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Multilayer emulsions stabilized by vegetable proteins and polysaccharides

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ABSTRACT

There is great interest in the food, cosmetic and pharmaceutical industry in the use of proteins and polysaccharides as natural hydrocolloids to create novel emulsion systems with improved stability and functionality. For example, the electrostatic interaction between proteins and polysaccharides may be used to form oil-in-water (O/W) emulsions with multilayered interfacial membranes around oil droplets or multilayer emulsions. This type of emulsions have been developed using the layer-by-layer (*LbL*) technique, which consists of direct adsorption of an oppositely charged polyelectrolyte layer (e.g. polysaccharides) on a primary layer of ionic emulsifiers (e.g. proteins). The polymeric structure and electrical charge of proteins make them a special class of compounds very suitable for its utilization in the *LbL* technique. In recent years, the utilization of proteins as emulsifiers in food and pharmaceutical industry has been turning towards plants as a preferred alternative to animal-based sources. This article reviews the current understanding of the utilization of different vegetable proteins as emulsifier in order to stabilize O/W multilayer emulsion systems. Additionally, it highlights some potential applications of the multilayer emulsion technology in the industry, for improving the stability of emulsions to environmental stresses and for developing controlled or triggered release systems.

1. Introduction

Current technological development in industry demands new emulsifiers or methodologies to obtain stable emulsions, thus creating novel products or improving its Shelf-life associated with an adequate delivery activity, efficiency and yield of active principles. Oil-in-water emulsions (O/W) are widely used in the cleaning, cosmetics, pharmaceutical and food industries for encapsulating different bioactive compounds and increasing their solubility and stability. Homogenization of oily and aqueous phases is achieved in the presence of one or more emulsifiers. The emulsifier is adsorbed to the surface of just formed droplets reducing the interfacial tension and facilitating droplet disruption.

In recent years, proteins from animal and vegetables sources, have been considered as natural emulsifiers in different industrial processes [1]. Their biodegradability, compatibility and excellent characteristics are remarkable properties of these natural polymers. Moreover, industries in their search for protein ingredients have been turning towards plants as a preferred alternative to animal-based sources, e.g. in vegetarian diets, due to increased consumer concerns over the safety of animal-derived products [2•].

Many types of proteins can be used as emulsifiers due to their amphiphilic character, polymeric structure, and electrical charge characteristics [3]. The amphiphilic character means that they can be adsorbed to the droplet surfaces during homogenization. Despite their good features, protein-stabilized emulsions are highly sensitive to environmental stresses such as pH, ionic strength and temperature, affecting encapsulated compounds [4••]. In this regards, it has been shown that these emulsions are particularly sensitive to pH and ionic strength. They tend to flocculate at pH values close to the isoelectric point of the adsorbed proteins and when the ionic strength exceeds a particular level, because the electrostatic repulsion between the droplets is then no longer sufficiently strong to overcome the various attractive interactions. This instability may limit their application in some commercial products [5]. As a way to overcome this disadvantage, the strategy is the incorporation of additional polysaccharide coating layers that stabilize the O/W emulsions by means of electrostatic interaction with the protein layer. Along these lines, Guzey and McClements [4••] convey that, one strategy to improve the physical stability of O/W emulsions to environmental stresses is to form multilayer emulsions. Multilayer

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emulsions consist of various interfacial layers of proteins (emulsifier) and/or polysaccharides around oil droplets which are deposited using the *LbL* electrostatic technique. *LbL* has been shown to have potential applications in foods, including controlled/triggered release, stabilization of emulsions, processing and storage conditions. The production of stable multilayered emulsions using this technique depends on biopolymer properties (e.g., charge density, molecular weight and conformation), emulsifier layer thickness and bulk physicochemical conditions [1]. Additionally, multilayer emulsions have the potential to decrease lipid oxidation rates due to their ability to alter both the emulsion droplet charge and thickness of the interfacial region [6]. The electrical properties of the first layer of a multilayer emulsion are determined by the emulsifier, and they can therefore be controlled by selecting different types of emulsifiers [4••].

Several studies have shown that vegetable proteins can be used to prepare multilayer emulsions using the *LbL* technique [1,3,7]. The use of vegetable proteins as emulsifier reflects the present "green" trend in the pharmaceutical, cosmetics and food industries. In food applications, vegetable proteins are known to be less allergenic compared to animal derived proteins [2•]. This review presents the recent works dealing with the use of vegetable proteins in the formation of multilayer emulsion systems. The influence of the combination of different biopolymers, proteins, and polysaccharides on stability of O/W multilayer emulsions, and the industrial applications in several industrial processes will be particularly discussed. Besides, the review is focused on the utilization of *LbL* technique in the formation on multilayer emulsions. The information collected to prepare this revision follows the description of main published experimental studies in the last ten years.

2. Vegetable proteins as emulsifier

Proteins are commonly used in the food and pharmaceutical industry as emulsifiers in the stabilization of O/W emulsions. These natural polymers present several advantages: biocompatibility, biodegradability, good amphiphilic and functional properties such as water solubility, emulsifying and foaming capacity. Proteins are amphiphilic compounds, and therefore, they are able to adsorb strongly at the oil–water interface, favoring emulsion formation [8]. The amount adsorbed and the conformation adopted at the oil–water interface will depend very much on the protein amino acid composition since adsorption occurs through hydrophobic groups present within their structure [9].

