REVIEW

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Nanoparticulate carriers (NPC) for oral pharmaceutics and nutraceutics

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Received October 20, 2009, accepted October 27, 2009

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Pharmazie 65: 75–82 (2010) *doi: 10.1691/ph.2010.9338*

The introduction of nanoparticulate carriers (NPC) in the pharmaceutic and nutraceutic fields has changed the definitions of disease management and treatment, diagnosis, as well as the supply food chain in the agri-food sector. NPC composed of synthetic polymers, proteins or polysaccharides gather interesting properties to be used for oral administration of pharmaceutics and nutraceutics. Oral administration remains the most convenient way of delivering drugs (e.g. peptides, proteins and nucleic acids) since these suffer similar metabolic pathways as food supply. Recent advances in biotechnology have produced highly potent new molecules however with low oral bioavailability. A suitable and promising approach to overcome their sensitivity to chemical and enzymatic hydrolysis as well as the poor cellular uptake, would be their entrapment within suitable gastrointestinal (GI) resistant NPC. Increasing attention has been paid to the potential use of NPC for peptides, proteins, antioxidants (carotenoids, omega fatty acids, coenzyme Q10), vitamins, probiotics, for oral administration. This review focuses on the most important materials to produce NPC for oral administration, and the most recent achievements in the production techniques and bioactives successfully delivered by these means.

1. Introduction

Over the past few decades, new science disciplines and technologies, particularly bio- and nanotechnology, have deeply influenced the latest achievements in various areas including pharmaceutical and agri-food sectors. Several research lines have shown that the distribution profile of bioactive molecules (drugs and nutrients) can be modified at cell and tissue levels by their entrapment in submicron colloidal systems well-known as nanoparticulate carriers (NPC).

NPC are colloidal systems usually depicting a mean particle size below 1000 nm, being able to entrap bioactive molecules within their matrix and/or absorbed onto their surface (functionalized and/or coated). NPC have successfully been applied to overcome the physiological difficulties encountered in oral administration of bioactive molecules including pharmaceutically active compounds (peptides, proteins, genes, nucleic acids), nutraceutics, and probes/dyes usually applied for diagnostic purposes.

If directly coupled with the bioactive molecule (functionalized and/or coated) the NPC is solely used as a carrier system. Nevertheless, if NPC is produced from a bioactive raw-material the system itself, may reveal some biological activity, i.e. therapeutic function as its own carrier (Baran et al. 2002; Cascone et al. 2002; Duncan 2003; Kipp 2004).

NPC can be prepared from a variety of materials such as synthetic or semi-synthetic polymers, proteins, and also polysaccharides. The source materials may be of biological origin, such as albumin, gelatine, lactid acid, dextran, chitosan, or have "chemical" characteristics, e.g. synthetic polymers, carbon, silica, as well as metals (De Jong and Borm 2008).

To search for an appropriate NPC the main criteria to bear in mind upon the selection of a material include: (i) the required mean size and the inherent physicochemical properties of the loaded bioactive (e.g. aqueous solubility, chemical degradation) which influence the formulation stability and shelf life; (ii) the surface characteristics (e.g. charge and permeability) to improve their targeting; (iii) degree of biodegradability, biocompatibility and toxicity, (iv) drug release profile; and (v) antigenicity of the final product (Kreuter 1994). Based on these criteria, biodegradable raw-materials, with a limited life span as long as therapeutically needed, are considered an optimal option for NPC development.

The present review is focused on the most common raw-materials used to produce NPC intended to deliver bioactives (pharmaceutics and/or nutraceutics) by oral administration. Several local or systemic applications may be foreseen providing successful examples of bioactives entrapped and delivered by means of NPC.

