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Multilayer Oil-in-Water Emulsions: Formation, Characteristics and Application as the Carriers for Lipophilic Bioactive Food Components – a Review

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This review article demonstrates fundamentals regarding the manufacturing of multilayer oil-in-water (M-O/W) emulsions and factors affecting stability of these systems. Moreover, characteristics of major bioactive lipophilic components and ingredients mostly applied to form multilayered membranes as well analytical methods used to examine properties of M-O/W emulsions are specified. It has been shown that production of M-O/W systems is based on the layer-by-layer (LbL) electrostatic deposition technique which makes use of the electrostatic attraction of oppositely charged surfactants and biopolymers to form multicomposite protective layers around emulsion droplets. Finally, limitations regarding studies of M-O/W systems which should be developed are specified.

INTRODUCTION

Lipophilic bioactive food components (nutraceuticals) comprise a variety of ingredients such as fatty acids, carotenoids, phytosterols and oil soluble vitamins. Their inclusion is a prerequisite for the manufacturing of functional foods designed to improve the long-term health and well-being of consumers worldwide [Wackerbarth *et al*., 2009; McClements & Li, 2010a; Yang & McClements, 2013]. The oil-in-water (o/w) emulsions are very suitable for many practical applications where transfer, delivery and/or controlled release of different active species are needed [Grigoriev & Miller, 2009; McClements & Li, 2010b; Bortnowska, 2012]. For example, the incorporation of lipophilic bioactive components into o/w emulsions may increase compatibility with aqueous environment, retard their chemical degradation as well as enhance their activity [McClements *et al*., 2007; Shchukina & Shchukin, 2011]. However, the o/w systems, containing oil droplets coated with single-layered membranes, are often prone to physical instability, especially when they are exposed to environmental stresses such as heating, refrigeration, freezing, drying, pH and ionic strength changes [Chuah *et al*., 2009; Fredrick *et al*., 2010; Wang *et al*., 2011]. Moreover, their capacity to both protect and control the release of encapsulated lipophilic compounds may be limited due to the low thickness of the interfacial membranes and consequently their high rate of molecular diffusion [Gharsallaoui *et al*., 2012].

Recent studies have shown that the application of interfacial engineering technology to manufacture multilayer oil-in-water (M-O/W) emulsions can lead to the formation of versatile delivery systems with improved stability towards physical and chemical changes at different environmental conditions [Klinkesorn & McClements, 2010; Tokle *et al*., 2010; Zeeb *et al*., 2011]. M-O/W emulsions typically consist of oil droplets (the core) surrounded by nanometer thick layers (the shell) comprised of different surfactants and biopolymers (polyelectrolytes), *i.e*. proteins and polysaccharides [Weiss *et al*., 2006; Wang *et al*., 2011]. This technology is based on the electrostatic layer-by-layer (LbL) deposition technique which involves repeated adsorption of oppositely charged biopolymers onto the oil droplets coated with ionic emulsifiers [Li *et al*., 2010; Dickinson, 2011]. The successful formation of these interfacial complexes depends on careful control of system conditions such as: (i) droplet characteristics (*e.g*. concentration, size, charge), (ii) biopolymer properties (*e.g*. concentration, molecular weight), (iii) solution composition (*e.g*. pH, ionic strength), and (iv) mixing conditions (*e.g*. order of addition, stirring speed) so as to prevent bridging and depletion flocculation [Pallandre *et al*., 2007; McClements, 2012]. Similarly as classic o/w emulsions, the M-O/W ones can be transformed into powder form [Gharsallaoui *et al*., 2012; Serfert *et al*., 2013]. For many spray-dried capsules, the ultimate purpose is to have the encapsulated material released at a controlled rate when the powder is dispersed in a product or comes into contact with saliva during ingestion [Jones & McClements, 2010]. However, the relatively high temperature, needed to facilitate water evaporation, may induce chemical interactions

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in the material as well as lower the viability of the encapsulated components and consequently reduce their activity in the final product [de Vos *et al*., 2010; Nazzaro *et al*., 2012].

Depending on the affinity, nutraceuticals can be incorporated into the oil phase prior to homogenization or into one or more of the biopolymer layers surrounding oil droplets [McClements *et al*., 2007; Lesmes *et al*., 2010]. Components trapped within the core of M-O/W emulsion could be released in response to a specific environmental triggers such as dilution, pH, ionic strength, temperature, or enzyme activity due to, *e.g*. complete shell dissociation or modulation of its porosity [Li *et al*., 2010; McClements & Li, 2010a; McClements, 2012; Benjamin *et al*., 2012]. It has been therefore assumed that the M-O/W systems can be widely used as the delivery systems for bioactive lipophilic components in the food, pharmaceutical, and biopharmaceutical industries [Johnston *et al*., 2006; Klinkesorn & McClements, 2010; Li *et al*., 2010; McClements & Li, 2010b; Hu *et al*., 2011].

