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Bioavailability of nanoparticles in nutrient and nutraceutical delivery

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Abstract

The field of nanoparticle delivery systems for nutrients and nutraceuticals with poor water solubility has been expanding, almost exponentially, over the last five years, and some of these technologies are now in the process of being incorporated in food products. The market projections for these technologies suggest a multifold increase in their commercial potential over the next five years. The interest in the pharmaceutical and food-related applications of these technologies has sparked tremendous developments in mechanical (top-down) and chemical (bottom-up) processes to obtain such nanoparticle systems. Mechanical approaches are capable of producing nanoparticles, typically in the 100–1000 nm range, whereas chemical methods tend to produce 10–100 nm particles. Despite these technological developments, there is a lack of information regarding the basis of design for such nanoparticle systems. Fundamental thermodynamic and mass transfer equations reveal that, in order to generate a broad spectrum delivery system, nanoparticles with 100 nm diameter (or less) should be produced. However, experimental data reveal that, in some cases, even nanoparticles in the 100–1000 nm range are capable of producing substantial improvement in the bioavailability of the active ingredients. In most cases, this improvement in bioavailability seems to be linked to the direct uptake of the nanoparticle. Furthermore, direct nanoparticle uptake is controlled by the size and surface chemistry of the nanoparticle system. The use of this direct nanoparticle uptake, in particular for soluble but poorly absorbed ingredients, is one of the areas that needs to be explored in the future, as well as the potential side effects of these nanoparticle carriers.

Keywords: Lipid nanoparticles; Bioavailability; Dissolution; Uptake; Peyer's patches; BCS dissolution models; Vitamins; Minerals; Food fortification

1. Introduction

The field of food nanotechnology has experienced significant growth over the last five years. Such growth has been fuelled by the potential of harnessing the large surface area to volume ratio of these materials to improve the bioavailability of active ingredients, introduce controlled/target release, improve sensory aspects, and others [1•,2,3•,4••,5]. The growth of the field is partially quantified in Fig. 1, where the cumulative number of articles and patents containing the keywords "food" and "nanoparticles" in their abstract or the claims (in the case of patents) is presented as a function of year of publication. As indicated by the trends in Fig. 1, most of the growth in the food nanotechnology field has taken place after the year 2000 as a result of numerous nanotechnology initiatives of the late nineties, and the development of food-grade additives suitable for nanoparticle production.

Currently, the market of nanotechnology products in the food industry approaches the US$ 1 billion (most of this on nanoparticle coatings for packaging technologies, health promoting products, and beverages) and has the potential to grow to more than US$20 billion in the next decade [1•]. Recent reviews present an excellent summary of the research groups, private and government organizations that have been spearheading the field of food nanotechnology [1•,4••]. Most of the work that these research groups have generated over the last five years on nanoparticle vehicles has concentrated on developing production methods inspired on pharmaceutical drug delivery systems [4••]. The challenge in developing such production methods has been to replace some of the polymers and surfactants used in the pharmaceutical industry with food-grade alternatives.

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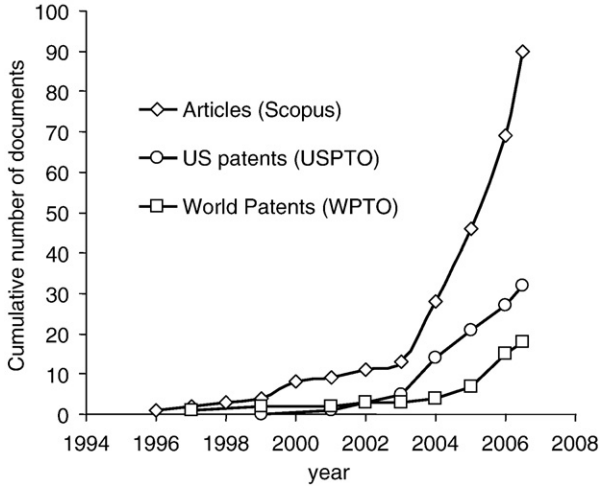

Fig. 1. Cumulative number of articles (Scopus July 10, 2007), United States patent (USPTO July 10, 2007), and world patents (WIPO July 10, 2007) containing the keywords "food" and "nanoparticle" in the abstract and/or in the claims.

Recent technological advances that make use of lipids, proteins and polysaccharides as additives are contributing to meet this challenge, and they have open the door to new functionalities and applications for nanoparticle delivery systems.

To design the next generation of nanoparticle vehicles it is necessary to reflect on the mechanisms of active ingredient uptake, and on how to modify or optimize the properties of these nanoparticles to maximize the bioavailability of different ingredients. The purpose of this review is to help fill, at least in part, that knowledge gap, and identify some of the elements that are still missing. Based on that information, new opportunities and challenges for nanoparticle vehicles will be discussed.

2. Nanoparticle vehicles in nutrient and nutraceutical delivery

There are two basic approaches to generate nanoparticle systems, one is the "top-down" approach, whereby small particles are produced through different size reduction (mechanical) processes, and the other approach is the "bottom-up" approach where the nanoparticle is produce by the self-assembly of smaller molecules such as lipids and proteins (chemical processes) [4••,6–11]. However, there is a growing trend to combine bottom-up and top-down approaches to produce nanoparticle systems [12••]. Here, only the most common mechanical and chemical processes are described, as well as the characteristics of the particles produced.

2.1. Mechanical processes

The term mechanical process, in this article, refers to processes that use shear or particle collisions as the energy source to break down larger entities into smaller nano-scale aggregates. Such mechanical processes could be considered as part of the "top-down" approach mentioned above. The potential advantage of mechanical processes over chemical processes is that mechanical processes require minimal use of chemical additives, which mitigates the concerns regarding the regulations imposed on such formulation ingredients. There are two types of mechanical processes — mills for the nanonization (as opposed to micronization) of solid particles, and microfluidic processes for the nanonization of liquids or melts. Fig. 2 presents selected examples of mills and microfluidic processes.

