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REVIEW

POLYMERIC NANOPARTICLES WITH BIOMEDICAL APPLICATIONS

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Some of the newest findings in the domain of polymeric nanoparticles are presented, focusing on the synthesis and biomedical applications of polymer based nanoparticles. The nanoparticles are extensively studied due to their almost unlimited potential of providing drug targeting within areas non accessible by other means. Delivery of genes into neurons can be achieved by optimization the size of nanoparticles, as well as the conformation of their surface. Another advantage of their using is the possibility to obtain vaccines for oral administration. Finally, the nanoparticles can assure a therapeutic concentration of drug at the target tissue, thus decreasing the systemic toxic effects.

INTRODUCTION

Classical therapy proved itself useless many times, due to the random distribution of the drug into human body, high systemic toxicity usually associated with drugs (especially anticancer drugs), high hydrophobicity of some biological active substances and low tissues permeability. To overcome all these problems, a number of drug targeting techniques were developed: liposomes, [1] microparticles, [2] nanoparticles, [3] drug-polymer conjugates [4] and polymeric micelles.[5]

Some studies showed that it is possible to use nanoparticles for the targeting of highly hydrophobic drugs. The pharmacological studies were confirmed by clinical trials, and some of the formulations are in general use nowadays *i.e.*: in January 2005, FDA approved the use of Abraxane™, a suspension of paclitaxel loaded nanoparticles for breast cancer treatment).[6]

In order to be usable in the therapy, nanoparticles should meet several requirements: stability in time, so they can be stored for several months; a long circulating time; assure the biodistribution according to the aim they were developed for; allow the passive or active targetting in the desired area; stimuli responsive (pH, temperature, etc.); usable as a contrast substance for the medical imaging (scintigraphy, ultrasonography, magnetic resonance imaging, computer tomography).[7]

The use of nanoparticles has a number of advantages: targetting, decreasing of the doses, availability for parenteral administration, maintaining the therapeutic concentration, limiting the toxic effects. Sometimes, in order to meet all the requirements several constituents are added: antiproteases (for the delivery of insulin), modifiers of gastrointestinal tract permeability (cyclodextrins, bile salts, enzimes – hyaluronidases).

NANOPARTICLES SYNTHESIS

1. Solvent evaporation

The polymer is dissolved into an organic solvent [dichloromethane (DCM), chloroform, ethyl acetate]. The drug is dissolved or dispersed in

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the polymer solution and the final mixture is emulsified in an aqueous solution containing a surfactant, resulting in an oil in water emulsion.

Lee *et al.*[3] have synthesized polylactide - tocopheryl polyethylene glycol succinate (PLA-TPGS) nanoparticles by the double emulsion method. A solution of bovine serum albumin (BSA) is emulsified (by sonication 55W, 30 minutes) in an organic phase (PLA in DCM) resulting a primary water in oil emulsion. The resulting emulsion was re-emulsified in an aqueous solution of poly(vinyl alcohol) (PVA) by sonication (2 minutes). The organic solvent was evaporated at room temperature and atmospheric pressure. Increasing of TPGS content lowers the average molecular weight and viscosity, leading to a decrease in the nanoparticles diameter.

Poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles were synthesized by Gomez-Gaete.[8] A solution of PLGA and dexamethasone (DXM) in organic solvent was pre-emulsioned with an aqueous PVA solution (0.25 %). After sonication, the solvent was evaporated and the nanoparticles were recovered by ultracentrifugation.

The authors have studied the influence of the copolymer composition and molecular weight on the DXM encapsulation efficiency (Table 1).