In many respects M-O/W emulsions may have similar bulk physicochemical properties as classic ones with similar particle characteristics, *e.g*. concentration, size distribution and net charge. Nevertheless, the possibility of precise engineering the properties of the nanolaminated shells that coat the droplets provides great scope of options to improve their stability and functional performance [Aoki *et al*., 2005; Guzey & McClements, 2007; McClements & Li, 2010a; Cho *et al*., 2010; Wang *et al*., 2011; Schmelz *et al*., 2011].

This review article is focused on the recent achievements in the development of M-O/W emulsions in relation to main factors that affect their formation. Moreover, characteristics of major lipophilic bioactive components, potential applications of these emulsions and analytical methods used to study properties of M-O/W systems are considered.

CHARACTERISTICS OF MULTILAYERED MEMBRANE COMPONENTS

A variety of different food-grade emulsifiers and biopolymers can be used to assemble nanolaminated coatings around lipid droplets, including various surfactants, phospholipids, proteins and polysaccharides [Gu *et al*., 2005; de Vos *et al*., 2010; McClements & Li, 2010a].

Relatively large number of food-grade small molecule emulsifiers (surfactants and phospholipids) is offered by the industry to prepare emulsions with negatively charged oil droplets, *e.g*. lecithins, fatty acid salts, diacetyl tartaric acid esters of mono- and diglycerydes, sodium stearoyl lactylates, and citric acid esters of mono- and diglycerydes (Table 1). Lecithin is a zwitterionic emulsifier, however in emulsified systems (acid environment) it forms negatively-charged layers on the oil droplets [McClements *et al*., 2007; Klinkesorn & McClements, 2009]. In some experiments, sodium dodecyl sulfate (SDS) with relatively high hydrophilic-lipophilic balance (HLB = 40) has also been used as a model anionic surfactant. SDS is a non-food-grade emulsifier, but it does represent a number of small-molecule anionic surfactants that are commonly used in foods, can be obtained in high purity and its properties are generally well understood [Stauffer, 2001; Surh *et al*., 2005; Thanasukarn *et al*., 2006]. It has been found that surfactant-coated droplets remain highly negative across a wide pH range [Hong & McClements, 2007]. This severely limits the application of food-grade polysaccharides that can be used to produce multilayered membranes, since most of them are also anionic [McClements *et al*., 2007]. Small molecule emulsifiers differ in their values of hydrophilic-lipophilic balance and this enables to prepare mixtures of surfactants exhibiting differentiated thermodynamic surface activity (Table 1). Surfactants are usually more surface-active than proteins because they can pack more efficiently at the oil-water (o-w) interface, therefore competitive adsorption between these two components at the o-w interface may occur [Stauffer, 2001; Bortnowska, 2008; Grigoriev & Miller, 2009; Dickinson, 2011].

Proteins are biological polymers comprised of 20 common amino acids linked together through peptide bonds. The type, number and sequence of amino acids along the polypeptide chain determine the molecular weight, conformation, hydrophobicity, electrical charge, physical interactions and chemical reactivity of proteins [Jones & McClements, 2010]. Selected characteristics of proteins commonly used to manufacture multilayered membranes are demonstrated in Table 2. Food proteins exhibit surface-active properties due to a distinct distribution of hydrophobic and hydrophilic moieties, and therefore may be used as emulsifiers to formulate o/w emulsions [Zeeb *et al*., 2011]. The primary driving force, for the o-w adsorption of proteins, is the removal of the non-polar side-chains of the biopolymer away from the unfavourable environment of the aqueous solution. Whereas, the secondary driving force is associated with the unfolding of the protein molecule on adsorption. The level of proteins unfolding determines the cohesivity of the adsorbed layer, because the higher the unfolding the more probable is the crosslinking between neighbouring proteins [Stauffer, 2001; Dickinson, 2011]. Protein hydrophobicity is divided into two functional categories: surface hydrophobicity and internal one. Surface hydrophobicity is important molecular characteristic property influencing strongly interactions with: (i) nonpolar components (*e.g*. fatty acids, flavors), (ii) nonpolar surfaces (oil or air), and (iii) other proteins. Whereas, internal hydrophobicity results from globular conformations and is relatively ineffective in bulk protein interactions [Grigoriev & Miller, 2009; Jones & McClements, 2010]. Electrical charge of proteins may be both positive and negative and is driven by both isoelectric point (pI) of protein and pH of solution [Guzey & McClements, 2006]. At relatively high H^+ concentrations (pH << pI), the amino groups are positively charged ($–NH_3^+$) and the carboxyl groups are neutral (–COOH), so the net protein charge is positive. Whereas, at relatively low H^+ concentration (pH >> pI), the carboxyl groups are negatively charged ($–COO^-$), and the amino groups are neutral ($–NH_2$), thus the net protein charge is negative [Schmelz *et al*., 2011; Charoen *et al*., 2012]. However, the electrical charge distribution on protein surfaces is heterogeneous with varying amounts of positive and negative regions and even though the net charge on protein is zero at its pI, the protein still has both positive and negative regions on its surface and thus it can be involved in attractive and/or repulsive electrostatic

