Review Article

Theme: Develop Enabling Technologies for Delivering Poorly Water Soluble Drugs: Current Status and Future Perspectives
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Engineered Nanoparticulate Drug Delivery Systems: The Next Frontier for Oral Administration?

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Abstract. For the past few decades, there has been a considerable research interest in the area of oral drug delivery using nanoparticle (NP) delivery systems as carriers. Oral NPs have been used as a physical approach to improve the solubility and the stability of active pharmaceutical ingredients (APIs) in the gastrointestinal juices, to enhance the intestinal permeability of drugs, to sustain and to control the release of encapsulated APIs allowing the dosing frequency to be reduced, and finally, to achieve both local and systemic drug targeting. Numerous materials have been used in the formulation of oral NPs leading to different nanoparticulate platforms. In this paper, we review various aspects of the formulation and the characterization of polymeric, lipid, and inorganic NPs. Special attention will be dedicated to their performance in the oral delivery of drug molecules and therapeutic genes.

KEY WORDS: biodegradable; nanoparticles; natural; oral; polymer.

INTRODUCTION

Owing to its noninvasive nature, the oral route is the most preferred route for drug delivery by patients and clinicians alike. Nevertheless, effective oral drug delivery remains challenging owing to the drug physicochemical properties and to the nature of the gastrointestinal (GI) tract. Considering the GI tract, obstacles to drug absorption mainly include P-glycoprotein (P-gp) pump-mediated efflux, the enzymatic barrier, the stomach's highly acidic environment, and first-pass elimination by hepatic and/or intestinal cytochrome P450 (CYP450) (1), while solubility, molecular weight, and partition coefficient are the major physicochemical concerns dictating drug dissolution and permeability through the GI barrier (2).

In order to circumvent the above-mentioned issues, attention has been driven towards nanotechnology approaches. Nanoencapsulation allows drug oral bioavailability to be enhanced due to increased solubility and permeability and/or by shielding the entrapped drug from harsh conditions of the GI tract.

Nanoparticles (NP) development for circumventing oral medication concerns, from our point of view, falls into three major fields: cancer therapy, long-term treatment, and vaccination.

Indeed, oral chemotherapy represents a great challenge, and its success is expected to revolutionize cancer chemotherapy. Unfortunately, most anticancer drugs are Biopharmaceutics Classification System (BCS) IV (*e.g.*, paclitaxel and docetaxel) or BCS III drugs (*e.g.*, anthracyclines and nucleoside analogs) that are not orally bioavailable being substrates for P-gp and/or CYP450 (3) or because of their high polarity and low intestinal permeability (4). Polymeric NPs bear a great potential to address this problem by avoiding the recognition of P-gp and intra-enterocytic CYP450. In addition, NPs could adhere to the luminal surface of M cells that have a thin glycocalyx layer and thus increase the chance of the drug to be delivered directly through them by transcytosis. Furthermore, taking into account the side effects of cancer chemotherapy such as mucositis, ulceration of the GI tract, and diarrhea, drug association with particulate carriers could address this concern by limiting the direct contact between the drug and the GI mucosa.

In the case of chronic diseases, such as diabetes mellitus, hyperlipidemia, hypertension, inflammatory bowel diseases, etc., oral medication for patients treated throughout their lives represents a crucial demand in order to improve their quality of life and to assure their adherence to treatment (5). The poor oral bioavailability of many drugs used in long-term treatments remains till now the focus of numerous research works aiming to overcome this drawback. Such limitations have provided the impetus for the development of NPs allowing numerous goals to be achieved: first, shielding the

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entrapped drugs from the harsh GI environment enabling them to reach intact the site of absorption (the gut wall) (6); second, enhancing drug apparent water solubility being encapsulated inside colloidal nanocarriers (7); third, enhancing intestinal permeability of drugs once carried by NPs that are chiefly taken up by M cells known for their high transcytotic capacity and low lysosomal hydrolase activity (8); and fourth, reducing dosing frequency because of the controlled and sustained release of the nanoencapsulated drugs and thus resolving the problem of nonadherence to treatment (9).

Another major application domain of oral NPs is oral vaccination based on antigen or DNA delivery. Oral vaccination is a public health issue considering the lower costs of oral vaccination compared to a systemic one. In addition, both mucosal and systemic immune responses could be induced by mucosal administration of the vaccine (10). Nanoencapsulation can make oral vaccination possible as they safeguard antigen or DNA-based vaccines from the premature degradation by the hydrolytic GI environment and promote their uptake by M cells, key players of the mucosal immunity induction. In addition, NPs display a sustained release of the encapsulated vaccine increasing the duration of contact between the vaccine and immune cells, thus favoring an effective immune response (11).

In fine, oral NPs could be exploited as vectors for GI targeting to treat local diseases. The direct contact of the carriers with the target tissues (inflamed or cancerous tissues) leads to high local concentration and thus to higher drug effectiveness. This strategy has been already highlighted in previously published reviews (12,13).

Moreover, some examples in the literature indicated that systemic targeting could also be considered using oral NPs. In the research work conducted by Löbenberg and coworkers (14), hexylcyanoacrylate NPs were used as colloidal drug carriers for azidothymidine labeled with ^{14}C . Besides enhanced bioavailability, radioactivity could be detected in the blood, brain, and several organs containing a large number of macrophages after oral administration of drug-loaded NPs compared to a solution of ^{14}C -azidothymidine. In

another example, antitubercular drugs were detected in the lungs, liver, and spleen for 15 days after the oral administration of the encapsulated drugs in lecithin-functionalized poly(lactide-co-glycolide) NPs (15).

The use of NPs of different drug classes for oral delivery has been previously well reviewed (11,16,17). In this review, we aim to establish a global vision of the state of the art based on the nature of the constituting matrix, as it represents beside NP size the main factor dictating the fate and the safety of NPs in the GI tract (Fig. 1) and their interaction with the intestinal epithelium and thus therapeutic applications that can be considered.

NATURAL POLYMER-BASED NANOPARTICLES

Chitosans and Derivatives

Over the past 30 years, the use of colloidal carriers made of naturally occurring polysaccharides such as chitosan, pectin, alginate, carageenans, and starch has been increasing because of their low production costs, biocompatibility, and very low toxicity (18). In addition, these polymers generally show mucoadhesive properties offering the advantage of prolonged persistence at the absorption site of the drug, thus making them an interesting matrix for oral NP. Among these natural biopolymers, chitosan, owing to its absorption-promoting effect and its biodegradability, has gained considerable attention as a potential carrier for improving the intestinal transport of drugs and macromolecules (peptides, proteins, oligonucleotides, and plasmids). These properties seem to be due to the mucoadhesion providing a prolonged contact time between the drug and the absorption site, as well as a transient opening of tight junctions in the mucosal epithelium promoting a paracellular transport. Both behaviors have been found to be resulting from electrostatic interaction between positively charged chitosan and negatively charge components of the mucosal epithelium (19).

Indeed, the pH of the absorption site is of great importance to the effectiveness of chitosan as absorption enhancer, as only protonated soluble molecules can efficiently interact with the epithelium components (20). Indeed, at pH values below its pK_a (6.5), chitosan bears a positive charge thanks to its amine groups. These positively charged amine groups interact with the anionic tight junction-associated glycoproteins, such as sialic acid, resulting in a structural reorganization of the latter and thereby producing a transient opening of the tight junctions in the mucosal epithelium (21). Hence, the intestinal permeability enhancement is dependent on the pH altogether with chitosan molecular weight and deacetylation degree (22), which determine the degree of ionization of chitosan. A pH value above 6.5 is only encountered at the distal ends of the gut and is expected to be of concern only when NPs are targeted to these portions of the GI tract.