Table 1

Influence of copolymer composition on nanoparticles size

Copolymer	Particle size (nm)	BSA loading (%)	Efficiency (%)
PLA-TPGS 97:3	362	12,6	75,6
PLA-TPGS 94:6	323	11,5	68,8
PLA-TPGS 88:12	274	7,4	44,3
PLGA	330	10,8	64,5

The nanoparticles diameter varies between 274 and 362 nm. The maximum loading degree was achieved for PLA. The maximum loading rate was observed for PLGA 75:25 (170 μg/100 mg polymers). The molecular weight doesn't seem to affect the bioactive principle loading, as pointed by some authors.[9]

The DXM encapsulation is not the result of the hydrophobic interactions as PLA is more hydrophobic than PLGA 50:50. The solvent has an important role, as in the case of DXM, values between 112 and 226 μg/100 mL being found for a methanol/chloroform mixture and acetone: DCM mixture respectively.

PEG-b-PLA:PLGA nanoparticles were obtained by des Rieux *et al.*[10] by double emulsion method. In a copolymer solution in DCM (PEG-b-PLA:PLGA = 1:1 by weight) a PBS or helodermin solution was added and then sonicated. An aqueous solution of sodium cholate was poured over the primary emulsion, leading to a double emulsion water/oil/water.

2. Spontaneous emulsification

It is a modified version of the solvent evaporation method, in which the water miscible solvents, such as acetone, methanol as well as water non miscible solvents are used as organic phase. Because of the rapid diffusion of the water miscible solvent at the interface, strong turbulences are generated, leading to the formation of nanoparticles.[11,12] By increasing the quantity of water miscible solvent it is possible to decrease the diameter of the obtained nanoparticles.

The same technique was used by Sun[13] for the synthesis of PLA nanoparticles in a surfactant free system. A solution of PLA in acetone was added dropwise onto an aqueous solution of ethanol (50% v) under stirring. By dissolving a small quantity of copper chlorophyl in the PLA in acetone solution, copper chlorophyl labeled nanoparticles are obtained. These nanoparticles can be traced *in vivo* by analytical electron microscopy. The AFM and TEM analyses shown a particle diameter between 20 – 50 nm.

Iyer *et al.*[14] have synthesized nanosized micelles of poly(styrene–*co*-maleic acid)–zinc protoporphyrin. A zinc protoporphyrin solution was added to hydrolyzed SMA (1/1 weight) which forms instantaneous micelles. The micelles were centrifuged, washed with distilled water, then the pH was adjusted to 7.4 and finally lyophilized. DLS analysis pointed a mean diameter of 176.5 nm, with a polydispersity of 0.145. The CMC was determined to be 4mg/mL by DLS. By IR spectrometry the authors proved that the nanoparticles loaded ZnPP is not chemically modified, preserving its heme Oxygenasis-1 inhibitory potential.

Hypericine loaded PLA and PLGA nanoparticles were obtained by Zeisser *et al*.[15] An organic phase, consisting of a PLA or PLGA solution in acetone, in which the hypericine was also dissolved, was poured onto an aqueous PAV solution (0.4% by weight) after the formation of nanoparticles, the solvent was evaporated at atmospheric pressure and room temperature. The nanoparticles diameter was between 200 and 300 nm with a polydispersity lower than 0.1.

Using the same method, nanoparticles of PLGA loaded with anticancer drugs (9-nitrocamptothecin) (NC) were obtained by Derakhshandeh *et al.*[16] The organic phase (PLGA and 9-NC in acetone) was poured onto an aqueous PVA solution leading to formation of nanoparticles. The acetone was evaporated at room temperature. The nanoparticles were purified by centrifugation and then resuspended several times in order to remove completely the PVA and excess drug. The average dimension of the nanoparticles was 207±26 nm, with a polydispersity of 0,01%.

3. Dialysis

Jeon *et al.*[17] presented a modality to obtain nanoparticles by dialysis. The advantage of this method is that no surfactant is required, leading to an increased biocompatibility of the polymer-drug system. The authors have obtained PLGA nanoparticles by dissolving it into different organic solvents followed by dialysis for 34 hours. In order to obtain drug loaded nanoparticles, the bioactive principle was dissolved in the organic solvent in the same time as PLGA. After dialysis (24h) the nanoparticles were lyophilized. As organic solvents the authors used DMSO (dimethyl sulfoxide), DMAc (dimethylacetamide), DMF (dimethylformamide) and acetone. For the first three solvents the diameter, measured by SEM and TEM, ranged between 200 and 400 nm, and for acetone it was between 500 and 1000 nm.

