Historical perspective

Encapsulation, protection, and delivery of bioactive proteins and peptides using nanoparticle and microparticle systems: A review

David Julian McClements

Department of Food Science, University of Massachusetts Amherst, Amherst, MA 01003, USA

Abstract

There are many examples of bioactive proteins and peptides that would benefit from oral delivery through functional foods, supplements, or medical foods, including hormones, enzymes, antimicrobials, vaccines, and ACE inhibitors. However, many of these bioactive proteins are highly susceptible to denaturation, aggregation or hydrolysis within commercial products or inside the human gastrointestinal tract (GIT). Moreover, many bioactive proteins have poor absorption characteristics within the GIT. Colloidal systems, which contain nanoparticles or microparticles, can be designed to encapsulate, retain, protect, and deliver bioactive proteins. For instance, a bioactive protein may have to remain encapsulated and stable during storage and passage through the mouth and stomach, but then be released within the small intestine where it can be absorbed. This article reviews the application of food-grade colloidal systems for oral delivery of bioactive proteins, including microemulsions, emulsions, nanoemulsions, solid lipid nanoparticles, multiple emulsions, liposomes, and microgels. It also provides a critical assessment of the characteristics of colloidal particles that impact the effectiveness of protein delivery systems, such as particle composition, size, permeability, interfacial properties, and stability. This information should be useful for the rational design of medical foods, functional foods, and supplements for effective oral delivery of bioactive proteins.

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There is great interest in the oral delivery of various types of bioactive proteins and peptides because of their potential health benefits, such as hormones, enzymes, vaccines, antimicrobials, and nutraceuticals [1–7]. For the sake of concision, these types of biologically active proteins and peptides will be referred to collectively as “bioactive proteins” for the remainder of this article. Bioactive proteins exhibit a broad spectrum of biological activities, which make them of interest for application in foods, supplements, and medicines. For instance, oral delivery of lipase aids in the breakdown of lactose into galactose and glucose within the small intestine, which is important for individuals with lactose intolerance [8,9]. Similarly, oral delivery of lipase can help patients with pancreatitis, i.e., the inability to breakdown lipids within the small intestine [10]. Bioactive proteins may also include various kinds of hormones, such as insulin and glucagon-like peptide-1 (GLP-1) which are used to treat diabetes [11] or angiotensin converting enzyme (ACE) inhibitors which are used to treat hypertension [12]. Certain peptides have strong antimicrobical activity, and can therefore be utilized as therapeutic agents [5]. However, there are a number of important technical challenges that have to be overcome before these bioactive proteins can be successfully delivered through the oral route. Typically, bioactive proteins must have a specific three-dimensional structure to exhibit their beneficial biological activities [13]. Proteins may undergo appreciable changes in their molecular structure within commercial products (such as functional foods, supplements, and drugs) during manufacturing, transport or storage, as well as inside the gastrointestinal tract (GIT) after ingestion. These structural changes may be brought on by alterations in environmental conditions, such as pH, ionic strength, denaturants, temperature, and enzyme activity [14]. In particular, many proteins are susceptible to degradation within the highly acidic and protease-rich environment of the human stomach [15]. Consequently, bioactive proteins often have to be encapsulated so as to protect them during storage and after ingestion, but then release them at the appropriate site of action within the human body [3,16,17]. Numerous types of colloidal delivery systems with different structural designs have been developed to encapsulate bioactive proteins (Fig. 1), with each system having its own advantages and disadvantages. The selection of the most efficacious oral delivery system for a specific application depends on a thorough understanding of the factors that impact the loading, retention, stability, and release of the proteins in that specific system. The aim of this review article is therefore to provide a critical evaluation of some of the most important factors that impact the development of oral delivery systems for bioactive proteins based on drug-grade nanoparticles and microparticles. This type of colloidal particle is assembled from drug-grade ingredients (such as proteins, polysaccharides, lipids, surfactants, and mineral oils) using drug-grade processing operations. These colloid systems could therefore be widely utilized in medical foods, functional foods, or supplements specifically designed to deliver bioactive proteins via the oral route.

2. Protein characteristics

The first factor to consider when identifying an appropriate colloidal delivery system for a specific application is the molecular and physicochemical properties of the bioactive proteins to be encapsulated.
Bioactive proteins vary considerably in their molecular weights, conformations, electrical characteristics, polarities, and stabilities [13], which will impact their loading, retention, stability, and release in colloidal delivery systems. In this section, a brief overview of the impact of molecular and physicochemical properties of bioactive proteins that may impact the design of colloidal delivery systems is given.

2.1. Molecular dimensions

The dimensions of proteins in aqueous solutions depend on their molecular weight, conformation, and aggregation state, and may have a major impact on their retention and release within colloidal delivery systems. The molecular weight of individual bioactive proteins may vary from around 1 kDa for relatively small peptides to around 100 kDa for relatively large proteins. The conformations of bioactive proteins are mainly determined by their specific biological functions, and can be conveniently classified as globular, random coil or helical [13]. At the same molecular weight, the dimensions of proteins in solution depend strongly on the configuration they tend to adopt, with globular proteins being considerably smaller than random oil or helical proteins. Proteins may exist as individual molecules, small clusters, or large aggregates depending on solution conditions, such as pH, ionic strength, and temperature [18,19]. Consequently, proteins may vary considerably in their molecular dimensions, from a few nm (for small isolated globular proteins) to a few 100 nm (for aggregated proteins). Knowledge of the molecular dimensions of proteins under different solution conditions is therefore important for developing appropriate delivery systems. For W/O microemulsions or emulsions (Fig. 1), a bioactive protein should be smaller than the hydrophilic domains (water droplets) inside this kind of colloidal delivery system if it is going to be successfully encapsulated [20]. Conversely, for polymeric colloidal particles such as microgels (Fig. 1), a bioactive protein should be considerably larger than the pore size if it is going to be trapped inside the particles through steric hindrance effects. The impact of the molecular dimensions of proteins on their retention and release from polymeric colloidal particles is discussed in a later section.

2.2. Electrical characteristics

The electrical characteristics of proteins are also important in determining their encapsulation properties, as changes in electrostatic interactions between proteins and colloidal particles are often used to tune their retention and release properties [21–23]. The electrical properties of proteins depend on the number of exposed anionic (e.g., $\text{COOH} \leftrightarrow \text{COO}^- + \text{H}^+$) and cationic (e.g., $\text{NH}_2 + \text{H}^+ \leftrightarrow \text{NH}_3^+$) groups on their surfaces, and the pH of the surrounding solution. Typically, the electrical charge goes from positive to neutral to negative as the pH is increased from below to above the isoelectric point (pI) of the protein (Fig. 2). Some of the isoelectric points of common bioactive proteins are included in Table 1. Experimentally, the electrical characteristics of proteins can be conveniently characterized by measuring the change in $\zeta$-potential with pH using micro-electrophoresis methods.

Knowledge of the electrical characteristics of bioactive proteins can be extremely important in designing effective colloidal delivery systems. For instance, the retention and release of a bioactive protein from a polymeric colloidal particle depends on the charge characteristics of the biopolymers from which it is constructed. Thus, proteins will be attracted to networks consisting of anionic biopolymers (such

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Fig. 1. Schematic diagrams of some common types of colloidal delivery systems for encapsulation of hydrophilic bioactive proteins so that they can be incorporated into aqueous-based products.
as alginate, carrageenan, or pectin) at pH values below their pI, but released at higher pH values [24,25]. Conversely, it would be expected that proteins would be attracted to networks consisting of cationic biopolymers (such as chitosan or polylysine) above their pI, but released at lower values. It should be highlighted that the strength of electrostatic interactions is weakened in the presence of salts due to electrostatic screening effects [26]. Consequently, for practical applications, it may be difficult to retain bioactive proteins inside colloidal particles using this approach because of the relatively high salt contents found in many commercial products.

### 2.3. Polarity, solubility, and surface activity

The polarity of proteins is important because it determines their solubility characteristics, as well as their interactions with each other and with other substances. Proteins vary from being very hydrophilic to very hydrophobic depending on the relative proportion of polar and non-polar groups exposed at their surfaces [27]. Consequently, bioactive proteins may be either soluble or insoluble in aqueous solutions depending on their surface polarities (Table 1). Some bioactive proteins have good surface activity because they have an appropriate balance of polar and non-polar groups on their surfaces, i.e., they can adsorb to air-water, oil-water, or solid-water interfaces [28]. Knowledge of the polarity of proteins can be particularly important for designing effective colloidal delivery systems.

### 2.4. Stability

The native structure of proteins may be altered appreciably when environmental conditions are changed, such as pH, ionic composition, or temperature [29,30]. Changes in protein conformation often lead to a loss in biological activity, and therefore it is important to clearly establish the major physical and chemical factors that impact the stability of the protein to be encapsulated. Globular proteins undergo appreciable conformational changes when they are heated above their thermal denaturation temperature ($T_m$), whose value depends on protein type and local environmental conditions (such as pH, ionic strength, and dielectric constant) [27]. In addition, they may undergo conformational changes when they adsorb to certain interfaces, which is known as surface denaturation, and can also lead to a loss of activity [31,32]. They may also become denatured under highly acidic or alkaline conditions [33], when exposed to certain types of salts [34], or in the presence of certain types of surfactant [35]. These changes in structure and activity may be reversible or irreversible depending on the nature of the protein and the environmental conditions. Consequently, it is important to identify the range of temperatures, pH values, and ingredient interactions where a bioactive protein retains its activity. Colloidal delivery systems can sometimes be designed to extend this range of conditions, and therefore enhance protein stability and functionality.