2. Nanoparticulate carriers for pharmaceutics and nutraceutics

2.1. Polymeric-based NPC

Polymeric-based NPC may be prepared from synthetic or semi-synthetic polymers, e.g. polyamines, poly(amino acids), polyesters, poly(alkyl cyanoacrykates), polyorthoesters (POE) and polyanhydrides (Linhardt 1989; Schwendeman et al. 1997). The bioactive can be entrapped (dissolved or dispersed) within the NPC matrix, adsorbed onto their surface, or even attached

in case a binding molecule is placed between the NPC surface and the bioactive. Furthermore, depending on the preparation procedure applied, two different structures may be obtained, i.e. nanospheres or nanocapsules. These will influence the NPC properties including their loading capacity, entrapment efficiency, yield of production, mean size, zeta potential, as well as the bioactive release profile. Nanospheres have a matrix-like structure, in which the bioactive is physically and uniformly dispersed or dissolved (Couvreur et al. 1995); whereas nanocapsules are composed of a polymeric shell and an inner oil core (Mainardes and Silva 2004). In these latter, the presence of oil leads to a vesicular structure in which the bioactive is confined to a cavity surrounded by a unique polymer membrane.

Polymeric-based NPC are of special interest from the pharmaceutical point of view due to their desirable properties such as biocompatibility, biodegradability, surface modification and functionalization facilities (Rawat et al. 2006). In addition, these carriers are more stable in the gastrointestinal (GI) tract than other colloidal carriers (e.g. liposomes), and can protect the entrapped bioactive molecules against GI tract environment. Moreover, modifying the type of polymer, well defined physicochemical characteristics may be accomplished according to the therapeutic needs, such as hydrophobicity, zeta potential, bioactive release profile (e.g. delayed, prolonged, triggered), and biological behaviour (e.g. targeting bioadhesion, improved cellular uptake) (Galindo-Rodriguez et al. 2005).

The main drawback of biodegradable NPC is their non-specific interaction either with cells and/or plasma proteins, leading to accumulation of the bioactive in non-target tissues. For a number of applications, surface-modified NPC have been developed to control their interactions with biologic milieu and therefore their biodistribution. In fact, it is possible to modify the NPC surface by adsorption or chemical grafting of hydrophilic molecules such as poly(ethylene glycol) (PEG) (Blummel et al. 2007; Ramachandran et al. 2008), polyethylene oxide (PEO) (Santander-Ortega et al. 2007; Tai et al. 2008), poloxamers (Petri et al. 2007; Kreuter and Gelperina 2008), poloxamine (Moghimi and Gray 1997), polysorbates (Ambruosi et al. 2006; Teeranachaideekul et al. 2008), and also phospholipids (des Rieux et al. 2006). In this context, PEG has received considerable attention as coating polymer. PEG-coated NPC could successfully avoid the mononuclear phagocyte system sequestration, thus increasing NPC blood circulation time in comparison to uncoated NPC (Gref et al. 1994; Gref 2002). These type of systems are well known as long-circulating particles (Kommareddy et al. 2005). They have been successfully produced for various pharmaceutical purposes such as control bioactive release profile, target particular organ/tissue, and deliver bioactive molecules like protein, peptides, and genes through a peroral route of administration (Langer 2000; Bhadra et al. 2002; Lee et al. 2005). The most suitable polymer may be chosen to achieve high entrapment efficiencies for a particular bioactive. Several methods are well described in the literature to prepare polymeric-based NPC, being usually selected according to the physicochemical characteristics of the bioactive to be entrapped (Montasser et al. 2000; Soppimath et al. 2001; Couvreur et al. 2002). Some polymers are less sensitive to processing conditions than others, due to their chemical characteristics, molecular weight and crystallinity (Lemoine et al. 1996).

From a general point of view, polymeric-based NPC have been developed mainly by three methods: dispersion of performed polymers, polymerization of monomers, and ionic gelation or coacervation of hydrophilic polymers. Nevertheless, several other techniques have emerged in the last years, being possible nowadays to obtain NPC by e.g. supercritical fluid technology (Reverchon and Adami 2006; Jensen et al. 2007; Moisan et al. 2008) and particle replication in non-wetting templates (PRINT) (Roland et al. 2005).