As expected, there are challenges in the production of nanoparticle systems with mechanical processes. These challenges include creating high energy density "bursts" to break down the particle, preventing the re-aggregation of the particles,

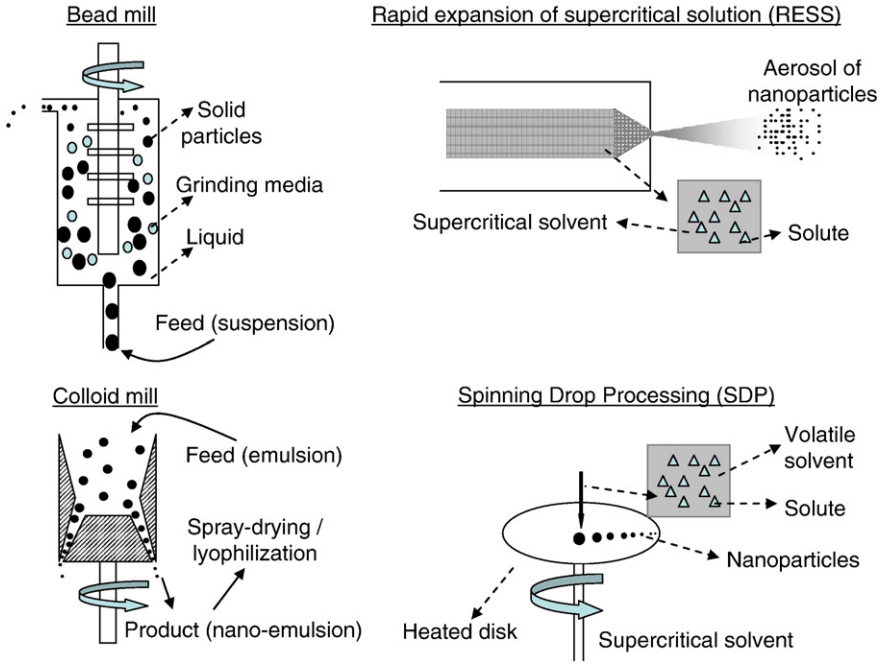

Fig. 2. Schematic of selected mechanical processes used to produce nanoparticle formulations.

and segregating large and small particles. In the case of mill processes, the first challenge is typically met by shearing or colliding either the particles onto themselves (jet mill) or using other small and hard particles as "grinding media" (bead and ball mills).

Ball mill processes have been used for the production of iron nanoparticles in aqueous suspension [13–15]. In those cases the presence of a stabilizer, typically a surface active molecule like oleic acid or sodium oleate are used to prevent the re-aggregation of the particles. The smallest particle size attainable with ball mill technology is close to 20 nm, but this is only achievable if the particle is produced by the precipitation of the solid from a supersaturated solution [13,14]. In most cases, the ball mill process is used to regulate the growth of the crystal. Perhaps the best known application of a ball mill process in the presence of suspension additives is the NanoCrystal® technology used to produce 100 nm–200 nm nanoparticles of poorly soluble drugs [16–18].

The bead mill process, on the other hand, is capable of producing nanoparticles as small as 20 nm from micrometer-size crystalline drugs [19]. Due to the relative simplicity and the ability to process a wide range of materials, this process is considered to be one of the most promising milling methods for the production of solid nanoparticles [10,19,20,21]. To date, there are no reported applications of bead mills to produce nanoparticles of solid forms of nutrients (such as colloidal iron) and nutraceuticals. Furthermore, it has been proposed that bead mills could be used to carryout chemical reactions (surface modification) during the milling process, which is another potential tool for the formulator [21].

Microfluidization (colloid mills) processes and related liquid-based technologies use the flow-induced shear of liquids, hot melts and other soft aggregates to produce or maintain nano-sized dispersion of the processed material. Such flow-induced shear is typically obtained by inducing large pressure drops across small nozzles. In the case of the colloidal mill presented in Fig. 2, the shear is produced by the rotation of the central cone. Colloid mills also face the challenge of stabilizing the product against aggregation (coalescence). Various alternatives such as rapid cooling, spray drying, solvent evaporation, and the use of hydrocolloid coatings, liquid crystals or surfactants as stabilizing agents can be considered to protect the product against aggregation. Microfluidization is an established technology in food processing, specially for dairy products [22,23], and it has been used in the production of submicron liposomes for the delivery of ferrous sulfate, ascorbic acid and for the delivery of other poorly absorbed hydrophilic compounds [24,25]. Microfluidization is also an important technique for encapsulating probiotic cultures [26]. Furthermore, microfluidization constitutes the basis for the production of solid lipid nanoparticles (SLN) [27]. Chen and Wagner used microfluidization to produce 100 nm vitamin E nanoparticles stabilized by a starch coating, suitable for fortified beverages [28]. Tan and Nakajima prepared 60–140 nm β-carotene nanodispersions by microfluidization–emulsification followed by solvent evaporation [29].

Another version of the microfluidization process is the use of supercritical fluids as the solvent media. The principle of the rapid expansion supercritical solutions (RESS, one of the first supercritical solvent-based processes) consists on dissolving the active ingredient (mostly hydrophobic compounds) in the supercritical fluid (such as carbon dioxide), followed by an expansion of the solution through a small orifice. The high shear rates across the orifice creates a fine mist where the supercritical fluid quickly evaporates, and as it evaporates, it induces the precipitation/solidification of the solute into nanoparticles [30,31]. Fig. 2 presents a schematic of this process. It has been shown that after rapid expansion, β-sitosterol nanoparticles initially dissolved in carbon dioxide form particles as small as 2–8 nm but that due to the agglomeration of those particles after they are produced, the final product tends to grow to sizes of 100–500 nm if no stabilizing additive is added [32]. Upon addition of sodium dodecyl sulfate (used to prevent the growth aggregation of particles) the final particle size could be controlled to 10–100 nm [32]. Other forms of supercritical fluid-based technologies include rapid expansion into a second liquid (known as RESOLV or RESAS if the second liquid is an aqueous solutions), precipitation with a compressed antisolvent (PCA), and a combination of RESAS with microfluidization/homogenization (RELGSH) [33•].

Another methods of inducing the precipitation/solidification into a disperse state with mechanical shear include: the spray freezing into a cryogenic liquid (SFL), atmospheric freeze-drying (ATMFD) [33•], and the spinning disk processing (SDP) method [34].

The spinning disk processing (SDP) method is illustrated in Fig. 2. In this type of process a jet is impinged onto a heated rotating disk. The centrifugal force breaks down the jet into small particles and the heat transferred from the rotating disk into the liquid induces a fast evaporation of the solvent, leaving behind a fine mist of particles. Similarly to the RESS method, the SDP technology also requires the use of surfactants to control particle growth and minimize the agglomeration of particles. SDP has been used to generate 40–100 nm β-carotene particles stabilized by polyglycerol esters of fatty acids [12••,34].

Other mechanical processes such as ultrasound-based technologies, membrane emulsification, and electrified coaxial liquid jets have also been proposed as alternatives to produce nanoparticle systems [4••]. In the case of ultrasound and membrane emulsification processes, the presence of surfactant and polymers is necessary to produce the desired nanoparticle systems.