The mechanism of micelle formation in the absence of the surfactant is not yet fully understood, but several explanations have been proposed. One of them is that the PLGA nanoparticles can be formed due to hydrophobic self-aggregation of polymer macromolecules. These nanoparticles would be stabilized only by the presence of electrostatic charged groups on the surface of the PLGA nanoparticles.[18] The major drawback of this method is the weak loading efficiency.

4. Salting out

Another method of obtaining nanoparticles was described by Cirstoiu-Hapca.[19] A solution of 15% PAV and 60% magnesium chloride hexahydrate was mixed with an organic phase – 18% PLA in acetone. A fluorescent marker – 3,3′-dioctadecyloxacarbo-cyanine perchlorate (DiO) (0.01%) – was also included in the nanoparticles, in order to trace the nanoparticle location in the human cells. Nanoparticles were purified by centrifugation 3 times (resuspended after each centrifugation) and finally lyophilized. Tiol groups were grafted on the nanoparticles surface by a two step carbodiimide reaction (Figure 1).

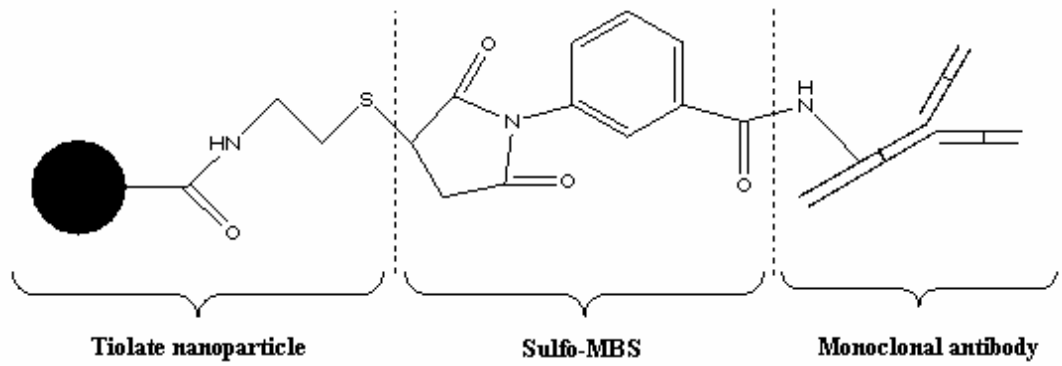

Fig. 1 – Schematic representation of the antibody modified nanoparticles.

Monoclonal antibodies were grafted to the surface, leading to nanoparticles with a diameter of around 170 nm, allowing them to act as good targeting systems. Depending on the antibody, the position of the nanoparticles inside the cell was different.

5. Emulsion polymerization

Emulsion polymerization was one of the first methods of obtaining nanoparticles ever described. The main condition for this method is that the

monomer is emulsionable. The surfactant plays a very important role in this case.[20, 21]

Depending on the nature of the aqueous phase, two types of emulsion polymerization can be described: conventional emulsion polymerization where the continuous phase consists in water (oil/water emulsion) and inverse emulsion where the continuous phase is organic (water/oil emulsion). In both cases the monomer is dispersed in the phase in which it is insoluble, in micelles or surfactant stabilised droplets. The polymerization is initiated by a physical or chemical agent. The initiator leads to formation of free radicals that will collide with the monomer molecules and will start the reaction. Generally the reaction stops when the monomer is completely finished.

In order to obtain drug loaded nanoparticles, the drug can be dissolved in the polymerization environment before adding the monomer or after the polymerization.