The pharmacokinetic behaviour of the bioactive compound (e.g. absorption, biodistribution pattern and elimination) is influenced by the polymeric composition of the NPC (e.g. hydrophobicity, surface charge and biodegradation profile), by any adjuvant substances included, and by the loaded bioactive (e.g. molecular weight, charge, localization in the nanosphere by adsorption or incorporation) (Reis et al. 2006). In addition, the nature of polymers influences both NPC size and the bioactive release profile. Although natural polymers generally give a relatively fast release, synthetic polymers enable controlled bioactive release over a longer period of time. Among the different classes of biodegradable polymers, the thermoplastic aliphatic poly(esters) like poly(lactide) (PLA), poly(glycolide) (PGA), and especially the copolymer of lactide and glycolide, poly(lactide-co-glycolide) (PLGA), poly(ε-caprolactone) (PCL), are most commonly used for the NPC production. They have outstanding favourable properties, such as good biocompatibility, biodegradability (not requiring surgery for removal), and mechanical strength (Jain 2000). In addition, these polymers are easy to formulate into different carriers for a variety of bioactives e.g. vaccines, peptides, and proteins. They protect the loaded bioactive against degradation and control its site specific delivery. Due to their strongly hydrophobic nature, they are more efficient for the entrapment of hydrophobic rather than hydrophilic bioactive molecules.

Poly(ε-caprolactone) (PCL) biodegradation suffers slow biodegradation, thus making this polymer a suitable candidate for long term delivery extending over a period of more than one year. This has led to its application in the preparation of different delivery systems in the form of microspheres, nanospheres and implants (Sinha et al. 2004). PCL also shows compatibility with a wide variety of polymers when it is used in the development of NPC (Gref et al. 2002; Lemarchand et al. 2003). It is promising to combine them with natural chitosan to develop amphiphilic copolymers which can be used to produce spherical or elliptical NPC suitable for bioactive delivery and targeting (Yu et al. 2006).

2.2. Protein-based NPC

Relevant proteins for oral administration of pharmaceutics and nutraceutics include albumin and gelatine. Albumin is one of the major proteins of human plasma, being up to approximately 60% of the total plasma proteins. This protein shows solubilizing properties in dilute salt solutions and precipitates at relatively low temperatures. The thermal treatment at elevated temperatures (95–170 °C) or the use of chemicals agents are on the basis of the preparation of albumin to form NPC (Gayathri 2003).

Albumin NPC have been prepared by coacervation and chemical cross-linkage with glutaraldehyde, and interferon γ was either adsorbed or entrapped in those albumin NPC (Segura et al. 2005). These NPC were able to load, mainly by electrostatic interactions, high amounts of interferon in its bioactive form. Other successful examples include albumin-based NPC for the delivery of DNA and oligonucleotides (Vogel et al. 2005). It was found that the presence of albumin during oligonucleotides-protamine complexation led to remarkable improvements in particle stability. Furthermore, the introduction of albumin reduced considerably the cytotoxic side effects of the NPC compared with those made solely of oligonucleotides and protamine (Weyermann et al. 2005). After cellular uptake the oligonucleotides were distributed within the cytoplasm.

The use of albumin NPC to deliver oligonucleotides offers a number of advantages. This negatively charged system is able to load oligonucleotides, without requirements of positive

compounds. Moreover, albumin NPC promote the nuclear accumulation of the oligonucleotide, without additives destabilizing endosomal membrane (Arnedo et al. 2004). Furthermore, NPC are quickly removed from the circulation by opsonization with various serum components followed by phagocytosis. Thus, oligonucleotides entrapped into these NPC will most likely be protected for enough time to get intact into macrophages, where these systems would be able to promote the adequate intracellular uptake to exert their activity.

Cationic albumin coated PLA-PEGylated conjugated NPC, containing an active fragment analogue of arginine vasopressin, were developed by the double emulsion/solvent evaporation procedure (Lu et al. 2005; Xie et al. 2006) to promote the substance to penetrate through blood brain barrier via intravenous injection (Xie et al. 2006).