### 3. Challenges to oral protein delivery

There are a number of major hurdles that must be overcome before bioactive proteins and peptides can be successfully delivered via the oral route [36,37]. In this section, some of the most important hurdles are highlighted, as well as some possible strategies to overcome them.

#### 3.1. Product stability

##### 3.1.1. Challenge

Bioactive proteins may be incorporated into functional foods, supplements, or medical foods that have different physicochemical properties and storage requirements. For instance, proteins may be delivered in the form of fluids, gels, pastes, powders, or bulk solids, which may be exposed to different temperature, light, oxygen, and humidity levels. As a result, the proteins may become denatured, aggregated, or hydrolyzed during the manufacture, storage, transport or utilization of commercial products, thereby reducing their biological activity and efficacy [38–41]. Consequently, knowledge of the composition and structure of the matrix surrounding proteins in commercial products is important, as well as information about the environmental stresses that they might encounter during the lifetime of the product. In addition, the range of conditions where the bioactive proteins maintain their structure and activity should also be clearly defined.

##### 3.1.2. Potential solutions

Knowledge of the environmental factors and ingredient interactions that adversely alter the structure and activity of a specific bioactive protein can be utilized to design a product matrix and processing operations that will minimize any damage to it. For instance, if the temperature, pH, and water-activity ranges that promote protein denaturation are known, then the product can be designed to avoid these

<table>
<thead>
<tr>
<th>Protein</th>
<th>MW (Da)</th>
<th>Conformation</th>
<th>pI</th>
<th>$T_m$ (°C)</th>
<th>Polarity</th>
<th>Biological activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipase (pancreatic)</td>
<td>51,000</td>
<td>Globular</td>
<td>4.9</td>
<td>70</td>
<td>Amphiphilic</td>
<td>Digestive enzyme: hydrolyzes lipids</td>
</tr>
<tr>
<td>Lactase</td>
<td>465,400</td>
<td>Globular (tetramer)</td>
<td>4.61</td>
<td>86</td>
<td>Amphiphilic</td>
<td>Digestive enzyme: hydrolyzes lactose</td>
</tr>
<tr>
<td>Amylase</td>
<td>55,000</td>
<td>Globular</td>
<td>6.5–7.0</td>
<td>61</td>
<td>Amphiphilic</td>
<td>Digestive enzyme: hydrolyzes starch</td>
</tr>
<tr>
<td>Insulin</td>
<td>5808</td>
<td>Dimer</td>
<td>5.3</td>
<td>76</td>
<td>Amphiphilic</td>
<td>Hormone: modulates glucose levels in the blood</td>
</tr>
<tr>
<td>GLP-1</td>
<td>3298</td>
<td>Flexible coils</td>
<td>4.6</td>
<td>–</td>
<td>Amphiphilic</td>
<td>Hormone: enhances insulin secretion</td>
</tr>
<tr>
<td>Ghrelin</td>
<td>3371</td>
<td>Flexible coils</td>
<td>11.5</td>
<td>–</td>
<td>Amphiphilic</td>
<td>Hormone: regulates appetite</td>
</tr>
<tr>
<td>Nisin</td>
<td>3354</td>
<td>Flexible coils</td>
<td>8.5</td>
<td>–</td>
<td>Hydrophobic</td>
<td>Antimicrobial: inhibits microorganisms</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>14,000</td>
<td>Globular</td>
<td>11</td>
<td>72</td>
<td>Amphiphilic</td>
<td>Antimicrobial: inhibits microorganisms</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>77,000</td>
<td>Globular</td>
<td>8.7</td>
<td>61 and 93</td>
<td>Amphiphilic</td>
<td>Antioxidant: inhibits oxidation of lipids</td>
</tr>
</tbody>
</table>
environmental stress factors. In some cases, encapsulation of bioactive proteins in delivery systems can be used to improve the stability of bioactive proteins by altering their local environment [42,43].

3.2. Gastrointestinal stability

3.2.1. Challenge

Many bioactive proteins are highly susceptible to denaturation, aggregation, and hydrolysis when exposed to gastrointestinal fluids [39,41]. In particular, the highly acidic environment of the gastric fluids within the stomach may promote protein denaturation and aggregation [44,45]. The extent of these effects depends on the structure and properties of the bioactive proteins, as well as the nature of any foods consumed with them. In addition, digestive enzymes (such as proteases) in the mouth, stomach, and small intestine can promote hydrolysis of proteins [46,47]. The extent, rate, and nature of hydrolysis depend on the molecular structure of the bioactive proteins involved, as well as their environment. Consequently, many bioactive proteins may lose their biological activity when they are exposed to the fluids within the gastrointestinal tract.

3.2.2. Potential solutions

Knowledge of the impact of specific GIT conditions on the structure and activity of a bioactive protein can be utilized to design an effective delivery system that inhibits these changes. For instance, if a bioactive protein is normally denatured and hydrolyzed in the stomach due to the high acidity and enzyme activity of the gastric fluids, then a delivery system can be developed to isolate it from these stressors. For instance, it has been shown that digestive enzymes (such as lactase and lipase) can be trapped inside biopolymer microgels that maintain a neutral internal pH under gastric conditions, which greatly enhances their stability and activity [42,43].

3.3. Absorption

3.3.1. Challenge

Another major factor that limits the efficacy of bioactive proteins is their relatively low absorption within the gastrointestinal tract [39,41]. The proteins must diffuse through the gastrointestinal fluids and across the mucus layer before they reach the surfaces of the epithelium cells (Fig. 3). The rheological properties of the gastrointestinal fluids impact the mixing and transport of the bioactive proteins, thereby impacting their residence time within certain regions of the GIT, as well as their absorption rate. The gastrointestinal fluids may vary from relatively low viscosity fluids to highly viscous gels depending on the type and amount of foods consumed [48–50]. The mucus layer consists of a highly viscous network of cross-linked mucin molecules and other substances (e.g., enzymes, lipids, and mineral ions) that coats the GIT and protects it from damage [51–53]. Bioactive proteins (or the colloidal particles containing them) may not be able to enter the mucus layer, or they may be trapped within the mucus layer and hydrolyzed by digestive enzymes before reaching the epithelium cells [37]. Moreover, once the bioactive proteins do encounter the epithelium cells they must be absorbed, which is often challenging because of their relatively large size and polarity. In principle, absorption may occur through a variety of physiological mechanisms, including transcellular (passive or active), paracellular (T-junctions), and endocytosis mechanisms [37]. Proteins (or colloidal particles containing them) may be absorbed by enterocytes or M-cells depending on their dimensions and surface characteristics. Typically, the overall extent of intact protein absorption is relatively low because of these barriers, and so special approaches must be developed to increase it.

3.3.2. Potential solutions

A number of different methods can be used to increase the absorption of bioactive proteins by the epithelium cells. First, permeation enhancers can be included in a protein delivery system that increase the absorption of bioactive proteins by the epithelium cells before they can reach the systemic circulation.

Fig. 3. Proteins or protein-loaded particles must move through the lumen and mucus layer and be absorbed by the epithelium cells before they can reach the systemic circulation.
the permeability of the cell membranes (transcellular) or that open up the tight junctions separating the cells (paracellular), thereby promoting greater protein absorption [54]. Second, efflux inhibitors can be included in a protein delivery system that blocks the active transport mechanisms within the cell membrane that normally expel proteins or particles out of the epithelium cells [36]. Third, the proteins can be encapsulated within colloidal particles that are absorbed by the cells, and then released into the systemic circulation [37,55]. The design of colloidal delivery systems to achieve this goal depends on a good understanding of the various cellular absorption mechanisms, and the factors that impact them (such as particle size, shape, charge, and polarity).

4. Product requirements

Once the molecular and physicochemical properties of the bioactive proteins have been clearly defined, and the challenges to their delivery have been identified, it is then necessary to specify the requirements of the end product, which will depend on the particular application. In the case of medical foods, functional foods, or supplements, one should consider the required appearance, texture, mouthfeel, and stability characteristics of the end product. For example, the bioactive protein may be delivered in the form of a cloudy low viscosity beverage, a transparent gummy type product, or an opaque solid tablet. In addition, it is important to define the functional attributes of the end product. For instance, it may have to protect the bioactive protein from degradation within the end product, mouth and stomach, but then release it within the small intestine. Some of the most important factors that should be considered when developing a delivery system for bioactive proteins intended for oral ingestion are highlighted in this section [56].

4.1. Matrix compatibility

If the delivery system is going to be incorporated into a medical food, functional food, or supplement intended for oral ingestion, then it should not adversely impact the desirable quality attributes of the end product, such as its appearance, texture, mouthfeel, taste, or shelf-life (see later). Particle characteristics, such as their concentration, size, shape, and charge, will determine their impact on end product properties.

4.2. Product stability

The delivery system should prevent any undesired changes in the activity of the bioactive protein during the manufacture, storage, and utilization of the end product. The approach used to ensure protein stability will depend on the nature of the product, e.g., whether it is a fluid, gel, or solid. Moreover, the delivery system itself should be resistant to any undesired changes in its properties throughout the lifetime of the end product. Consequently, the delivery system may have to be designed to be resistant to changes in pH, ionic strength, temperature, light, oxygen, and mechanical stresses. This can be achieved by careful selection of the composition and structure of the colloidal delivery system, as well as by controlling the composition and structure of the food matrix and packaging material.

4.3. Dose

The delivery system should be capable of encapsulating the level of bioactive proteins required to have the intended biological effect, and then consistently delivering the proteins to the intended site of action at this level. The level of bioactive proteins delivered will depend on the loading capacity, retention, and release properties of the colloidal particles. Factors that may affect the reliability of the dose received should also be carefully considered, and the delivery system should be designed to overcome any problems, e.g., variable processing or storage conditions, or food matrix effects.