Besides the interesting absorption enhancement properties, the ease of NP preparation in aqueous media makes chitosan the polymer of choice to deliver hydrophilic macromolecules and explains the increasing number of published research works about chitosan-based oral NP. Almost all the preparation methods of chitosan NP are based on the dissolution of chitosan in acidic aqueous media (at pH values

Fig. 1. Classification of oral NPs according to current nanotoxicological findings

below 6.5). A direct advantage of this characteristic is the avoidance of the use of organic solvents, which may alter the encapsulated drug during NP manufacturing. In the same vein, the water solubility of chitosan mostly leads to efficient entrapment of polar hydrophilic drugs which are generally dissolved in the aqueous phase where they will be associated with chitosan *via* electrostatic interaction or hydrogen bonding. It is noteworthy to mention that polar drugs are usually poorly encapsulated in the commonly used polymers, such as poly(ϵ -caprolactone) and polylactic acid, because of the lack of affinity to these hydrophobic polymers. Moreover, chitosan being available in a wide range of molecular weight and degree of deacetylation enables formulators to design NPs with adjustable size and surface charge that will, in turn, affect their pathway through the GI barrier and the release profile of the entrapped drug. The different techniques currently used in chitosan NP manufacturing are summarized in Table I.

Numerous studies support the concept that chitosan NPs lead to a strongly improved bioavailability of many orally given peptide nucleic acids and drugs. Sarmiento and coworkers reported that the insulin-loaded NPs could effectively reduce the blood glucose level in a diabetic rat model (23). In this study, confocal microscopic examinations of FITC-labeled insulin NPs showed clear adhesion to the rat intestinal epithelium and internalization of insulin within the intestinal mucosa (23).

In other studies, enhanced antimicrobial activities have been reported for several antibiotics, such as streptomycin (24), when they are loaded in chitosan NPs. In this study, streptomycin-loaded chitosan NPs showed potential activity when administered orally in an *in vivo* mouse model of *Mycobacterium tuberculosis*. When administered at a dose of 100 mg/kg, the streptomycin-loaded chitosan NPs showed similar activity equivalent to their subcutaneous injection (24).

Over the last decade, nanoencapsulation into chitosan network have been increasingly applied to nucleic acids for vaccination and therapeutic gene expression purposes. Chew and coworkers have encapsulated the plasmid DNA containing house dust mite allergen genes in chitosan NPs in order for it to be administered *via* the oral route rather than the parenteral one (25). According to data shown in this work, the oral feeding of chitosan–DNA NPs induced specific immune responses against the major dust mite allergen, Der p 1, whereas intramuscular immunization alone could not.

Borges and coworkers (26) have co-encapsulated the hepatitis B recombinant antigen with a synthetic oligodeoxynucleotide containing immunostimulatory CpG motif (CpG ODN) in chitosan NPs. Following their oral administration, the prepared NPs induced both local and systemic immune responses, thus making this delivery system a promising one to prevent or overcome hepatitis B infections.

Recently, DNA vaccines encapsulated in chitosan NPs were prepared by a complex coacervation method to enhance the efficacy of a DNA vaccine against swine influenza (27). In this study, the authors reported that chitosan NPs improved the delivery of DNA to antigen-presenting cells by efficient transfection through local lymphoid tissue and uptake by dendritic cells.

Despite the encouraging results, the transfection level of chitosan-based delivery systems is still too low for clinical application. Because of the quite low toxicity of chitosan compared to cationic lipids that are widely used for gene transfection, many efforts are being pursued to better understand the chitosan–NP transfection efficiency-related parameters. It has been found that chitosan molecular weight and degree of deacetylation are critical factors affecting stability and transfection efficiency of nucleic acid-loaded chitosan NPs, as recently reviewed by Mao (28). Higher molecular weight and degree of deacetylation promote the

Table I. The Preparation Methods of Chitosan NPs

Preparation method	Principle	Examples	Mean size	References
Ionotropic gelation “polyelectrolyte complexation”	Electrostatic interaction between amine groups of chitosan and negatively charged groups of a polyanion such as tripolyphosphate or an anionic polymer (poly gamma-glutamic acid, Arabic gum, alginate, or pectin)	Chitosan/alginate NPs for insulin oral delivery	750 nm	(23)
Chitosan–drug ionic complexation	Electrostatic interaction between anionic drugs like oligonucleotides and plasmids and chitosan	Chitosan NPs as DNA vehicles for oral vaccination	100–400 nm	(24)
Precipitation	Desolvation of chitosan in its water solution by adding a flocculant (sodium sulfate) making hydrogen bonds between chitosan molecules	Alginate-coated chitosan NPs loaded with hepatitis B antigen for oral vaccination	300–600 nm	(25)
Emulsion cross-linking	Formation of water in oil simple emulsion followed by a chemical cross-linking of chitosan by glutaraldehyde	5-Fluorouracil chitosan NPs	0.5–1 μ m	(29)
Electrospray deposition	The process relies on coulomb repulsion to break the chitosan solutions (in acetic acid) into fine charged droplets, which can be described by an electrochemical model	–	124 nm	(30)

formation of stable nucleic acid–chitosan complexes. Higher molecular weight chitosans are long and flexible with a higher degree of deacetylation enhancing its electrostatic interaction with nucleic acids, thus synergically reducing the size of complexes and increasing their surface positive charge along with their stability and transfection efficiency.

Moreover, NPs based on chemically modified chitosan are being widely studied for colonic drug targeting in order to treat local diseases such as colorectal cancer (31) as well as for systemic drug delivery (32). Considering the relatively low enzymatic activities in the colon, if drugs could be delivered to this site, a greater efficiency of absorption would be expected (32). The use of chitosan has often been limited in colonic drug targeting because chitosan is highly soluble in the acidic environment, sometimes resulting in a burst release of the drug at the stomach. Besides, upon reaching the colon, chitosan undergoes degradation by chitinases produced by microflora, leading to a reduction in their molecular weight and thereby loss of mechanical strength, making them unable to hold the drug any longer (33). These limitations, among others, have provided the impetus for the development of a wide range of chemically modified chitosan bearing quaternary ammonium, thiol, or polyethylene glycol groups or coupled to ligands such as hyaluronic acid or folate.

These chitosan derivatives display the characteristics of native chitosan and other specific properties as well. For example, quaternized chitosan derivatives have been used as carriers for colon delivery of insulin (32). Quaternized chitosan overcomes the drawback of limited solubility of chitosan at neutral and weakly alkaline pH, being positively charged independently of the medium pH value. As expected, *in vivo* studies in rats have shown enhanced colon absorption of insulin by using these NPs compared to free insulin in diabetic rats. Furthermore, NPs based on hyaluronic acid-coupled chitosan have been developed in order to specifically deliver oxaliplatin to the colorectal carcinoma cells that overexpress hyaluronic acid receptors (34).

Besides, thiolated chitosan showed improved mucoadhesive properties due to the formation of disulfide bridges with the cysteine residues in the intestinal mucus glycoproteins. Atyabi and coworkers demonstrated that thiolated polymers inhibit the efflux pump (35). In this work, thiolated chitosan NPs have been developed as an oral delivery system for amikacin, a polycationic aminoglycoside, which is poorly available probably due to the efflux by the P-gp pump. *In vitro* permeation studies of amikacin NPs conducted using rat gut reported that 82% of the entrapped amikacin permeated through the rat gut membrane (35).