2.2. Chemical processes

The term chemical processes refer to those methods of nanoparticle preparation where either chemical reactions and/or the self-assembly of surfactant and polymers are the primary drivers of the process. In these processes, the input of mechanical energy is typically limited to keeping the suspension fully mixed and to prevent agglomeration and settling. There are typically five components involved in chemical methods: the solute of interest, an internal (dispersed) solvent, an external solvent (typically water), a surfactant that is dispersed in the

external solvent(s), and in some cases a polymer that is soluble in the internal solvent, but insoluble in the external one.

Horn and Rieger classified the chemical methods of producing organic nanoparticles according to the nature of the internal solvent [12••]. According to their system, there are three types of internal solvents: a lipophilic solvent, an amphiphilic solvent and a hydrophilic solvent. These processes are illustrated in Fig. 3. The lipophilic solvent method is basically a process of emulsification/homogenization where the presence of a surfactant and/or polymers reduces the energy required for emulsification (by reducing the interfacial tension) and protects the nanodroplets against coalescence. The external solvent is later evaporated through spray drying or lyophilization technologies. Solid lipid nanoparticles (SLN) are prepared using this lipophilic solvent method [35,36]. Other lipophilic solvent methods involve the use of self-emulsifying systems that rely in microemulsion phase behavior, which is briefly described later in this article.

The amphiphilic solvent method consists of dissolving the solute in a polar organic (internal) solvent such as acetone or methylene chloride (containing a pre-dissolved lipophilic polymer), and mixing this system with an aqueous solutions containing a surfactant or hydrocolloids. The affinity between the internal and external solvent is such that emulsification occurs spontaneously and, upon addition of more water, the internal solvent diffuses out of the emulsion drop and into the aqueous phase, inducing the precipitation of the lipophilic polymer and the solute in nano-scale aggregates. This method has been recently used to produce 20–80 nm β-carotene nanoparticles using acetone as the amphiphilic solvent, poly-(lactic-co-glycolic) acid as stabilizing polymer and either Tween® 20 or gelatin as emulsifier [37]. The spontaneous emulsification solvent diffusion method (SESD) is a variant of this amphiphilic solvent method [38–40].

The hydrophilic (internal) solvent method involves the use of water-soluble alcohols as the internal solvent. In this case, the organic solute and a stabilizing polymer are dissolved in alcohol, and upon mixing with an aqueous solution containing the emulsifier, nanodroplets are spontaneously formed. Soon after emulsification, the alcohol (internal hydrophilic solvent)

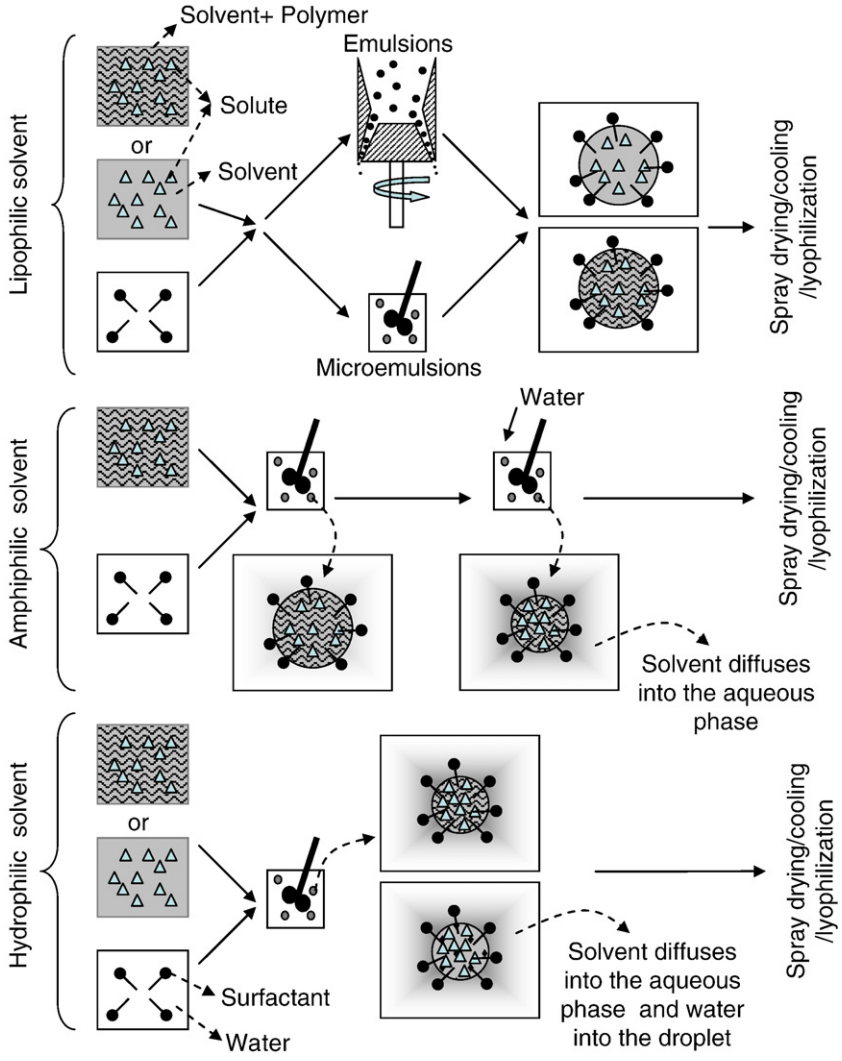

Fig. 3. Schematic of chemical processes used to produce nanoparticle formulations based on the classification of Horn and Rieger [12••].

diffuses into water (without the need of adding external water). As the alcohol diffuses out of the droplet, the solute precipitates in the lipophilic polymer matrix [12••]. Another take on the hydrophilic solvent method is the use of polymers or hydrocolloids that could precipitate upon changes of pH, temperature, or electrolyte composition. In recent years, there is growing interest in using these reactive methods to transform globulin proteins and casein micelles in nanocapsules for the delivery of hydrophobic nutraceuticals and hydrophilic minerals [3•,41].

In all these chemical processes there are two important objectives, the first one is to find a quick way to produce a solid network that will make up the body of the nanoparticle, and the second objective is to protect that the nanoparticle from agglomeration using surfactants or other emulsifiers. In most of the cases reviewed above, the solid network is produced by a polymer such as polylactic acid, poly-(lactic-co-glycolic) acid or a similar polymer, and the hydrophobic drug (e.g. β-carotene) is deposited as a small nano-sized crystal aggregate in the particle. In such cases, the solubility of the crystal-forming active ingredients could be further improved if the active ingredient is dissolved as a solid solution in a solid lipid matrix [42•].

