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Nanoencapsulation: A New Trend in Food Engineering Processing

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Abstract Nanoencapsulation is defined as a technology to pack substances in miniature making use of techniques such as nanocomposite, nanoemulsification, and nanoestructuration. It provides final product functionality (including controlled release of the core) which is expected to be maintained during storage. Within the food engineering field, protection of bioactive compounds such as vitamins, antioxidants, proteins, and lipids as well as carbohydrates may be achieved using this technique for the production of functional foods with enhanced functionality and stability. In this paper, the different techniques that have been developed for the production of nanocapsules are discussed, and examples of their application are provided including regulatory aspects on products of nanotechnology. Also, it is illustrated a proposal of classification and characterization of the different structural arrangements of core–shell materials in nanoencapsulates composites found in the literature.

Keywords Nanotechnology · Nanoencapsulation · Nanoemulsion · Size of particles

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Introduction

The term nanotechnology was coined in 1974; Nario Taniguchi used it to describe the manipulation of particles of less than one micrometer, and in 1959, the physicist, Richard Feynman spread the concept within the atomic engineering field [1]. Nanotechnology involves research, technology development, and control of structures within sizes from 1 to 100 nm. Nowadays, nanotechnology is applied to creating structures with special properties which strongly depend on size and structure such as quantum confinement in semiconductor particles, surface plasmon resonance in some metal particles, and superparamagnetism in magnetic materials [2, 3].

A number of advances in the food processing fields have been made on nanotechnology grounds. Preparation of nano-sized materials can be carried out using "Top down" methodologies which consist in decreasing the size of macrostructures down to the nano-size scale or by "Bottom up" techniques in which arrangements of atoms, molecules, or single particles are induced. The large surface area per unit mass of nano-sized biomaterials may increase their biological activity [4] as in the formulations of herbaceous plants as a result of nanotechnology-based studies [5]. Nanostructures have been used for the inhibition of thermal polymerization and for eliminating bad odors by catalytic refining of frying oil using nanoceramics [6]. Also, the preparation of milk protein nanotubes with potential applications as carrier materials and enhancers of properties such as jellification and viscosity has received special attention [7]. As far as packaging is concerned, there have been advances on the preparation of nylon and titanium dioxide nanocomposites as barriers to oxygen in fried foods packs [8]. Worldwide sales of nanofoods have increased from 150 millions of dollars in 2002 to 860 in 2004, and it

is foresight that this figure will reach 20.4 billions in 2010 [9, 10].

Is within the nutraceutical and food safety fields that applications of nanotechnology have made important progress; the FDA approved methodologies and techniques supporting the inclusion of nanotechnology-based ingredients for mass consumption [11]. Examples of these are the production of vitamins in the form of micelles and of small (nano) detectors of viruses. These developments provide the pattern for the further progress in the creation of new nanostructured products that are capable of releasing at controlled rates, different compounds of improved functionality [12, 13].

In this revision, it is aimed to highlight basic and applied aspects of encapsulation processes in nanotechnology, stressing production of functional foods. Also, issues on characterization and elaboration and regulatory issues on nanoencapsulation-related fields are described.

Regulation

Legal aspects related to the handling and consumption of nano-sized materials in food applications are important for deciding on the consumption of these composites and will have in years to come an important role in mass production of nanofoods. Currently available information of risks associated to manipulation of nano-sized products is limited, and there are not internationally agreed regulatory parameters related to manipulation of nano-sized food materials, and thus, many products reach markets without prior evaluation on safety aspects related to their manipulation [11, 14].

Impact of nanoparticles over human physiology depends on their chemical nature, surface properties, and manner of self-assembly [15]. Particles enter human body by inhalation, contact, or ingestion. Particles having a size within the range of 4–10 um can reach human lungs, whereas particles having a size smaller than 400 nm may reach alveoli [16]. There is a lack of information on aspects related to development of foodstuffs which contain manufactured nanoparticles and on physicochemical properties of nano-sized particles used as additives during their transit through the gastrointestinal tract [17].

Regulatory aspects related to nanoproducts are led by the United States of America. The National Nanotechnology Initiative (NNI) describes the uses and the applications of nanotechnology in three main areas: (1) Research and technology on the development of products at the atomic, molecular or macromolecular scales in the length of 1–100 nm. (2) Creating and using structures that have novel properties and functions because of their small sizes. (3) Ability to control or manipulate on the atomic scale [11].

It is important to mention that although the "nano" term refers to a magnitude 10^{-9}, study of materials of bigger size but with pores, cracks, incrustations, etc. of nano-size which add functionality are included in the nanotechnology study field. This has been illustrated by introducing thermodynamic considerations in water sorption analysis of food-related systems as in the work by Flores-Andrade et al. [18]. Improved functionality may be observed in food applications at particle sizes somehow larger than the 1–100 nm range reported earlier. For example, Wullf-Pérez et al. [19] demonstrated that the colloidal stability of some nanoemulsions can be precisely controlled by the chemical structure of its interface. In their work, they used the amphiphilic uncharged tri-block copolymer Pluronic F68 as surfactant and natural oils from soybean, sesame, and olive as the organic phase. The more stable resulting emulsions resulted very appropriate for parenteral administration and had an average particle size, determined by dynamic light scattering (DLS), of approximately 500 nm.

