A survey about release mechanism of bioactive compound through microencapsulation; Characterizing food proteins

Abstract—Microencapsulation is a useful approach to improve delivery of bioactive compounds into foods, particularly probiotics, minerals, vitamins, fatty acids, and antioxidants. Several microencapsulation techniques have been discussed for use in the food industry which show a promotion for the production of functional foods. Moreover, these technologies could improve the successful delivery of bioactive ingredients to the gastrointestinal tract. Proteins as natural polymers are heterogeneous mixtures of different sizes with a wide range of molecular weights. Studies revealed that protein nanoparticles successfully controlled the release rate of drugs for prolonged periods suggesting protein nanoparticles as efficient controlled release vehicles. Protein-based nanoparticles offer various possibilities for surface modification due to the presence of functional groups (i.e. carboxylic and amino groups) on the surface of the nanoparticles thus enabling specific targeting to the site of action. Concerning the cost; proteins are much less expensive compared to other functional carriers. This study tries to take a review at common release models of bioactive compounds and finally remarks the protein based food carriers of this compounds.

Keywords; Microencapsulation; bioactive compound; release mechanisms; Food proteins

I. INTRODUCTION

Encapsulation technologies have gained increased interest to the food industry as they are applied to sustain stability of the bioactive compounds during processing and storage also to prevent undesirable interactions with the food matrix [3]. Further, a real benefit of encapsulation is due to controlled release of the incorporated ingredients and deliver them to a specific target in a suitable time. Protein hydro gels have been used as a particle carrier because of their high nutritional value and generally recognized as safe; also proteins would compensate potentiometric changes because of their buffer based characteristics [1].

II. ENCAPSULATION METHODS

A. Extrusion

To form microcapsules by the extrusion method, a bioactive compound suspension is mixed with a solution of hydrocolloids, and the mixture is dripped into a solution containing multivalent cations (CaCl₂). By addition of sodium alginate droplets to a calcium solution, interfacial polymerization appears by precipitation of calcium alginate followed by a more gradual gelation of the interior as calcium ions permeate through the alginate systems. High payload up to 30% can be expected. The size and the shape of the capsules depend on the diameter of the orifice, the distance of free fall into the hardening solution, the concentration of the calcium chloride in the hardening solution, and the viscosity of the hydrocolloid-compound mixture. Predominantly relatively large capsules in size ranges above 0.5 mm up to 5 mm are produced [1,3].

B. Emulsion

In the emulsion technique, a small bulk of compound-polymer suspension (discontinuous phase) is added to a large volume of a vegetable oil (continuous phase) such as sunflower oil. The mixture is homogenized to form water in oil (w/o) emulsion. In some cases emulsifiers are added to form more stable emulsions, since these agents lower the surface tension of droplets leading to smaller spheres. Once the emulsion is formed, solidification occurs after the addition of an adequate solidifying agent to the emulsion. Adjustment of agitation speed and phase ratio enables production of the targeted bead size [1]. The emulsion technique is the most commonly used method for the microencapsulation of probiotic cells which by this technique generally smaller capsules can be produced, as compared to the extrusion technique. While the extrusion method leads to a very narrow particle size distribution, given by the geometry of the nozzle, a wider distribution of particle sizes is a general limitation of the emulsion technique [2].

C. Spray drying

Spray-drying is known as a routine process in food technology, converting liquids into dry powders. In this case, mixtures of compound concentrates with aqueous solutions of various polymers, such as modified starch, gum acacia, gum Arabic, gelatin whey protein isolate and β-cyclodextrin. The advantage of spray-drying is that sufficient small capsules with average diameters below 100 μm are usually generated at comparably low costs and the process is widely established in food technology [3].

Drying takes place at relatively high temperatures (210°C inlet and 90°C outlet), though the emulsion’s exposure to these temperatures lasts only for few seconds. The process results in free flowing, low bulk density powders of 10–100 micron sizes. Optimal payloads of 20% can be expected for flavors encapsulated in starch matrices. However, it must be considered that microcapsules prepared by this method are water soluble in most cases and therefore not suitable to protect probiotic cells in liquid formulations and in the human gastrointestinal tract. Furthermore, it must be considered that the high temperatures and rapid dehydration during spray-drying generally leads to a deterioration of the cells, resulting in a significant loss of living cells and a diminished resistance against following adverse environmental conditions [3].