Out of these chemical processing methods, the lipophilic solvent method, and in particular the fabrication of solid lipid nanoparticles (SLN) has received more attention due to their ease of preparation and for being amenable to a wide range of active ingredients. There are various ways to produce solid lipid nanoparticles (SLN), however the simplest method involves melting a lipid matrix (e.g. saturated fatty acids), and dissolving the hydrophobic active ingredient in this hot melt. This hot melt is then emulsified in an oil-in-water nanoemulsion produced by either microfluidization (high pressure homogenization) or by inducing a Type II–I microemulsion phase transition upon dilution with an aqueous solution. This hot-melt nanoemulsion is then spray congealed to produce submicron particles containing a solid solution of the active ingredient [43,44•]. The solid solution maintains the active ingredient in a glassy state in which this ingredient is more active [45]. SLN technology is applied in the formulation of cough syrups [44•]. Recently, microemulsion-based solid nanoparticles have been formulated for the controlled release of tea polyphenols [46,47].

Microemulsions are considered the ideal nano-reactors to produce nanoparticles, in particular nanoparticles of inorganic systems, and as explained above, they can also be used to produce lipid nanoparticles [48–50]. Microemulsions are composed of oil and/or water nanodomains that coexist in thermodynamic equilibrium. Perhaps the best known application of microemulsion in oral formulations is Neoral™ for the delivery of the hydrophilic peptide cyclosporine A, and HIV protease inhibitors Ritonavir and Saquinavir [51]. In these last two formulations, a microemulsion precursor is dosed in a gel form, and the microemulsion is formed during the digestion/absorption state. These formulations are referred to as self-microemulsifying drug delivery systems (SMEDDS). SMEDDS are used to increase the bioavailability of otherwise poorly adsorbed drugs by reducing the drop size of the emulsified system to less than 100 nm [51,52]. SMEDDS have been recently used to deliver Isoflavones extracted from Pueraria Lobata (a traditional Chinese medicinal herb), finding that this form of delivery increased the bioavailability (absorption) of this active ingredient by 2.5 folds when compared to a tablet formula [53]. Microemulsion aggregates have also been stabilized (against phase changes) through the use of complex coacervation. According to this approach, an oil-in-water microemulsion was coated with a coacervate phase formed after combining gum Arabic and gelatin [54]. The encapsulation efficiency of this complex was 60%, and the size of the coated aggregates ranged between 30 and 100 nm.

3. Bioavailability enhancement with nanoparticles

The term bioavailability refers to the fraction of a dose that is available at the site of action in the body. For most oral doses this definition is interpreted as the fraction of the dose that enters the bloodstream. Uptake (or intestinal absorption), on the other hand, refers to fraction of the dose that is absorbed through the intestinal walls. Although both definitions are related, the entire dose that is absorbed through the intestine (uptake) may not be bioavailable due to the various processes involved in the absorption of nutrients. To design effective nanoparticle delivery systems for nutrients, nutraceuticals and related active ingredients, it is necessary to understand the biological processes that regulate uptake and bioavailability.

The schematic of Fig. 4 illustrates some of the main processes involved in the absorption of nutrients and active ingredients. After the food/dose has been partially digested (mainly by mastication) in the oral cavity, the food goes through a dissolution process in the stomach at acidic conditions (pH ~ 1 to 2) during a period of time that ranges from 1 to 3 h. Various enzymes (pepsin and others) are released in the stomach to help break down some of the proteins and carbohydrates. Dissolution in the stomach of the nanoparticle may or may not be desirable depending on the stability of the active ingredients in the acidic pH. If the nanoparticles require protection against the acidic environment of the stomach, they can be microencapsulated using enteric coatings [55]. As the digested food (now in the form of a suspension) leaves the stomach and enters the duodenum, it mixes with the bile salts (such as sodium glycocholate, sodium taurocholate, and lecithin) released by the gall bladder. These bile salts emulsify the fats and other hydrophobic compounds present in the suspension.

The average size of bile salt aggregates ranges from 4 nm (for bile salt micelles) to 60 nm (for bile salt vesicles) [56]. In some ways, bile salts micelles and vesicles are nature's own nanoparticle delivery system. In fact, the need for manufactured nanoparticle delivery systems is questionable in certain cases. For example, Faulks and Southon indicate that the solubility of most carotenoids in triglycerides is 100–200 mg/g and that up to 70 mg of apolar and 44 mg of polar carotenoids could be absorbed in a single meal without the use of nanoparticle delivery systems [57]. However, these authors indicate that carotenoids (as nutraceutical extracts without a delivery formulations) are not bioavailable if they are not consumed with

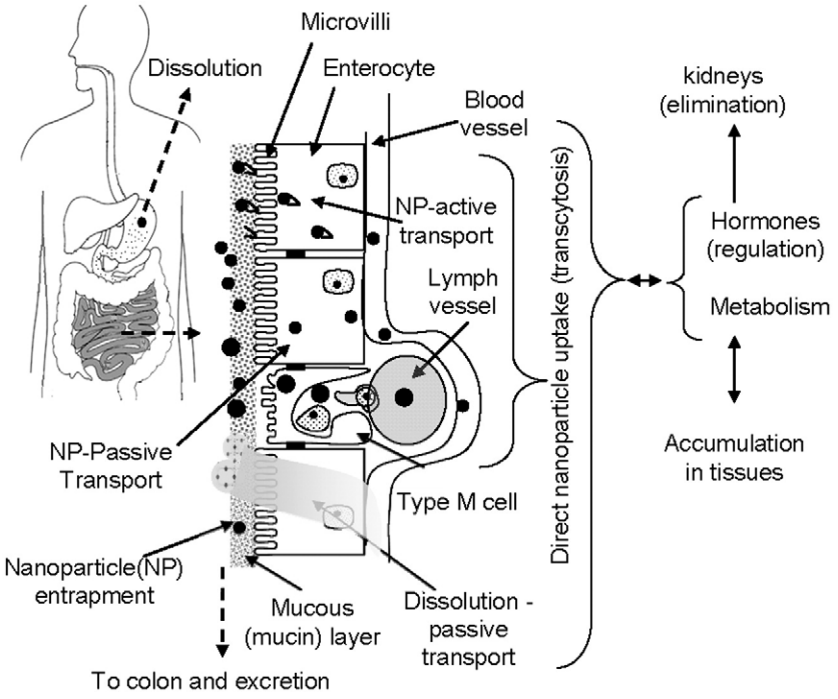

Fig. 4. Schematic of the mechanisms of active ingredient uptake using nanoparticle systems.

food. This observation reflects the fact that bile salts can emulsify (and serve as nanocarriers) carotenoids dissolved in triglycerides, but that bile salt micelles cannot solubilize pure carotenoids.

