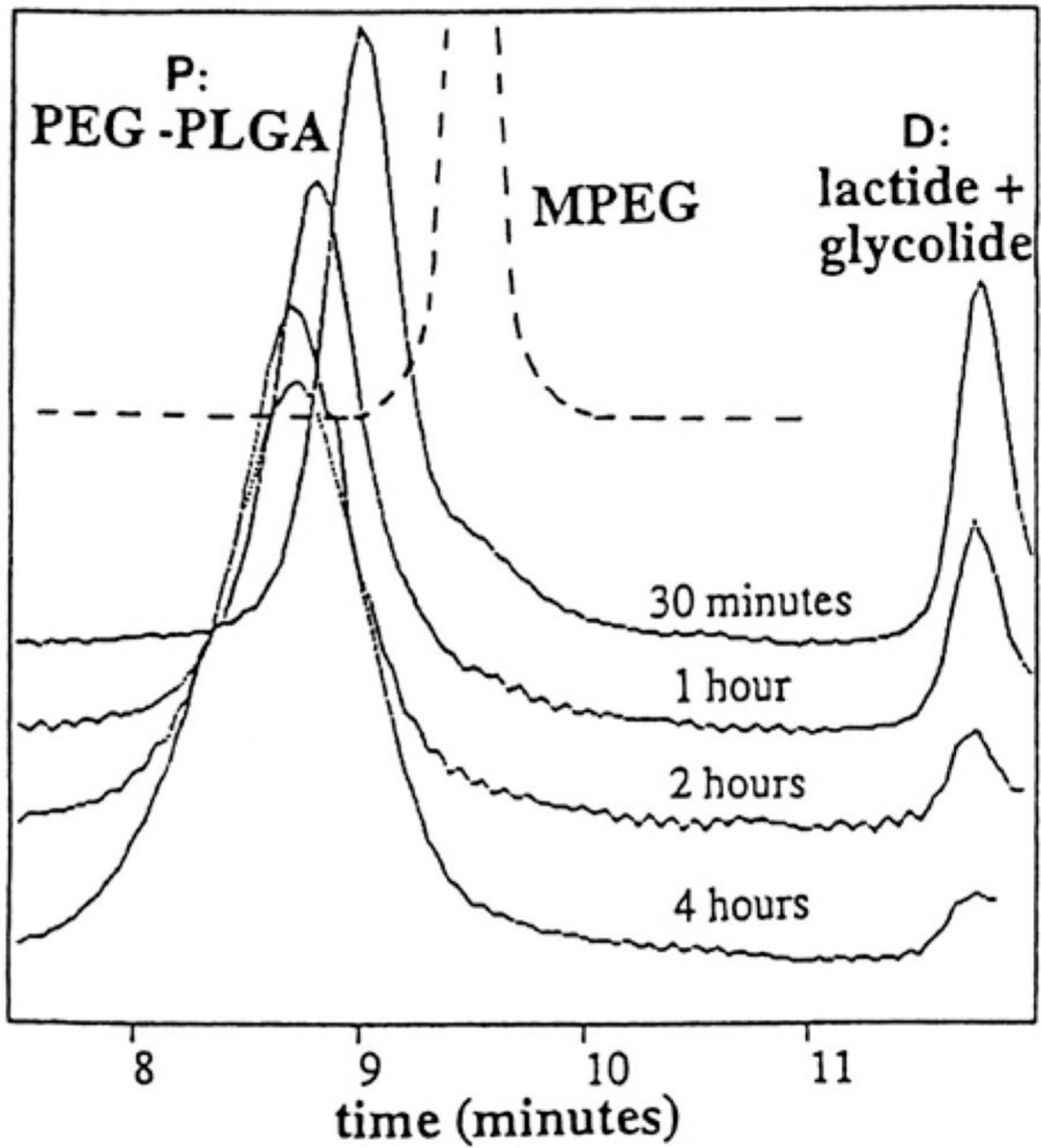


**Fig. 3.**
Time course of the polymerization reaction between MPEG and lactide/glycolide followed by gel permeation chromatography (adapted from [25]). Peak P: PEG-PLGA copolymer; peak D: starting monomers (lactide/glycolide).