TABLE 1. Hydrophilic-lipophilic balance (HLB), acceptable daily intake (ADI) and solubility of selected food-grade ionic small molecule emulsifiers used to produce multilayer M-O/W emulsions. Derived from: Stauffer [2001], Rutkowski *et al*. [2003], Fernandes *et al*. [2012].

Name	Abbreviation	EU number	HLB	ADI (mg/kg)	Solubility
Lecithins	Lecithins	E 322	9–11	ND	oil/water
Fatty acid salts	FA	E 470a	18–20	ND	oil/water
Sodium stearoyl-2-lactylate	SSL	E 481	10–12	0–25	water
Citric acid esters of mono- and diglycerydes	CITREM	E 472c	10–12	ND	water
Diacetyl tartaric acid esters of mono- and diglycerydes	DATEM	E 472e	8–10	0–50	oil/water

ND, not determined.

TABLE 2. Molecular weight (MW), main structural type and isoelectric point (pI) of proteins used to produce multilayered membranes. Derived from: Sikorski [2007], Shutava *et al*. [2009], Matalanis *et al*. [2011].

Name	MW (kDa)	Main structural type	pI
β-Lactoglobulin	18–18.3	globular	4.9–5.4
Bovine serum albumin	66.3–68	globular	4.7–5.1
Caseins	19–25.2	rheomorphic	4.9–6.1
Ovalbumin	44–45	globular	4.5–4.7
Soy glycinin	150–170[a]; 350–354[b]	globular	4.8–5
Lactoferrin	76–80	globular	8.1–9
Gelatin	15–250[c]	linear	7–9.4[d]; 4.7–5.5[e]

[a] 7S fraction. [b] 11S fraction. [c] depending on the source and hydrolysis conditions. [d] Type A gelatin. [e] Type B gelatin.

interactions [Matalanis *et al*., 2011]. Proteins interact with other components through a variety of physical or chemical interactions. Physical interactions (*e.g*. electrostatic or hydrophobic forces) play a role in the interchange with solvents, cosolvents, phospholipids, surfactants, polysaccharides, sugars and minerals. Chemical reactions among proteins include: (i) disulfide interchanges, (ii) dehydratation, (iii) phenolic oxidation as well as (iv) Maillard and transglutaninase reactions [McClements, 2006; Jones & McClements, 2010; Ye *et al*., 2011; Benjamin *et al*., 2012].

Polysaccharides are polymers of monosaccharides differing from one another chemically in terms of the type, number, sequence and bonding of the monosaccharides within the polymer chain. These chemical differences lead to variations in molecular properties, such as molecular weight, degree of branching, structure, flexibility, electrical charge, and interactions [Stauffer, 2001; Matalanis *et al*., 2011]. Molecular conformations of polysaccharides are limited to random coil or helical structures. Linear structures are favoured as glycosidic bond rotation and chain flexibility is restricted. Helical polysaccharides (*e.g*. carrageenan) are constructed from hydrogen bonds and undergo conformational changes with heating. Helix-to-coil transition occurs at a specific temperature (T_m) whereupon the polysaccharide may reassociate into intermolecular junctions [Jones & McClements, 2010]. The electrical charge on polysaccharides depends on the nature of the ionic groups along the chain background as well as solution conditions. These compounds can be: (i) neutral (*e.g*. starch, dextran, agar, galactomannans, cellulose), (ii) anionic (*e.g*. alginate, pectin, gum arabic, carrageenan, xanthan, gellan), and (iii) cationic (*e.g.* chitosan) [McClements *et al*., 2007; Li *et al*., 2012; McClements, 2012]. Anionic polysaccharides tend to be neutral at pH values sufficiently below their pK_a values and negative above, whereas cationic polysaccharides tend to be neutral at pH values sufficiently above their pK_a values but positive below. The most common charged groups on polysaccharides are: (i) sulfate groups (*e.g*. carrageenan), (ii) carboxyl groups (*e.g*. pectin, alginate, xanthan, carboxymethylcellulose) and (iii) amino groups (*e.g*. chitosan): $-SO_4H \leftrightarrow -SO_4^-$ ($pK_a \sim 2$); $-CO_2H \leftrightarrow -CO_2^-$ ($pK_a \sim 3.5$); $-NH_3^+ \leftrightarrow -NH_2$ ($pK_a \sim 6.5$) [Aoki *et al*., 2005; Matalanis *et al*., 2011]. In an acid environment chitosan behaves as polycationic electrolyte and this yields antifungal or antimicrobial activities since cations can bind to anionic sites on bacterial and fungal cell wall surfaces [de Vos *et al*., 2010; Weiss *et al*., 2006]. However, at higher pH, it tends to lose its charge and may precipitate from solution due to deprotonation of the amino groups [Hong & McClements, 2007]. It should be also underlined that the pK_a of the ionizable groups on a polyelectrolyte can be shifted from their values in solution due to their local electrostatic environment. This change in pK_a can alter the pH where one would expect a polyelectrolyte layer to become desorbed from an o-w interface [Guzey & McClements, 2006]. Some of the polysaccharides exhibit satisfactory surface-activity. This has its molecular origin in either the non-polar character of chemical groups attached to the hydrophilic backbone (*e.g*. hydrophobically modified starch/cellulose) or the presence of a protein component linked covalently or physically to the polysaccharide (*e.g*. some gums, pectins) [Dickinson, 2009; Wang *et al*., 2011]. These hydrocolloids, when adsorbed at the oil-water interface, can be more effective than surfactants and proteins