D. Protein-based microcapsules

In contrast to the commonly used hydrocolloids, even highly concentrated aqueous solutions from many proteins
still have a relatively low viscosity and can therefore be used as precursor for microencapsulation. This enables the formation of microcapsules with a high-density gel network [4].

E. Comparison Of methods

In addition to the matrix material, the chosen encapsulation technique determines the physical characteristics of the resulting capsules. While spray drying processes are relatively cheap and comparably small capsules are created, as a disadvantage they are mostly water soluble. In contrast to this, predominantly large, water insoluble capsules are generated by the extrusion method. In comparison, the emulsion technique has the advantage that smaller capsules can be created. However, the resulting capsules must be separated from oil which is more difficult, instead of separation from an aqueous hardening solution [2,5].

III. BIOACTIVE COMPOUND RELEASE MECHANISM

A. Diffusion

Diffusion is the most common mechanism of bioactive compounds release; in which the dissolution fluid penetrates the shell then the core material comes into the contact with the dissolution fluid and leak out through the interstitial channels or pores. Basically, the release of core material depends on:

[1] the rate of bioactive compounds dissolution in the dissolution fluid
[2] the rate of penetration of dissolution fluid to the microcapsules
[3] the rate at which the dissolved bioactive compounds escape from the microcapsule.

The kinetics of such bioactive compounds release follows Higuchi’s equation:

\[ Q = \frac{D}{J} (2A - \varepsilon, C_s) \right ] \cdot C_i \cdot t^{1/2} \]

Where \( Q \) is the amount of bioactive compounds released per unit area of exposed surface in time \( t \); \( J \) is the tortuosity of the capillary system in the wall; \( D \) is the diffusion coefficient of the solute in the solution; \( A \) is the total amount of bioactive compounds per unit volume; \( \varepsilon \) is the porosity of the wall material and; \( C_i \) is the solubility of bioactive compounds [5].

B. Dissolution

The release rate of bioactive compounds from the microcapsule depends on the dissolution rate of polymer coat, when the coat is soluble in the dissolution fluid. The solubility in the dissolution fluid and thickness of coat influence the release rate.

C. Osmosis

Another method of bioactive compounds release is through osmosis. The essential requirement of osmosis is semi permeable membrane and in microcapsule polymer coat serve the purpose. As the process progress an osmotic pressure is created between the outside and inside membrane of microcapsule which result in release of bioactive compounds through small pores [1].

D. Erosion

Erosion of coat generally occur due to pH or enzymatic hydrolysis and causes bioactive compounds release with certain coat materials like stearyl-alcohol and glyceryl monostearate. Here are some common model which can predict and describe the release rate: [5,7]

Applied release models

Zero order : \( M_i - M_t = Kt \)
First order: \( \ln M_i - \ln M_t = K.t \)
Hixson – Crowell : \( (M_i)^{1/3} - (M_t)^{1/3} = K.t \)
Peppas : \( M_t / M_i = k.t^n \)

E. Controlled release mechanisms

Delayed release

Delayed release is a mechanism whereby the release of an active substance is delayed from a finite “lag time” up to a point when/where its release is favored and is no longer hindered. Examples of this category include encapsulating probiotic bacteria for their protection from gastric acidity and further release in the lower intestine, flavor release upon microwave heating of ready-meals or the release of encapsulated sodium bicarbonate upon baking of a dough or cake batter [7,8].

Sustained release

Sustained release is a mechanism designed to maintain constant concentration of an active at its target site. Examples of this release pattern include encapsulating flavors and sweeteners for chewing gum applications so that their rate of release is reduced to maintain a desired flavor effect throughout the time of chewing [5].

Burst Release Mechanism

Burst release is simply described by a high initial delivery of an entrapped active, before the release reaches a stable profile, thus reducing the system’s effective lifetime and complicating the release control. Although burst release may be preferred for flavor high- impact applications.
Burst release can most often take place in reservoir and hydrogel systems, though it can still take place in matrix designs. Reasons for this range from cracks in the protective capsule shell to storage effect where the membrane becomes saturated with the active substances or due to very high active loading. When placed in a release medium, the active can quickly diffuse out of the membrane surface causing a burst effect. Low-molecular-weight actives frequently undergo burst release, a result of high osmotic pressure and increased concentration gradient [8]. Finally it is essential to mention that Particle size is one of many parameters that may be adjusted to control release rates of encapsulated ingredients.