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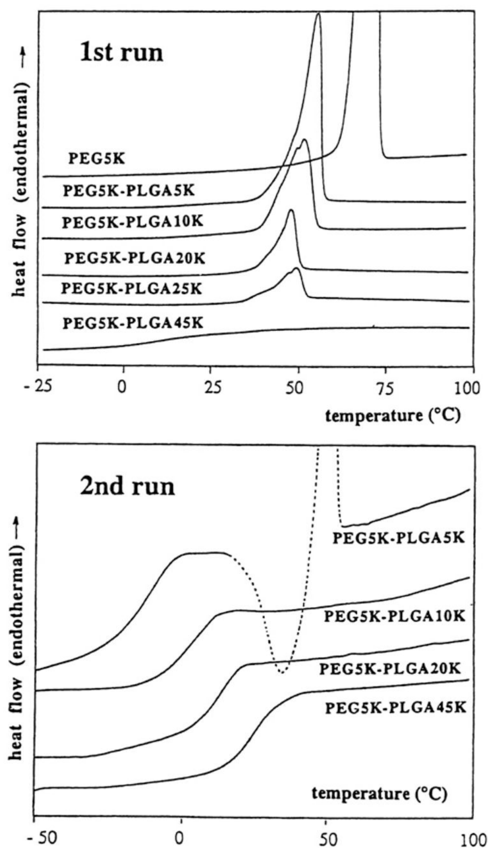


**Fig. 4.**

DSC thermograms of PEG5K-PLGA copolymers with increased chain length of PLGA. The first run (heating rate 10°C/min) was obtained with purified polymers. The samples were rapidly quenched and a second run (10°C/min) was enregistered.

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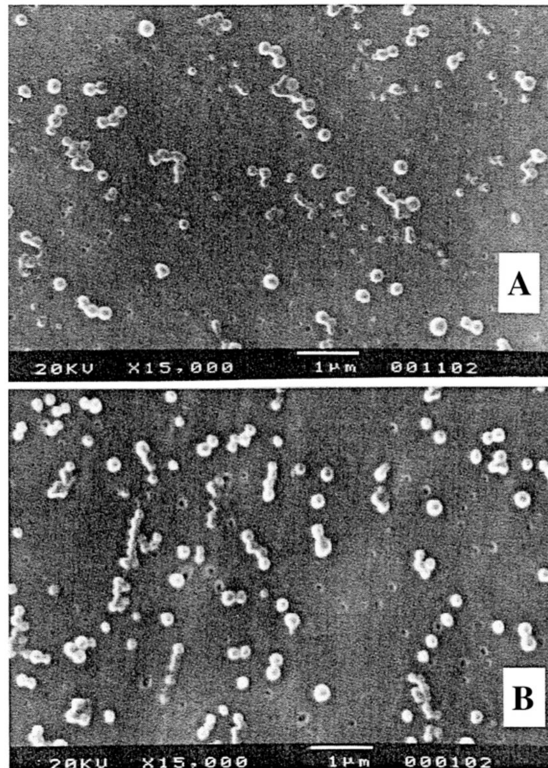


**Fig. 5.**

Scanning electron microscopy of PLGA40K (A) and PEG5K-PLGA45K (B) nanospheres.

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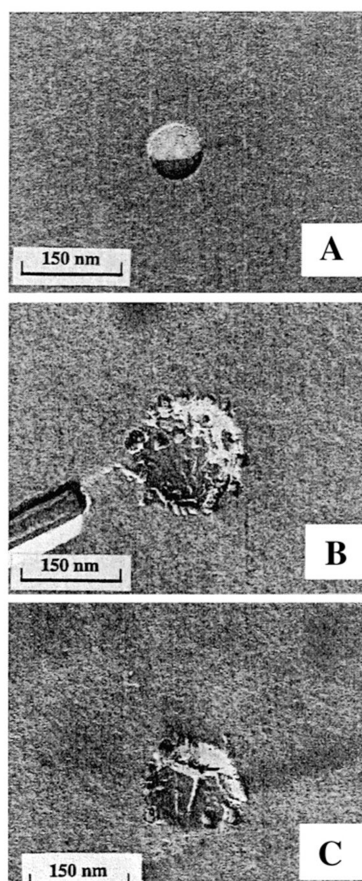


**Fig. 6.**

Freeze-fracture electron microscopy of PLGA40K (A), PEG20K-PLGA180K (B) and lidocaine-loaded (45 wt%) PEG20K-PLGA180K (C) nanospheres.