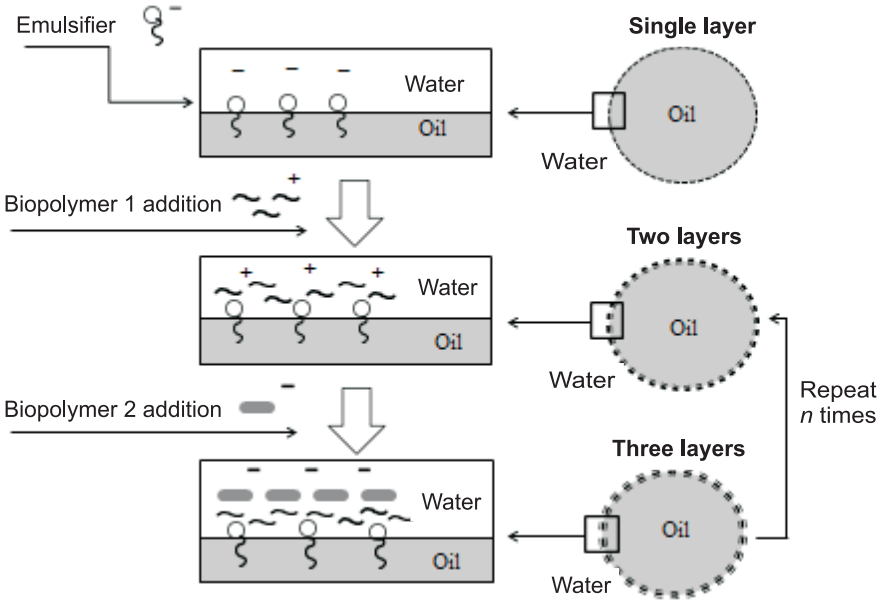

FIGURE 1. Scheme of the formation of multilayered membranes on oil droplets (anionic-droplet approach).

in conferring long-term emulsion stability due to the formation of a thicker and stronger secondary layer facilitating electrostatic and steric stabilization [Chuah *et al*., 2009; Charoen *et al*., 2012]. However, they can be not sufficiently effective as proteins to prevent release of volatile lipophilic compounds [Bortnowska, 2012].

MULTILAYER OIL-IN-WATER EMULSIONS FORMATION

The multilayer oil-in-water (M-O/W) emulsions are formed applying a layer-by-layer (LbL) electrostatic deposition technique which involves repeated adsorption of ionic biopolymers onto the surfaces of oppositely charged oil droplets coated by an emulsifier [Humblet-Hua *et al*., 2011; McClements, 2012]. Depending on the number of interfaces surrounding droplets, the primary, secondary, tertiary, quaternary, *etc*., emulsions can be produced [Hu *et al*., 2011]. The formation of multilayered membranes on the oil droplets is presented in Figure 1.