The mechanism of polymer particles formation during the polymerization is not fully understood. Initially the process has been described as a simple polymerization inside the surfactant stabilized particles but the polymer particles at the end of the polymerization being much smaller (100 – 300 nm) than the monomer droplets (1 – 10 μm), other mechanism were advanced.[22]

The first mechanism proposed is that of micelle polymerization. According to it, the micelles form the polymerization site. The micelles have nanometric dimensions, therefore a much higher surface than polymer particle. It is assumed that once generated in the aqueous phase, the activated monomers will initiate the polymerization inside the micelles. Being slightly soluble in the continuous phase, the monomer molecules will migrate from the droplets to micelles through continuous phase, allowing the propagation of the reaction. In this case, the monomer droplets act as true monomer reservoirs.

Another mechanism was proposed by Vanderhoff [23] and Kreuter.[24] If the monomer is rather soluble in the continuous phase, the nucleation and polymerization can take place right in this phase, leading to oligomers. In this case, along the all chain growth process the reservoir role is assumed by both micelles and monomer droplets. When the oligomers reach a certain length they precipitate, forming the primary particles, stabilized by the surfactant molecules coming from micelles or from monomer droplets. Depending on the system stability the final nanospheres will be obtained either by the diffusion of monomer inside the primary particles or by the fusion of primary particles.

No matter the mechanism involved each spherical particle obtained during the emulsion polymerization contains a high number of macromolecular chains.[25,26]

One of the most studied systems in the emulsion polymerization is that of alkylcyanoacrylates. The polyalkylcyanoacrylates have been used for many years as adhesives in surgery, due to their increased biocompatibility and biodegradability.

Couvreur[27] has obtained nanoparticles with a mean diameter of 200 nm by the mechanically initiated polymerization of methyl methacrylate or cyanoacrylate in the presence of Polisorbat 20 as surfactant.

In order to obtain stable nanoparticles, the reaction must be carried out at a low pH (1.0 – 3.5). This acid pH represents a drawback, as no acid sensitive drug can be used in the formulation process. The particles dimensions are determined by the surfactant used and pH.

Other particles were obtained by rather similar technique from poly(dimethylidene malonate) – non biodegradable nanoparticles.[28] Derivates were obtained as nanoparticles at a pH 5.5 – 6.0.[29]

Castaldello *et al.*[30] have obtained copolymeric nanoparticles starting from three monomers [methyl methacrylate and two water soluble monomers: ethylene glycol and methyl ether methacrylate by emulsion polymerization using potassium persulfate as initiator. The product was purified by dialysis in order to remove all the residual monomers. Nanoparticles were dried under vacuum, at room temperature. Core-shell nanoparticles were obtained, with a PMMA core, surrounded by a highly hydrophilic shell, consisting in PEG brushes and cationic moieties. The nanoparticles were coupled with DNA in order to use them as vaccines delivery systems. The particle size was measured by SEM, resulting in values of about 960 nm. Zeta potential analysis proved that the cationic comonomer is covalently bound to the surface of the formed nanoparticles.

6. Electrohydrodynamic atomization

A rather new method was used by Xie *et al.*[31] who obtained nanoparticles by electrohydrodynamic atomization (EHDA) of a PLGA solution in acetonitrile. The basis of this method have been laid by sir Raileigh who described the possibility to dispers a fluid in small electrically charged

droplets under the influence of an electrostatic field. Because of the droplets electrostatic charge, a maximum charge is displayed on the surface of the particles – the Rayleigh limit, when the Coulomb fission could occur. The Coulomb fission was proven by the experimental results, but in this study the authors succeeded to avoid it, in order to obtain nanoparticles of uniform diameters.

The maximum electrostatic charge is given by the relation:

$$Q = 8\pi\sqrt{\varepsilon_0 \gamma R^3}$$

where Q is the surface charges of the particle, ε_0 – dielectric constant of the liquid, and γ – surface tension of the liquid.