Chi-Fai et al. [11] reported 42 bodies worldwide that support development of nanotechnology and involved in regulatory aspects. Asia was reported to have 23 entities, nine of them in Japan; Taiwan and Korea had five each one and China, four. Europe was the second continent in the list having four integrated entities, plus other five by United Kingdom, three by France, and two by Germany and Italy. United States was reported to have six groups at the time of releasing the survey; Canada with one and finally, Australia with three.

Background

Nanoencapsulation is defined as a technology to pack substances in miniature and refers to bioactive packing at the nanoscale [20]. Nanocapsules can often release their content at controlled rates [21] as in the case of hydrophobic ion pairing for incorporating lysozyme into electrospun poly(ε-caprolactone) (PCL)/poly(ethylene glycol) (PEG) non-woven membranes. Li et al. [22] reported that the addition of PEG into the PCL nanofibers not only improved the hydrophilicity of the membrane but also played an important role on in vitro lysozyme release rate. It was also found that the release rate of lysozyme was enhanced with the increase in PEG content and with salt concentration in the medium accelerating the liberation of the enzyme which kept most of its activity. In another work, Niu et al. [23] studied the effects of tissue engineering scaffold on the controlled releasing of a synthetic peptide from porous nano-hydroxyapatite/collagen/poly (L-lactic acid)/chitosan microspheres (nHAC/PLLA/CMs) containing different quantities of chitosan which were prepared by a thermally induced phase separation method.

Rehydration of food materials can be improved by encapsulation. Generation of pores, extent of hydrophobic–hydrophilic balances within them, and capsule–disolvent large contact areas aid to improve wetting processes. Zimet and Livney [24] investigated on the use of β-lactoglobulin (β-Lg) as natural molecular nanocarrier for hydrophobic molecules and reported the formation of colloidally stable nanocomplexes of fatty acids (docosahexaenoic) DHA-loaded β-Lg and low methoxyl pectin below the isoelectric point of β-Lg (5.2). These authors found that by adding excess of pectin, the formation of dilutable nanoparticle dispersions was enabled, and transparent solutions containing 0.05% b-Lg and DHA at a 1:2 (b-Lg:DHA) molar ratio were formed, having a very good colloidal stability and average particle size of 100 nm. These nanocomplexes provided good protection against degradation of DHA during accelerated shelf life stress tests.

It is also important the encapsulation and delivery of sensitive health-promoting substances using natural GRAS (generally regarded as safe) ingredients. Semo et al. [25] studied systems containing the oil-soluble vitamin D2, which is essential for calcium metabolism. They demonstrated the possibility to load this vitamin into casein micelles (CM), utilizing the natural self-assembly tendency of bovine caseins. The vitamin was about 5.5 times more concentrated within the micelles than in the serum. Also, the morphology and average diameter of the re-assembled micelles were studied. This study suggested that CM may be useful as nanovehicles for entrapment, protection, and delivery of sensitive hydrophobic nutraceuticals within food products. Also, Preetz et al. [26] prepared three-layer polyelectrolyte shell capsules and a core consisting of medium-chain triglycerides. The preparation was based on a high-pressure homogenized emulsion that was stabilized by a modified starch. The nanocapsules with an average size of 130 nm were characterized by Zeta potential, nuclear magnetic resonance, transmission electron microscopy, and light scattering techniques. All ingredients were non-toxic and biocompatible.

Preparation of capsules by dehydration and induction of nanopores on the surface of shell materials can be observed in the work by Drusch and Berg [27]. The aim of the study was to investigate the localization of the extractable oil in spray-dried microencapsulated fish oil prepared under different spray-drying conditions and the impact on lipid oxidation upon storage. Authors used confocal laser scanning microscopy, scanning electron microscopy, and different extraction procedures and revealed that the extractable oil in microencapsulated fish oil is mainly located on the surface and in oil droplets close to the surface. It is noteworthy that the inlet/outlet spray-drying air temperatures (210/90 °C y 160/60 °C) not only had effects on surface lipidic contents. Besides, it is possible to observe formation of nanopores on the surface of the materials.

The main technological trends in the preparation of nanocapsules are development of nano-sized capsules, structuring nano or micro capsules with nano-sized pores [28], and encapsulation of nano-size particulate compounds. Encapsulation of nanoparticles prepared from nanoemulsions of different sources of oils and produced by different homogenization methodologies followed by spray drying has received a good deal of attention within this field [29–35].