The primary emulsion containing single-layered charged droplets is manufactured by homogenizing an oil and aqueous phase together in the presence of an ionized hydrophilic emulsifier that rapidly adsorbs to the surface of the droplets formed during homogenization [Klinkesorn & McClements, 2010]. Emulsions containing oil droplets covered with surfactants are stabilized mainly electrostatically. These systems are considered to have a good stability against particle aggregation when the droplet surface charges are either more negative than -30 mV or more positive than + 30 mV [Lesmes *et al*., 2010]. The electrical charge of the oil droplets in primary emulsion may also be affected by the presence of charged impurities in the oil phase (*e.g*. phospholipids or free fatty acids) as well as due to preferential adsorption of small ions from the aqueous phase, *e.g*. OH^- and H_3O^+ [Guzey & McClements, 2006]. Two different approaches can be used to form primary emulsion, it means by making oil droplets negatively or positively charged [Hu *et al*., 2011]. The application of surfactants allows to produce negatively charged oil droplets, whereas using proteins the coatings can exhibit positive or negative potentials [Jones & McClements, 2010].

The driving force for the deposition of a second, third, and further layers is inherent to the system and based on the electrostatic attractions between the components of each couple of templating and forming monolayers, respectively. Due to overcharging effects, each deposited layer does not only fully compensate the charge of the previous templating layer but imparts an uncompensated counterchange allowing for further LbL deposition. Components used to form outer layers in M-O/W emulsions could be any food-grade biopolymers that have an electrical charge such as proteins and polysaccharides [Grigoriev & Miller, 2009; Dickinson, 2011]. The biopolymer layers can be deposited around the oil droplets using either one- or two-step mixing procedure depending on the charge-pH characteristics of the polyelectrolytes used [McClements & Li, 2010a]. In the one-step mixing approach, an o/w emulsion containing electrically charged droplets is prepared and it is then directly mixed with a solution containing oppositely charged polyelectrolyte molecules that adsorb to the droplets surfaces through electrostatic attraction [Aoki *et al*., 2005; McClements *et al*., 2007; Gudipati *et al*., 2010]. In the two-step mixing, an o/w emulsion is made containing a polyelectrolyte at a pH where there is not a strong electrostatic attraction between the droplets and the polyelectrolyte molecules (*e.g*. a pH where they are both either negatively or positively charged). The pH of the solution is then adjusted to change the electrical charge on either the droplets and/or the polyelectrolyte molecules so that the polyelectrolyte adsorbs to the droplets surfaces through electrostatic attraction (*e.g*. a pH where the droplet and polyelectrolyte have opposite charges) [Hong & McClements, 2007; Hu *et al*., 2011; McClements, 2012]. A washing step may be required between each electrostatic deposition step in order to remove any excess of non-adsorbed biopolymer remaining

in the continuous phase [McClements *et al*., 2007]. Application of M-O/W emulsions may be limited when the interfacial protein-polysaccharide complexes are held together only by electrostatic attraction. Changes in pH and ionic strength values may decrease the magnitude of electrostatic interactions between the biopolymers and in some cases the polysaccharide layer may dissociate from the protein-coated lipid droplet surfaces [Littoz & McClements, 2008; Weiss *et al*., 2006]. Therefore, in certain applications of LbL technology, it would be beneficial to produce droplets coated by multilayers that remain intact by using additional stabilizing effects such as enzymatic cross-linking between adsorbed biopolymers [Zeeb *et al*., 2011; Li *et al*., 2012; McClements, 2012].

MAJOR LIPOPHILIC BIOACTIVE FOOD COMPONENTS PROPERTIES

The definition of bioactive food molecules is very often interchangeably used with the term of nutraceutical components. Nutraceuticals are dietary supplements that deliver a concentrated form of presumed bioactive agents from a food, presented in a non-food matrix and are used with the aim to promote health in doses that exceed those that could be obtained from normal foods [Bernal *et al*., 2011; Das *et al*., 2012]. The lipophilic nutraceuticals that may be incorporated into foods are for example: fatty acids, carotenoids, tocopherols, phytosterols, oil soluble vitamins, and co-enzyme Q10 [de Vos *et al*., 2010; McClements & Li, 2010a; Yang & McClements, 2013].

Fatty acids, belonging to lipophilic nutraceutical components, are carboxylic acids with a variable unbranched aliphatic chain which is either saturated (*e.g*. butyric acid) or unsaturated in fatty acids such as: eicosapentaenoic acid (EPA, C20:5), docosahexaenoic acid (DHA, C22:6), α-linolenic acid (ALA, C18:3) and conjugated linoleic acid (CLA, C18:2) [Aydin, 2005; Akalin *et al*., 2006; Klinkesorn & McClements, 2009]. EPA, DHA and ALA are the major omega-3 (ω-3) fatty acids and ALA is the precursor of EPA and DHA. However, since humans are not efficient at converting ALA to the long chain ω-3 fatty acids, health benefits have been mainly attributed to dietary EPA and DHA [McClements *et al*., 2007; Das *et al*., 2012]. Studies suggest that ω-3 fatty acids are essential for human metabolism and have many beneficial effects including the prevention of a number of diseases, such as coronary hearth diseases, inflammation, autoimmune disorders, hypertension and hypotriglyceridemic effect [Bernal *et al*., 2011]. Conjugated linoleic acid (CLA) is a mixture of geometrical and positional isomers of linoleic acid (C18:2, cis-9, cis-12). In contrast to linoleic acid, double bonds in CLA are usually located at positions 9 and 11 or 10 and 12 and each double bond can be either in the *cis* or *trans* configuration. The isomers may exhibit different activities or work synergistically and the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 ones are currently considered that exhibit anticancer activity [Aydin, 2005; Park & Pariza, 2007]. Butyric acid (BA) is one of the short-chain fatty acids produced by anaerobic bacteria, is part of the normal flora, *e.g.* of gut and mouth. BA is an extracellular metabolite that helps the intestine maintain colonic health, serves as an energy source for colorectal cells, and positively influences immune responses [Akalin *et al*., 2006; Cueno *et al*., 2013].