Nowadays, the utilization of vegetable proteins as emulsifiers has increased even surpassing the use of proteins from animal sources. In food applications, for example, vegetable proteins are known to be less allergenic compared to animal derived proteins. Vegetable proteins consist of several fractions: the major fraction is glutenin, followed by globulin fraction, albumin and prolamin [2•].

Among vegetable proteins used as emulsifiers, we can mainly find soy protein isolate, pea protein isolate, lupin protein isolate, broad beans and cereal proteins (such as wheat proteins) [10,11]. These types of proteins have been used to facilitate the formation, improve the stability, and provide specific physicochemical properties to emulsions [2•]. Many vegetable proteins are surface-active molecules that can be used as emulsifiers because of their ability to facilitate the formation, improve the stability and produce desirable physicochemical properties in O/W emulsions [12•].

Despite their functional properties and benefits, emulsions stabilized by proteins are highly sensitive to environmental stresses such as pH, ionic strength and temperature [13]. For example, at pH values close to the isoelectric point of protein and/or high salt concentration in the emulsion, the electrostatic repulsion of the protein adsorption layers decreases and therefore, coalescence and flocculation happen [13]. Besides, when emulsion is subjected to heat treatment, for pasteurization or sterilization purposes, flocculation happens because of the protein denaturation which holds the droplets together [14]. For this reason, several strategies have been developed to improve the stability of protein-stabilized emulsions to droplet flocculation induced by pH or ionic strength effects. For example, (1) the incorporation of multivalent counterions, such as Ca^{2+}, Fe^{2+} or Fe^{3+}, to emulsions systems; (2) the addition of ionic surfactants to protein-stabilized emulsion to change the pH dependence of the ζ-potential of the droplets, thereby changing the range of pH values so that the emulsion is stable to flocculation [12•]; and, (3) the addition of electrically charged biopolymers to the surface of oppositely charged droplets to a protein-stabilized emulsion in order to increase its physical stability to environmental stresses [15•]. The *LbL* electrostatic technique is normally utilized for forming this type of systems. This last topic will be explained in detail in Sections 3 and 4 of the present review.

3. Design and preparation of multilayer emulsions

Multilayer emulsions consist of small oil droplets, dispersed in an aqueous medium, surrounded by a multilayered interfacial membrane generally composed of an emulsifier (surfactant or protein) and a charged biopolymer (polysaccharides) (Fig. 1) [7]. Emulsions containing oil droplets stabilized by protein–polysaccharide membranes are formed using *LbL* deposition process. In this method, a primary emulsion is prepared by homogenizing oil and water phases in the presence of a positively or negatively charged emulsifier. The resulting primary emulsion is then mixed with an oppositely charged polyelectrolyte solution to create a secondary emulsion. The secondary emulsion is then mixed into another solution containing polyelectrolytes that have an opposite charge to the previous one to create a tertiary emulsion (Fig. 2) [4••].

The *LbL* deposition technique offers a promising way to prepare emulsions using electrostatic attraction of charged biopolymers to oppositely charged droplets [4••]. Emulsions prepared using this method have been shown to have enhanced stability in respect of ionic strength [4••], pH [16] and temperature [15•]. Moreover, this method provides a new powerful tool to improve resistance of food emulsions to extreme environmental stresses [4••].

According to Burgos-Díaz and coworkers [15•], to create stable multilayer emulsions with the required physicochemical properties, it is essential to choose a suitable combination of emulsifier and biopolymers. Thus, the ionic behavior (electrical charge) should be evaluated as a function of pH between protein (emulsifier) and each polysaccharides used in the system, to determine the highest protein–polysaccharide electrostatic attraction. It should be noted that, the electrical properties of the first layer of multilayer emulsions are determined by the charge of the emulsifier, and they can thus be controlled by selecting different types of emulsifiers. Each of these emulsifiers has different electrical characteristics, which can influence the formation and properties of multilayer interfaces [4••]. There are three methods of preparation that have been developed to produce stable multilayer systems: (i) saturation method, (ii) centrifugation method, and (iii) filtration method [4••]. For all of the methods mentioned above the (i) saturation method is an easy and rapid way to prepare multilayer emulsions. This technique consists mainly in the determination of the concentration in which oil droplets of emulsion are completely surrounded by polyelectrolyte. According to Guzey and McClements [4••], the saturation concentration for a particular system has to be determined empirically (for example, using ζ-potential measurements). For example, Burgos-Díaz and coworkers [15•] used the saturation method to prepare secondary emulsions using lupin protein isolate from Alu*Prot*-CGNA and chitosan. Fig. 3 shows that the ζ-potential values of emulsions (at pH 5) increased significantly when the chitosan concentration was increased between 0.02 and 0.06 (wt.%), indicating that chitosan molecules were adsorbed onto lupin protein. Additionally, the net charge on the droplet changed from negative to positive as the chitosan concentration was increased until reaching a relatively constant value, which indicates that cationic chitosan molecules