In addition to the release of bile salts, a bicarbonate solution containing a cocktail of enzymes (trypsin among others) is also released in the duodenum, increasing the pH of the solution to 6–7. The suspension then enters the largest part of the small intestine (4–7 m) where it resides for about 3 to 5 h before entering the large intestine. The inner surface of the small intestine is covered with small "finger-like" protuberances called vili. Each epithelial cell is covered with even smaller protuberances called microvilli that helps increase the area for nutrient absorption. A mucous layer of an anionic glycoprotein (mucin) typically covers the surface of the microvili and represents a key factor in the uptake of nanoparticles. The microvili and the mucous (mucin-rich) layer are illustrated in Fig. 4.

The absorption of nutrients through the small intestine occurs through two main mechanisms, active and passive transport. Active transport involves the uptake of the active ingredient through specific channels on the surface of the epithelial cells. The cells use their own energy to capture and absorb nutrients even when the concentration of the nutrient inside the cell is higher than the concentration outside the cell. Active transport is the main mechanism by which cell captures and absorb highly soluble minerals like calcium and iron. This active uptake is controlled by hormones that regulate the concentration of minerals and other nutrients in the body. Fig. 4 also presents a simplified schematic of the control systems that regulate the absorption of nutrients and nutraceuticals. These control systems maintain a certain homeostatic level of substances in the blood, in a way that, if there is any excess of the active ingredient in the blood, additional doses are not adsorbed (active adsorption mechanism), and that this excess is accumulated in tissue or secreted after the compound has entered the bloodstream. Therefore, when assessing the effectiveness of nanoparticle carriers it is important to consider the level of "deficiency" of the nutrient in the intended users.

Passive transport occurs by a simple diffusion across the epithelial tissue. In this case the rate and extent of uptake is a function of the difference in the activity of the mineral or nutrient across the epithelial tissue. The activity of a solute is calculated as the product of its concentration times its activity coefficient. The activity coefficient reflects the solubility of the solute in the solvent. Poorly soluble ingredients (e.g. hydrophobic compounds in water) have a large activity coefficient, thus inducing a large driving force for the nutrient (active ingredient) to permeate. Most hydrophobic compounds are highly permeable through the intestines and transport using passive and active diffusion, however highly hydrophilic substances tend to have low permeability and absorb via active transport. For a more detailed review of the mechanisms of active ingredient absorption there are various reviews available [58–60].

There is agreement that formulating nanostructured delivery systems yields an increase in drug uptake, however the mechanisms by which this occurs are not well understood. These mechanisms may involve increasing the apparent solubility of the active ingredient, increasing the rate of mass transfer, increasing the retention time or increasing the absorption via direct uptake of the nanoparticle carrier [52,61,62••,63,64••]. To illustrate the effect of particle size on bioavailability and uptake, Fig. 5 presents a review of relative uptake/bioavailability reported by five different research groups as a function of particle size. In each case the relative uptake/bioavailability is obtained by dividing

the value at a given particle size by the maximum uptake/bioavailability obtained in each study. Out of these five systems, there are three groups that studied the plasma concentration of the active ingredient and obtained the bioavailability from the area under the curve (AUC) for their systems [12••,65,66] and two groups that studied the uptake of polymeric nanoparticles by the surface of the small intestine [63,67].

In the study described by Horn and Rieger, the concentration of β-carotene in calve's blood is reported as a function of time, after a dosage of β-carotene nanoparticles prepared by the amphiphilic (internal) solvent method [12••]. This data was integrated to obtain the AUC value, and the AUC values were normalized according to the criteria indicated above. Wu et al. presented the data of AUC for the absorption of the poorly soluble drug MK-0869 prepared using three different milling procedures — Nanocrystals® (120 nm), wet-milled particles (480 nm) and jet-milled particles (1850 nm) [65]. Luo et al. reported the AUC of vinpocetine prepared using solid lipid nanoparticles (SLN) [66]. From the nanoparticle uptake series, the data from Desai et al. presents the uptake of poly(lactic-co-glycolic) acid particles produced by four different methods — sonication (100 nm), microfluidization (500 nm), high pressure homogenization (1000 nm) and simple vortex mixing (10000 nm) [63]. These researchers used an in-situ rat model to load the particles in a section of the intestine, and determine the number of particles absorbed per unit of area of the intestinal tissue, and the fraction of particles absorbed was calculated based on the initial dosage. The work of Jani et al. was conducted in a similar way, but they used a continuous (chronic) exposure, and determine the number of polymer particles (latex in their case) absorbed by various tissues [67].

As shown in Fig. 5, reducing the particle size to values below 500 nm produces higher absorption of the active ingredient and higher particle uptake, however, the value of relative uptake or bioavailability for large particles (larger than 500 nm) depends on the system. It is remarkable that the slopes of the linear portion of the absorption/uptake curves in Fig. 5 are relatively similar, considering the fact that the data come from a variety of sources, which suggests that the uptake of the nanoparticle carrier is an important factor in enhancing the bioavailability of the active ingredient. Unfortunately, no data on bioavailability or uptake was found for particles smaller than 50 nm in order to determine if these linear portions of the curve could be extended to smaller particle sizes.