Different techniques have been developed for the production of nanocapsules. In general, these methodologies may be divided into three main groups [36]: (a) Physical processes such as spray drying–coating, extrusion, and spray drying; (b) Physiochemical processes such as simple or complex coacervation and entrapment into liposomes; (c) Chemical processes: interfacial polymerization and molecular inclusion. In order to select the most suitable encapsulation process for a given purpose, it is necessary to know the required size of the particles, the physicochemical properties of the core material as well as the nature of the substance to encapsulate. Other parameters to be taken into account are the desired or recommended releasing mechanism and associated costs [36]. Techniques for achieving nanoencapsulation are more complex than those used for microencapsulation chiefly due to the fact that it is, in general, more difficult to attain a good nanoencapsulation process given the complex morphology of the capsule and core material and the demands of releasing rates [11]. Chemical and physical natures of the core and shell materials, evaluation of their interactions as well as their proportion in the formulation of the capsules are important parameters which determine the final properties of the particles [37]. Castellanos et al. [38] evaluated the effect of molecular radius and chemical composition of wall materials onto -chymotrypsin with methoxy poly(ethylene glycol; PEG) encapsulated in poly(lactic-*co*-glycolic) acid (PLGA) microspheres by a solid-in-oil-in-water method. Encapsulation was evaluated through enzyme kinetics, thermal stability, and tertiary structure intactness.

Nanoencapsulation is efficient for the development of functional products and may help to solve difficulties such as loss of functionality during processing or in storage, incompatibilities between core and shell materials, generation of bad odors and flavors, deterioration of texture, and in the case of enzymes, lose of activity [39].

The formation of polymeric nanoparticles in water which are used for food or medicine is the result of the auto assembling of nanocomposites containing hydrophobic complexes in the center of the capsule and the even more hydrophobic shell [29, 40]. One way to produce amphiphilic structures is attaching or “grafting” dextran with

synthetic polymers [41]. This assembly can also be modified by the addition of hydrophobic functional groups [42].

Emulsions are mixtures of immiscible fluids. Usually, one of them, in the form of small droplets, forms the dispersed phase, and although the natural tendency of the emulsions is to coalesce, the rate and the extent of these phenomena will depend mainly on the droplet size of the emulsion (DSE) and composition of the phases. Depending on the DSE, emulsions can be classified into micro (10–100 nm), mini (100–1,000 nm), and macroemulsions (0.5–100 μm) [43]. Nano-submicron emulsions are kinetically stable systems that can be either transparent (DSE $<$ 200 nm) or "milky" (DSE $\approx$ 500 nm) [43, 44].

Interfaces between two immiscible fluids generate a tension also called interfacial energy which is caused by the difference in cohesion between the molecules of both phases. The generation of boundaries or interfaces implies energy costs proportional to the amount of generated interface, and the size distribution of droplets forming the emulsions plays a key role in determining the specific interfacial tension [45].

Colloidal stability of nanoemulsions can be controlled by handling the chemical nature and structure of the phases, and by handling the process of elaboration [19].

Formation of Nanoemulsions

Methods and principles for the preparation of nanoemulsions are those based on processes available for the fabrication of traditional-sized emulsions and on the applications of flow fields, and by using membranes and micro-channels [46]. In Table 1, a selection of works describing nanoemulsification techniques and applications in food-related areas is presented.

Preparation of Emulsions Using Flow Fields

One of the most frequent methods to produce emulsions is by inducing a very strong flow by agitation, intense mixing, or by flow through a small opening. Flow around a droplet induces a shear force that makes the droplets to break up, forming smaller droplets [55].

- Rotor–stator systems: the flow field between two elements becomes very intense within a very short distance. The most important equipments used are the stirred tanks as well as the colloid and toothed mills [56].
- High-pressure homogenizers: the mixture is pumped through a very small hole or gap. The extensional flows break up the large droplets into small ones. Valve and nozzle systems are used in these cases [57].
- Ultrasound systems: high frequency sound waves follow a pattern which propagates turbulence and induces fluctuation of pressure generated by an actuator which vibrates at specific frequencies. This technology has not yet been proved as efficient for industrial scale applications [58].

Preparation of Emulsions by Flow through a Membrane

In membrane treatments, fluid is forced through a membrane having pores of a certain diameter to decrease the size of droplets in the downstream side of the membrane. The main characteristics of these techniques are that flow fields applied are mild, and energy consumption is lower than for homogenization through a single orifice [59].

- Cross flow membrane emulsification [60]: if the purpose is to prepare oil in water emulsions, the membrane used should be hydrophilic, causing the oil to emerge out of a pore and forming small droplets on top of the mouth of pore as shown in Fig. 1.
- Dead-End ("Premix") membrane emulsification: coarse pre-emulsions need to be passed a number of times through the membrane to homogenize size of the emulsion [59, 61].

Preparation of Emulsions by Flow through Micro-Channels (Microfluidics)

In this technique, streams of fluids are forced to pass through a system of micro-channels arranged in a number of geometries in such a way that the dragging force exerted by the fluids when moving in the system and contact to each other causes the breaking down of the droplets. The operating principle of these equipments is based on shear stresses developed when the streams get into contact at high velocities. In all geometries of micro-channel devices depicted in Fig. 2, the relationship between radiuses of conducts for different fluids subjected to processing are, besides working pressure, key factors for obtaining good quality nanoemulsions with the desired size distribution. T-Junction geometry is usually applied for oil in water O/W emulsions, whereas cross and concentric junction arrangements are used for a wider range of needs such as preparation of water–oil–water or oil–water–oil emulsions and also to avoid coalescence of droplets and production of non-spherical particles.