Gelatine is also a well-known protein widely used in medical applications. It is suitable to develop NPC since it exhibits biodegradability, weak antigen activity, and superior biocompatibility compared to other natural proteins, such as albumin (Lee et al. 2001). Gelatine is a natural macromolecule derived from collagen by prolonged boiling of animal bones and skin, being inexpensive, easily to cross-link and to be chemically modified, showing therefore a huge potential for the preparation of NPC.

One of the first approaches to develop gelatine-based NPC was reported to a system based on gelatine NPC entrapped in PLGA microspheres (Li et al. 1997). Gelatine NPC containing bovine serum albumin (BSA) as model protein were previously prepared, and then entrapped within the hydrophobic PLGA microspheres to create nanoparticle-microsphere assemblies. A phase separation method followed by a solvent extraction method was applied to produce such assemblies. Release experiments showed that this new system possesses sustained release characteristics for proteins, demonstrating as well the ability to protect proteins against integrity loss or denaturation. The loading of BSA in gelatine-based NPC was conducted using a modified w/o emulsion method, without recurring to emulsifiers (Li et al. 1998). The release of BSA from those particles was also observed to follow a diffusion-controlled release mechanism.

A coacervation process was applied to load DNA into gelatine-based NPC with the cell ligand transferrin covalently bound to the gelatine (Truong-Le et al. 1999). This system was developed for active gene delivery and targeting. After encapsulation within NPC, DNA was partially resistant to DNAse I and its biological integrity was successfully demonstrated. This gene delivery device also allowed the incorporation of lysosomolytic agents to reduce degradation of the DNA in the endosomal and lysosomal compartments of cells, improving the bioavailability of DNA molecules (Leong et al. 1998). Furthermore, NPC could be lyophilized for storage without loss of DNA bioactivity (Leong et al. 1998).

It was also demonstrated that gelatine-based NPC can easily be surface modified with sulfhydryl groups, involved in the covalent attachment of proteins, such as avidin (Coester et al. 2000). Avidin-conjugated gelatine NPC were pointed out as potential carrier system for peptide nucleic acid derivatives in antisense therapy (Coester et al. 2000; Balthasar et al. 2005).

Modified gelatine has also been synthesized and used to prepare NPC. Example are thiol-modified gelatine NPC prepared by desolvation, which have been employed to entrap DNA with greater transfection efficiency than regular gelatine NPC (Kommareddy and Amiji 2005). PEGylated gelatine NPC were also found to be more stable against DNAase degradation than regular gelatine NPC (Kaul and Amiji 2005), and also with higher transfection efficiencies and high desirable application for systemic delivery of gene material to solid tumours (Goldie and Mansoor 2005).

2.3. Polysaccharide-based NPC

Polysaccharides are diverse in their structure and properties since these compounds have a larger number of reactive groups, and a wide range of molecular weight, which contribute to their variable chemical structure (Liu et al. 2008). Polysaccharides can be divided into polyelectrolytes and non-polyelectrolytes. The polyelectrolytes group include positively charged polysaccharides (chitosan) and negatively charged polysaccharides (e.g. alginate, pectin, heparin, hyaluronic acid). The presence of chemically very different groups on the molecular chains allows them to be chemically and biochemically modified resulting in many types of polysaccharide derivates.

Polysaccharides are an important class of physiological materials with a very special properties like biocompatible, biodegradable, and protecting properties, which make them a promising choice to prepared NPC for bioactive entrapment and delivery (Sinha et al. 2004). In addition, most of natural polysaccharides have hydrophilic groups, such as hydroxyl, carboxyl and amino groups, enabling them to set up non-covalent bonds with biological tissue following bioadhesion (Lee et al. 2000). NPC prepared with bioadhesive polysaccharide (e.g. chitosan, alginate) could prolong the residence time, and therefore increase the absorption of the loaded bioactive.

Several studies have been published reporting the application of polysaccharides and their derivates as for bioactive delivery of several types of proteins (Li et al. 2007; Bayat et al. 2008; Rawat et al. 2008; Thirawong, Thongborisute et al. 2008).