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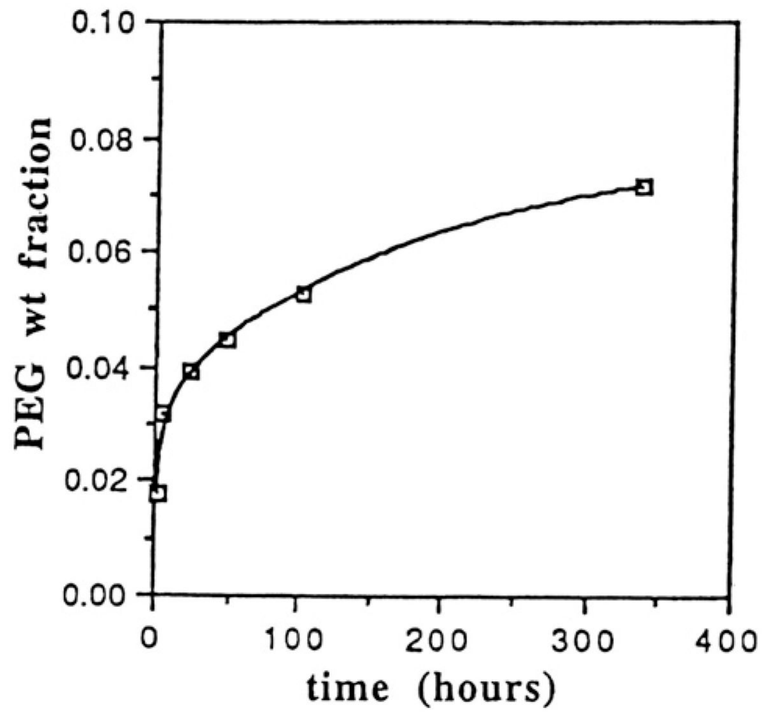


**Fig. 7.**
Fraction of PEG detached from PEG5K-PLGA45K nanospheres during incubation at 37°C in phosphate buffer solutions (pH 7.4).

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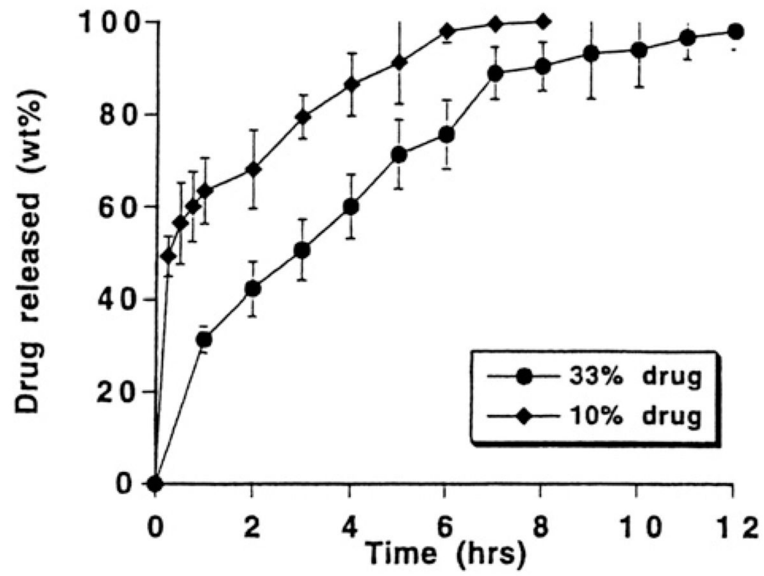


**Fig. 8.**

Lidocaine release from PEG20K-PLGA180K nanospheres (10 and 33 wt% loading) (reproduced with permission from [22]).