EHDA has different applications: electrospray ionization in mass spectroscopy, thin films deposition by electrospray, pharmaceutical productions and polymeric particle fabrication for drug encapsulation.[32,33] The authors have obtained particles with diameters ranging between 800 nm and 2 μm. The diameter can be changed by modifying the solution composition, by adding surfactant or by changing the atomization nozzle.

7. Nanoparticles from hydrophilic polymers

Some of the most used hydrophilic polymers are chitosan, gelatine and sodium alginate. Some methods of nanoparticle formation were described until now. Hydrophilic drug carriers with a limited capacity of loading proteins were described by Greff *et al.*[34]

Calvo *et al.* [35-38] presented a method to obtain nanoparticles of chitosan by ionic gelation. This method involves two phases: one of the phases contains chitosan and a diblock copolymer and the second one a polyanion – sodium tripolyphosphate (STPP). The positively charged amino moieties of the chitosan interact with STPP ions. The average size of the nanoparticles can be varied between 200 and1000 nm, depending on the chitosan and PEO-PPO copolymer percentage. These nanoparticles present a good capacity of bonding bovine serum albumin, [35,36] insulin,[37] tetanus and diphtheria toxins and nucleotides.[38]

Mao *et al.*[39] have prepared chitosan-DNA nanoparticles by a complex coacervation technique. That can be used for oral gene delivery. The same coacervation technique was used to prepare gelatine-DNA nanoparticles but with a lower loading capacity for antineoplazic proteins than chitosan-DNA nanoparticles.[40]

Chitosan nanoparticles were also obtained by the emulsion-coacervation technique.[41] Chitosan and the bioactive drug were dissolved into water. A water in liquid paraffin emulsion was stabilized in the presence of a surfactant. A NaOH solution in liquid paraffin emulsion was added onto the first emulsion. Chitosan forms nanoparticles in contact with NaOH, by coacervation.

Biodegradable polyesters with short polylactone chains grafted on the PVA backbone were synthesized by bulk polymerization of monomers in the presence of polyol groups. By changing the composition, branched biodegradable polyesters can be obtained. These copolymers undergo self assembling processes, generating stable nanoparticles that can complex proteins (human serum albumin, cytochrome C, tetanus toxin). The advantage of these copolymers is that no surfactants and no solvents are required.[42,43]

The main methods for synthesis of polymeric nanoparticles are summarized in Table 2.

Table 2

The most used polymers in nanoparticles formation/the most common techniques for obtaining nanoparticles

Polymer	Nanoparticles preparation technique
Synthetic polymers	
Poly(alkyl cyanoacrylate)	Polymerization
Poly(alkyl methacrylate)	
Polystyrene	
Poly(vinyl pyridine)	
Poly(ε-caprolactone)	Nanoprecipitation
PLA	
PLGA	
Poly(methacrylate)	
Poly(ε-caprolactone)	Solvent evaporation
Polylactic acid	
Poly(lactic-co-glycolic acid)	
Poly(β hydroxy butyrate)	

Ethyl cellulose Poly(alkyl methacrylate) Ethyl cellulose	Salting out
PLA PLGA PLA	Supercritical fluids technology
Natural polymers Albumins Casein Gelatine Alginate Chitosan Ethyl cellulose	Solubilization, denaturation, ionic gelation

NANOPARTICLES CHARACTERIZATION

Several techniques are commonly used to characterize nanoparticles: size determination methods – DLS, SEM, TEM, AFM; drug loading techniques – HPLC, NMR, IR, UV-Vis, GPC; physical characterization techniques – DSC, TGA and other techniques from the organic and macromolecular chemistry.

PHARMACEUTICAL CONSIDERATIONS

As nanoparticles are synthesized to be used as drug delivery systems, they have to comply with several requirements: biocompatibility, stability (allowing the storage for a long period) and the possibility to sterilise them (when parenteral administration is desired).