Among various polysaccharides, chitosan and their derivates have early been used to prepare NPC. Chitosan, a non-branched polyamine of D-glucosamine and *N*-acetyl glucosamine molecules, is a naturally occurring linear polysaccharide, which is characterized for its biodegradable, non-toxic and biocompatible properties (Hirano 1996). Positively charged chitosan will bind to cell membranes and is reported to decrease the transepithelial electrical resistance and transiently opening tight conjunction between epithelial cells (Artursson et al. 1994). The safety of the chitosan, its mucoadhesive ability allowing to prolong residence time in the GI tract, and its ability to enhance absorption by increasing cellular permeability, have all been relevant properties to highlight its suitability for oral drug delivery (Bowman and Leong 2006). Due to its properties, chitosan allows the preparation of organic solvent free mucoadhesive NPC (Janes et al. 2001). Water soluble chitosan derivatives, easily dissolved in neutral aqueous medium, can also be used to produce NPC, aiming to avoid the potential cytotoxicity induced by acidic solution and, thus maintaining the bioactivity of the loaded molecules (Amidi et al. 2006; Sandri et al. 2006; Sandri et al. 2007; Chen et al. 2008).

Other polysaccharides used to prepare NPC, alone and in combination with other polysaccharides, include alginate (You and Peng 2004; Zahoor et al. 2005; Ahmad et al. 2006; Borges et al. 2007), dextran and dextran sulphate (Chauvierre et al. 2003; Bertholon et al. 2006; Tiyaboonchai and Limpeanchob 2007), heparin (Passirani et al. 1998; Labarre et al. 2005; Choi et al. 2006; Liu et al. 2007), amylose (Lalush et al. 2005), hialuronic acid (Chauvierre et al. 2003; Han et al. 2005), pectin (Chauvierre et al. 2003) and pullulan (Nishikawa et al. 1996; Akiyoshi et al. 1997; Akiyoshi et al. 2000; Kuroda et al. 2002; Na et al. 2003; Choi et al. 2006).

Liu et al. (2008) published a comprehensive review focusing on the mechanisms of polysaccharide-based NPC preparation. According to structural characteristics, polysaccharide-based NPC are prepared mainly by four different mechanisms, namely covalent cross-linking (Bodnar et al. 2005; Zhi et al. 2005; Liu et al. 2007), ionic crosslinking (Lu et al. 2006; Jain and Banerjee 2008; Tsai et al. 2008; Zhang et al. 2008), polyelectrolyte

complexation (Lee et al. 2008), and self-assembly of hydrophobically modified polysaccharides (Jeong et al. 2006; Opanasopit et al. 2007; Yang et al. 2008). This latter method seems to be particularly suitable to develop NPC with targeting properties, e.g. polyester or polyakylcyanoacrylate NPC coated with PEG. To reveal an active targeting, specific ligands have to be attached to NPC surface to enable molecular recognition (Lemarchand et al. 2004). However, at the surface of PEG-coated NPC there are no reactive groups, which limits any chemical coupling of such ligands (Stella et al. 2000). Therefore, polysaccharide coatings have been considered as an alternative to the PEG coating because they have specific receptors in certain cells or tissues (Listinsky et al. 1998; Stahn and Zeisig 2000). In addition, several mucosal surfaces are good targets for polysaccharide recognition such as nasal, pulmonary and peroral. Lemarchand et al. (2004) reviewed different strategies to coat the surface of polymeric as well as inorganic NPC with polysaccharide and their medical application, mainly for imaging cancer approaches.

3. Uptake of oral pharmaceutics- and nutraceutics-loaded NPC

Oral drug delivery is the preferable route for drug administration because it is non-invasive, avoids pain and discomfort associated with injections, and it decreases the contamination risk. It is also physiologically desirable, since the exogenous bioactive imitates their physiological pathway undergoing first hepatic bypass. Moreover, among the non-invasive routes of administration that have been evaluated for the delivery of bioactives, the oral route remains the most convenient, although it is not the most efficient for peptides and proteins due to their low absorption rate. The main reasons for the low oral bioavailability of such bioactives are related to chemical and conformational stability, cellular and luminal enzymatic degradation in the GI tract and poor intrinsic penetration of the intestinal membrane (Hamman et al. 2005; des Rieux et al. 2006; Morishita and Peppas 2006).