Finally, pegylated chitosans have been developed to improve the water solubility of chitosan and to enhance the transfection efficiency with a minimal cytotoxicity. Malhotra and coworkers have reported the encapsulation of siRNA in pegylated chitosan-based NPs (36). The authors reported that suitable NPs could be synthesized using this polymer and that they have the capacity to carry genes and provide adequate transfection efficacy with no toxicity when tested in neuronal cells (36).

Alginates

Alginate is one of the most studied and applied natural polysaccharides in oral controlled delivery systems. Specifically,

the simple aqueous-based gel formation of sodium alginate in the presence of divalent cations such as Ca^{2+} has been used for drug delivery and cell encapsulation (37). It is a hydrophilic linear polymer composed of β -mannuronic acid monomers and α -L-glucuronic acid units, extracted from a marine brown alga. Alginate has been reported to be mucoadhesive, biodegradable, and biocompatible.

Due to its polylactic structure, alginate-based delivery systems are pH sensitive. Drug release is significantly reduced in low-pH solutions. Upon contact with the intestinal fluid, the alginate matrix will undergo an ion exchange between divalent cation and sodium resulting in disintegration of the matrix and release of the drug. Indeed, alginate biodegradation occurs *via* a slow and unpredictable dissolution process *in vivo*, mainly due to its sensitivity towards calcium chelating compounds (e.g., phosphate, citrate, and lactate) (38). Hence, the limited control on gel dissolution and the frequently observed significant burst release effect have limited the use of alginate as a sole component in oral controlled release delivery systems. Composite matrix NPs of alginate and chitosan (37) or polymethacrylates (39) were used to optimize the release rate.

The preparation of alginate NPs is based on the ionic gelation induced by divalent cations, such as calcium and zinc, which can cooperatively bind between the glucuronic acid units to form ionic bridges between adjacent polymer chains (40). A critical adjustment in the relative concentrations of alginate and the cation results in a pregel state, *i.e.*, alginate NPs. Composite NPs are obtained by adding a small volume of the aqueous solution of the second polymer (chitosan or cationic polymethacrylates) to the initial alginate aqueous solution with calcium chloride (37,39).

Hydrophilic drugs (e.g., rifampicin and gliclazide) are generally efficiently entrapped in NPs made of alginate, which is usually blended with another polymer, and display an enhanced oral bioavailability and biological performance, as supported by several studies (39,41). Ahmad and coworkers reported high encapsulation efficiency of antituberculosis drugs in alginate–chitosan NPs ranging from 80–90% for rifampicin, 88–95% for ethambutol, and 70–90% for isoniazid/pyrazinamide (41). In this work, *in vivo* studies in mice revealed a significant enhancement in the relative bioavailability of encapsulated drugs when compared to free drugs. In addition, the treatment of tuberculosis-infected mice with three oral doses of the formulation spaced 15 days apart resulted in complete bacterial clearance from the organs, compared to 45 conventional doses of orally administered free drugs.

Moreover, gliclazide encapsulation in alginate–Eudragit® E NPs have been studied using different formulation parameters. The optimized particles displayed a high encapsulation efficiency (82%), high drug loading (49%), and a particle size of 525 nm (39). In addition, a pharmacokinetic study in rabbits revealed better bioavailability of gliclazide with sustained release in plasma from gliclazide NPs compared to plain gliclazide.

Proteins

Nanoparticulate delivery systems made from proteins (e.g., albumin, gelatin, and gliadin) exhibit biodegradability, biocompatibility, and low antigenicity. Besides, the primary structure of

NP-constituting proteins and the different accessible functional groups allow targeting ligands, shielding substances or drugs to be covalently attached to the NP surface.

Gelatin is obtained by controlled hydrolysis of collagen, the major component of skin, bones, and connective tissues. There are two different types of gelatin: A and B showing a difference in their respective isoelectric points (7–9 for gelatin type A and 4–5 for gelatin type B). Albumin, a major plasma component, is widely available in pure form.

Gliadin, a vegetable protein extracted from gluten of wheat, has a distinct edge over the above-mentioned proteins for oral delivery because of its mucoadhesive properties. Being mainly composed of neutral and lipophilic amino acids, gliadin shows hydrophobic character promoting an efficient encapsulation of lipophilic drugs and a prolonged drug release when used as a matrix for NP (42).

Protein-based NPs are generally manufactured by the emulsification, desolvation, or coacervation methods. In the first method, water/oil is prepared starting from the protein water solution and vegetable or organic oil. The protein-containing water droplets are hardened either by thermal treatment or by covalent cross-linkage. In the case of albumin, the prepared emulsion is heated (95°C and 120°C), resulting in coagulation and stabilization of albumin droplets (43), while in the case of gelatin, the emulsion is cooled at low temperature (below the gelation point) resulting in gelation of gelatin droplets (44). The emulsification method involves the use of organic solvents in order to remove the oil and highly toxic cross-linker residues as well as the surfactants used during the emulsification step.

In the second method, the protein is precipitated in its water solution by adding a desolvation agent, such as alcohol or natural salts, resulting in the formation of protein aggregates, which will be subsequently cross-linked by adding a cross-linker such as glutaraldehyde (45). The obvious drawbacks of the desolvation method are (1) the formation of large aggregates whose size falls outside the nanoscale and (2) lack of stability in aqueous dispersion leading to phase separation.

The coacervation method is very similar to the desolvation technique; it consists on mixing of the aqueous protein solution with an organic solvent like acetone or ethanol to yield tiny coacervates and cross-linking of the obtained coacervates (46). In this method, the pH of the starting aqueous solution is adjusted in order to ionize the formed coacervates leading to NPs with a reduced size, which could be attributed to the increased repulsion between aggregates during particle formation. In addition, better stability of protein NPs could be achieved by introducing a polysaccharide to the NP matrix. Kumar and Jain have encapsulated ciprofloxacin in HSA–pectin NPs (47). NPs were prepared by pH-coacervation method starting from a buffer solution (pH 8.0) containing HSA, pectin, and ciprofloxacin which was subsequently desolvated by acetone added dropwise and then cross-linked with glutaraldehyde. Adding pectin to the matrix was found to suppress NP aggregation, usually observed for HSA NPs, which is probably due to pectin segments present on the surface of NPs.

The concept that protein-based NPs, especially those made of gliadin, significantly enhance the oral bioavailability of drugs is supported by several studies. In a research work conducted by Arango and coworkers (42), gliadin NPs have been prepared as an oral carrier for carbazole, a highly lipophilic drug with a

low solubility in aqueous milieu, and thus, the oral administration of the aqueous suspension provided negligible plasma concentrations. Gliadin NPs were prepared by the desolvation method followed or not by a subsequent cross-linking step with glutaraldehyde. NPs displayed nearly the same size, surface charge, and drug loading (460 nm, +28 mV, 13 µg/mg, and 453 nm, +27 mV, and 12 µg/mg) for nonhardened NPs and cross-linked NPs, respectively. Both types of NPs dramatically increased the carbazole oral bioavailability up to 49% and provided sustained plasma concentrations of carbazole (42).

In another study (48), lectin was covalently conjugated to gliadin NPs as a targeting ligand to the carbohydrate receptors of *Helicobacter pylori*. NP antimicrobial activity was assessed a growth inhibitory percentage of the isolated *H. pylori* strain (48). The inhibitory efficacy of lecithin-conjugated gliadin NPs was found to be twofold higher than that of gliadin NPs.