One of the fundamental equations used to support the design of nanoparticle systems is the equation of Ostwald–Freundlich that establishes the increase in solubility of a given substance based on the increase of interfacial energy at high curvatures (small particle size) [42•,68]:

$$\ln\left(\frac{Cs}{Cso}\right) = \frac{2Mw \times \gamma}{\rho \times R \times T} \times \frac{1}{r} \quad (1)$$

where Cs is the solubility of the solute for a given particle size, Cso is the solubility of the solute for large r values (a flat interface), Mw is the molecular weight of the solute, γ is the interfacial tension between the solute and the solvent, ρ is the density of the solute, R is the Universal Gas Constant, T is

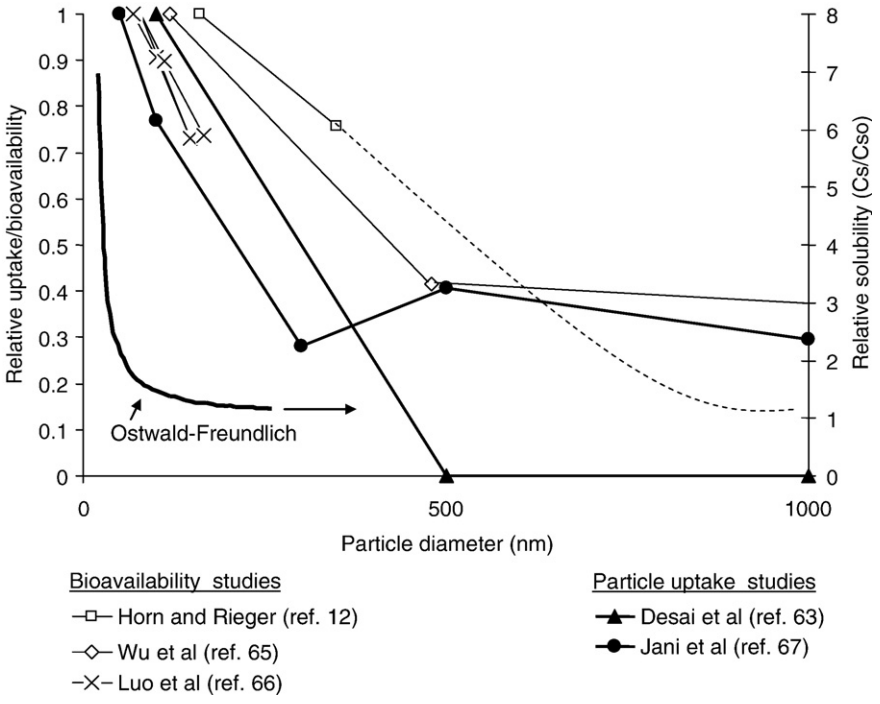

Fig. 5. Relative bioavailability of the active ingredient delivered using nanoparticle formulations and relative uptake of polymeric nanoparticles as a function of particle size. The right abscissa presents the relative solubility of an example compound (molecular weight of 500 g/mol, surface tension of 50 dyn/cm and density 1 g/cm^3) calculated using the Ostwald–Frendlich equation (Eq. (1)).

the absolute temperature of the system, and r is the radius of the droplet/particle.

Fig. 5 also includes a curve of relative solubility (Cs / Cso) versus particle size calculated using Eq. (1) for a hypothetical compound with MW = 500 g/mol, γ = 50 dyn/cm, and ρ = 1 g/cm^3 and T = 310 K (37 °C). If one assumes that the solubility of the active ingredient in the intestinal fluid is the limiting step for the uptake of the active ingredient, then the Ostwald–Freundlich equation plotted in Fig. 5 would suggest that when the nanoparticles are smaller than 100 nm it is when significant improvements in bioavailability are expected [42•]. The data in Fig. 5 suggest that other mechanisms may also be at play in the enhancement of relative bioavailability with 100–500 nm nanoparticles.

It has been proposed that other factors such as the increase in the rate of release (due to the large surface area), the increase in the retention time due to the small size of the nanoparticles (entrapment in the mucous layer), or the direct uptake of the particle (as suggested before) are important elements that explain the improved absorption with nanoparticle systems [12••,69].

3.1. Pharmacodynamics (mass transfer) of nanoparticle delivery

Oh et al. and Amidon et al. [62••,70] studied the mass balance of particles dissolving in the intestine and absorbing through the intestinal wall. Fig. 6 presents a schematic of the processes of dissolution and absorption considered in their model. The basic assumptions of their model are: (a) the administered dose enters the intestine in the form of monodisperse spherical particles or droplets, and no aggregation or attrition occurs during intestinal transit; (b) the intestine is a cylindrical tube of radius R and length L; (c) there are no reactions (metabolism) in the intestine; (d) the solubility of the active ingredient (C_s) is independent of particle size (r_Z) or changes in pH through the intestinal transit; (e) the suspension of the particles moves in plug flow conditions (no dispersion, high Peclet numbers); (f) the mass transfer coefficient (for the dissolution of the active ingredient from the particle) obeys the limiting Sherwood Number for viscous flow ($k_l = D/r_Z$, where D is the diffusion coefficient of the active ingredient in the luminal solution); (g) the concentration of the dissolved active ingredient in the lumen (C_Z) is several folds larger than the concentration of the active ingredient in plasma (C_P) such that $C_Z >> C_P$, and the flux across the intestinal membrane is P_{eff}* C_Z, where Peff is the effective permeability of the active ingredient through the intestinal membrane.

Using these assumptions, the mass balance around the particles yield:

$$\frac{dr^*}{dz^*} = -\frac{\mathrm{Dn}}{3}\frac{(1-C^*)}{r^*} \tag{2}$$

where r* is a normalized form of the particle radius ($r^* = r_Z/r_0$, r_0 is the initial particle size), z* is a normalized form of the position or transit of the particle through the intestine ($z^* = z/L$), C* is the normalized concentration of the active ingredient in solution ($C^* = C_Z/C_S$), and Dn is defined as the dissolution