The development of an effective oral delivery system for proteins requires a comprehensive perception of their physicochemical properties, such as molecular weight, hydrophobicity, ionization coefficient and pH stability, as well as of the biological barriers that limit protein absorption through the GI tract (Mahato et al. 2003). Strategies to improve the oral bioavailability of bioactives have ranged from changing their physicochemical properties by modification of their lipophilicity and enzyme susceptibility, to add novel functionality using transport-carrier molecules that are recognized by endogenous transport-carrier systems in the GI tract and/or to their inclusion in specially adapted NPC (Morishita and Peppas 2006). Bioactive association with colloidal carriers, such as polymeric NPC, is one of several approaches proposed to improve their oral bioavailability. Such NPC are more stable in the GI tract than other colloidal carriers (e.g. liposomes), and can protect entrapped bioactives from GI environment. The use of different polymers allows the modulation of physicochemical and bioactive release properties and consequently the biological behaviour (Galindo-Rodriguez et al. 2005). Additionally, the NPC surface can be easily modified by adsorption or chemical grafting of certain hydrophilic molecules as previously mentioned. Moreover, their submicron size and their large specific surface area favour absorption compared to larger carriers. Consequently, it has already been widely shown that nanoencapsulation of bioactives protect them against the harsh environment of the GI tract and increases their transmucosal uptake and absorption (des Rieux et al. 2006).

Paracellular and transcellular routes have been explored to predict the intestinal absorption of proteins. Paracellular pathway is commonly pointed out as limited to protein absorption, due to the low surface area and tightness of the junctions of the intercellular spaces (Salamat-Miller and Johnston 2005). Strategies to modify the physicochemical properties of the intestinal wall and to modulate the tight junctions associated with the paracellular pathway have been mentioned (Salamat-Miller and Johnston 2005). A particular case has been reported to chitosan NPC because this natural polymer shows the special feature of adhering to mucous surface and transiently opening or widening the tight injunction between epithelial cells (Janes et al. 2001; Pan et al. 2002). Adhesive chitosan NPC were helpful in increasing the relative pharmacological bioavailability of insulin (Pan et al. 2002) and calcitonin (Prego et al. 2006) associated with the ability to weaken the intestinal barrier in a reversible way. A distinct advantage of proper particle size helped to increase the bioactive effects (Pan et al. 2002).

One of the advantages of NPC, when administered orally, is that they can be absorbed transcellularly, although in small quantities, not only through the membranous epithelial cells (M-cells) of the Peyer's patches in the Gut-Associated Lymphoid Tissue (GALT), but also through the much more numerous gut enterocytes (Hussain et al. 2001). The uptake of NPC carrying proteins by enterocytes is a limited but capable process (des Rieux et al. 2006). To improve the uptake of NPC, surface modifications (Vila et al. 2004; Bhattarai et al. 2006) and enhancing mucoadhesion (Tobio et al. 2000; Garcia-Fuentes et al. 2005) properties are usually explored with efficiency to promote the contact of proteins with the intestinal epithelium, increasing the concentration at the site of absorption. In the GI tract, the cationic NPC are favoured to bind to the mucous layer negatively charged, thus cationic polymers are selected as preferential mucoadhesive coating.