More recently, Bhavsar and Amiji have developed a novel hybrid oral carrier based on gelatin and poly(epsilon-caprolactone) matrix for oral gene delivery and called it “NPs-in-microsphere oral system NiMOS” (49). NiMOS were formulated by encapsulating plasmid DNA in type B gelatin NPs of around 200 nm in diameter and then further protection of the NPs with a poly(epsilon-caprolactone) matrix to form microspheres of less than 5 µm. When administered orally, the NiMOS have shown longer residence in the small and large intestines in fasted rats compared with gelatin NPs. In addition, when plasmid-expressing betagalactosidase or green fluorescent protein was encapsulated in NiMOS, transfection was observed in the small and large intestines following a single 100-mg DNA dose for up to 5 days post-administration (49).

SYNTHETIC POLYMER-BASED NANOPARTICLES

Over the last few decades, synthetic polymers have been developed in order to offer a wide range of drug delivery matrix enabling to tailor different release profiles and to design intelligent systems. Today, polymeric materials provide the most important avenues for research, primarily because of their ease of processing and the ability of researchers to readily control their physicochemical properties *via* molecular synthesis.

Indeed, many considerations have to be taken into account concerning the developed polymers, such as biocompatibility, degradation behavior, and toxicity of degradation products and formulation issues and challenges. Hence, any polymer selected for drug delivery formulation is commonly classified according to chemical nature, such as polyester, polymethacrylates, backbone stability (biodegradable, non-biodegradable), biocompatibility, and water solubility (hydrophobic, hydrophilic).

Currently, research is being focused on fast-degrading, biocompatible, and amorphous matrix. Besides, mucoadhesive polymers are highly desirable for oral delivery systems since they can adhere to mucosal linings within the GI tract for extended periods, releasing their entrapped drugs slowly over time.

Polymethacrylates

Acrylates are a family of polymers, which are a type of vinyl polymer. Acrylate monomers are esters which contain vinyl groups, that is, two carbon atoms double-bonded to each other, directly attached to the carbonyl carbon. Some acrylates have an

extra alkyl group attached to the alpha carbon, and these are called alkylacrylates (in the case of methyl, they are called methacrylates).

Acrylic polymers such as polymethacrylates have excellent film-forming characteristics and are used for coating tablets, pellets, capsules, and granules. They are pharmacologically inactive and have a good compatibility with mucosal membranes. However, polymethacrylates are nonbiodegradable polymers and therefore are not suitable for parenteral uses. Eudragit® is a trade name of copolymers derived from esters of acrylic and methacrylic acids, whose properties are determined by functional groups. Eudragit® grades differ in their proportion of neutral, alkaline, or acid groups resulting in different physicochemical properties and offer excellent choice for the formulating scientists.

Enteric-type methacrylates such as Eudragit® L/S are pH-responsive polymers providing a barrier against drug release in the stomach and enabling controlled release in the intestine. These polymers are weak acids not dissolving in acidic gastric juice but are readily soluble in aqueous media at pH 6 and 7, respectively, which may be equivalent to drug release to the distal ileum, in which they bear a negative charge.

Eudragit® RS and RL types are widely used as controlled release matrix. The amount of quaternary ammonium groups in RS type is between 4.5% and 6.8% and in RL type is between 8.8% and 12%. Eudragit® RL are more permeable than Eudragit® RS due to its higher quaternary ammonium group content. Both RS and RL types are insoluble at physiological pH values and capable of swelling. In addition, owing to their polycationic nature, these polymers display a mucoadhesive character and thereby could favor intestinal absorption (50).

The cationic types of Eudragit®, such as Eudragit® RS, RL, or E, have been used as matrix for the encapsulation of polyanionic macromolecules that are the nucleic acids into NPs. In a research work conducted by Wang and coworkers, antisense oligodeoxynucleotides (ODN) were encapsulated in Eudragit® RL100 or RS100 NPs for oral delivery (51). The NPs were prepared by nanoprecipitation and then mixed with oligonucleotides. Spherical NPs of an average size of 127 nm were obtained, and almost the antisense ODN were loaded when NPs/ODN ratio was 6.6. The cell uptake of ODN was significantly increased when loaded by NPs, which well depended on the NP concentration. Meanwhile, slight cytotoxicity was observed when a high dose of NPs was used.

In another study, the DNA plasmid has been encapsulated in Eudragit® RS or RL NPs as nonviral vectors (52). NPs were prepared by two methods: nanoprecipitation and double emulsion. Cytotoxicity tests based on mitochondrial activity (MTT test) revealed that the NPs had limited cytotoxicity which depended on both the cell type and the NP concentration. According to the authors, Eudragit® RS or RL NPs can introduce the transgene into different types of cells but are generally less effective than the lipofectamine control. Interestingly, it has been found that NPs prepared by nanoprecipitation were slightly more efficient than NPs prepared from a double emulsion. Finally, particle size was not an important factor for transfection, since no significant difference was observed with size between 50 and 350 nm (52).

Cationic types of Eudragit® have been successfully used for the elaboration of nano- and microparticles loaded with

heparin, a polyanionic macromolecule, for oral medication purposes (53). Heparin NPs were prepared by the double emulsion and solvent evaporation method using Eudragit® RS or RL alone or blended with a polyester (poly(caprolactone) or poly(lactide-co-glycolide)). Encapsulation efficiencies as high as 60% and 98% for Eudragit® RS and RL NPs, respectively, were obtained along with maintaining the heparin biological activity, as measured by the anti-Xa activity (53). The *in vivo* performance of these two formulations has been assessed in rabbits (54). Increases in both anti-factor Xa activity and activated partial thromboplastin time were detected after the oral administration of each formulation confirming their oral absorption (54).

Furthermore, cationic types of Eudragit® were also used as matrix for oral nanocarriers of therapeutic peptides and proteins. A blend of Eudragit® RS and poly(caprolactone) was used for insulin nanoencapsulation by the double emulsion/solvent evaporation method (55). The formulation of insulin-loaded NPs has been studied and optimized using the experimental design methodology. The optimized formulation was based on a blend PCL/RS 50:50 enabling formulators to obtain relatively small NPs (322 nm) with a narrow particle size distribution, containing at least 25 IU of insulin per 100 mg of polymer and delivering at least 4 IU after 7 h of release. Besides, optimized NPs displayed a positive surface charge (+43 mV) which could favor intimate contact with the GI tract and therefore insulin absorption (55).

Cationic types of Eudragit® were also used for the encapsulation of small hydrophilic molecules in order to achieve prolonged drug release and to improve patient compliance, especially in long-term therapies. Accordingly, lamivudine, an antiviral drug used in the treatment of human immunodeficiency virus HIV and chronic hepatitis B, was encapsulated into Eudragit® RS 100 NPs aiming to design controlled and sustained release once-daily formulations of lamivudine that can maintain systemic drug levels consistently above its target antiretroviral concentration throughout the course of the treatment (which is crucial for the success of AIDS therapy) (56). As the authors expected, lamivudine-loaded NPs displayed a zero order *in vitro* release profile and provided a sustained release of lamivudine over a period of 24 h (56).

In addition, the elaboration of NPs using anionic types of Eudragit® has been reported in numerous studies as pH-sensitive systems achieving a selective release of the drug close to its absorption site and thus improving its oral bioavailability. Consistently, the oral bioavailability of a poorly water-soluble HIV-1 protease inhibitor has been dramatically improved *via* its encapsulation in Eudragit® L100, when assessed in mice and dogs (57). More recently, insulin-loaded NPs have been elaborated using the anionic Eudragit® L 100-55 and chitosan of various molecular weights by complex coacervation (58). The obtained NPs were nonspherical and displayed an encapsulation efficiency of 30.5% with a positive surface charge increased by increasing molecular weight of chitosan. The circular dichroism spectroscopy studies indicated that the nanoencapsulation process did not significantly disrupt the internal structure of insulin; additionally, pH sensitivity of NPs was preserved, and the insulin release was pH dependent.