IV. CHARACTERIZING FOOD PROTEINS

A. Gelatin
Gelatin is a denatured protein that is obtained from collagen by acid and alkaline hydrolysis. It is considered as GRAS material by the FDA. It has a relatively low antigenicity because of being denatured. Its functional groups are accessible for various chemical modifications, which may be especially useful in developing targeted drug delivery vehicles. Gelatin has both cationic and anionic groups along with hydrophobic groups. The repeating sequences of Gly-Pro-Ala amino acid triplets are responsible for its triple helical structure. In terms of sustained release, less initial burst and safety and more stability [6].

B. Collagen
Collagen is the most abundant mammalian protein accounting for about 20–30% of total body proteins. Some disadvantages of collagen-based systems arose from the difficulty of assuring adequate supplies and their poor mechanical strength. Due to their small size with a large surface area, high adsorptive capacity and ability to disperse in water to form a clear colloidal solution, collagen-based nanoparticles have been used as a sustained release formulation for antimicrobial agents or steroids [4, 6].

C. Casein
Casein, the major milk protein, is inexpensive, available, nontoxic and highly functional. As a natural food product, this GRAS protein is biocompatible and biodegradable. Many of the structural and physicochemical properties of caseins facilitate their functionality in drug delivery systems including binding of ions and molecules, exceptional surface-active stabilizing properties, excellent emulsification and self-assembly properties together with super gelation and water binding capacities [4]. Additionally, caseins are not sensitive to temperature, whereas whey proteins show important denaturation at temperatures above 70 °C [9].
Mainly four casein phosphoproteins, αS1-, αS2-, β- and κ-casein in cow milk with molecular weights between 19 and 25 kDa and an average isoelectric point (pI) between 4.6 and 4.8. Casein-based nanoparticles as drug delivery systems were previously reviewed. Caseins are amphipilic proteins that can be thought as block copolymers with high levels of hydrophobic or hydrophilic amino acid residues. Therefore, caseins exhibit a strong tendency to self-assemble into spherical casein micelles 50–500 nm in diameter [9]. Casein micelles effectively protected vitamin D2 and the ω-3 polyunsaturated fatty acid docosahexaenoic acid (DHA) against UV-light-induced degradation and oxidation, respectively [4].

D. Whey proteins
Beta lactoglobulin (βLG), the major whey protein in cow milk and its principal gelling agent, is a small (18.3 kDa) globular protein with two disulfide bond and a free thiol group which is not accessible to solvent at or below neutral pH. Because it can maintain a stable globular conformation, β-LG is known to be stable at low pH and highly resistant to proteolytic degradation in the stomach. Alpha-lactalbumin (α LA), the second most prevalent whey protein in cow milk, is a smaller globular metalloprotein with four disulfide bridges. Commercially, albumins are obtained from egg white (ovalbumin), bovine serum (bovine serum albumin, BSA) and human serum. [10]

E. Elastin
Elastin is the dominant extracellular matrix protein deposited in the arterial wall conferring the properties of extensibility. Elastin undergoes a self-aggregation process in its natural environment where it is produced from a water soluble precursor, tropoelastin, which spontaneously aggregates into a covalently cross linked fibrillar polymeric structure [4]. Another advantage is that they may be engineered to undergo a rapid phase transition in response to temperatures suitable for adjuvant, clinical therapies such as microwave thermal ablation. At temperatures above the transition temperature (Tc), recombinant thermo-sensitive ELPs undergo a reversible phase transition where they hydrophobically self-assemble into an insoluble aggregate, forming nano and micro particles which could be applied as controlled release devices [9].
F. Zein

Zein is a water-insoluble but alcohol-soluble protein with a molecular weight of about 40 kDa that is predominantly present in the endosperm of corn kernels. It contains three quarter of lipophilic and one quarter of hydrophilic amino acid residues. Commercial zein is currently separated from corn gluten meal, a co-product of corn wet milling and is a mixture of at least four types of proteins: α-, β-, γ- and δ-zein, each with a different amino acid sequence, molecular weight and solubility. Zein has been employed as an edible coating for foods because it shows low water uptake, high thermal resistance and good tensile properties [4,3].