4.4. Gastrointestinal stability

After ingestion, the delivery system should be designed to protect the bioactive proteins from degradation within certain regions of the GIT (such as the mouth and stomach), but then release them in other regions (such as the small intestine). In addition, the delivery system may have to be designed to have a prolonged residence time in the region of the GIT where the bioactive proteins are supposed to be absorbed, which may require that the colloidal particles have mucosal properties.

4.5. Ingredient selection

Colloidal delivery systems intended for oral ingestion may be fabricated from a variety of synthetic and/or natural constituents, including surfactants, phospholipids, proteins, polysaccharides, and lipids. For certain applications, it may be important to select particular types of ingredients, e.g., for individuals who have vegan, vegetarian, Kosher, or non-allergenic dietary requirements. In addition, the cost, shelf-life, ease of use, and reliability of the ingredients used to assemble the colloidal delivery system should be considered.

4.6. Production economics and feasibility

The colloidal delivery system should be capable of being consistently produced at an appropriate scale and cost. Many of the methods of producing colloidal delivery systems described in the literature involve ingredients or processing operations that are too costly or inappropriate for commercial applications.

5. Particle characteristics

Once the properties of the bioactive proteins to be encapsulated have been clearly defined, and the requirements of the end product have been specified, then it is necessary to establish the particle characteristics required to create an appropriate oral delivery system [56]. In this section, some of the most important particle properties that may impact the efficacy of colloidal delivery systems for bioactive proteins are highlighted.

5.1. Composition

Colloidal particles can be assembled from a variety of food-grade ingredients, including proteins, polysaccharides, lipids, surfactants, and minerals [57–59]. The ingredients used to fabricate the colloidal particles impact their functional attributes, and so ingredient selection is an important consideration when developing colloidal delivery systems for bioactive proteins. For instance, the composition of colloidal particles impacts the region they are digested in the GIT, as well as their ability to inhibit protein degradation. Some of the most important ways that particle composition impacts the encapsulation, protection, and release of bioactive proteins are discussed in later sections.

5.2. Size and shape

The size and shape of the colloidal particles used to encapsulate bioactive proteins should also be carefully selected for the particular application. The size of colloidal particles may vary from around 10 nm for small nanoparticles (such as microemulsions) to around 1 mm for large microparticles (such as hydrogel beads), and depends on the ingredients and processing operations used to fabricate them. The colloidal particles in delivery systems are often spherical, but they can have other shapes, such as ellipsoid, cylindrical, or irregular, which can impact the optical, rheological, stability, and release characteristics of colloidal dispersions. The impact of the size and shape of colloidal
particles on their ability to encapsulate and deliver bioactive proteins is discussed in later sections.

5.3. Interfacial properties

The interfacial properties of colloidal particles can be manipulated by fabricating them from different ingredients, or by coating them with other ingredients after they have been formed. Consequently, the thickness, composition, charge, and permeability of the interfacial layer can be controlled, which allows one to manipulate the retention, protection, and release of encapsulated bioactive proteins. The interfacial properties of colloidal particles with some lipophilic character (such as lipid droplets or hydrophobic protein nanoparticles) can be modified after fabrication by adsorbing surface-active emulsifiers to their surfaces, such as surfactants, phospholipids, proteins, or polysaccharides [60,61]. The interfacial properties of colloidal particles with an electrical charge can be altered by depositing oppositely charged substances, such as biopolymers or solid particles, onto their surfaces [62]. This electrostatic deposition method can be used to coat colloidal particles with multiple layers of charged substances, which allows the thickness, permeability, and electrical properties of the interfacial layers to be manipulated.

5.4. Aggregation state

Colloidal particles may be present within a colloidal dispersion as individual entities or as clusters ("flocs"). The aggregation state of the particles in a system may have a major impact on their functional attributes. For example, it influences the stability of the particles to gravitational separation (flocs usually move faster than individual particles because of their larger size) and rheology (flocs usually give a higher viscosity than individual particles because they trap more solvent). Moreover, aggregated particles may be digested more slowly that individual particles in the GIT, which can impact the stability and release of bioactive proteins [63,64]. Consequently, it is often important to control the aggregation state of the colloidal particles in the product, as well as in the GIT.

6. Particle functionality

In this section, the most important functional attributes of the particles in colloidal delivery systems are highlighted, with special reference to their relevance to the encapsulation, protection, and delivery of bioactive proteins.

6.1. Loading

An important property of any protein delivery system is the maximum amount of bioactive protein that can be successfully loaded into the colloidal particles, i.e., the loading capacity (LC) [60]. The LC will determine the level of colloidal particles required to achieve the intended dose of the bioactive protein. Moreover, the fraction of bioactive protein that is actually incorporated into the colloidal particles (rather than remaining outside of them) during the encapsulation process is also important, i.e., the encapsulation efficiency (EE). The EE will determine the fraction of bioactive protein that is lost during the manufacturing process, which obviously has important economic consequences. The following expressions can be used to calculate these values for a particular bioactive protein-colloidal particle combination:

\[ \text{LC} = \frac{m_{B,E}}{m_p} \]  
\[ \text{EE} = \frac{m_{B,E}}{m_{B,T}} \]  

Here, \(m_{B,E}\) and \(m_{B,T}\) are the masses of the encapsulated and total bioactive protein used to produce the colloidal delivery system, and \(m_p\) is the total mass of the colloidal particles (carrier material + bioactive proteins).

The loading capacity and encapsulation efficiency depend on the molecular and physicochemical properties of the bioactive proteins, as well as of the carrier materials used to assemble the colloidal delivery system. Most bioactive proteins are predominantly hydrophilic and so colloidal particles should have some hydrophilic domains within them, which means they must be assembled from ingredients that have appreciable numbers of polar groups (such as hydroxyl, carboxyl, sulfate, phosphate, or amino groups). Some examples of colloidal delivery systems with hydrophilic domains (usually water) are reverse micelles, W/O microemulsions, W/O emulsions, W/O/W emulsions, liposomes, and microgels (Fig. 1). Bioactive proteins may also be held inside colloidal particles by electrostatic interactions between oppositely charged groups or by hydrophobic interactions between non-polar groups (see next section). In this case, the ingredients used to form the interior of the colloidal particles should have electrically charged or non-polar groups that are strongly attracted to the bioactive proteins. In some cases, the sign or magnitude of the interactions between bioactive proteins and carrier materials can be altered by changing environmental conditions (such as pH, ionic strength or temperature), which can be used to develop triggered release mechanisms.

As a specific example, the encapsulation of bioactive proteins within W1/O/W2 emulsions is considered, where W1 and W2 are the internal and external aqueous phases, and O is the oil phase (Fig. 1). Assuming that the bioactive proteins cannot diffuse through the oil phase and that the internal water droplets are not disrupted during the second homogenization stage, the encapsulation efficiency would be relatively high (EE ≈ 100%) because all of the proteins would remain trapped within the internal water phase. The loading capacity depends on the highest level of protein that can be dissolved in the internal water phase and the highest level of water droplets that can be incorporated in the W/O emulsion. If it is assumed that the maximum amount of protein that can be dissolved into an aqueous solution is around 20% and the maximum level of water droplets that can be packed into an oil phase is around 50%, then the loading capacity (LC) of the capsules in a W/O/W emulsion would be around 10%. Similar kinds of calculations could be carried out for other kinds of colloidal delivery systems. However, in practice it is always important to measure the masses of encapsulated and non-encapsulated bioactive protein to determine the EE and LC values more accurately.

6.2. Retention/release

Colloidal delivery systems are often designed to retain bioactive proteins under one set of environmental conditions, but then release them when exposed to another set of conditions, such as a change in pH, ionic strength, temperature, or enzyme activity [60]. A number of physicochemical mechanisms can be utilized to control the retention and release of bioactive proteins (Fig. 4).

6.2.1. Simple diffusion

In the simplest case, bioactive proteins may be released from polymeric colloidal particles by simple diffusion. To a first approximation, the release of a bioactive protein from a spherical particle due to simple diffusion can be described by the following expression [65]:

\[ M(t) = M(\infty) \left[1 - \exp\left(-\frac{1.2\pi^2D_p}{r^2}t\right)\right] \]  

Here, \(M(t)\) and \(M(\infty)\) are the concentrations of the bioactive protein trapped within the colloidal particles at time \(t\) and at equilibrium (infinite time), \(r\) is the radius of the colloidal particles, and \(D_p\) is the diffusion coefficient of the bioactive proteins through the colloidal particles. Bioactive proteins are often encapsulated within biopolymer microgels whose interior consists of a network of cross-linked polymer molecules with a certain pore size. In this case, the diffusion coefficient can be obtained using the following expression,
which describes the diffusion of small molecules through a network of polymer chains [66,67]:

\[
D_P = D_W \exp\left(-\pi \frac{r_H + r_f}{\zeta + 2r_f}\right)
\]  

(3)

Here, \(D_P\) and \(D_W\) are the diffusion coefficients of the bioactive protein through the polymer network and through pure water, \(r_H\) is the hydrodynamic radius of the bioactive protein, \(r_f\) is the cross-sectional radius of the polymer chains, and \(\zeta\) is the pore diameter. The translational diffusion coefficient of proteins through water can be estimated using the following equation:

\[
D_w = \frac{k_B T}{6\pi \eta r_H}
\]

(4)

Here, \(k_B\) is Boltzmann’s constant, \(T\) is absolute temperature, and \(\eta\) is the viscosity of water. These equations can provide valuable insights into the impact of the pore size and external dimensions of polymeric colloidal particles on protein retention and release. A prediction of the influence of the protein dimensions relative to the pore size \(r_H/\zeta\) on the normalized diffusion coefficient \((D_P/D_W)\) is shown in Fig. 5. This prediction indicates that the bioactive proteins must have dimensions appreciably larger than those of the pores in the polymer network before their diffusion is appreciably retarded. Consequently, polymeric colloidal particles would only retain proteins when they have pore sizes appreciably smaller than that of the bioactive proteins. This may be difficult to achieve for small peptides \((d < 1\ \text{nm})\), but may be possible for larger proteins or protein aggregates. This equation also predicts that the release of bioactive proteins from colloidal particles decreases as the size of the particles increases (Fig. 6), since the proteins have a greater distance to travel before they reach the external environment. Conversely, the retention of proteins will increase as the particle size
increases for the same reason. Predictions made using the above equation show that the release of proteins from polymeric colloidal particles would be quite rapid (<5 min), even if they had relatively small pore sizes ($\zeta = 1$ nm) and large external radii ($r = 1000$ μm) (Fig. 6). This simple calculation therefore suggests that other physicochemical mechanisms are required to ensure that proteins are retained within smaller particles, e.g., specific molecular interactions or physical barriers.