Carotenoids are the widest distributed group of pigments in nature with over 600 indentified compounds. They are classified into two types of compounds: carotenes, which are unsaturated hydrocarbons, *e.g*. lycopene, α- and β-carotenes, and oxycarotenoids or xanthophylls, which possess one or more functional groups containing oxygen, *e.g*. lutein, astaxanthin, and canthaxanthin [Santipanichwong & Suphantharika, 2007]. The most typical feature of the carotenoid molecules is a polyene chain, which is responsible for their physical and chemical properties. Nevertheless, this chain of conjugated double bonds also allows carotenoids to be degraded *via* oxidation processes originated by reactive species (singlet oxygen and free radicals often generated during lipid peroxidation) that are added to the polyene chain [Rascón *et al*., 2011; Fernández-Garcia *et al*., 2012]. Certain carotenoids such as β-carotene, lycopene, lutein, astaxanthin exhibit provitamin-A activity and antioxidant properties [Hou *et al*., 2012; Das *et al*., 2012]. These compounds show inmunomodulating activities and therefore can prevent degenerative diseases, such as cardiovascular ones, and several types of cancer, *e.g*. prostate and digestive tract tumors [Bernal *et al*., 2011].

Phytosterols and phytostanols and their esters are a group of steroid alcohols that occur naturally in plants [Bacchetti *et al*., 2011]. More than 250 phytosterols have been identified, however the most abundant are stigmasterol, β-sitosterol and campesterol, which differ in the number of methyl or ethyl groups in the side chain attached to their steroid ring. Stanols are saturated sterols with no double bond attached in their steroid ring and are less abundant in nature [Cohn *et al*., 2010]. Fortification of food with phytosterols may be difficult due to their high melting point and tendency to form insoluble crystals. Esterification of phytosterols to polyunsaturated fatty acids increases sterol solubility, and upon ingestion of phytosterols esters, lipases hydrolyze the fatty acids to produce free phytosterols. These compounds are susceptible to oxidation, however it is not clear whether the oxidized phytosterols lose their bioactivity or are toxic similarly to oxidized cholesterol [McClements *et al*., 2007; García-Llatasa & Rodríguez-Estrada, 2011]. Supplementation of human diets with phytosterols and their esters can decrease serum low-density lipoprotein-cholesterol concentration, which is regarded as modifiable risk factor for atherosclerosis [Brown *et al*., 2010].

Vitamins are organic compounds with a relatively low molecular weight and no energetic value that are found in the diet in small quantities (<1 g per d) yet are essential to ensure body growth, reproduction and functioning. As the body cannot synthesize its own vitamins (or at least not in sufficient amounts), adequate amounts of vitamins have to be provided by diet [Reboul & Borel, 2011]. It has been reported that the main fat-soluble vitamins which may be incorporated into foods are: A, D and E [McClements & Li, 2010a]. Vitamin A refers to a group of polyunsaturated hydrocarbons with important nutritional roles in humans. The main compounds in this group are the retinoids, which are chemical derivatives of retinol, and provitamin A carotenoids, being partially converted to retinoids *in vivo* [Loveday & Singh, 2008]. Vitamin A is needed for scotopic and color vision in the retina

of the eye [Miyazaki *et al*., 2012]. Vitamin D is a seco-sterol that modifies various biological functions in the body. Researchers have identified 37 target organs for vitamin D. Low maternal vitamin D status or its inadequate dietary intake during pregnancy predisposes children to asthma and allergic rhinitis and imparts bone mass growth [Viljakainen *et al*., 2011]. Vitamin E comes in eight different molecular forms that have a common structural feature: a chromanol ring and a phytol side chain. The different molecular forms of vitamin E are classified as either tocopherols (α, β, γ, and δ) or tocotrienols (α, β, γ, and δ), with α-tocopherol being the most biologically active form [Yang & McClements, 2013] Vitamin E or its derivatives are frequently added to the oil phase of o/w emulsion products for fortification reasons or to block the production of reactive oxygen species formed when lipids undergo oxidation [Sagalowicz & Leser, 2010]. The major biological function of vitamin E appears to be as an oil-soluble antioxidant [Yang & McClements, 2013].