Kawakatsu et al. [62], who developed this procedure, found that the size of produced droplets was related to the

Table 1 Examples of different methods of nanoemulsification in food-related areas

Method	Studied system	Reference
Phase inversion composition	Meyhod used to prepare O/W nanoemulsions in the W/oleylammonium chloride–oleylamine–C12E10/hexadecane ionic system, where the oleylammonium acted as a cationic surfactant. Nanoemulsions were prepared by continuous addition of HCl watery solutions to the mixtures formed by hexadecane, oleylamine, and C12E10 or by progressive addition of potassium hydroxide watery solutions to hexadecane–oleic acid–C12E10 mixtures. Progressively the pH decreased, resulting in a progressive ionization of the oleylamine. Likewise, the oleic acid was gradually ionized when an increase in pH is produced by the addition of KOH. The preparation conditions were maintained constant at a stirring rate of 750 rpm and an addition rate of 1.6 mL/min	[47]
High pressure homogenization	Oil-in-water (O/W) emulsions were prepared using β-carotene as the dispersed phase and Milli-Q water as the continuous phase. The premix was homogenized using a high-speed homogenizer at 5,000 rpm for 10 min to form a coarse emulsion, followed by a two-stage high pressure homogenization. Temperature and pressure, β-carotene concentration, the quadrics of emulsifier concentration and the interactions between β-carotene and emulsifier concentrations and between homogenization temperature and emulsifier concentration $p < 0.05$ had a significant effect on the stability of the emulsions	[48]
Ultrasonic	Nanoemulsion was prepared from a lipid mixture composed of 40 mg cholesteryl oleate, 20 mg egg phosphatidylcholine, 1 mg triolein, and 0.5 mg cholesterol	[49]
	Emulsification of lipids by prolonged ultrasonic irradiation in aqueous media and the procedure of two-step ultracentrifugation of the crude emulsion with density adjustment by addition of KBr to obtain the nanoemulsion	[50] [51] [52]
	The authors investigated the preparation of O/W nanoemulsion containing surfactants and oleoresin capsicum(OC) in four component systems, O/W nanoemulsions of OC could be prepared by the ultrasonication process at the ration of mixture of OC:Tween 80(1:0.7) and by self-assembly method at the ratio of mixture of OC:Tween 80(1:3) with a particle size of 20–100 nm and having a good stability during storage	
	Multibond metal alkoxides and ultrasonic pretreatment were used to produce nanoemulsions with large interfacial area which had the potential to be easily separated. The micelle size was observed to be as low as 5.1 nm. Viscosity and apparent vapor pressure reduction were also observed. The alcohol/soybean oil molar ratio was a main factor for apparent vapor pressure reduction	
	In this work, the authors created remarkably small transparent O/W nanoemulsions with average diameters as low as 40 nm from sunflower oil. This was possible using ultrasound or high shear homogenization and a surfactant/co-surfactant/oil system that is well optimized. The minimum droplet size was obtained when both droplet deformability (surfactant design) and the applied shear (equipment geometry) were optimal. Oil stability was unaffected by the sonication process	
Microfluidization	Pre-emulsions were prepared by dissolving sodium dodecylsulphate, (0.–5.6 wt%) and polyethylene glycol (0–18.9 wt%) in distilled deionized water and then adding the oil (15 vol%) using mixer Emulsions were prepared using a Microfluidics M-110Y MicrofluidizerTM by subjecting pre-emulsions to five passes at 1,000 bar, which was the maximum practical limit with the air supply of the Microfluidizer. Examination of the Ostwald ripening of mixed oil nanoemulsions found that the entropy gain associated with oil demixing provided a thermodynamic barrier to Ostwald ripening	[53] [54]
	The authors prepared nanoemulsion with a MicroFluidizer Processor®-containing delta, alpha, and gamma tocopherol, these nanoemulsions have enhanced anti-inflammatory properties and increased bioavailability, with gamma tocopherol, in particular compared to their suspensions	

geometry of the channel but independent of the flow. Micro-channels can be found on different sizes with various geometries. Microfluidics devices deal with small amounts of fluids and have the potential to significantly change the way of processing dispersed food systems [63].