In fine, it can be concluded that Eudragit® is a very widely used polymer in oral drug delivery systems. More and more examples of drug nanoencapsulation in Eudragit® are being

reported in the literature. These works are mainly focused on drugs that are poorly absorbed and/or poorly water soluble such as cyclosporine (59), beclomethasone (60), etc.

Polyesters and Their Copolymers

Aliphatic polyesters, such as poly(ϵ -caprolactone) (PCL), poly(lactic acid) (PLA), and their copolymers, are the most common being biodegradable polymers. Some are approved by regulatory agencies for clinical use (*e.g.*, PLA and its copolymer with PGA). These polymers are completely degraded into small molecules through hydrolytic and/or enzyme degradation, which are subsequently eliminated mainly by renal clearance (61). Hence, since their synthesis, they are being widely used in tissue engineering and drug delivery systems manufacturing.

Polyester-based NP could be easily prepared by several methods, such as solvent evaporation, solvent displacement, salting out, dialysis, and emulsion-solvent diffusion technique, which are well described and illustrated in a recent review (62). For NP manufacturing, the choice of the polymer is based on two principal characteristics: its crystallinity and molecular weight, in other words, its hydrophobicity. Indeed, polymer hydrophobicity has a direct impact on NP degradation rate and consequently on the drug release kinetics. Polyesters are generally partially crystalline hydrophobic polymers that are suitable for lipophilic and hydrophobic drug encapsulation. PCL is more hydrophobic than PLA, thus showing slow degradation, making it especially interesting when a prolonged drug release up to several months is desired.

In oral delivery, polyester-based NPs have been designed for several purposes: drug protection against the GI harsh environment, oral absorption enhancement, and systemic targeting along with avoiding the intravenous route. Some examples are given.

In literature, PCL (14600 Da)-based NPs were developed and characterized tamoxifen targeting breast cancer cells after oral administration (63). Tamoxifen-loaded NPs were spherical, sized 250 to 300 nm, with a positively charged surface of +25 mV probably due to a significant presence of the drug on the NPs' surface. The maximum tamoxifen loading efficiency was 64%. According to published data, a large fraction of the administered NP dose was taken up by MCF-7 cells through nonspecific endocytosis (63). The significant uptake of NPs, leading to an enhanced local concentration of the drug, indicates that PCL NPs could serve as a useful form of targeted drug delivery system towards breast cancer (63). Furthermore, the oral bioavailability of cyclosporine (CyA) was significantly enhanced *via* its encapsulation in PCL NPs leading to specific immunosuppression although without increasing the adverse effects (64).

Being hydrophobic and thus inconvenient for hydrophilic drug encapsulation, polyesters have been coupled with hydrophilic polymers such as poly(glycolic acid) (PGA), polyethylene glycol (PEG), polyethylene-polypropylene glycol (commercialized as Pluronic®), or D- α -tocopheryl polyethylene glycol 1000 succinate (TPGS).

Block copolymers of PLA with Pluronic® F127 (PLA-F127-PLA) were synthesized by Xiong and coworkers (65) in order to be used as matrix for oral NP delivering insulin (65). Insulin-loaded NPs were prepared by the dialysis method. Obtained NPs had an average size of 100 nm and a vesicular

structure. Hypoglycemic behavior of insulin-loaded NPs was examined after their oral administration to fasted diabetic mice. Blood glucose was efficiently and gradually reduced to a minimum of (about 4.5 mmol/L) over 5 h and maintained at this level for 18.5 h. These results demonstrate that PLA-F127-PLA NP provides a good protection of insulin in the GI tract and an enhanced intestinal absorption as well as a controlled and prolonged release (65).

Poly(lactide-co-glycolide) (PLGA), PLA, and PGA copolymers are commercially available and commonly used for NP manufacturing. According to the PLA to PGA molar ratio, molecular weight, and the end group (carboxyl or ester), PLGAs exhibit different degradation rates leading to NPs with different drug release kinetics and encapsulation efficiencies. For instance, PLGA NPs prepared for oral delivery of cystatin, a protein with potential anticancer activity, have shown to be able to preserve drug activity depending on the type of PLGA used in formulation (66). In this study, three types of PLGA of 50:50 molar ratio were used: Resomer RG® 503 H and Resomer RG® 502 H have a carboxyl end group with molecular weights of 48 and 12 kDa, respectively, and Resomer RG® 502 with ester end group and molecular weight of 12 kDa. The encapsulation efficiency was clearly affected by the type of PLGA used in formulation. The more hydrophilic types (*i.e.*, RG® 502 H having the lower molecular weight and carboxyl end group) were more suitable for cystatin encapsulation. However, they produced burst drug release compared with smaller release observed from NPs prepared with RG® 502, the esterified type. Moreover, both RG® 502 H and RG® 503 H-based NPs were able to maintain cystatin activity during 5 days of incubation in phosphate buffer solution at 37°C, whereas the stability of cystatin encapsulated in RG® 502-based NPs dramatically decreased over the same period. The authors attributed this result to the free carboxyl end groups in RG® 502 H and RG® 503 H that probably interact with cystatin, thereby preserving its activity (66).

Furthermore, PEG blocs covalently attached to PCL, PLA, or PLGA were used for oral NP elaboration. It has been shown that PEG improves the stability of PLA NPs in the GI fluids and facilitates NP transport through GI mucosa to the lymphatic system (67). Therefore, polyester-PEG-based NPs represent promising vehicles for oral vaccination. Moreover, ligand grafting to PEG results in further enhancement of NP transport across the GI barrier as reported by Garinot and coworkers (68). In this work, PEG-PLGA NPs were developed as oral nanocarriers for vaccines and compared with NPs bearing RGD peptides covalently linked to PEG. After oral administration to mice of NPs loaded with ovalbumin as a model antigen, targeted NPs have shown to be slightly more efficient to produce immunization than nontargeted ones as evidenced by the number of mice producing IgG in their sera (68).

LIPID-BASED NANOPARTICLES

Liposomes

Liposomes are vesicular carriers sizing from several micrometers to tens of nanometers. They are composed of one or more phospholipid bilayers in which lipophilic drugs

could be inserted and an inner aqueous core, which is a suitable compartment for encapsulating hydrophilic drugs (69). The tolerability and safety of liposomes have been widely verified in animals as well as in human volunteers. No untoward effects have been recognized; liposomes offer good biocompatibility, low toxicity, and high safety (69).

Liposomes bilayers can be composed of natural and/or synthetic phospholipids such as egg phosphatidylcholine, soy phosphatidylcholine, dipalmitoyl phosphatidylcholine, hydrogenated soy phosphatidylcholines, dimyristoylphosphatidylcholine, etc. Cholesterol is usually included to stabilize lipid bilayers. In addition, they may include positively (stearylamine) or negatively (dicetyl phosphate) charged molecules and/or PEG-attached lipids (70). Factors affecting drug encapsulation efficiency within liposomes depend on both liposomes and encapsulated drug properties. Concerning the encapsulated drug, the entrapment efficiency is affected by hydrophilic or lipophilic properties and its tendency to interact with the lipid bilayer. As for the liposome properties, it has been reported that aqueous volume, membrane rigidity, surface area, and preparation methods have an influence on the encapsulation efficiency (70).