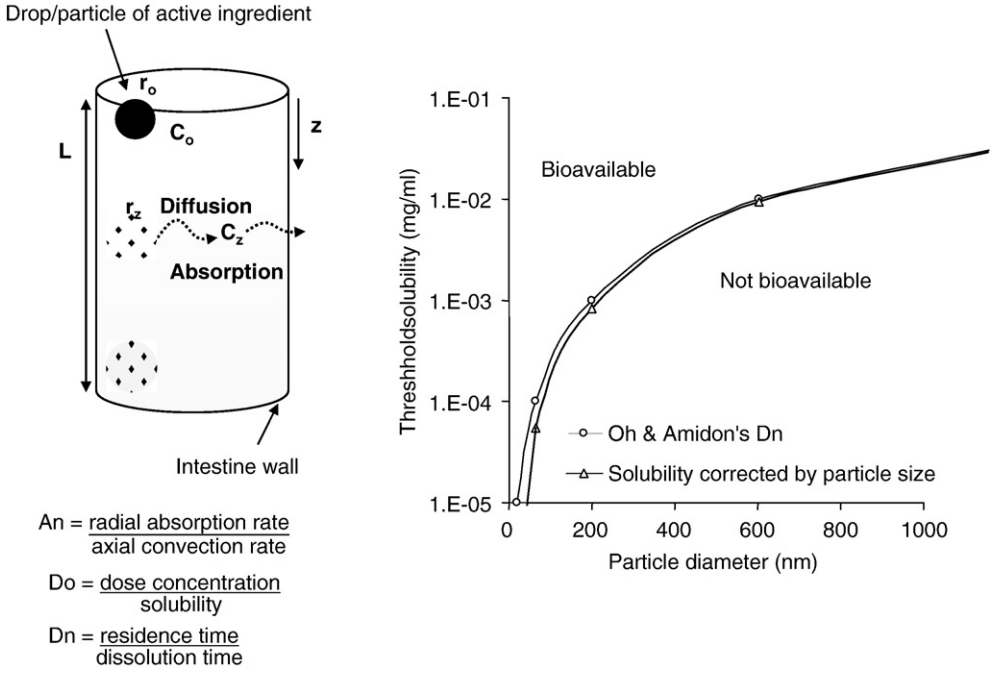

Fig. 6. Left — schematic of the mechanism of dissolution–absorption used by Oh and Amidon to setup the mass balance equations (Eqs. (2)–(7)) to determine the fraction of active ingredient absorbed. Right— threshold solubility of the active ingredient for absorption as a function of particle size calculated using Eq. (3), and a critical dissolution number Dn = 3.3. A diffusion coefficient of the active ingredient of 1×10^{-5} cm^2/s, and a residence time of 3 h were assumed in these calculations. The corrected solubility values were calculated on the basis of Eq. (1) (same basis used in Fig. 5).

number (Fig. 6), and is calculated using the following expression:

$$\mathrm{Dn} = \frac{D}{r_0}\frac{C_S\left(4\pi \times r_0^2\right)}{\left(4/3\pi \times r_0^3\rho\right)}\frac{\pi R^2 L}{Q} \quad (3)$$

where ρ is the density of the particle, and Q is the flow rate of the intestinal fluid (assumed constant). The mass balance for the dissolved active ingredient in the intestine is:

$$\frac{dC^*}{dz^*} = \mathrm{Dn} \times \mathrm{Do} \times r^*(1 - C^*) - 2^*\mathrm{An}^*C^* \quad (4)$$

where Do is the "dose number", and is calculated as:

$$\mathrm{Do} = \frac{\mathrm{Mo}}{\mathrm{Vo}}\frac{1}{C_S} \quad (5)$$

where Mo is the mass of the active ingredient dosed. Vo is the volume of liquid taken with the dose. The absorption number, An, is defined as:

$$\mathrm{An} = \frac{P_{\mathrm{eff}}\pi \times R \times L}{Q} \quad (6)$$

Using the total mass balance of the active ingredient, the fraction absorbed (F) is:

$$F = 1 - r^{*3} - \frac{C^*}{\mathrm{Do}}. \quad (7)$$

Eqs. (2–7) constitute the mathematical framework for the Biopharmaceutical Classification System (BCS) used to determine when an active ingredient is fully absorbed. These equations also help to understand the effect of different formulation strategies, including nanoparticle delivery, on the absorption of the active ingredient. The dissolution number (Dn), in particular, increases as the particle radius (r_0) decreases, which in some cases represents an increase in the absorption of the active ingredient. Based on the simulations of Oh et al. [62••], only when the dose number increases in the range of 1 to 10 is when a significant increase in the fraction of active ingredient absorbed is obtained. Typically a value of Dn between 3 and 4 could be used as the threshold point between almost complete absorption (Dn>4) and negligible absorption (Dn<3).

Using Eq. (3), and a critical dose number of 3.3 (Dn=3.3) it is possible to calculate a "threshold" solubility for drug absorption as a function of particle size. It is expected that active ingredients whose solubility is higher than that threshold concentration would be completely absorbed (assuming that the active ingredient is permeable). Fig. 6 presents the calculated values of that threshold concentration as a function of particle size (r_0) assuming that the residence time in the intestine is 3 h ($\pi R^2 L/Q$=3 h). Fig. 6 may serve as a "map" to design nanoparticle delivery systems since, for a given active ingredient solubility, it is possible to determine the particle size below which a significant solubilization enhancement would be observed. For example, the solubility in water of vitamins E and K3 has been reported to be 20 mg/ml and 150 mg/l respectively [71]. Using Fig. 6 one would predict that nanoparticle delivery systems with less than 600 nm would yield significant enhancement in the absorption of vitamin E and that even particles of 1 μm size would be sufficiently small to guarantee maximum absorption of Vitamin K3.

It is possible to expand the equations of Oh and Amidon to include the solubilization enhancement predicted by the Ostwald–Freundlich equation (Eq. (1)). In this case a simple correction could be used to recalculate the value of the threshold solubility. This corrected threshold solubility is also presented in Fig. 6. According to the data in Fig. 6 there is little difference between the threshold solubility calculated by the Oh and Amidon equations and the values corrected by the effect of curvature on solubility. The difference becomes apparent for nanoparticle systems of 100 nm in diameter or less, which is consistent with previous discussions regarding the Ostwald–Freundlich equation.

Besides particle size and solubility, the other important parameter that defines the dose number is residence time. The longer the residence time in the intestine, the higher the dose number. There are two alternatives to increase the residence time of the nanoparticle. The first alternative involves the use of bioadhesives (e.g. chitosan) that will be explained in the next section, and the second alternative is reducing the particle size to increase the retention by the mucous layer of the intestines [72•]. Unfortunately, the effect of particle size on residence time has not been studied for nanoparticle systems in contact with intestinal mucosa. However, Lai et al. have recently carried out studies on diffusion coefficients of nanoparticles in contact with vaginal mucous layers (similar to intestinal mucous layers) finding that the diffusion coefficient of 100 nm particles is up to three orders of magnitude lower than the diffusion coefficient of 200 nm nanoparticles [73]. Lai et al. propose that the principles of size exclusion chromatography (SEC) can explain the slower movement of 100 nm particles along mucous layers [73]. In SEC the retention time of smaller particles is extended due to the tortuous path that the particle can take when it moves through a reticulated gel media, however, for larger particles only a limited number of large channels are available for these structures, thus producing a shorter diffusion path. Lai et al. also point out that the reticular structure of the gel maybe modified by the interactions between the surface of the nanoparticle and the mucin gel.

3.2. Nanoparticle uptake

Direct nanoparticle uptake is yet another method of improving the bioavailability of active ingredients, especially for compounds that are soluble in water but that have low permeability. The topic of nanoparticle uptake has been the object of controversy over the last ten years and several reviews have been devoted to clarify some of the inconsistencies in the experimental data [63,64••,67,69,74,75].