Coenzyme Q10 (CoQ10) also known as ubiquinone or ubidecarenone is essential as bioenergetic, being a fundamental cofactor in mitochondrial respiratory chain required for ATP production. Apart from its significant role in maintaining mitochondrial function, ubiquinol, the reduced form of CoQ10, is an influential endogenous lipophilic antioxidant that acts directly by protecting cellular components from free radicals and indirectly *via* regenerating other antioxidants, viz., α-tocopherol and ascorbate [El-Abhar, 2010]. It has been suggested that CoQ10 incorporated into o/w emulsions demonstrated slightly greater bioavailability than in standard commercial CoQ10 products [Thanatuksorn *et al*., 2009].

STABILITY OF M-O/W EMULSIONS AND LIPOPHILIC NUTRACEUTICALS

A number of studies have shown that the physical and chemical stability of M-O/W emulsion-based systems as well as incorporated into them bioactive lipids can be improved by engineering their interfacial properties. For example, good stability to droplets aggregation over a wide range of pH and ionic strength values, thermal processing as well as freeze-thaw cycling has been reported by: Aoki *et al*. [2005]; Surh *et al*. [2005]; Thanasukarn *et al*. [2006]; Gu *et al*. [2007]; Pallandre *et al*. [2007]; Tokle *et al*. [2010]; Mun *et al*. [2010]; Lesmes *et al*. [2010]; Wang *et al*. [2011]; and Zeeb *et al*. [2011]. The found effects have been generally explained as follows: the formation of thick highly charged interfacial layers decreased the attractive (van der Waals) and increased (or prevented decrease) the repulsive (electrostatic or steric) colloidal interactions between multilayer-coated oil droplets thereby improving their stability to flocculation and coalescence [Guzey & McClements, 2007; Zeeb *et al*., 2011; Schmelz *et al*., 2011; McClements, 2012]. Regarding chemical stability, the increase of oxidative stability of ω-3 fatty acids has been demonstrated by: Gudipati *et al*. [2010]; Lesmes *et al*. [2010]; Serfert *et al*. [2013] and others. Hou *et al*. [2012] reported that multilayered membranes had a significant impact on the increase of β-carotene stability. The observed improvement concerning chemical stability of bioactive lipids has been postulated to be due to the minimized interactions between encapsulated components and chemically-reactive aqueous phase substances. For example, by increasing the positive interfacial charge it could be possible to prevent transition metals such as iron or copper from entering into close contact with sensitive to oxidation lipophilic components [Gudipati *et al*., 2010; McClements, 2012]. Moreover, it has been suggested that lipid oxidation could be decreased by the use of materials containing low amount of transitions metals, *e.g*. in commercial oil the iron and copper concentration has been recommended to be < 0.1 ppm and < 0.2 ppm, respectively [Katsuda *et al*., 2008]. However, it has to be stressed, that the magnitude and range of electrostatic interactions between charged interfacial membranes and transition metals can be reduced as the ionic strength of the solution increases because of the accumulation of counter-ions around the surfaces, which is usually referred to as electrostatic screening [Surh *et al*., 2005; Guzey & McClements, 2006; Zeeb *et al*., 2011].

Several authors have reported the possibility to control release of encapsulated components by appropriate designing the thickness and permeability of multilayered membranes [Shchukina & Shchukin, 2011; Delcea *et al*., 2011; Humblet-Hua *et al*., 2011]. However, it has also been demonstrated that release of encapsulated lipophilic component in spray-dried capsules increased upon raising water activity. This was related to the fact that higher water content resulted in damage of the matrix structure, which caused greater diffusion of encapsulated volatile molecules from oil droplets to the hydrated matrix and then their greater release from the powder [Gharsallaoui *et al*., 2012].

Moreover, some of the authors suggested that multilayered membranes can modulate the digestion of encapsulated lipids under gastrointestinal conditions. These effects have been assessed that M-O/W emulsions may be used for rational design of delivery systems for lipophilic nutraceuticals that need to be encapsulated within foods but released in human body [Klinkesorn & McClements, 2010; Li *et al*., 2010; McClements & Li, 2010a; Hu *et al*., 2011].

INSTRUMENTAL METHODS CHARACTERIZATION

A wide variety of different analytical methods and experimental procedures have been developed to characterize: oil droplets electrical properties, structural organization within biopolymer interfacial layers and emulsions [Guzey & McClements, 2006].