Nanoencapsulation Procedures

Polymerization

Polymerization of miniemulsions causes the encapsulation by separation of phases during the process. Amphiphilic

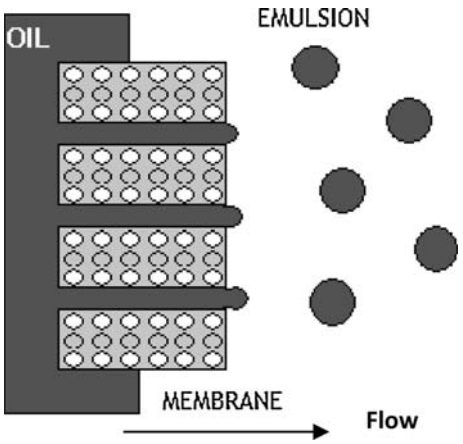

Fig. 1 Cross flow membrane emulsification

oligomers are used as surfactant agents to prepare the miniemulsion (oil particles from 50 to 500 nm dispersed in water). Due to the properties of amphiphilic oligomers, the molecules can auto assembly in the water/oil interface through ultrasonication. To form these products, a water-soluble initiator such as potassium persulfate may also be added, and from the mixture, the water-soluble radicals will emerge. After several additions of monomers, oligo-radicals begin to be active on the surface and are captured by mini packets of oil, causing that the active radicals of the surface link to the interface of those mini packets and water. Polymerization is carried out on the interface where the chains of the polymer gradually grow, allowing the formation of the core. Important factors during this process are the hydrophobicity and the structure of the oligomers which also have an influence on the morphology of the nanocapsules [64]. Ren et al. [65] prepared liquid-core nanocapsules by cross linking an amphiphilic copolymer at an oil–water interface. The hydrophilic copolymer poly[(ethylene oxide)-*co*-glycidol] was first obtained by anionic polymerization of ethylene oxide and ethoxyethyl glycidyl ether, then the hydroxyl groups on the backbone were recovered after hydrolysis and partially modified by hydrophobic conjugated linoleic acid. The copolymer with multiple linoleate pendants was absorbed at an oil–water interface and then cross linked to form stable nanocapsules. The mean diameter of the nanocapsule was below 350 nm, and the particle size could be adjusted by manipulating the emulsification conditions. It was found that the nanocapsules were stable in water for at least 5 months.

Another work highlighting several advantages of polymerization was carried out by Liang et al. [66] who investigated whether the entrapment of peptides within poly(alkyl cyanoacrylate) nanoparticles could be increased by functionalizing the peptide, so that it could copolymerize with the alkyl cyanoacrylate monomer; nanoparticles were prepared using both an aqueous micellar and a water-in-oil micro-emulsion polymerization template. Using the micellar template, nanoparticles could not be produced in the presence of acryloyl peptide, and rather, an agglomerated mass was formed on the stirrer. In contrast, nanoparticles could be prepared using both acryloyl and parent peptide using the water-in-oil micro-emulsion template. Encapsulation efficiency was more than twofold greater for the functionalized peptide.

Assembling Using Layer by Layer Techniques

By using layer-by-layer (LBL) techniques, it is possible to manufacture nano-scale films achieving a precise control of their thickness and uniformity [67]. LBL is based on adsorption of opposite-charged polyelectrolytes contained in a solution with a solid substrate having a charged surface [67–72]. Interactions among layers are affected by the pH and ionic strength of the system [73, 74]. In food areas, this technique has a wide range of applications such as the development of new edible coatings with improved functionality for fresh and minimally processed fruits as well as

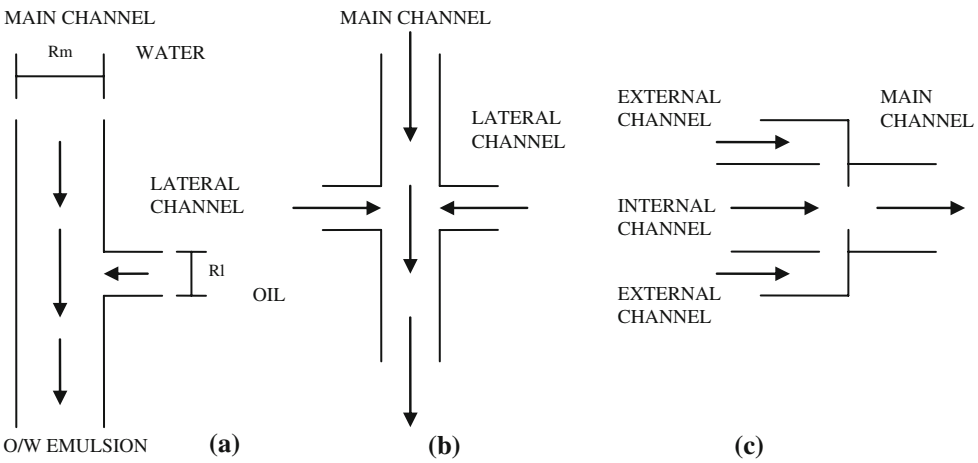

Fig. 2 Channel arrangements in microfluidics devices. **a** T-junction **b** Cross junction **c** Concentric junction

for the protection of aromatic compounds from those fruits [74].