It should be noted that different kinds of mathematical models may be required to describe the release of proteins from other kinds of colloidal delivery systems, such as W/O/W emulsions, emulsified microemulsions, or liposomes (Fig. 1). Indeed, it is likely that simple diffusion is not the most important release mechanism for these systems.

6.2.2. Swelling

The pore size in a polymeric colloidal particle can sometimes be altered by changing the environmental conditions (such as pH, ionic strength, or temperature) to induce swelling or shrinkage of the particles [68,69]. For instance, polymer networks comprised of ionized polyelectrolytes tend to swell under conditions where there is a strong electrostatic repulsion between the polymer chains, i.e., at low ionic strengths or at pH values where the polymer has a high charge density. Conversely, these types of polymer networks tend to shrink when the solution conditions reduce the electrostatic repulsion between the polymer chains, i.e., at high ionic strengths or at pH values where the polymer has a low charge density. This phenomenon has been reported in alginate microgels that tend to swell under neutral conditions because of the high negative charge density on the alginate chains, but shrink under acid conditions because the carboxyl groups on the alginate chains lose some of their charge [70,71]. Changing the ionic strength of the aqueous solution surrounding polymeric particles containing charged polymers may also lead to swelling or shrinking. For instance, it has been shown that adding salts (sodium or calcium chloride) to transglutaminase cross-linked caseinate gels caused them to shrink, due to a reduction in the electrostatic repulsion between the biopolymer chains [72]. This phenomenon can be utilized to develop delivery systems that can trigger the release of bioactive proteins in response to a change in ionic strength. Polymeric particles can also be prepared that will swell or shrink when the temperature is changed, because this alters the conformation of the polymer chains from expanded to collapsed [69]. A classic example of natural food-grade colloidal particles that undergo swelling upon heating is starch granules. Native starch granules have dense structures with small pore sizes at low temperatures, but they swell when they are heated in the presence of water [73,74]. Starch granules have been used to encapsulate various types of bioactive components by incubating them with a solution of the bioactives [74–76].

The swelling and shrinking of polymeric particles can be utilized to load, retain, and release bioactive proteins. For instance, a protein-loaded biopolymer microgel could be prepared under conditions where the polymer network is shrunk and has small pores, thereby inhibiting the release of the proteins. The solution conditions could then be changed to cause the polymer network to swell, thereby facilitating the release of the proteins due to simple diffusion (see previous section).

6.2.3. Specific molecular interactions

An alternative mechanism for controlling the retention and release of proteins from polymeric colloidal particles is to utilize specific attractive or repulsive interactions between the proteins and the polymer molecules [23,26]. If there is a strong attraction between the proteins and polymers, then the protein will tend to be retained inside the colloidal particles, but if there is no attraction or a repulsion then the proteins will tend to be released. One of the most commonly used means of controlling molecular interactions is based on changing the pH or ionic strength to weaken or strengthen the electrostatic interactions within the colloidal particles [22]. In particular, the net charge on proteins usually goes from positive to negative as the pH is increased from below to above their isoelectric points (Fig. 7a). Consequently, the proteins tend to be attracted (retained) to anionic polymers at low pH values, but repelled (released) at high pH values. To a first approximation, the strength of the electrostatic interaction can be estimated using the following expression [22]:

$$ EI = -\tilde{S}_{\text{protein}} \times \tilde{S}_{\text{polymer}} $$

(Eq. 5)
Here, $\zeta_{\text{Protein}}$ and $\zeta_{\text{Polymer}}$ are the effective surface potentials ($\zeta$-potentials) of the protein and polymer, respectively. The value of the electrostatic interaction will be attractive when this term is positive, and repulsive when it is negative. The change in the electrostatic interaction parameter with pH for the whey protein–alginate system is shown in Fig. 7b. The strongest electrostatic attraction occurs around pH 3.5, and therefore this would be expected to be the pH where the protein was held inside the particles most strongly. In practice, one must also take into account the change in charge in the polymer molecules due to any ion-binding effects associated with their cross-linking inside the colloidal particles. In addition, bioactive proteins typically have a mixture of both cationic and anionic groups on their surfaces, and so they may be able to bind under conditions where both the protein and polymer have similar net charges [77]. An example of the retention/release of proteins from polymeric colloidal particles due to a change in electrostatic interactions is highlighted in Fig. 8, which shows the impact of pH on the retention of a model globular protein (whey protein) from alginate microgels [25]. The proteins are held inside the alginate microgels at low pH values where the two biopolymers have opposite charges (positive and negative), but are released at high pH values where they have similar charges (both negative). It should be noted that the strength of the electrostatic attraction may be

![Fig. 7](image1.png)

**Fig. 7.** The electrical surface potential ($\zeta$-potential) of bioactive proteins and of the polymers used to construct colloidal particles often depends on pH: (a) $\zeta$-potential versus pH profile of whey protein and alginate solutions; (b) Effective electrostatic interaction versus pH for a mixed whey protein and alginate system, calculated from $\zeta$-potential measurements.

![Fig. 8](image2.png)

**Fig. 8.** Confocal fluorescence microscopy images of the impact of pH on the retention and release of a model protein (whey protein isolate) encapsulated in calcium alginate microgels. The protein was stained green.

Data from Zhang et al. (2016), Food Hydrocolloids, 58, 308–315.
considerably weakened in the presence of salts due to electrostatic screening effects [26], which may promote the release of protein molecules in functional foods or other products with relatively high ionic strengths.

6.2.4. Particle dissociation

In some applications, colloidal particles are designed to dissociate or degrade under a particular set of environmental conditions and thereby release any encapsulated bioactive proteins. (Fig. 4) [24]. Colloidal particles fabricated from different kinds of ingredients are responsive to different environmental conditions. For example, many starches are mainly broken down in the mouth by amylases, many proteins are mainly degraded in the stomach by proteases, many lipids are mainly digested in the small intestine by lipases, and many dietary fibers are mainly broken down in the large intestine by enzymes secreted by colonic bacteria [24]. Thus, colloidal particles may be designed to breakdown in the mouth, stomach, small intestine, or colon by fabricating them from ingredients with different susceptibilities to digestive enzymes. Certain types of colloidal particles can also be triggered to dissociate when exposed to changes in the pH or ionic strength of their environment, which can be attributed to weakening of the electrostatic interactions between the molecules involved [78]. For example, biopolymer microgels assembled from sodium caseinate and pectin have been shown to remain intact at low pH values where the biopolymers have opposite charges and therefore attract each other, but fall apart at high pH values where the biopolymers have similar charges and therefore repel each other [79]. Some colloidal particles can also be triggered to dissociate in response to specific temperature changes, such as cooling or heating. For instance, biopolymer microgels formed by electrostatic complexation of gelatin and beet pectin were shown to be stable at ambient temperature, but to dissociate upon heating (Fig. 9), which was attributed to a conformational change of the gelatin molecules [80–82]. This kind of system may therefore be useful for developing temperature-triggered release systems for bioactive proteins.

6.3. Bioactive protection

The microenvironment of the bioactive proteins inside a colloidal particle can have a major impact on their chemical and physical stability. For example, some substances have good antioxidant properties and can therefore retard protein oxidation, e.g., free radical scavengers and chelating agents [83,84]. Other substances (such as antioxidants and buffers) can inhibit local pH changes inside colloidal particles, and thereby stabilize proteins from acid- or alkaline-induced changes in their conformation [42,43]. Some constituents are able to stabilize the native structure of bioactive proteins, and thereby protect against activity losses normally caused by denaturation, e.g., some sugars, polyols, salts, and surfactants [30,85]. Consequently, it may be advantageous to co-encapsulate these stabilizing molecules with the bioactive proteins within the colloidal particles, thereby improving their stability and activity.

The ability of a colloidal particle to protect a bioactive protein from environmental degradation often increases as the particle size increases, because then a greater fraction of the proteins is present within the interior of the particles and so isolated from the surroundings. The fraction of proteins located within a thin shell (δ) close to the surface of colloidal particles comprised of pure proteins is given by:

$$\Phi = 1 - \left(1 + \frac{\delta}{r}\right)^{-3}$$

If it is assumed that δ is equal to the diameter of the protein molecules, and that the proteins are evenly dispersed throughout the particles, then the change in Φ with the radius (r) of the colloidal particles can be calculated (Fig. 10). When the radius of the colloidal particles is less than about 100 nm, then a significant fraction of the protein molecules may be present at the particle surfaces, and therefore more susceptible to chemical degradation.