Fig. 5 presents two sets of data that show the direct uptake of nanoparticles in in-vivo studies. The data of Jani et al. [67], shown in Fig. 5 as relative uptake of polystyrene nanoparticles, has been probably the most discussed and referenced set of data

to illustrate the direct uptake of nanoparticles [64••,69,74,75]. The data of Jani et al. [67], Desai et al. [63] and other researchers [64••,69,72•,74] strongly support the idea that direct nanoparticle uptake, especially for systems in the 10–100 nm range is a viable route for the delivery of active ingredients. These and other researchers have proposed three main routes of nanoparticle uptake: paracellular uptake, transcellular (transcytosis) uptake by enterocytes (which represent 90–95% of the epithelial cells of the intestine) and transcellular uptake by M (microfold) cells [69]. The paracellular uptake (uptake through the interstitial space between epithelial cells) is considered to be the least effective of the three mechanisms because the space between cells ranges between 0.3 and 1 nm, which is too small for most nanoparticles to permeate [76].

The transcellular (transcytosis) uptake of nanoparticles is illustrated in Fig. 4. There are two methods of nanoparticle transcytosis, one is passive in which case the nanoparticle diffuses through the epithelial cell and the second method of involves the presence of receptors on the surface of the cells (represented as small ">" marks in Fig. 4) that capture nanoparticles with specific surface chemistry. The same basic mechanisms of transport apply to enterocytes and M cells. However the M cells are more permeable than enterocytes, and therefore M cells have been used as the target for numerous micro- and nanoparticle delivery strategies [64••,69]. Such M cells are located in the Peyer's patches of the lower intestine, and its role is to "sample" potential antigens present in the intestinal track.

Although M cells are the ideal portal for micro- and nanoparticle delivery, the problem with this approach is that these M cells represent typically less than 1% of the total intestine area, which makes the selective delivery to these sites more difficult [64••]. There is currently intense activity, in the pharmaceutical arena, on surface modification technologies to incorporate selective ligands for M cell delivery, including lectin-coated nanoparticles, and nanoparticles coated with exopolymers produced by toxic bacteria [77–80]. The principle of this target uptake is that M cells, due to their antigen monitoring activity, have developed receptors that capture particles coated with glycoproteins such as those used in target delivery. Recent studies suggest that lectins, in particular, may be the key to improve the absorption of hydrophilic (and poorly absorbed) nutraceuticals such as isoflavones [81], however, there is little progress made, to date, in exploring those possibilities in nutrient and nutraceuticals applications.

Another approach to promote nanoparticle uptake by M cells and enterocytes is the use of coatings to modify the surface chemistry of the nanoparticle systems. For example, chitosan coatings have been used for numerous researchers to as a mucoadhesive to enhance the entrapment of nanoparticles [64••,69,72•,82,83]. In that case, chitosan, a weak cationic polysaccharide, introduces hydrophilic groups on the surface of the particle and establishes weak ionic interactions with the negatively charged mucin layer that coats the surface of enterocytes and M layers [69]. It has been found that increasing the hydrophilicity of the surface of the nanoparticle typically promotes the translocation of nanoparticles across cellular cytoplasm [64••,84]. Another popular alternative to introduce hydrophilic moieties to the surface of the nanoparticle is the use of polyethylene glycol coatings [69,85]. Perhaps one of the most effective methods of increasing the adhesion of nanoparticles to the mucin layer that line the surface of the intestines is the use of strong cationic coatings produced with synthetic polymers such as Eudagrit® RS or cationic surfactants such as alkyl trimethyl ammonium salts [69,86–88]. However, if the interaction between the cationic nanoparticle and the mucin proteins is too strong, the particle will remain adhered to the mucin and will not permeate through the epithelial tissue [64••].

Currently, the idea of introducing nanoparticle systems in nutrient and nutraceutical delivery systems is mainly focused on improving the dissolution mechanisms discussed in Section 3.1 and illustrated in Fig. 6, but the direct nanoparticle uptake has been largely overlooked. However, if one turns to bile salts micelles and vesicles as example of nanoparticle systems, one finds that the uptake of the solutes encapsulated in these systems occurs by active transcellular transport (transcytosis) of the nanoparticle [89,90]. The similar behavior of the relative bioavailability and the relative nanoparticle uptake between completely different formulations presented in Fig. 5 suggest that nanoparticle uptake contributes to a significant fraction of the active ingredient uptake. Furr and Clark suggest that the mechanism of uptake of carotenoids is mediated through their solubilization in bile salts aggregates and that these aggregates directly transport the carotenoids to the chylomicrons [91•]. In principle, it is possible to use these principles of direct nanoparticle uptake to improve the bioavailability of soluble but poorly absorbed nutrients and nutraceuticals. Ratnam et al. have proposed the use of nanoparticle delivery systems for the delivery of more polar compounds such as isoflavones, and suggested that micro- and nanoparticles are among the most promising systems to accomplish this objective [92•].

4. Outlook

The field of food nanotechnology is experiencing significant growth due to the confluence of interests of industry, government and academia. In the area of nutrient and nutraceutical delivery there have been important advances made in nanoparticle formulations designed to improve the bioavailability of poorly water-soluble ingredients. However, very little has been done on the improvement of the uptake of hydrophilic compounds such as some soluble minerals (like calcium and iron) and soluble antioxidants (such as isoflavones). Most researchers have worked under the assumption that improvement in bioavailability comes from improvement in apparent solubility and have neglected the impact that mass transfer issues and direct nanoparticle uptake play in enhance bioavailability. More fundamental studies on nanoparticle-mediated nutrient and nutraceutical transport are needed to understand this technology and engineer new nanoparticle delivery systems.

There are exciting new developments in self-assembled protein and lipid micro- and nanoparticle systems that, if properly targeted to active sites, such as M cells, could produce technologies that

would improve the bioavailability of soluble but poorly absorbed nutrients and nutraceuticals. The confluence of pharmaceutical, nutrition, and colloid sciences with food engineering will be the key to unlock the full potential of nanoparticle delivery systems in food applications.

In addition to the potential technological impact of nanoparticle delivery systems in the food industry, there are also concerns about unforeseen side effects of the technology. The fact that these carriers are designed with food-grade ingredients does not mean that they might not cause undesired effects such as transporting or depositing active ingredients or excipients in tissue that they are not supposed to, or enhancing the absorption of substances that they are not meant to transport but that are present in the food matrix. Regulations on food nanotechnology are a likely development in the near future that may have a significant impact on the methods of preparations, dosages, and ingredients used in these systems.

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