In other works, this technique was applied as strategy to fabricate the nano-multilayer wall for micro and submicrocapsules by the LBL of polyelectrolytes, particularly polymers such as chitosan (CHI) and alginate (ALG) for drug-controlled release. By means of the LBL technique, immobilized enzymes of urease and superoxide dismutase on polystyrene nanoparticles presented a decrease in enzyme bioactivity but an increase in their storage stability. LBL self-assembly of CHI and ALG was used directly on indomethacin (IDM) microcrystals to reduce the releasing rates. The LBL multilayers have been used to encapsulate the drug-loading microparticles made from solvent evaporation or adsorption using porous $CaCO_3$ microparticles to enhance the loading capacity and suppress the initial burst. Increasing the number of layers, raising the deposition temperature, and cross linking of the neighboring layers slowed down the enzymatic desorption of polyelectrolyte multilayer films and the releasing rate of encapsulated drug [75].

Liposomes

Liposomes trapped in water-soluble compounds have been used in pharmacology and cosmetology. The encapsulation of antimicrobials in liposomes helps preserving their effectiveness and stability in various applications [13]. Principles involved in the formation of liposomes are those based on hydrophobic/hydrophilic interactions between lipid/lipid and lipid/water. The added energy (ultrasonication, homogenization, agitation, or heat) generates settlement of lipids in the form of bicapillar vesicles to attain thermodynamic equilibrium in the aqueous phase [76] as shown Fig. 3.

The capacity of liposomic carriers to encapsulate antimicrobial compounds depends on the interaction between the liposome membrane and the membrane of the bacteria.

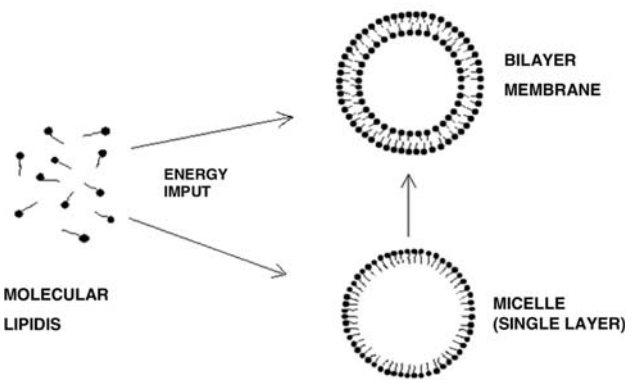

Fig. 3 Forming a liposome membrane (bi and single layer arrangements)

It has been observed that addition of positively charged nisin to a phospholipid anion such as diglycerol phosphate generates electrostatic interactions causing neutral liposomes to group together, thus generating hydrophobic interactions [77]. In another work, nanoliposomes were prepared from different lipids (Phospholipon 90H, Phospholipon 100H, dipalmitoylphosphatidylcholine (DPPC), stearylamine (SA), dicetyl phosphate (DCP), and cholesterol) and were tested for their capacity to encapsulate nisin Z and target bacteria (*Bacillus subtilis* and *Pseudomonas aeruginosa*). Nisin was entrapped in different nanoliposomes with encapsulation efficiencies (EE) ranging from 12 to 54%. Anionic vesicles possessed the highest EE for nisin, while increasing the cholesterol content in the lipid membranes up to 20% molar ratio resulted in a reduction in EE. Stability of nanoliposome-encapsulated nisin was demonstrated for at least 14 months at 4 °C (DPPC:DCP:CHOL vesicles) and for 12 months at 25 °C (DPPC:SA:CHOL vesicles) [78].

Other works such as those by Xia et al. [79] showed the effectiveness of food-related compounds to elaborating nanoliposomes. Coenzyme Q10 (CoQ10) was incorporated into nanoliposomes composed of egg yolk phospholipid, cholesterol, and Tween 80, and the influence of CoQ10 on the nanoliposomal structure was investigated. CoQ10 incorporation can suppress the increase in the *z*-average diameter of nanoliposomes during storage for 8 months at 4 °C. The liposomal lipid peroxidation caused by Fe(III)/ascorbate was also significantly inhibited. Fluorescence probe studies indicated that CoQ10 incorporation increases microviscosity of the nanoliposomes. Results suggested that CoQ10 might intercalate between lipid molecules and perturb the bilayer structure.

Dialysis

In this process, the core compound is added to the copolymer into an organic solvent. After repeated dialysis procedures against water, encapsulation is achieved [80]. Nanoparticles formed by using amphiphilic copolymers have been used as carriers of hydrophobic compounds [12] which form a shell that serves as a carrier of lipophilic drugs and stabilizes particles in aqueous dispersions, thus improving the kinetic stability of the capsules over those obtained by other methodologies [37]. Final product may be subjected to dehydration to stabilize capsules [81, 82]. There are not many application of dialysis in the nanofoods field, however, fundamentals of this methodology can be applied to situations such as those described by Min et al. [83] who prepared by chemical conjugation, hydrophobically modified glycol chitosan (HGC) nanoparticles containing water insoluble camptothecin (CPT). Insoluble anticancer drug, CPT, was easily encapsulated into HGC

nanoparticles by a dialysis method, and the drug-loading efficiency was above 80%. CPT-encapsulated HGC (CPT-HGC) nanoparticles formed nano-sized self-aggregates in aqueous media (280–330 nm in diameter) and showed sustained release of CPT for 1 week.