Depending on the synthesis technique, different impurities can exist in the nanoparticles (monomer molecules, initiator, solvent, surfactant, electrolytes, polymer aggregates). The elimination of polymer aggregates by ultrafiltration is quite easy, but some of the small molecules can be very difficult to remove. The most widely used methods of purification are: gel filtration, lyophylization and ultracentrifugation. Some of them had drawbacks: for example, dialysis allows only the removal of small molecules and it is very slow while ultracentrifugation leads to particle aggregation.[22] An industrial method to purify nanoparticles was described by Allemann *et al.* [44] – the cross flow filtration technique. Nanoparticles are filtered through a membrane into a cross flow, parallel with the membrane surface. In this way the clogging of the filter is avoided. The technique is relatively simple and can be applied on an industrial scale. For example, 6 g of PLA nanoparticles, obtained by salting out can be purified in approximately 3 hours.

APPLICATIONS OF POLYMERIC NANOPARTICLES

1. Corticoids release

Corticoids are anti-inflamatory drugs with high efficiency in the treatment of posterior segment eye diseases such as uveitis. It has also been proved that corticoids can improve the wound healing and they may be effective in the case of fibrosis (proliferative vitreoretinopathy and subretinal neovascularization).

Systemic administration of corticoids determines a series of side effects, topic administration being preferred. In the eventuality of topic administration, only a small amount of the drug reaches the posterior segment of the eye. Direct injections in the vitreous can increase the therapeutic efficiency but usually repeated injections are required, generating a great discomfort for the patient.[45,47] Some risks are also associated with this technique, such as vitreous haemorrhage or retinal detachment. Some local toxic effects have been also observed.

Another modality to insure a therapeutic concentration of the drug is to use drug releasing implants.[45] There are also disadvantages associated with this technique: a large surgical incision is required to install the implant;[48] the implant is very difficult to remove and it exists the possibility that the implant would migrate, endomaging the epithelium.[49]

An alternative to these rather complicated methods is to use corticoids loaded nanoparticles. Some of the most promising polymers are PLGA due to their very low toxicity.[50]

Gomez *et al.*[8] presented the synthesis of dexamethasone loaded PLGA nanoparticles. Dexamethasone is a poorly soluble crystalline corticoid generally used in the treatment of diabetic macular edema (as an implantable device).

2. Anticancer therapies

In the anticancer therapy, one of the worst problems is the low tumour answer to treatment, because of the non specific bioavailability of the administered anticancer agent. By using nanoparticles it is possible to achieve the bioaccumulation of the drug in the target tissue. In the most cases, the EPR (enhanced permeation and retention) effect is responsible for the accumulation of drug in the tumour tissue.

Hapca *et al.*[19] synthesized PLA nanoparticles on which they have grafted monoclonal antibodies with a high specificity for the treatment of ovarian cancer and lymphomas.

A current application of nanoparticles carriers is the controlled release of heme oxygenase (HO-1) inhibitors.[14] HO-1 is an enzyme involved in the oxidation of heme, by cleavage of the porphyrin ring leading to formation of biliverdin, carbon monoxide and free iron. Biliverdin is subsequently hydrogenated by the cytosolic enzyme biliverdin reductase to form bilirubin (a potent antioxidant). Although high levels of bilirubin in the blood can cause toxic effects to central nervous system, in a normal quantity, the bilirubin protects the human cells against oxidation.[51-54]

Tumors cells can also use HO-1 to protect themselves from oxidative processes. Especially the renal and prostatic tumors are characterized by a high concentration of HO-1. These observations led to the hypothesis that the administration of a heme inhibitor could increase the tumor's sensibility to oxidative processes. The oxidative processes can then be easily increased by certain usual drugs: cisplatin, anthracyclin, camptothecin etc.