Because of its high hydrophobicity, zein has been successfully applied as a promising carrier for encapsulation and controlled release of fat-soluble compounds (e.g. gitoxin). In addition, the protein structure allows zein to function as a polymeric amphiphile where it exists as small globules with diameters between 150 and 550 nm in aqueous ethanol solution. The zein molecule has a very special bricklike shape and thus has a potential to carry other molecules inside them. Sustained release of water-soluble drugs such as heparin was observed in vitro over 9 and 20 days, respectively, from the films made of zein microspheres. Liquid–liquid dispersion process was used to produce zein nanoparticles. In this process, zein was dissolved in 70% v/v aqueous ethanol, followed by shearing zein solutions into deionizer water using a high-speed homogenizer [4]. This process was later used to microencapsulate spice essential oilsoregano, red thyme. Zein nanocapsules showed sustained release of water-soluble lysozyme from at neutral pH. Similarly, zein nanoparticles were used to encapsulate fish oil in solid as an alternative to emulsions. In a recent study, zein nanoparticles coated with carboxymethyl chitosan provided better controlled release of vitamin D₃ and improved its photo stability against UV light compared to uncoated nanoparticles [6, 2].

G. Gliadin

Gliadin is a protein complex of which is index of cereal grains, respectively wheat. Two main fractions are present: gliadins, which is soluble in 70% ethanol, structured of single chain polypeptides with an average molecular weight of 25–100 kDa linked by intra-molecular disulphide bonds and glutenin, an alcohol insoluble part consisting of gliadin-like subunits stabilized by intermolecular disulphide bonds with molecular weight greater than 106 kDa. All fractions have remarkably low solubility in aqueous solution except at extreme pH [6]. This low water solubility has been attributed to the presence of disulphide bonds and to the cooperative hydrophobic interactions which cause the protein chains to assume a folded shape. The amino acid composition shows that gliadin has equal amounts of polar and neutral amino acids, mainly glutamine (about 40%) in addition to high proline content (14%).

A biphasic pattern of release was observed with an initial burst effect followed by zero-order diffusion. Gliadin nanoparticles were shown to be suitable controlled release systems for hydrophobic and amphiphilic drugs [4]. The high bioadhesive capacity of gliadin nanoparticles may be explained by gliadin composition being rich in neutral and lipophilic residues where neutral amino acids can promote hydrogen bonding interactions with the mucosa. It was observed that gliadin nanoparticles dramatically increased the carbazole oral bioavailability up to 49% and provided sustained release properties related to the bioadhesive capacity of gliadin nanoparticles with the stomach mucosa after oral administration [8].

H. Soy proteins

Soybean, from the most cultivated plant in the world, is rich in proteins (40–50%), lipids and carbohydrates. Soy protein isolates (SPI) are also used as emulsifiers in food emulsions because of their surface active properties of their constitutive proteins; the storage globulins 7 S (β-conglycinin) and 11 S (glycinin) [6]. Electrospray nanofibers (200 nm to 2 μm) fabricated from soy protein isolate (SPI)/poly (ethylene oxide) (PEO) blend and poly (lactic acid) were used for controlled release of a naturally occurring antimicrobial compound, allyl isothiocyanate (AITC). Release of AITC was negligible under dry conditions, but increased dramatically as relative humidity increased [4].

V. CHALLENGES

One of the main drawbacks of the animal protein-based nanoparticles is their inability to achieve a sustained drug release due to their hydrophilic nature and rapid solubilization in aqueous environments. When the system absorbs water and swells, bioactive compounds may rapidly diffuse out. Chemical cross linkers (e.g. glutaraldehyde and formaldehyde) usually used to harden protein nanoparticles suffer from the presence of residual untouched cross linker inside the nanoparticles together with the risk of formation of toxic products by reaction with the tissues during in vivo biodegradation this problem could be overcome by using hydrophobic plant proteins with no need for cross linking.

VI. CONCLUSION

Protein nanoparticles are likely to be well tolerated in vivo without deleterious side effects. They offer several advantages over synthetic polymers being GRAS drug delivery devices with high nutritional value and abundant...
renewable sources. As related to safety, they are metabolizable in vivo by digestive enzymes into innocuous peptides whereas synthetic polymers may give harmful degradation products. Additionally, protein nanoparticles exhibit high loading capacity of various bioactive compounds due to multiple binding sites present in their molecules. They exhibit a variety of possible drug loading mechanisms including electrostatic attractions, hydrophobic interactions and covalent bonding. Finally in order to maximize the best delivery of bioactive compounds in body, it is necessary to know right release kinetic model of them for preventing them from the intrinsic stresses.

REFERENCES