6.4. Particle stability

Colloidal particles are exposed to variations in pH, ionic composition, ingredient interactions, enzyme activities, light, oxygen, and temperatures during their manufacture, storage, and utilization, as well as when they pass through the gastrointestinal tract. Consequently, it is important to carefully design colloidal particles so that they remain stable within the different environments that they may encounter before they release their protein payloads.

6.4.1. Gravitational stability

The stability of colloidal particles to gravitational separation depends strongly on their particle size. For instance, the velocity (v) that a spherical colloidal particle moves through an ideal (Newtonian) fluid is given by Stokes’ law:

$$v = -\frac{2gr^2(\rho_p - \rho_f)}{9\eta}$$

![Fig. 9. Optical microscopy images of the impact of temperature on the dissociation of biopolymer microgels fabricated from gelatin and pectin in the absence and presence of covalent cross-linking with glutaraldehyde (2 mM). Data from Wu and McClements 2015. Food Research International 78, 177–185.](image)
Here, $g$ is the acceleration due to gravity, $r$ is the particle radius, $\rho_p$ is the particle density, $\rho_f$ is the fluid density, and $\eta$ is the fluid viscosity. A positive sign for the velocity means that the colloidal particle moves upwards ("creaming") due to gravitational forces, whereas a negative sign means that the colloidal particle moves downwards ("sedimentation"). Stokes’ law indicates that the velocity that a colloidal particle moves through a fluid increases with increasing particle size, increasing density contrast, and decreasing fluid viscosity. This expression is only strictly applicable for describing the movement of non-interacting monodisperse rigid spheres in relatively dilute colloidal dispersions ($\phi < 5\%$). More comprehensive models have been developed to account for polydispersity, aggregation, non-sphericity, non-rigidity, particle interactions and non-Newtonian fluids [86]. Theoretical predictions of the sedimentation velocity of colloidal particles with different compositions and sizes are shown in Table 2. These predictions show that the sedimentation velocity is relatively fast (>5 mm/day) even for quite small polymeric colloidal particles (1 μm). This may limit the application of larger colloidal particles to viscous, gelled, or solid products where particle movement is retarded by the surrounding matrix. Alternatively, it may be possible to utilize density matching methods to inhibit gravitational separation, i.e., using a mixture of lipid and biopolymer inside the colloidal particles [87].

### 6.4.2. Aggregation stability

The aggregation stability of colloidal particles can be manipulated by controlling the attractive and repulsive colloidal interactions that operate between them and with other components in their environment, such as van der Waals, hydrophobic, bridging, depletion, steric, and electrostatic interactions. For instance, the overall colloidal interactions operating between a pair of particles can be summarized as follows [86]:

$$w(h) = w_{VDW}(h) + w_{S}(h) + w_{E}(h) + w_{H}(h) + w_{D}(h) + w_{F}(h)$$

Here, $w(h)$ is the interaction potential, and the subscripts, V, D, S, E, H, D and B stand for total, van der Waals, steric, electrostatic, hydrophobic, depletion, and bridging, respectively. Each interaction can be characterized by a sign ([$+$] repulsive or [−] attractive), magnitude (weak to strong), and range (short to long). The overall stability of the colloidal particles to aggregation depends on the balance of attractive and repulsive forces operating in the system. When the attractive interactions (commonly van der Waals and hydrophobic) outweigh the repulsive interactions (commonly electrostatic and steric), the particles tend to aggregate, otherwise they tend to remain as individual entities. Aggregated particles may keep their original integrity (flocculation) or merge together (coalescence) depending on the nature of the particles (solid or liquid) and interfacial coating (soft or rigid). Aggregation reduces the surface area of the particles exposed to the surrounding fluids, and may therefore decrease the enzymatic digestion rate under gastrointestinal tract conditions [64,88]. Protein-loaded colloidal particles therefore have to be carefully designed to control their aggregation state in the end product and GIT.

### 6.5. Particle permeability

The ability of protein molecules to travel through colloidal particles impacts their retention and release characteristics. Moreover, the ability of other types of molecular species, such as ions (e.g., $H^+$, $OH^-$ or Fe$^{2+}$), enzymes (e.g., proteases or lipases), or surface-active molecules (e.g., surfactants or bile salts), to permeate into colloidal particles may impact the stability of the encapsulated proteins. The passage of proteins and other molecular species through colloidal particles depends on a number of factors: (i) their solubility in the phases inside the particles; (ii) the rheological properties of the phases inside the particles; (iii) the size of any pores in the colloidal particles (e.g., in polymer networks); and, (iv) the number and strength of any molecular interactions between the molecular species and the particles [89,90]. The relative importance of these different factors depends on the composition and internal structure of the colloidal particles. In this section, we mainly focus on the properties of colloidal particles whose interior consists of a network of cross-linked polymers (usually proteins or polysaccharides).

As discussed earlier, the size of the pores within a polymeric colloidal particle should be appreciably smaller than the dimensions of a protein in order to retard its diffusion into the surrounding environment (Fig. 5). Individual bioactive protein and peptide molecules are usually quite small, with molecular dimensions ranging from about 1 to 10 nm. Consequently, the pore size in polymeric colloidal particles should be very small to inhibit protein diffusion. In some situations, the pore size of a polymeric colloidal particle can be made to change in response to a specific environmental trigger, such as pH, ionic strength, or temperature [24,91]. Consequently, it may be possible to retain the proteins inside the colloidal particles under one set of conditions, but then release them under another set of conditions. In cases where it is not possible to encapsulate individual bioactive protein molecules within polymeric colloidal particles because of their small size relative to the pores, it may be possible to encapsulate large protein aggregates, proteins adsorbed to particle surfaces (such as oil droplets or solid particles), or proteins trapped in other colloidal structures (such as W/O/W emulsions). In this case, the bioactive proteins may be in a form that is large enough to remain trapped inside the pores.
7. Particle impact on end product quality

A factor that is often not considered when developing delivery systems for bioactive proteins is their impact on the quality attributes of the commercial products that they will be incorporated into. Delivery systems may alter the appearance, texture, mouthfeel, and stability of functional foods by an amount that depends on the concentration and nature of the colloidal particles used. Obviously, it is important that the delivery system is compatible with the food matrix, and does not cause any adverse effects on the desirable quality attributes of the end product.

7.1. Appearance

The optical properties of suspensions of colloidal particles depend on their size, concentration, and refractive index [92]. Colloidal particles may alter the appearance of a material due to their ability to both absorb and scatter light. The selective absorption of certain wavelengths of light by chromophores in a colloidal particle mainly determines the color of a product (e.g., blue, green, or red), whereas the scattering of light waves by the colloidal particles mainly determines its opacity (e.g., clear, turbid or opaque). The opacity of a product increases as the refractive index contrast between the colloidal particles and surrounding medium increases, and as the particle concentration increases, because this leads to greater light scattering. The size of the colloidal particles relative to the wavelength of light also determines the degree of light scattering and opacity: very fine particles (\(d < 50\) nm) lead to products that are optically transparent; intermediate sized particles (100 nm < \(d < 100\) mm) lead to cloudy or optically opaque products; and relatively large particles (\(d > 100\) mm) can be discerned as individual objects by the human eye [92]. The appearance of an end product intended for oral ingestion is often an important quality attribute that determines consumer or patient acceptance. If the bioactive proteins are going to be delivered through an optically transparent product (such as a soft drink or fortified water), then it will be important that the colloidal particles are relatively small so that they do not scatter light strongly. Conversely, if the bioactive proteins are going to be delivered using an optically cloudy or opaque product (such as a nutritional beverage or yogurt), then the colloidal particles can be larger.

7.2. Texture

The incorporation of a colloidal delivery system into an end product may also impact its rheological properties [60]. Each end product, such as a functional food, medical food, or supplement has its own unique textural attributes. For instance, a beverage should be a low viscosity fluid, a yoghurt a viscoelastic fluid, and a capsule a soft solid. Consequently, it is important to consider the potential impact of incorporation of protein-loaded colloidal particles into an end product. The impact of the colloidal particles on the rheological properties of an end product will mainly be determined by their concentration and aggregation state.

For fluid colloidal dispersions, the shear viscosity \(\eta_0\) increases with increasing particle concentration according to the following semi-empirical expression:

\[
\eta = \eta_0 \left(1 - \frac{\phi}{\phi_c}\right)^{-2}
\]

Here, \(\eta_0\) is the viscosity of the fluid surrounding the colloidal particles, \(\phi\) is the colloidal particle volume fraction, and \(\phi_c\) is the critical volume fraction at which the colloidal particles become close-packed. This equation assumes that the colloidal particles are non-interacting rigid spheres, and that the surrounding fluid is ideal (Newtonian). More comprehensive theories have been developed to take other factors into account. The viscosity of a colloidal dispersion typically increases when the particles become aggregated because this leads to an increase in the effective volume fraction of the system. The viscosity may also increase when the size of the particles decreases because the volume occupied by the interfacial layer becomes more important, or because particle-particle interactions become more important [86]. Predictions made using the above equation show that the viscosity of a colloidal dispersion increases with increasing particle concentration (Fig. 11). Incorporating a relatively low level of colloidal particles into a liquid beverage (<10%) would be expected to cause only a slight increase in viscosity, but any higher level may cause an appreciable increase in viscosity.