Peyer's patches are specific structures dispersed through the lymphoid nodules of the intestinal mucosa called O-MALT (Organized Associated Lymphoid Mucosa). They are rich in M cells, cells specialized for antigen sampling but also a potential portal for oral delivery of peptides and proteins since they possess high transcytotic capacity, and are able to transport a broad range of materials (Clark et al. 2001; Florence 2005). Uptake by M-cells have demonstrated to result in enhanced physiologic action of proteins after oral administration of NPC (Pinto-Alphandary et al. 2003; Borges et al. 2006). Furthermore, adsorptive endocytosis seems to occur through clathrin coated pits and vesicles, fluid phase endocytosis and phagocytosis (Qaddoumi et al. 2003). NPC size, electrical charge and surface hydrophobicity, are the main factors that influence their uptake by Peyer's patches. It is well accepted that hydrophobic, negatively charged protein-loaded NPC smaller that 1 μm potentially show the best absorption rate (Jung et al. 2000; Yoo and Park 2004), however no generalities must be taken because other condition can rule the final absorption process.

Bioactive natural compounds, such as vitamins, antimicrobials, antioxidants, probiotics, bioactive peptides, can be incorporated into food matrices to developed innovative functional food, known as nutraceutic products. In the latest years, nutraceutics have been gathering attention from the scientific community, food manufacturers and consumers. Even though the involvement of these compounds in physiological functions is not yet fully understood, it is widely accepted that their addition to food matrices may have physiological benefits or disease preventing properties (Elliott and Ong 2002). One example of these benefits is the antihypertensive effect of dietary peptides derived from milk protein, mediated by angiotensin converting enzyme inhibition (Groziak and Miller 2000).

Considering oral administration, the most important key point for the effectiveness of a nutraceutic is keeping its bioavailability through dietary supplementation depending on several factors. The development of nutraceutic oral formulations is a great challenge mainly due to insufficient gastric residence time, low permeability and/or poor solubility within the gut, as well as instability under environment conditions encountered in food processing (temperature, oxygen, light) or in the GI tract (pH, enzymes, presence of other nutrients) (Bell 2001). Many approaches have been explored and even applied to protect and deliver bioactive molecules by oral route, such as chemical modifications, coupling agents, hydrogels and polymer-based NPC.

Although nanotechnology applications for pharmaceutical systems are well investigated and documented in which various systems have been developed for intelligent, modulated, and selective delivery of drugs to specific areas, the use of NPC in food sector are new emergent, but they are predicted to grow rapidly in the near future.

In order to facilitate the most favourable nanotechnological responses, the effective delivery of nutraceutics delivered by means of NPC will require appropriate food formulation and manufacturer process able to maintain the bioactive form fully functional until the time of consumption and deliver this integrate form to the physiological target (Chen et al. 2006).

Due to their submicron-meter size NPC seems to be promising to improve the bioavailability of nutraceutics, especially poorly soluble and high molecular weight bioactives, such as natural antioxidants, functional lipids (e.g. carotenoids, phytosterol, omega 3 fatty acids), proteins and numerous other compounds that are widely used as active ingredients in various food products (Chen et al. 2006). In the same way of oral pharmaceutical administration, in food matrices the NPC can dramatically prolong bioactive residence time in the GI tract by decreasing the influence of intestinal clearance mechanisms and increasing the specific surface available to interact with the biological support (Kawashim 2001; Arbos et al. 2002). In addition, NPC can penetrate deeply into tissues through fine capillaries, cross the epithelial lining fenestration and are generally taken up efficiently by cells (Desai et al. 1996, 1997; Lamprecht et al. 2004; des Rieux et al. 2006), thus allowing the nutraceutics delivery to target sites in the body. Nutrient digestion or absorption may increase or decrease depending on the structural, chemical, and physical states of NPC and the nutrients bound within them.