One of the most interesting inhibitor of HO-1 is the zinc protoporphyrin (ZnPP). Apart from inhibiting HO, ZnPP also induces cell death by a secondary mechanism. The protoporphyrin derivates are also known to be efficient photosensitizers. These derivatives absorb light in the UV- visible region and being excited to a long-lived triplet state can interact with molecular oxygen, which on becoming singlet oxygen exerts cytotoxic effect.

The use of ZnPP is limited by its poor solubility in water. Macromolecular porphyrin compounds were also synthesized but the molecular weight was not high enough to allow the tumor targeting. A product with an adequate average molecular weight was obtained by Iyer *et al.*[14], by conjugation of ZnPP with PEG. The ZnPP-PEG conjugate showed a good releasing kinetic for *in vivo* and *in vitro* models, accumulating in the target tissue – by EPR. The main disadvantage was represented by the poor loading capacity (only 1.5% weight of ZnPP). The increased amount of PEG induces a high viscosity even for a minimal loading with ZnPP. Because of the high viscosity, the parenteral administration it is not available.

Another method to achieve the targeting of HO inhibitors is the synthesis of amphiphilic copolymers [poly(styrene-*alt*-maleic anhydride) (SAM)] that can load high amounts of ZnPP (up to 60%). Besides, the SAM copolymer proved to be biocompatible and it also has a stimulating effect for the immune system – by activating the macrophages (T and NK cells) (Figure 2).

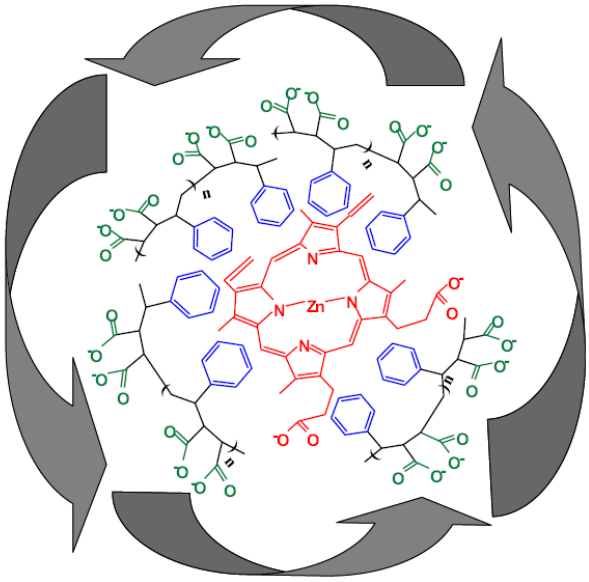

Fig. 2 – Schematic representation of SAM-ZnPP micelles formation by hydrophobic interactions.
ZnPP IX is surrounded by 4 SAM chains (n = 3 – 11). Aromatic rings of SAM
are involved in hydrophobic interactions with ZnPP while the carboxyl moieties (from anhydride) confer water solubility.

Other drugs that have been successfully used in the treatment of cancer are paclitaxel and doxorubicin.[55] In the case of paclitaxel, after the oral administration, the bioavailability is as low as 1%.[54] Paclitaxel is eliminated from bloodstream at the first hepatic passage, by cytochrome P-450. By synthesizing small enough nanoparticles, it is possible to attach and maintain the nanoparticle-paclitaxel complex to the intestinal mucosa until the complete absorption of paclitaxel to portal vein.

The particles dimensions have an important role in their capacity to cross through biological membranes. Several studies confirmed that particles with a diameter of 5000 nm can reach the lymphatic circulatory system. Particles with a diameter lower than 500 nm can cross the epithelium membrane by endocytosis and nanoparticles with diameter lower than 50 nm can reach the interstitial spaces.[56] These observations allowed the development of novel pharmaceuticals formulations for oral administration of anticancer drugs.