7.3. Mouthfeel

The mouthfeel of a colloidal system intended for oral delivery of bioactive proteins may impact its desirability, and therefore impact consumer acceptance or patient compliance [93, 94]. Colloidal particles less than about 50 \(\mu\)m cannot usually be sensed as individual entities within the human mouth, therefore leading to a “smooth” mouthfeel. Conversely, larger particles can be discerned and may lead to a “rough” or “gritty” mouthfeel depending on their size, shape, and hardness, as well as the viscosity of the surrounding medium. For instance, a recent study found that the perceived “grittiness” of orange drinks increased with increasing particle size when they were fortified with microcrystalline cellulose spheres [93].

8. Delivery system selection

A broad spectrum of colloidal delivery systems has been developed to encapsulate, protect, and release bioactive molecules, and many of these are suitable for application to proteins or peptides [59, 60, 95]. In this section, a brief review of some of the most common colloidal delivery systems is given, including a description of their structural characteristics, encapsulation properties, advantages, and disadvantages.
8.1. Microemulsions and emulsified microemulsions

Microemulsions are thermodynamically stable colloidal dispersions consisting of clusters of surfactant, oil and/or water molecules held together by physical forces, particularly hydrophobic interactions [96,97]. Microemulsions can be categorized as oil-in-water (O/W) or water-in-oil (W/O) types depending on the relative location of the polar and non-polar domains in the system. The colloidal particles in O/W microemulsions consist of small surfactant-oil clusters dispersed in water, where the non-polar tails of the surfactants are directed towards the hydrophobic interior, and the polar head groups are directed towards the hydrophilic exterior (water). The mean diameters of the particles in these systems typically range from about 5 to 100 nm. This type of microemulsion is unlikely to be effective at encapsulating most bioactive proteins because the proteins are too hydrophilic to be trapped inside the hydrophobic interior of the microemulsion particles. Conversely, the colloidal particles in W/O microemulsions consist of surfactant-water clusters dispersed in oil, with the polar tails of the surfactants pointing towards the hydrophilic interior, and their non-polar tails pointing outwards towards the oil phase (Fig. 1). These kinds of microemulsions are more appropriate for encapsulating hydrophobic bioactive proteins, because the proteins can be incorporated into the hydrophilic interiors (water phase) of the microemulsion particles [98,99]. Studies have shown that enzymes (chymotrypsin and lysozyme) can be successfully encapsulated in W/O microemulsions and retain their activity [100], but the surfactants and oils used in this study were unsuitable for food applications. A major limitation of these types of colloidal dispersions are that they are only suitable for application in oil continuous functional foods, supplements, or drugs, such as salad oils, spreads, or oil-filled capsules. However, W/O microemulsions can be converted into a water-dispersible form by homogenizing them with an aqueous phase containing a hydrophilic emulsifier to form an emulsified microemulsion, i.e., a W/O/W system [101,102]. These systems consist of small hydrophilic domains trapped inside oil droplets that are themselves dispersed in water (Fig. 1). Studies have shown that model bioactive proteins (BSA and cytochrome C) can be trapped inside the internal hydrophilic domains of emulsified microemulsions, and thereby protected from the external water phase by an intervening oil phase [103]. Nevertheless, research is still required to determine whether this type of delivery system can be formulated from food-grade ingredients, and whether it will remain stable under the range of environmental conditions found in commercial products.

8.2. Emulsions, nanoemulsions, and multiple emulsions

Unlike microemulsions, emulsions are thermodynamically unstable colloidal dispersions formed from two immiscible liquids (typically oil and water) with one of the liquids being dispersed as small droplets in the other [86]. This type of colloidal system can also be categorized into O/W or W/O forms depending on the relative location of the oil and water phases. In addition, they can be classified as either nanoemulsions (d < 100 nm) or emulsions (d > 100 nm) depending on the mean diameter of the droplets they contain [104]. In this article, the term “emulsion” is used to refer to both nanoemulsions and emulsions since they are both thermodynamically unstable systems that have many similar structural and physicochemical characteristics. However, it should be recognized that nanoemulsions may have advantages over emulsions for some applications because they have better stability to gravitational separation and aggregation, they may be optically transparent, and they are often digested more rapidly [105]. O/W emulsions are unsuitable for encapsulating proteins inside of the oil droplets because the droplet interiors are too hydrophobic. However, bioactive proteins may be adsorbed to the surfaces of oil droplets (Fig. 1) [106]. It should be noted that adsorption of proteins to surfaces can change their structures and activities due to surface denaturation [107], and that proteins present at the exterior of oil droplets may not be protected against degradation when exposed to food or GIT conditions [108]. However, the protein-coated droplets could be encapsulated within some other types of colloidal particle (such as biopolymer microgels) to better protect them. Alternatively, bioactive proteins can be electrostatically deposited onto the surfaces of emulsifier-coated oil droplets, and can form one or more layers there [62]. The protein layers can then be covered by other types of biopolymer layers so as to modulate their encapsulation, protection, and release properties [109].

W/O emulsions are more suitable systems for encapsulating bioactive proteins because the droplets have a hydrophilic interior [55]. However, this type of system is only useful for delivering proteins in functional foods, supplements, or drugs that have a lipid continuous phase, such as salad oils, butters, spreads, or oil-filled capsules [86]. This limitation can be overcome by further homogenizing the W/O emulsions with an aqueous phase containing a hydrophilic emulsifier to form W/O/W emulsions [110]. Protein delivery systems produced using this approach would consist of protein-loaded water droplets trapped inside oil droplets, which are themselves dispersed in an aqueous continuous phase (Fig. 1). However, it is important that the bioactive proteins are not damaged or released during the formation of the multiple emulsions. Moreover, these systems are often time consuming and difficult to prepare, and are prone to breakdown in commercial products [110]. Previous studies have shown that W/O/W emulsions can be used to encapsulate insulin within the internal water droplets [111–113]. This type of system would be expected to protect the insulin from degradation within the oral and gastric fluids, but then release it within the small intestinal fluids when the lipid phase was digested by pancreatic lipase. It is not clear whether the bioactive proteins would then be protected from inactivation in the small intestinal fluids, or how they would reach the epithelium cells intact. However, the presence of digestible lipids in the oil phase of the W/O/W could increase the permeability of the cell membranes, thereby facilitating protein absorption [114].

Recently it has been proposed that bioactive proteins can be encapsulated in “solid-in-oil-in-water” (S/O/W) emulsions (Fig. 1), which consist of powdered proteins dispersed in an oil phase containing a lipophilic emulsifier, which is then homogenized within a water phase containing a hydrophilic emulsifier [115]. These systems may be able to overcome some of the problems associated with W/O/W emulsions, but further research is required to determine if their stability is sufficient for commercial applications.

8.3. Solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) are usually produced by preparing an O/W nanoemulsion or emulsion at a temperature above the melting point of the oil phase, and then cooling the system to promote crystallization of the lipid droplets [116,117]. However, this kind of simple O/W system has limited application for encapsulation of hydrophilic bioactive substances. For this reason, alternative methods of forming SLNs with more complex structures have been developed to encapsulate hydrophilic bioactive substances. One of the most widely investigated methods involves the formation of W/O/W emulsions using a high-melting lipid as the oil phase [118,119]. The high-melting lipid may be dissolved in an organic solvent that is evaporated after W/O/W emulsion formation to crystallize the remaining lipid (“solvent evaporation” method), or the W/O/W emulsion may be formed at a temperature above the melting point of the lipids and then cooled to crystallize the lipid phase (“hot homogenization” method). These processes lead to the formation of a solid fat shell around the water droplets containing the encapsulated bioactive proteins (Fig. 1). The main advantage of the solvent evaporation method over the hot homogenization method is that it does not involve the utilization of elevated temperatures that can denature some proteins. SLNs formed from W/O/W emulsion templates can be used to encapsulate and protect bioactive proteins. For instance, the solvent evaporation approach has been used to trap insulin inside SLNs.
Liposomes consist of concentric rings of phospholipid molecules organized tail-to-tail, which are mainly held together by hydrophobic interactions (Fig. 1) [122–124]. Liposomes can encapsulate hydrophilic substances within their hydrophilic water core, and amphiphilic or lipophilic substances within their hydrophobic lipid bilayers [125]. Consequently, they can be used to encapsulate both hydrophilic proteins and proteins that have large hydrophobic domains (such as those found embedded in cell membrane walls in nature). The major drawback of liposome-based delivery systems is that they tend to have a relatively low encapsulation efficiency, and they are highly susceptible to breakdown in food matrices and GIT environments [125–127]. However, the stability of liposomes may be improved by coating them with biopolymer layers using electrostatic deposition methods [128,129]. Alternatively, it may be possible to trap the liposomes within other delivery systems (such as biopolymer microgels) to improve their stability.

There have been numerous studies on establishing the potential of liposomes for encapsulating, stabilizing, and delivering bioactive peptides in the pharmaceutical industry, and a number of commercial products have been developed based on this type of technology [1,130,131]. In this section, only a brief overview of some systems that may be suitable for application in functional foods is given. Studies have shown that ghrelin, an appetite-stimulating hormone with a short GIT lifetime, can be encapsulated within phospholipid liposomes [132]. Encapsulation of the ghrelin was shown to improve its in vivo performance [118,120,121], which was recently shown to significantly improve its oral bioavailability using in vivo (diabetic rat) studies [118].

8.4. Liposomes

8.5. Biopolymer microgels

Biopolymer microgels are colloidal particles comprised of one or more types of biopolymer (usually proteins and/or polysaccharides) held together by physical and/or chemical bonds [135,136] (Fig. 1). Bioactive proteins can be encapsulated within biopolymer microgels by mixing them with a solution containing the biopolymer molecules prior to microgel formation, or by loading them into microgels after their formation. Biopolymer microgels can be fabricated from a wide range of different proteins and/or polysaccharides using various production methods, including injection, emulsion templating, coacervation, thermodynamic incompatibility, antisolvent precipitation, and molding methods [135,137]. Consequently, there is considerable flexibility in producing biopolymer microgels with compositions, sizes, structures, permeabilities, and interfacial properties tailored for specific applications (Fig. 12).