Some strategies are being explored to improve nutraceutics bioavailability. One of these approaches include the surface coating of NPC with a specific protein, which can modify the adhesive properties of NPC and their behaviour in the GI tract. In fact, some proteins (e.g. lectin) are able to bind specifically to sugar-residue-bearing sites located at the surface of epithelial cells (Goldstein et al. 1980). The application of this strategy is reported by covalent binding of lectin to polyvinylmethylether maleic anhydride NPC resulting in the decrease of terminal elimination rate in the GI mucosa, while BSA-coated NPC demonstrated high ability to adhere to the stomach mucosa (Arbos et al. 2002). Protein-coated surfaces can also be used to uptake the bioactive molecule by specific target-cell population (Goppert and Muller 2005) and protection of sensitive nutraceutics for the GI environment, e.g. β-lactoglobulin coated chitosan NPC (Chen and Subirade 2005). Due to its mucoadhesive properties, chitosan NPC should then adhere to the intestinal wall to facilitate the absorption of the nutraceutic. In another recent study (Jang and Lee 2008), the results clearly demonstrate the stability of chitosan NPC for L-ascorbic acid during the heat food processing and the possibility to enhance its antioxidant activity due to the prolonged release of L-ascorbic acid from chitosan NPC.

Other example of successful applications of NPC for nutraceutics is their use to deliver coenzyme Q10 (CoQ10), which is an antioxidant agent with well-established pharmacological activities. However, CoQ10 is only marketed as a nutritional supplement without any claim of therapeutic activity probably due to its poor physicochemical and biopharmaceutical properties leading to its low oral bioavailability. Ankola et al. (2007) documented the potential of NPC in improving the therapeutic value of molecules like CoQ10, facilitating its use as first line therapeutic agent for prophylaxis and therapy by overcoming the problems associated with its delivery in physiological conditions. These authors developed biodegradable NPC based on PLGA, using the quaternary ammonium salt didodecyldimethylammonium bromide as a stabilizer.

There are many other nutraceutics that are poorly absorbed, such as vitamin B12, vitamin K2, vitamin E and many phytonutrients, especially the polyphenols and terpenes, the latter of which include the carotenoids, chromonols, lemonoids and saponins, in which NPC seems to have great potential to enhance the bioavailability of those compounds and to generate new nutraceutic products.

Some nutraceutics-loaded NPC (e.g. vitamins, antimicrobials, antioxidants, etc) are commercially available (Chaudhry et al. 2008). Examples of these products include different Nanoceuticals™ from RBC Life Sciences® Inc. USA, Nano Calcium/Magnesium from Mag-I-Cal.com USA and nano-selenium-enriched Nanotea from Shenzhen Become Industry & Trade Co., Ltd., China. Another example is Nutri-Nano™ CoQ-10 from Solgar, USA. The product Novasol® from Aquanova® Germany contained nano-structured supplements based on "Nano-Sized Self-assembled Liquid Structures (NSSL)" from NutraLease Ltd. Israel, and NanoClusters™ delivery device for food products from RBC Life Sciences® Inc. USA. BioDelivery Sciences International has introduced their Bioral™ nanocochleate nutrient delivery system for micronutrients and antioxidants. The nanocochleates, with approximately 50 nm in size, are based on a phosphatidylserine carrier derived from soya bean.

4. Conclusions

There is a current need identify novel strategies to overcome limitations of oral delivery of sensitive bioactives being developed by biotechnology. A recently emerging scientific field – nanotechnology - works on a small scale to produce nanoparticulate carriers (NPC), but its implications in the pharmaceutical and agri-food markets are becoming massive. Besides the chemical nature of NPC, these submicron meter systems show promising features not only for pharmaceutical and cosmetics application, but also to develop innovate functional food. Delivery of therapeutic proteins/peptides and other nutraceutics have being receiving a considerable amount of attention worldwide, but several limitations to oral administration of such sensitive bioactives may still be pointed out. Intestinal membrane permeability, size, intestinal and hepatic metabolism and bioactive solubility are the major barriers playing an important role in decreasing bioavailability after oral administration. A number of approaches have been used to overcome these limitations. Non-conventional polymeric, protein and polysaccharide NPC have been developed which can be structurally modified to increase their targeting efficiency and delivery of entrapped bioactives in their matrix. Hydrophilic bioactives usually depict poor membrane permeability which may be overcome by increasing their membrane partitioning characteristics and their affinity to the mediated NPC. Likewise, surfacing NPC with mucoadhesive polymers seem to be an interesting strategy for the oral delivery

of sensitive bioactives enhancing their GI stability and bioavailability.

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