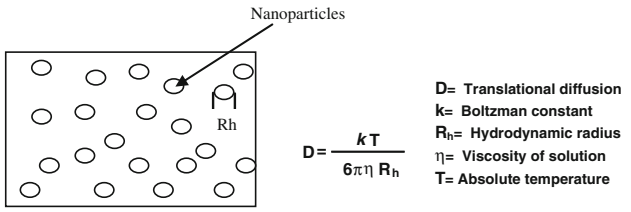

Fig. 5 Hydrodynamic radius and Stokes–Einstein equation

Proposal of a Classification and Characterization of Nanocapsules

The properties and appearance of nanocapsules depend, by far, on their size and on the size of particles forming the core. Particles called nanocapsules are those having a nano-size diameter, a micro porous structure or nano-sized core compounds, and combination of them as proposed in Fig. 4. The characterization of these materials includes the evaluation of their shape, texture, and appearance.

In the case of nanocapsules obtained by spray drying, it is important to characterize not only the nanoemulsion but also, the final dehydrated product. It is important to specify that it is not possible to assure that the final powder will have a similar distribution of sizes than the emulsion feed to the spray drier. Pre-emulsification, emulsification, and atomization in the drier may induce several phenomena including breakage or coalescence of particles [84].

There are various tools used to evaluate the particle size of nanoemulsions and nanopowders. The most commonly reported is the dynamic light scattering technique (DLS) which measures the Brownian motion of particles [85]. The smaller the particles, the faster will move; speed of motion is called translational diffusion coefficient (D) which is related to the radius of particle and obtained by means of the Stokes–Einstein equation as illustrated in Fig. 5.

The Zeta potential measures the overall charge a particle acquires in a specific medium giving an indication of the stability of a system. If particles have a large Zeta potential (negative or positive), they will repel each other, this means a higher stability than a neutral particles charge. The Zeta potential is a measure of the net charge, and there may be significant charge heterogeneities that can lead to aggregation, even though, the net Zeta potential suggests otherwise [86]. Gèze et al. [87] conducted pioneer works on characterization of nanoparticles by DLS, cryo-transmission electron microscopy, and Zeta potential within the food field. In their work, amphiphilic β-cyclodextrins (βCDa) were synthesized by statistically grafting hexanoyl carbon chains. The obtained derivative was used to prepare submicronic colloidal nanosphere suspensions using a nano-precipitation method. The fresh suspensions contained particles with diameters ranging from 60 to 100 nm. The long-term stability of the aqueous nano-dispersions was investigated. An unexpected good physical stability of the suspensions after 3-year storage at room temperature was observed. This behavior appears to be related to the small size and structural organization of the nanoparticles. The mean diameters determined from light scattering experiments were consistent with those measured from electron micrographs.

Different types of microscopes such as optic, confocal [88], transmission electronic [31], scanning electron (SEM), high-resolution transmission electron (HRETM), and atomic force (AFM) [40] have been used for the characterization of nanoparticles. Spectroscopic techniques

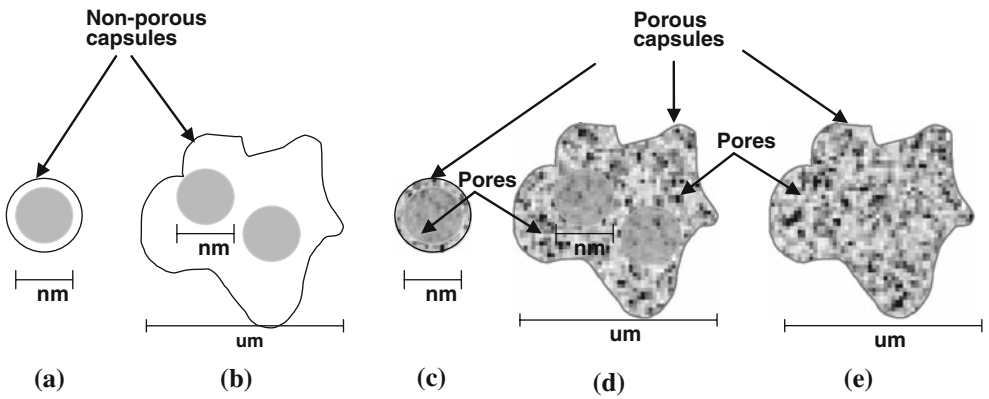

Fig. 4 Proposal of different nanostructuration patterns in capsules. **a** Non-porous nanocapsule containing a nano-sized core; **b** Non-porous microcapsule containing nano-sized cores; **c** Porous nanocapsule containing a nano-sized core; **d** Nanoporous microcapsule containing nano-sized cores; **e** Porous microcapsule with nanopores

coupled to SEM provide chemical composition by applying high angle annular dark field imaging (HAADF) and energy dispersive X-ray spectroscopy (XRD). Besides these high spatial resolution techniques, the use of X-ray photoelectron spectroscopy (XPS) may provide a coarse but sensitive view of the surface of particles. Some of the advantages of optical microscopes over the electronic ones are that the latter cannot see the actual features of the sample, since they deliver a generated image by electronic interactions between sample and electrons. Moreover, when using electronic vision systems, sample is more prone to be disrupted [89, 90]. A non-destructive microscopic technique with potential use in nanofoods-related areas is the environmental scanning electron microscope (ESEM); by using ESEM, it is possible to acquire images involving a minimum of manipulation of the objects in such a way that the presence of artifacts is minimized [91].