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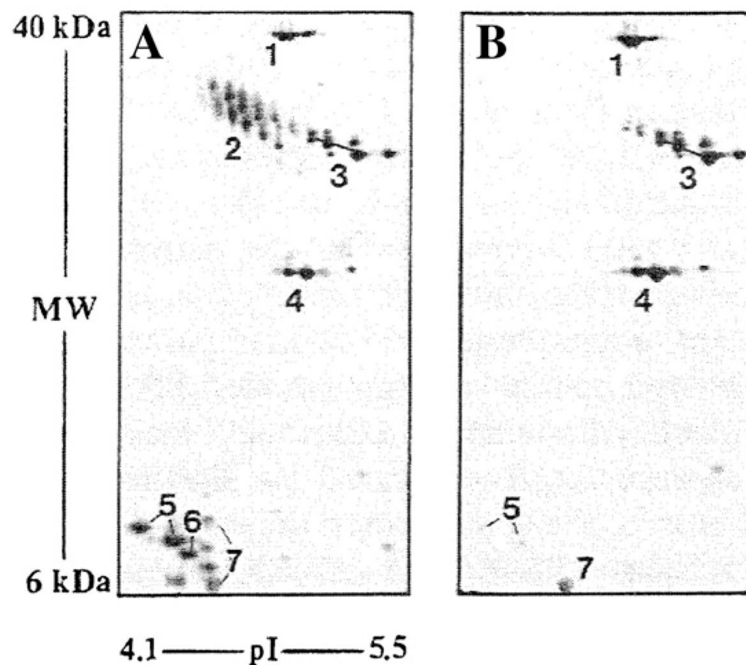
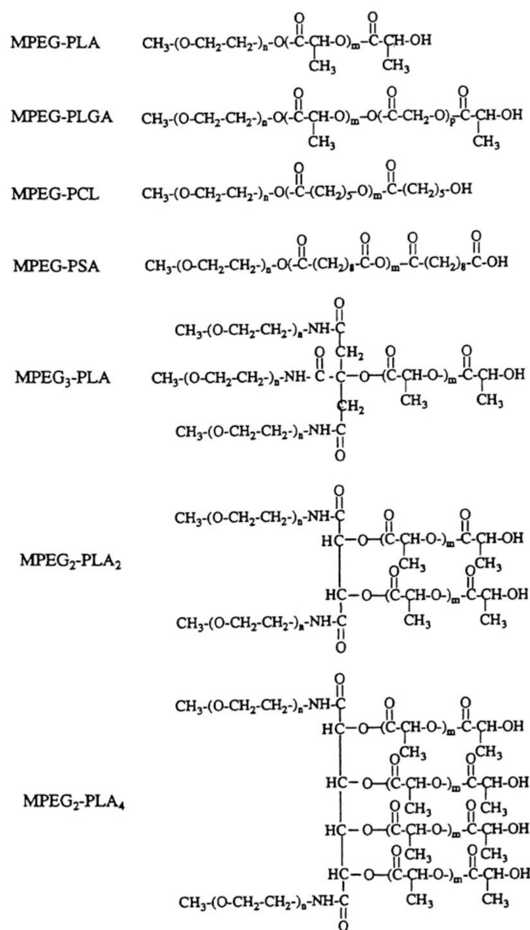



**Fig. 9.**

Plasma protein adsorption on PLGA40K (A) and PEG5K-PLGA45K (B) nanospheres (sample preparation and 2-D PAGE protocol after [72]). Close-up of the bottom left part of the 2-D PAGE gels. The proteines are separated on the basis of their molecular weights (MW) and isoelectric points (p*I*). (1) ApoA-IV, (2) ApoJ, (3) ApoE, (4) ApoA-1, (5) ApoC-III, (6) ApoC-II, (7) ApoA-II.




**Scheme 1.**

Chemical structure of some diblock PEG-R and multiblock $PEG_n$-$R_m$ copolymers used for the preparation of PEG-coated nanospheres.



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## Table 1

Influence of the surfactant on the hydrodynamic diameter *(d)*, polydispersity index (PI) and zeta potential (ZP) of PEG5KPLA45K and PLA100K nanospheres.

| Surfactant | | PEG5-PLA45 | | | PLA100 | | |
|---|---|---|---|---|---|---|---|
| | **cmc (mM)** | ***d*** **(nm)** | **PI** | **ZP (mV)** | ***d*** **(nm)** | **PI** | **ZP (mV)** |
| Cholic acid, Na salt | 14 | 148 | 0.049 | 1.1 | 117 | 0.021 | −9.3 |
| Glycocholic acid, Na salt | 7.1 | 154 | 0.084 | 0.7 | 130 | 0.082 | −12.3 |
| Taurocholic acid, Na salt | 3–11 | 164 | 0.078 | 0.3 | 135 | 0.067 | −12.4 |
| Deoxycholic acid, Na salt | 5 | 343 | 0.6 | ND | >800 | - | – |
| Poly(vinyl alcohol) 88% hydrolyzed, 13–23 K | | 192 | 0.138 | −1.2 | 203 | 0.134 | −4.2 |
| *n*-Octyl-*β*-D-glucopyranoside | 20–25 | 232 | 0.226 | ND | - | - | - |

Surfactants were used at the critical micellar concentration (cmc), with the exception of PVA (used at 0.6 wt% in water). ZP was measured with washed nanospheres resuspended in sodium chloride $10^{-3}$ M.