Methods of fabricating biopolymer microgels have been reviewed in detail elsewhere, including antisolvent precipitation, injection, coacervation, thermodynamic incompatibility, and templating methods [135–137], and so will not be covered here. Each of these methods has its own advantages and disadvantages, such as cost, simplicity, scale-up, production volume, and ability to produce microgel particles with different compositions, dimensions, and structures. Consequently, a manufacturer must select the most appropriate one for the particular application.

In this section, a few examples of the utilization of food-grade biopolymer microgels to encapsulate, protect, and deliver bioactive proteins is given. Studies have shown that microgels formed from alginate or carrageenan can be used to encapsulate enzymes (such as lipase and lactase), and improve their stability under simulated food matrix and gastrointestinal conditions [42,43,138]. In particular, it was shown that co-encapsulating the enzymes with an antacid (magnesium hydroxide) protected them from degradation under simulated stomach
developing effective strategies to improve the oral delivery of insulin. Consequently, there is great interest in developing oral delivery systems for insulin [11,143]. However, this has been challenging because insulin has a relatively low oral bioavailability due to its high susceptibility to degradation and low absorption in the human GIT [4,144]. Consequently, there is great interest in developing effective strategies to improve the oral delivery of insulin.

9. Applications

In this section, an overview of some key bioactive proteins and peptides that may benefit from incorporation into colloidal delivery systems intended for oral use is given. Emphasis is given to those delivery systems that can be fabricated from food-grade components.

9.1. Hormones: insulin

Insulin is a hormone that is used to treat individuals with diabetes by controlling blood glucose levels. Currently, insulin is mainly delivered by injection, which is clinically effective, but is inconvenient and uncomfortable for patients [142]. Consequently, there is great interest in developing oral delivery systems for insulin [11,143]. However, this has been challenging because insulin has a relatively low oral bioavailability due to its high susceptibility to degradation and low absorption in the human GIT [4,144]. Consequently, there is great interest in developing effective strategies to improve the oral delivery of insulin.

A good delivery system for insulin should: ensure it remains stable within commercial products (e.g., functional foods, supplements, or pharmaceuticals); protect it from degradation within the GIT; release it within the small intestine; and, enhance its permeability through the mucus layer and epithelium cells. There have been a number of review articles on the utilization of colloidal delivery systems to encapsulate insulin and deliver it through the oral route [2,11,144–146]. Many of these articles focus on pharmaceutical applications, but the materials utilized can often be used for food applications also, such as edible lipids, surfactants, proteins, and polysaccharides. For instance, a recent article reviewed the application of natural polymers (proteins and polysaccharides) for constructing colloidal delivery systems for insulin [144]. For this reason, only a number of recent studies that have potential for the oral delivery of insulin using functional foods are highlighted in this section.

Studies have shown that insulin can be successfully encapsulated within biopolymer microgels fabricated from alginate and chitosan using an emulsification-gelation method [147]. An alginate solution was passed through a membrane into an oil phase, which led to the formation of a W/O emulsion containing water droplets with alginate molecules trapped inside. This emulsion was then mixed with another oil phase containing calcium chloride, which led to cross-linking of the alginate molecules, presumably due to diffusion of calcium ions from one kind of water droplet to another. The resulting alginate microgels were then washed with an organic solvent and then dispersed in an aqueous solution containing chitosan and insulin, which led to the formation of a chitosan shell around an alginate-rich core, with insulin trapped inside the polymer matrix. This study showed that these microgels could protect insulin from degradation under simulated gastric conditions, but then release it under simulated small intestine conditions. In addition, they showed that oral administration of the insulin-loaded biopolymer microgels could control the blood glucose level in diabetic rats. These microgels may therefore have potential for application as insulin delivery systems in functional foods. However, the fabrication method used to prepare the microgels was fairly time-consuming and cumbersome, which may limit the practical application of this technology. Other researchers have shown that insulin can be

![Fig. 13. Impact of incubation in simulated gastric fluids (SGF, pH 2.5) on the internal pH of biopolymer microgels (calcium alginate) in the absence of presence of an antacid (magnesium hydroxide). (a) Change in internal pH of microgels after incubation in SGF; (b) Fluorescence microscopy images of microgels after incubation in SGF - the intensity of the fluorescence probe decreases with decreasing pH; (c) Neutralization reaction - the antacid is solid at neutral pH, but dissolves in acidic solutions, therefore releasing hydroxyl ions that can neutralize hydrogen ions. Data from Zhang et al. (2017), Food Hydrocolloids, 65, 198–205.](image)
encapsulated within biopolymer microgels fabricated from alginate and dextran sulfate also using the emulsification-gelation method [148]. These biopolymer microgels had a high encapsulation efficiency (~99%), and were shown to protect the biological activity of insulin in simulated gastric fluids.

Insulin-loaded chitosan nanoparticles have been prepared utilizing an ionic gelation method with tripolyphosphate (TPP) as a cross-linking agent [149]. These nanoparticles were then trapped inside W/O microemulsions formed from water, oil, surfactant, and co-surfactant. In vitro studies showed that insulin retained its structure after encapsulation, that its release was retarded under simulated gastric conditions, and that it had a relatively low cytotoxicity. An in vivo study showed that this type of delivery system was able to control blood glucose levels in rats after oral administration. This type of approach may therefore be suitable for developing oral delivery systems for insulin, but again the fabrication procedure is relatively complex, and the final microemulsions may not be suitable for application in functional food products.

Insulin has been loaded into calcium-alginate microgels formed using W/O emulsions as templates [150]. Two W/O emulsions were prepared, one with alginate in the water droplets and the other with calcium chloride in the water droplets. In both cases, the oil phase consisted of a hydrophobic polymer (poly(methyl methacrylate) dissolved in an organic solvent (1:1 chloroform-toluene). These two emulsions were then mixed together, which led to cross-linking of the alginate molecules. The calcium alginate microgels were then removed by centrifugation followed by washing with an organic solvent, and then they were freeze dried to form a powder. The dried microgels were then dispersed into an aqueous solution containing insulin, which caused the bioactive peptide to be absorbed into them. An in vitro study showed that the insulin was only released slowly under simulated gastric conditions, but more rapidly under simulated small intestine conditions. The microgels formed in this study were relatively small (<150 nm), which would be advantageous for some applications. However, the formation procedure used was again fairly complex, and used some ingredients and solvents that would not be appropriate for functional food applications.

Insulin has been encapsulated within solid lipid nanoparticles (SLNs) produced using a W/O/W emulsion solvent evaporation method [118]. Initially, the insulin was dissolved in water, which was then homogenized with a liquid oil phase containing a high-melting lipid (trimyristin), an organic solvent (dichloromethane), and a lipophilic surfactant (soy lecithin) to form a W/O emulsion. This W/O emulsion was then homogenized with an aqueous phase containing a hydrophilic surfactant (PVA) to form a W/O/W emulsion. The organic solvent was evaporated by mild heating, which caused the remaining oil phase (trimyristin) to crystallize, leading to the formation of insulin-loaded SLNs with diameters below 250 nm. In vitro studies showed that the insulin could be effectively encapsulated within the SLNs, and protected against enzyme degradation under simulated gastric conditions. In vivo studies showed that administration of the insulin-loaded SLNs to diabetic rats led to an appreciable increase (5-fold) in oral bioavailability compared to administration of non-encapsulated insulin. Although all the ingredients used in this study were not suitable for utilization in foods, it is possible to prepare this kind of delivery system using food-grade ingredients. Other researchers have used a similar approach to encapsulate insulin in SLNs formulated from another high-melting lipid (a triglyceride blend), and showed that the delivery systems formed had low toxicity using a fruit fly model [119]. The W/O/W emulsion solvent-evaporation method has also been used to encapsulate insulin within polymeric nanoparticles [151], but again the polymers used in this study were not food grade.

9.2. Digestive enzymes: lipase and lactase

Some individuals suffer from diseases related to the fact that they cannot produce sufficient quantities of digestive enzymes, such as lipase or lactase. In these cases, it may be useful to orally deliver encapsulated digestive enzymes to the appropriate region of the GIT. For instance, digestive enzymes could be delivered to the small intestine, such as lactase for individuals with lactose intolerance [8,9] or lipase for individuals with pancreatitis [10]. However, the structure and activity of digestive enzymes is highly sensitive to their local environment, which may lead to a loss of their potential health benefits. In particular, many digestive enzymes are denatured in the human stomach because of the high acidity and protease activity of the gastric fluids. Consequently, delivery systems are needed to encapsulate and protect the enzymes in the stomach, but then release them in the small intestine. In this section, a number of approaches that have been used to encapsulate digestive enzymes are described.

Solid-in-oil-in-water (S/O/W) emulsions (Fig. 1) have recently been developed as delivery systems for lactase [115]. Initially, a spray dried lactase powder was dispersed in an oil phase with a lipophilic surfactant (Span 80) to form a solid-in-oil phase. This S/O phase was then homogenized with a water phase containing a hydrophilic emulsifier (sodium caseinate) to form a S/O/W emulsion. The authors showed that the lactase could be encapsulated with a 75% efficiency, and that the capsules were stable in milk for up to 3 weeks. Encapsulation was also reported to retain the activity of lactase within simulated GIT fluids.