Freeze-fracture transmission electron microscopy was used to investigate the morphology of small unilamellar vesicles with a diameter of approximately 100 nm [92]. In this work, three different lecithins (SLP-WHITE, SLP-PC70 from Tsuji Oil Mill Co. Ltda Japan and PL30S from QP Co. Ltda Japan) were used for preparing liposomes. Also, their physicochemical properties were examined by using a confocal laser scanning microscopy (CLSM) and by measurements of trapping efficiency. CLSM observation showed that the particle size of liposomes prepared from SLP-WHITE was significantly smaller than those from other lecithins. In addition, liposomal solution prepared from SLP-WHITE remained well dispersed for at least 30 days, while two other liposomal solutions showed a phase separation due to aggregation and/or fusion of liposomes. SLP-WHITE liposomes were then prepared by using a homogenizer and a microfluidizer, aiming at improving the efficiency of preparation and the liposome stability.

Recently, Zabar et al. [93] studied amylase as vehicle for the nanoencapsulation of unsaturated fatty acids. Authors used three different structural strata of V-amylose, and nanostructures were examined by using XRD, DLS, nuclear magnetic resonance (NMR) as well as small angle x-ray scattering (SAXS). Using these methods, it was noted that decreased degree of fatty acid unsaturation induces the formation of organized and well-defined structures. SEM observations showed that structural trends extended even into the microscopic level. This study also showed that increased fatty acid unsaturation impairs structuration of amylase inclusion complexes.

Despite being able to observe and characterize samples obtained by different procedures, it is still difficult to identify the actual location of the different components of the nanoparticles. Possible interactions between core and wall materials are still difficult to evaluate [88]. Wall materials can act just as containers or may interact with core substances either holding them into the capsule or fixing them on their surface and even imbibing them as shown in Fig. 6. Confocal microscopy which delivers optical planes of the observed object may allow studying three-dimensional wall-core arrangements, and therefore the spatial location of their components.

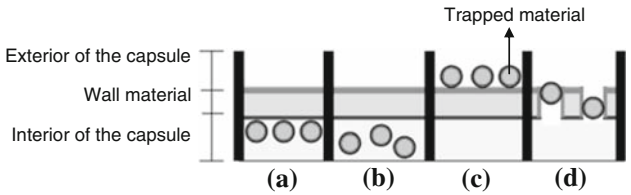

Fig. 6 Possible (*a*, *b*, *c*, *d*) locations of trapped material in capsules

Perspectives and Trends

Nanoencapsulation is a process that has been developed within the pharmaceutical field, and that has been applied on food-related fields for production of nanofoods and related products. There are, however, a number of issues that will have to be looked after for such application to become widely accessible by industry and consumers. Regulatory issues on nanofoods are still being developed, and it is expected that national bodies will increase initiatives to control, administrate, and promote proper development of nano-sized food-related products. Complex phenomena involved in nanotechnology and nanoengineering are related to interactions among different phases encountered in procedures in which, difficulties on solving turbulent–turbulent, laminar–turbulent multiphase interactions play key roles toward the developing of robust knowledge-base for designing pieces of equipments, processes, and products. Different disciplines and techniques are having increased and important roles in food nanotechnology such as advanced non-linear dynamics, solid state physics, biotechnology, computer vision systems together with novel interpretations of traditional concepts such as interfaces and product architecture. Also, the multiplicity of interactions among compounds frequently found in nanotechnology applications at molecular and atomic levels must be extensively and intensively studied with techniques that, in several cases, are still in the process of being developed, and that must be officially accepted by regulatory bodies. The main issues related to commercialization of nanoencapsulated composites are those derived from the evaluation of the practical use of nano-foods, including the appraisal of the advantages of the usage of these products against traditional ones of the same composition and bigger size.

Conclusion

Nanoencapsulation provides final product functionality (including controlled release of the core) which is expected to be maintained during storage. As far as production is concerned, stages of the process are important and determine structuring of the capsule. Trends in nanocapsule and nanoemulsion construction are related to manufacture, observation, and measurement of capsules as well as to the evaluation of the distribution of the size of the particle and interaction of wall and core materials and control of coalescence. It is expected that food engineering practice will make important profit of nanoencapsulated products in the near future given enhanced functional properties and stability. Diffusion of active compounds through the capsule has not been well understood and is, nowadays, a subject of major attention given the scale (nano) at which core substances migrate and interact with other components of the product. It is important to highlight that research and development on preparation of nanocapsules will include results of recent investigations on processes and equipments as well as on characterization techniques and will consider continuing adapting a number of tools from fields such as the medical, pharmaceutical and from physical, engineering, and chemical sciences.

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