Although parenteral administration is the first intended application of drug-loaded liposomes, liposomes for oral delivery have been developed. Poorly soluble drugs may benefit from improved water solubility and intestinal absorption when administered as liposomal colloidal suspensions. Table II lists some salient studies and recent publications describing drug-loaded liposome development for oral administration. Nevertheless, oral applications remain limited because of the destabilization of liposomes in the GI fluids especially in the presence of bile salts that can penetrate into liposomal lipid bilayers disrupting the vesicular structure (71). A higher concentration of bile salts can induce liposome transformation into small mixed micelles. Interestingly, the resultant mixed micelles can behave as excellent carriers for poorly water-soluble drug molecules and one of the most important mesophases before absorption (72). Accordingly, bile salts were introduced in oral liposome formulation for the delivery of many hydrophobic and poorly absorbed drugs (73). Recent research work have also used ligands composed of cellular nutrients, such as folic acid, as mediators of liposomal uptake by enterocytes (74).

Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLN) have been proposed as alternative nanocarriers showing distinct advantages over polymeric NPs and liposomes, such as increased drug stability, high drug payload, and an easier scaling up and sterilization (75). SLN show a mean size ranging from 50 to 1,000 nm and are composed of a lipid which is solid both at body and room temperature, dispersed either in water or in an aqueous surfactant solution (75). Their matrix is made from physiologically tolerated lipid components decreasing the potential for acute and chronic toxicity, such as triglycerides (e.g., tristearin, tripalmitin), partial glycerides (e.g., glyceryl mono-stearate, glyceryl behenate), fatty acids (e.g., stearic acid, palmitic acid), steroids (e.g., cholesterol), and waxes (e.g., cetyl palmitate) (76).

Table II. Recent Research Works on Liposomal Oral Delivery Systems

API	Lipidic excipient	Preparation method	Mean size	Encapsulation efficiency (%)	In vivo results	References
Midazolam	Hydrogenated phosphatidylcholine, dipalmitoylphosphatidic acid, cholesterol	Thin-film hydration	–	21–87	The absorption of orally administered midazolam liposome to the systemic circulation was $43.6 \pm 10.5\%$, which was significantly greater than that of midazolam solution.	(77)
Cefotaxime sodium	Soybean phosphatidylcholine, cholesterol	–	309–316 nm	22–28	The bioavailability of cefotaxime was found to increase 1.4–2.0-fold when administered via folic acid-coupled liposomes as compared with folic acid-free liposomes.	(74)
Oxymatrine (hepatitis B)	Phosphatidylcholine, phosphatidylserine, cholesterol	Double emulsification	17–22 μ m	53–65	The area under the plasma concentration–time curve obtained from the pharmacokinetics study of TMC-coated MVLs was found to be about 3.26- and 1.96-fold higher than that of drug solution and uncoated MVLs, respectively.	(78)
5-Fluorouracil (5-Fu)– N ₃ -O-tolyl-fluorouracil (TFu)	Soy lecithin, cholesterol	Modified film dispersion–homogenization technique	400 nm	88.87	In vitro drug release profile of TFu-loaded liposomes followed the bi-exponential equation. The results of the pharmacokinetic studies in mice indicated that the bioavailability of TFu-loaded liposomes was higher than the suspension after oral administration, and was bioequivalent comparing with TFu 50% alcohol solution after intravenous (i.v.) administration.	(79)
Hexamethylmelamine (HMM), an antitumor agent	Phosphatidylcholine cholesterol, sodium deoxycholate (NaDC)	Reverse phase evaporation	207–286 nm	6–46	The area under the plasma concentration–time curve obtained from the pharmacokinetics study of HMM NaDC-liposomes was found to be ~9.76- and 1.21-fold higher than that of HMM solution and HMM liposomes, respectively.	(73)

API active pharmaceutical ingredients, MVL multivesicular liposome, TMC N-trimethyl chitosan

The production of SLN could be handled using different methods, such as high-pressure homogenization (hot or cold homogenization), microemulsion technology, and solvent evaporation, which were previously well reviewed (75). Under optimized conditions, SLN may be suitable for encapsulating both lipophilic and hydrophilic drugs giving rise to well-stabilized formulations (80). Accordingly, SLN have drawn great attention as potential carriers for a large number of drugs. An overview of recent papers about drug-loaded oral SLN is presented in Table III. For instance, SLN-encapsulated peptides for oral delivery can benefit from the lipid-stabilizing and absorption-promoting effects associated with these materials (81).

Although SLN show increased stability when compared to liposomes, they still undergo degradation during their contact with GI fluids especially the lipolytic enzyme pancreatic lipase. It is noteworthy to mention that different degradation rates could be expected depending on SLN composition (lipid matrix and stabilizing surfactant). Indeed, fatty acids of longer hydrocarbon backbones displayed slower degradation than those with shorter chains (82). The choice of surfactants is crucial, being able to either accelerate SLN degradation (*e.g.*, cholic acid sodium salt) or slow it down (*e.g.*, Poloxamer 407) (82). The slowed degradation has been explained by the shielding effect exerted by the surfactant ethylene oxide chains at the SLN surface (82). Consistently, different degradations and thus drug release profiles could be conceived by adjusting SLN composition. Till now, the SLN dynamics have not been well elucidated. Further efforts are needed in order to better understand their *in vitro* and *in vivo* behaviors at the molecular scale.

Cubic Nanoparticles (Cubosomes)

The self-assembling of amphiphilic molecules including some lipids in an aqueous system is known to form a variety of liquid crystalline phases such as lamellar, inverted hexagonal, and inverted cubic phases (83). Lipids such as phosphatidylcholines, phosphatidylethanolamines, PEGylated phospholipids, and various monoglycerides have been confirmed to form bicontinuous cubic phases, which usually appear as isotropic bulk gels; those composed of surfactants of appropriate hydrophilicity/hydrophobicity balance can exist in equilibrium with excessive water and be dispersed into cubic nanoparticles (CNP) (84). Glyceryl monooleate (GMO), a synthetic monoglyceride approved by the FDA, in combination with poloxamer 407 or TPGS was frequently used in formulating bicontinuous cubic phase for oral delivery applications (84–86).

CNP seem to be advantageous as oral delivery carriers (84,85). Firstly, they are broken down into smaller particles by the intestinal lipases, but being lyotropic, CNP can hold drugs in their lipid bilayers avoiding their precipitation in the GI fluids. Secondly, their bioadhesive property increases the drug contact with the GI barrier. Thirdly, being the secondary vehicle during lipid digestion, CNP are assumed to play important roles in the process of lipid and drug absorption. The enhanced permeation effect of cubic phases has been already evidenced through the transdermal and transmucosal barrier. Similar mechanisms could be expected across the intestinal barrier (84,85). Despite the above-mentioned

advantages, few articles reported CNP for oral drug delivery. Some examples are given.