Hollow microparticles with pH-responsive pores have been prepared using an emulsion-evaporation method, and then used to encapsulate, protect and release lactase [152]. An oil phase was formed by dissolving a hydrophobic polymer (esters of acrylic and methacrylic acid) into an organic solvent/co-solvent mixture. The oil phase was then homogenized with an aqueous phase containing hydrophilic emulsifiers (polyvinyl alcohol and Tween 20) to prepare an O/W emulsion. The emulsion was then centrifuged and washed, and then the organic solvent was removed by evaporation. Finally, the samples were filtered and freeze dried to obtain empty polymer capsules. The capsules were then loaded with lactase by mixing them with an aqueous lactase solution and then placing the resulting mixture into a vacuum oven and switching the vacuum on/off multiple times. These capsules were shown to retain and protect the lactase under simulated gastric conditions, but then release them under simulated small intestine conditions. It was proposed that the release mechanism was the opening up of pores on the surfaces of the capsules under neutral conditions in the small intestine. The procedure used to form these capsules is quite complex and involves ingredients that would be unsuitable for functional food applications, but it may be possible to fabricate them from food-grade ingredients.

Another recent study showed that lactase could be encapsulated within gastro-protective alginate beads [138]. Conventional alginate beads have relatively large pore sizes, which means that gastric acids and proteases can easily diffuse into them and deactivate the encapsulated lactase [153]. An oil phase was formed by dissolving a hydrophobic polymer (esters of acrylic and methacrylic acid) into an organic solvent/co-solvent mixture. The oil phase was then homogenized with an aqueous phase containing hydrophilic emulsifiers (polyvinyl alcohol and Tween 20) to prepare an O/W emulsion. The emulsion was then centrifuged and washed, and then the organic solvent was removed by evaporation. Finally, the samples were filtered and freeze dried to obtain empty polymer capsules. The capsules were then loaded with lactase by mixing them with an aqueous lactase solution and then placing the resulting mixture into a vacuum oven and switching the vacuum on/off multiple times. These capsules were shown to retain and protect the lactase under simulated gastric conditions, but then release them under simulated small intestine conditions. It was proposed that the release mechanism was the opening up of pores on the surfaces of the capsules under neutral conditions in the small intestine. The procedure used to form these capsules is quite complex and involves ingredients that would be unsuitable for functional food applications, but it may be possible to fabricate them from food-grade ingredients.

Lactase has also been encapsulated within enteric-coated capsules formed from polyacrylic acid (PLA) and hydroxypropyl methycellulose phthalate (HMP) [155]. The lactase was first encapsulated within PLA nanoparticles formed using a W/O/W emulsion-evaporation method,
and then these lactase-loaded nanoparticles were filled into HMP capsules. The authors reported that the lactase remained inside the capsules and retained its activity under simulated gastric conditions, but was released under simulated small intestine conditions. Again, the polymers used to fabricate this kind of capsules may be unsuitable for application in many functional food products, but the same principles may be used to form food-grade versions.

9.3. Vaccines

It would be advantageous to deliver many protein-based vaccines orally because this is more convenient than giving injections. Ideally, orally ingested vaccines (antigens) should be taken up by M-cells in the Peyer's patches of the gastrointestinal tract so as to promote immunity responses against infections [156]. However, oral delivery of vaccines is challenging because of their tendency to be degraded in the GIT, and their relatively low absorption by the epithelium cells [157]. Consequently, there is considerable interest in orally administering vaccines in the form of colloidal delivery systems that can target M-cells [142,158].

A recent study showed that model vaccines could be encapsulated within polymer (methylacrylate-g-ethylene glycol) microgels, and that this increased the biological response of the vaccines when orally administered to mice [159]. Although the polymer used was not food-grade, this study highlights the potential of using biopolymer microgels for this purpose.

Another recent study utilized liposomes coated with an oppositely charged polyelectrolyte to encapsulate and deliver vaccines through the oral route [160]. The authors reported that the encapsulated vaccine gave a higher antigen-specific response in an in vivo mouse model than the non-encapsulated form. Biopolymer nanoparticles constructed from trimethyl chitosan and hydroxypropyl methylcellulose phthalate (HPMCP) have also been used to encapsulate protein-based vaccines i.e., hepatitis B surface antigen [161]. The nanoparticles formed were relatively small (d = 158 nm), and had a high encapsulation efficiency (EE = 86%). An in vitro study using a GIT model showed that the biopolymer nanoparticles could retain and protect the vaccine from degradation in a simulated gastric phase.

Researchers have utilized biopolymer microcapsules based on a synthetic polymer (thiolated Eudragit) to encapsulate vaccines (various protein antigens) so that they could be delivered orally [156]. These authors showed that the encapsulated vaccines were taken up by M-cells using an in vivo mouse model, and that they were able to elicit an immune response.

Some probiotics are able to secrete vaccines in the GIT after ingestion, but they are susceptible to degradation during gastrointestinal transit, which currently limits their application. For this reason, researchers have used colloidal delivery systems to encapsulate vaccine-producing probiotics and protect them from harsh GIT conditions. For instance, biopolymer microcapsules assembled from alginate and chitosan have been used to encapsulate a probiotic (Lactobacillus plantarum 25) that expresses M-cell homing protein-based vaccines [162]. Encapsulation was shown to increase the viability of the probiotics when exposed to simulated food storage and GIT conditions, but to release them after prolonged exposure to simulated small intestinal conditions. Moreover, the encapsulated probiotics were more effective at eliciting an immune response than the non-encapsulated ones.

A number of other types of colloidal particles have also been shown to be capable of encapsulating and improving the bioactivity of protein-based vaccines, including starch microparticles [163], archeosomes [164], liposomes [165], W/O/W emulsions [166,167], liposomes in W/O/W emulsions [168] and PLGA nanoparticles [169]. It should again be stressed, that many of these systems would be difficult to implement commercially in functional foods because they involve non-food grade ingredients, the fabrication processes are not economical, or the systems may not be stable enough.

9.4. Antimicrobials

Certain types of bioactive proteins have been shown to have good antimicrobial activity, e.g., nisin, lysozyme, cell-penetrating peptides [170]. However, the efficacy of these protein-based antimicrobials is often compromised because of their susceptibility to hydrolysis and molecular interactions with other components in complex food matrices. Consequently, there is considerable interest in the utilization of colloidal delivery systems to improve their efficacy [171,172]. Studies have shown that antimicrobial peptides can be incorporated into W/O micellar structures, and that their activity against bacteria (E. coli) depended on the composition and structure of the surfactant-oil-water system used [173]. In particular, the encapsulated peptides were shown to have a higher antimicrobial activity than the free peptides. Liposomes fabricated from phospholipids have been used to encapsulate nisin, and were shown to maintain a high encapsulation efficiency (70–90%), even when exposed to pH extremes and elevated temperatures [174]. Similar systems were shown to increase the antimicrobial activity of nisin and lysozyme against Listeria monocytogenes [175]. Biopolymer nanoparticles constructed from phytooligosaccharide have also been used as carriers of nisin, and were shown to prolong its antimicrobial activity against Listeria monocytogenes [176]. The activity of other antimicrobial proteins have also been shown to be improved after encapsulation in various types of colloidal particles, including nisin in liposomes [177], nisin in chitosan microcapsules [178,179], nisin in pectin microparticles [180], nisin in alginate/pectin microparticles [181], lysozyme in zein microparticles [182,183], and lysozyme in cross-linked starch microgels [184].

9.5. ACE inhibitors

A number of peptides have been shown to exhibit angiotensin I-converting enzyme (ACE) inhibitor activity, and can therefore be utilized to treat individuals with hypertension [185–187]. However, the oral delivery of these peptides is often challenging because they may have a bitter taste, and they are degraded during passage through the GIT, which reduces their activity. Consequently, delivery systems should mask their unpleasant taste in the mouth, protect them in the stomach, but then release them in the small intestine. Alternatively, encapsulation of proteins in delivery systems may alter the rate and extent of their hydrolysis in the GIT, thereby controlling the type and location of bioactive peptides generated within the gastrointestinal tract.

At present, there have been few studies on the utilization of colloidal delivery systems for this application. However, a recent study showed that an ACE inhibitor peptide (VLPVP) could be encapsulated within biopolymer microgels formed from chitosan and alginate using a membrane homogenizer [188]. An aqueous solution containing alginate and ACE inhibitor was injected into an oil phase to form a W/O emulsion, and then the alginate was cross-linked by adding calcium ions. Finally, the peptide-loaded alginate beads were washed with organic solvent, and then dispersed in an aqueous chitosan solution to coat them with this cationic biopolymer. It was shown that the peptide could be effectively trapped inside the microgels, and that the microgels protected the peptide from release and degradation under simulated gastric conditions, but released it under simulated small intestine conditions.

10. Conclusions

This article has provided a comprehensive review of the potential application of colloidal delivery systems to encapsulate, protect, and
release bioactive proteins and peptides, such as hormones, enzymes, vaccines, antimicrobials, and ACE inhibitors. The oral delivery of these bioactive proteins is often challenging because they are susceptible to denaturation, hydrolysis, or aggregation in commercial products and within the human GIT, and typically have a relatively low absorption across the epithelial layer. A broad spectrum of different colloidal systems has been investigated for their potential application as delivery systems for bioactive proteins, including microemulsions, emulsions, solid lipid nanoparticles, liposomes and biopolymer microgels. These delivery systems vary considerably in their cost, composition, ease of preparation, impact on product quality and sensory attributes, stability in products, and ability to increase protein activity. At present, there is still an urgent need to identify effective delivery system candidates for particular bioactive proteins, and to compare their relative advantages and disadvantages so that the optimum one for a particular application can be selected.

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