Particle size distribution can be determined applying scattering methods, *e.g*. static light scattering (SLS) and dynamic light scattering (DLS) [Littoz & McClements, 2008; Wang *et al*., 2011]. Each of these methods has a range of particle diameters that can be reliably detected. For example, SLS instruments typically measure particles from about 0.1 to 1000 mm, whereas DLS ones detect particles in the range of 1 nm to 5 mm [McClements & Li, 2010b; Hu *et al*., 2011]. The DLS method (Photon Correlation Spectroscopy) is a convenient tool for the detection of local fluctuations and *via* the Stokes-Einstein relation allows determination of the hydrodynamic radius of particles. A disadvantage of DSL is that it cannot distinguish between single particles and aggregates [Guzey & McClements, 2006; Sigel, 2009].

Electrophoretic mobility measurements provide useful information on the nature of the electrical charge distribution in the outermost region of the biopolymer stabilizing layer – the so-called "surface of shear" [Dickinson, 2011]. The electrical potential of this surface is related to zeta-potential (ζ) and defined as the distance away from the droplet surface below which the counter-ions remain strongly attached to the droplet when it moves in an electrical field [McClements *et al*., 2007; Ye *et al*., 2011]. Measurements of ζ-potential are commonly used to monitor the effects of pH and ionic strength of the bulk phase on the electrical potential of multilayered membranes [Lesmes *et al*., 2010; Hu *et al*., 2011; Zeeb *et al*., 2011].

The multilayer buildup on colloidal particles can be determined using: (i) scattering methods, *e.g*. single particle light scattering (SPLS), dynamic light scattering (DLS), small-angle neutron scattering (SANS); (ii) microscopy, *e.g*. optical microscopy (OM), scanning electron microscopy (SEM), transmission electron microscopy (TEM), confocal laser scanning microscopy (CLSM), atomic force microscopy (AFM), and (iii) spectroscopy, *e.g*. Fourier transform infrared spectroscopy (FTIR) or fluorescence spectroscopy [Guzey & McClements, 2006; Grigoriev & Miller, 2009; Shutava *et al*., 2009; Wackerbarth *et al*., 2009; McClements & Li, 2010b; Humblet-Hua *et al*., 2011]. Microscopy such as SEM, TEM and AFM can be used to provide visual evidence for the formation of multilayer interfaces around oil droplets [Guzey & McClements, 2006; Medeiros *et al*., 2012]. AFM measures the surface height through the vertical force between the probe and the specimen and provides topographical information through 3D images. This method is widely used for determining film thickness and surface morphology of protein-polyelectrolyte multilayers and brushes. Using AFM microscopy it is possible to determine surface roughness as low as 5–10 nm [Cooper *et al*., 2005; Dickinson, 2011]. FTIR allows the qualitative determination of organic compounds as the characteristic vibrational mode of each molecular group causes the appearance of bands in the infrared spectrum at a specific frequency, which is further influenced by the surrounding functional groups. Moreover, this method is an excellent tool for quantitative analysis as the intensities of the bands in the spectrum are proportional to the concentration (*i.e*. Beer's law is obeyed) [Vlachos *et al*., 2006]. The CLSM microscopy and fluorescence spectroscopy can be used to study permeability of multilayer microcapsules [Guzey & McClements, 2006; Klinkesorn & McClements, 2009].

CONCLUSIONS

This literature overview showed that layer-by-layer (LBL) electrostatic deposition technique is an interfacial engineering technology applied for the formation of multilayer oil-in-water (M-O/W) emulsions. This technology permits the step-wise adsorption of various components (*e.g*. proteins, polysaccharides, phospholipids and ionic surfactants) as the layer growth is governed by their electrostatic attraction and allows the formation of multilayer shells. The studies proved that it is possible to develop multilayer emulsions exhibiting different properties by using various combinations of surfactants and biopolymers. Application of the LbL technology resulted in the increase of environmental responsiveness of M-O/W systems. Moreover improvement regarding chemical stability of lipids and possibility to modulate release of lipophilic bioactive components and digestibility of lipids have been found. However, these systems exhibit also some disadvantages, which may limit their widespread commercial utilization. The main problem is connected with the fact that the maximum content of oil that can be used to formulate these systems is generally restricted, due to susceptibility to droplets aggregation with higher concentration of the internal phase. In addition, the range of food-grade cationic surfactants and polysaccharides that can be used currently is fairly limited, which restricts the opportunities to build interfacial membranes with specific characteristics. Summarizing, it seems that this method could be wider used by the food industry because the potential benefits of interfacial membranes formation may outweigh the costs for certain applications. However, these systems require further studies addressing physicochemical properties in relation to: (i) different internal phase concentration, (ii) membrane composition as well as (iii) physical and chemical stability of incorporated nutraceuticals.

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