Lai and coworkers investigated the CNP approach to enhance the oral bioavailability of the water-insoluble model drug simvastatin (84). Simvastatin-loaded CNP were prepared through fragmentation of the GMO/poloxamer 407 bulk cubic-phase gel using high-pressure homogenization. It has been found that both GMO/poloxamer 407 ratio and theoretical drug loading had no significant effect on particle size. The mean diameter of the CNP varied within the range of 100–150 nm. Almost complete drug entrapment was achieved with efficiency over 98% due to the high affinity of simvastatin to the hydrophobic regions of the cubic phase. Release of simvastatin from the CNP was limited with a total release lower than 3% at 10 h. Otherwise, pharmacokinetic profiles in beagle dogs showed sustained plasma levels of simvastatin for CNP over 12 h. The relative oral bioavailability of simvastatin CNP calculated on the basis of area under the curve was 241% compared to simvastatin crystal powder. It was concluded that the enhancement of simvastatin bioavailability was probably related to the facilitated absorption by lipids in the formulation rather than to the improved release (84). These results encouraged the research team to pursue their study by applying the CNP approach on a poorly absorbed drug, *i.e.*, CyA, aiming to improve its oral bioavailability (85). CyA-loaded CNP were prepared using a similar method to that used for simvastatin-loaded CNP manufacturing. CNP displayed a cubic inner structure, as evidenced by Cryo-TEM, an average size of about 180 nm, and a high entrapment efficiency of about 85%. Similarly to simvastatin-loaded CNP, *in vitro* CyA release from CNP was less than 5% at 12 h. *In vivo* studies in beagle dogs showed improved absorption of CNP-encapsulated CyA when compared to commercial microemulsion Neoral®. The relative oral bioavailability of CNP-encapsulated CyA was about 178% as compared to Neoral® (85).

Moreover, rapamycin-loaded CNP were formulated using GMO and vitamin TPGS as an emulsifier for oral delivery (86). CNP were efficiently taken up *in vitro* by MIA PaCa cells and demonstrated better cytotoxicity in comparison with native rapamycin. *In vivo* studies confirmed the enhanced bioavailability of rapamycin-loaded CNP with no toxicity and good biocompatibility at a higher dose of oral administration using a mouse model (86).

INORGANIC NANOPARTICLES

The rapid development of nanotechnology in recent years has created a myriad of engineered nanomaterials. Nanoscaled particles of metal (gold and silver NPs) and oxides (iron oxide, titanium dioxide, and silica) are increasingly being used in industrial production as well as scientific, biological, and medical research. In this review, attention has been focused on gold and silica NPs.

Gold Nanoparticles

Since the pioneering work of Faraday in 1857 on the synthesis of stable aqueous nanodispersions of gold, gold NPs (AuNP) have widely emerged as an attractive candidate for drug delivery. Due to their unique surface, electronic, and

Table III. Recent Research Works of Drug Encapsulation in Solid Lipid Nanoparticles

API	Lipidic excipient	Preparation method	Mean size	Encapsulation efficiency (%)	<i>In vivo</i> results	References
Vinpocetine	Glyceryl monostearate Tween80 Polyoxyethylene hydrogenated castor oil	Ultrasonic-solvent emulsification	70–167 nm	97–98.7%	The relative bioavailability of VIN in SLNs was significantly increased compared with that of the VIN solution. The amount of surfactant also had an important influence on the oral absorption of VIN.	(87)
All- <i>trans</i> retinoic acid (ATRA).	Compritol888 Pluronic F68 Tween 80 Soy lecithin	High-pressure homogenization	80–300 nm	95–97%	SLNs produced a significant improvement in the bioavailability of ATRA compared with ATRA solution.	(88)
Clozapine	Triglycerides (trimyristin, tripalmitin and tristearin) Soy lecithin 95% Poloxamer 188	Hot homogenization-ultrasonication	97–163 nm	89–97%	The amount of surfactant had an important influence on the oral absorption of ATRA. Bioavailability of clozapine SLNs were 2.45- to 4.51-fold after intraduodenal administration compared with that of clozapine suspension. In tested organs, the AUC and MRT of clozapine SLNs were higher than those of clozapine suspension especially in brain and reticuloendothelial cell-containing organs. These results indicate that SLN are suitable drug delivery system for the improvement of bioavailability of lipophilic drugs such as clozapine.	(89)
Lovastatin	Dynasan 114 Dynasan 116 Epikuron 200 Poloxamer 188	Hot homogenization-ultrasonication	60–119 nm	99%	The relative bioavailabilities of lovastatin and lovastatin hydroxy acid of SLN were increased by ~173% and 324%, respectively, compared with the reference lovastatin suspension.	(90)
Rifampicin, isoniazid and pyrazinamide	Stearic acid PVA (13,000–23,000)	Emulsion solvent diffusion	–	51% for rifampicin, 45% for isoniazid, and 41% for pyrazinamide	Therapeutic drug concentrations were maintained in the plasma for 8 days and in the organs (lungs, liver and spleen) for 10 days whereas free drugs were cleared by 1–2 days. In <i>M. tuberculosis</i> H37Rv-infected mice, no tubercle bacilli could be detected in the lungs/spleen after 5 oral doses of drug-loaded SLNs administered at every 10th day whereas 46 daily doses of oral free drugs were required to obtain an equivalent therapeutic benefit.	(91)

API active pharmaceutical ingredient, VIN vinpocetine, SLN solid lipid nanoparticles, AUC area under the curve, MRT mean residence time, PVA polyvinyl alcohol

optical properties, AuNP have been proposed for diverse medical applications, such as radiotherapy (92) and drug delivery (93).

For drug delivery, the payloads could be small or large biomolecules, like proteins, DNA, or RNA. Efficient release of these therapeutic agents that could be triggered by internal (e.g., glutathione or pH) or external (e.g., light) factors is a prerequisite for effective therapy.

In addition to their enhanced absorption and scattering (94), AuNP offer a good biocompatibility, relative inertia, facile synthesis, and conjugation to a variety of biomolecular ligands, antibodies, and other targeting moieties such as amino acids, proteins/enzymes, and DNA (93,95). They expose large surface areas for the immobilization of such biomolecules, without altering the biological activity of the conjugated species (93).

There are innumerable research works describing AuNP synthesis both in aqueous and organic solutions leading to NP sizing from one to few tens of nanometers which is unachievable in the case of polymeric NPs. Some examples of synthetic methods are the reduction of AuCl(PPh₃) with diborane or sodium borohydride (1–2 nm), the biphasic reduction of HAuCl₄ by sodium borohydride (1.5–5 nm), and the reduction of HAuCl₄ with sodium citrate in water (96). During NP synthesis, nucleation and successive growth are extremely sensitive to physical and chemical parameters. In some of the solution-phase metal NP synthesis procedures, the control of nucleation and growth steps is done by changing the reducing agent or stabilizer concentration; thus, NP size and shape can be controlled this way (97). Otherwise, the chemistry related to surface modification of AuNP has also evinced considerable interest; the demonstration that amine and thiol groups bind strongly to AuNP has enabled successfully surface modification (96,98).

AuNP may be good candidates for oral delivery of vaccines and/or other molecular species that may not normally be absorbed in their biologically active form by the oral route. Quantitative data on the uptake of 4-, 10-, 28-, and 58-nm-diameter metallic colloidal Au particles following oral administration to mice were published (94). It was found that colloidal Au uptake is dependent on particle size: smaller particles (4 and 10 nm diameter) cross the gastrointestinal tract more readily. Furthermore, it was shown that these small-sized particles are taken up by persorption in the small intestine through single, dead enterocytes in the process of being extruded from the villus (94).

AuNP bound to the insulin hormone for the therapeutic treatment of diabetes mellitus has been reported (93). Insulin was loaded onto bare AuNP and amino acid-capped AuNP and delivered in diabetic Wistar rats by oral and intranasal routes. The principal observation was that there is a significant insulin uptake which is mirrored by a significant fall in blood glucose levels. Furthermore, control of postprandial hyperglycemia by the intranasal delivery protocol is comparable to that achieved using the standard subcutaneous administration used for type I diabetes mellitus. Results confirmed considerable promise for the development of a novel noninvasive insulin delivery system based on AuNP (93).