3. Blood-brain barrier

It is known that between the blood streamline and the central nervous system there is a barrier known as the blood-brain barrier (BBB). BBB allows only the exchange of ions in order to maintain a constant osmotic pressure and the passage of nutrients. Its role is to protect the brain and the spinal axis from any chemical or bacteriological threats. The protection offered by the BBB comes at a certain price: it is impossible to get drugs through the barrier, so the therapy for the central nervous system is very difficult. In the brain, endothelial cells are packed more tightly together, due to the existence of tight zonulae occludentes junctions between them.

The blood-brain barrier recognizes therapeutic agents as foreign particles and doesn't allow their passage. Because of the blood-brain barrier, finding a way to deliver bioactive substances to brain has become a real challenge.

One of the methods to achieve drug delivery to the central nervous system is to entrap the drugs into nanoparticles. Because of their reduced size, nanoparticles are able to pass through the vascular endothelium of BBB. There are several studies that showed good result in the treatment of brain tumors by drug loaded nanoparticles.[57-60]

4. Vaccines and gene therapy

Another field where nanoparticles are very important is gene delivery. By encapsulating the genes into nanoparticles it is possible to protect them from degradation in the presence of certain factors (pH, bile, proteolytic enzymes). The entrapment of genes into nanoparticles has encountered some problems regarding the stability of the synthesized structures during the preparation as well as after administration. One method to ensure the stability is to bind the genes to the surface of nanoparticles or nanocapsules. The binding must be reversible in order to allow the cleavage of the complex once the target has been reached. In order to improve the biocompatibility of the product it is better to avoid the use of surfactants.[61-63]

A method to obtain nanoparticles without using a surfactant was proposed by Castadello *et al.* [30] who obtained nanoparticles able to bind DNA without using a surfactant. The nanoparticles have a PMMA core and a shell of PEG and positively charged groups. The PEG chains are both biocompatible and biodegradable and provide steric stability while the positively charged groups bind DNA. By using these complex structures, the risk of physical desorbtion is greatly decreased. Such a vaccine is also stable and non toxic while it is possible to administer it orally.

Gene therapy is a potential method for treating neurodegenerative diseases such as Parkinson. The controlled delivery of genes responsible for GNDF (Glial Cell Line-Derived Neurotrophic Factor) formation stops the disease evolution and maintains a constant level of dopamine despite de cells lost because of the disease. Also the delivery of genes involved in the tyrosine hydroxylase have shown good results.[64-66]

Initially viral vectors were used (recombining adenoviruses or retroviruses). An more efficient method is to entrap the genes into nanoparticles. One of the preferred polymers is poly(ethyleneimine). Polyethyleneimine (PEI) has been already used to deliver genes inside the neurons with promising results.[67] It protects the DNA inside the nanoparticle while the ionic character favours the membrane penetration by binding to the negatively charged heparan sulphate, expressed on the cell surface.

5. Diagnostic

Sun *et al.*[68] presented the modality to obtain copper chlorophyl labeled nanoparticles. These nanoparticles can be directly traced *in vivo* by analytical electron microscopy (AEM). Nanoparticles covered with Polisorbate T-80 have been detected in

the brain, what proves the existence of endocytosis and/or transcytosis at some extent. Therefore this type of nanoparticles can be used in the functional exploration of the brain.

CONCLUSIONS

The use of nanoparticles in the field of biomedical engineering represents one of the most promising advances in the field in the last years. Not only the time between two consecutive administrations is prolonged, but the systemic and, most of the times, local toxicity is substantially reduced.

The ease of use and versatility are doubled by the relatively cheap methods for producing the nanoparticles as compared to the development of a new "classic" drug. The concept of smart drugs seems less utopian than ever with the new advances in the science of nanoparticulate materials.

One of the most promising applications of nanoparticles is in gene therapy. Delivery of modified viral fragments inside the cells represents one of the most challenging tasks the bioscience undertook in the modern period.

The present review does not claim to be an exhaustive presentation of the uses of nanoparticles in medicine but rather an introduction in this inter-disciplinary field, focusing on the most susceptible to reach the mass production phase systems.

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