Besides, considering their small size and their nonbiodegradability, safety of AuNP was thoroughly studied. Shukla

and coworkers have conducted detailed studies on the interaction of AuNP with murine cells (RAW264.7 mouse leukemic monocyte/macrophage cell line). Their findings demonstrated the biocompatible properties of these particles such as nontoxicity, nonimmunogenicity, and high tissue permeability without hampering cell functionality (98). In addition, the antioxidant effect and the path of their eventual internalization in perinuclearly arranged lysosomes have been evidenced using various microscopy tools (98).

Further investigations about the AuNP–cell interactions have been performed by Connors and coworkers on human cells (K562 leukemia cell line) (99). Cellular uptake and cytotoxicity of AuNP have been studied for different particle types having average diameters of 4, 12, or 18 nm and containing a variety of surface modifiers and stabilizers (99). The cell viability was determined using the MTT assay. It was suggested according to data that spherical AuNPs with a variety of surface modifiers are not inherently toxic to human cells despite being taken up into cells (99).

Recently, the cellular uptake of AuNP was studied on mouse embryonic fibroblast cells, NIH3T3, and human glioma cell line, LN-229 (100). The cellular uptake study indicated that the gellan gum-reduced AuNP were located in human cancer cells (LN-229) while no uptake was observed in normal mouse embryonic fibroblast cells (NIH3T3) (100). This study was completed by *in vivo* subacute 28-day toxicity investigations (100). Subacute administration of gum-reduced AuNP to rat did not show any hematological or biochemical abnormalities. The weight and normal architecture of various organs did not change compared with the control. These observations established the specific uptake of AuNP into cancerous cells and also demonstrated that the gellan gum-reduced AuNP are devoid of toxicity in animals following oral administration (100).

Another *in vivo* toxicity study has been carried out in mice using AuNP of a well definite size (13.5 nm) at different concentrations (137.5–2,200 µg/kg) over 14–28 days and *via* three different administration routes (*i.e.*, oral, intraperitoneal, and intravenous) (101). The studied endpoints were: animal survival, body weight, hematology, morphology, and organ index. Results showed that low concentrations of AuNP did not cause an obvious decrease in body weight or appreciable toxicity, even after their breakdown *in vivo*. High concentrations induced slight decreases in body weight which was not statistically different from the control. However, obvious effects on organs have been observed at high concentration. Interestingly, of the three different administration routes, the oral and intraperitoneal injection showed the highest toxicity (101).

Silica Nanoparticles

As nonmetal oxides, silica, a major and natural component of sand and glass, has been employed in material sciences and engineering for many years. It is a versatile material due to the variety of chemical and physical properties. Silica is also a relatively benign material due to its biocompatibility and nontoxicity (102).

The application of silica NPs (SiNP) in the biomedical field has been progressing for several years with a main focus on drug and gene delivery (103). The major advantage of

SiNP is their toxicological safety that has been proven over 50 years of use as excipient for oral drug delivery. Therapeutic agents can be encapsulated, covalently attached, or absorbed onto such silica nanocarriers after surface modification. These approaches can easily overcome drug solubility and stability issues and also can help to control drug release (102).

Amorphous silica is generally regarded as safe and has been approved for use as a food or animal feed ingredient. However, recent literature revealed that amorphous silica may present toxicity concerns at high doses (104). The response of several normal fibroblast and tumor human cells to different doses of amorphous SiNP was investigated by performing MTT test as a cell proliferation assay and lactate dehydrogenase as a measure of plasma membrane leakage (104). Both tests indicated that low doses of SiNP are nontoxic, but at high doses, cell viability decreased, and cell membrane damage is induced. Considering exposure time, both assays revealed that fibroblast cells with long doubling times are more susceptible to injury induced by SiNP than tumor cells with short doubling times. Accordingly, it was suggested that the cytotoxicity of silica to human cells depends strongly on their metabolic activities (104).

Silica with a new molecular arrangement presenting hexagonal uniform pores called "mesoporous silica" has been synthesized in 1992 as molecular sieves (105). Since then, it has received an ever increasing attention, and many efforts have been done on its synthesis and applications. The most common types of mesoporous nanoparticles (MSNs) are MCM-41 and SBA-15 (105). MCM-41 materials are rather regular spheres (300–650 nm in diameter), while SBA-15 materials are irregularly shaped with dimensions of hundreds of nanometers. They were made by supramolecular self-assembly techniques under base- and acid-catalyzed conditions, respectively. Fifteen years later, Vallet-Regi and coworkers have first proposed the use of MSNs as drug delivery systems using ibuprofen as a drug model (106).

MSNs exhibit different structures and morphologies with pores of adjustable dimensions which make them potential drug delivery systems. First, the presence of pores maximizes their surface and thus the drug adsorption. Second, a well-controlled drug loading and release could be carried out by tuning the pore size. Third, the easy surface functionalization of silanol groups helps to load a wide variety of biomolecules (106).

Synthesis of MSNs is generally performed by self-assembly of silica and surfactant micelles used as structure directing. MSN materials are derived from supramolecular assemblies of surfactants, which template the inorganic component (commonly silica) during synthesis. Afterwards, the surfactant is removed, commonly by pyrolysis or dissolution with the appropriated solvent (107).

The MSN delivery approach was applied to poorly water-soluble drugs (e.g., ibuprofen and telmisartan) (107,108). For instance, telmisartan was loaded in MSNs functionalized with aminopropyl for oral delivery (108). MSN synthesis was done using an organic template method in an oil/water phase. Their findings indicated that drug load and release depend on both pore total volume and diameter. MSNs with a large pore (12.0 nm) exhibited high drug loading (60%) and enhanced dissolution rate. Bisphosphonates, well-known drugs showing very poor intestinal absorption, have been loaded in MSNs in an

attempt to design a controlled oral delivery (109). The surface of the pore was functionalized with amine groups resulting in a threefold increase of the drug load, despite the reduction in pore size. Accordingly, the load and delivery rate of bisphosphonate could be controlled through organic modification on the surface of the pore walls present in MSNs with the resultant intensification of the drug dosage (109).

On the other hand, oral gene delivery using MSN carriers was also evaluated (110). To this end, poly-L-lysine-modified MSNs were proposed as a novel nonviral vector for oral gene delivery. Efficient reporter gene expression was detected in the stomach and intestines where expression was mainly observed in mucous membrane cells. Glucose transporting tests showed that MSNs had no obvious toxicity to intestines of BALB/C mice. MSNs could efficiently deliver plasmid DNA and antisense oligonucleotides into cultured cells *in vitro* in the presence of serum-free medium (110).

CONCLUSION

Aiming to achieve an efficient oral delivery for numerous panels of therapeutics, considerable research works are being made over the last decades. Efforts are focused on NP development as the drug carriers of choice creating a sound basis to address the oral delivery concerns, such as poor drug solubility and/or stability in the GI juices and low intestinal permeability.

Depending on their constitutive materials (e.g., polymers, lipids, etc.) and physicochemical characteristics (e.g., size, degradation, and surface properties), NPs allowed formulators to design different release profiles and to achieve both local and systemic targeting of the encapsulated drug. However, the safety of materials and solvents used in NP manufacturing has to be considered with great attention. Indeed, the success of the nanocarriers in the coming years depends on the acquired knowledge about their toxicological aspects and the fate of their constituents in the body. In this respect, NPs based on biodegradable materials represent an attractive perspective.

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