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Review Article

Factor affecting the properties of water-in-oil-in-water emulsions for encapsulation of minerals and vitamins

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Abstract

The direct fortification of minerals and vitamins into food may induce chemical degradation, change the level of bioavailability or decrease the sensory quality of food products. The strategy to solve these problems is encapsulation technology. Numerous investigations described the use of water-in-oil-in-water (W/O/W) emulsions as encapsulation system. The properties and encapsulation efficiency of W/O/W emulsions are influenced by emulsion components, the emulsification processes, and environmental conditions. The recently published results of research done on the factors affecting the properties of W/O/W emulsions for encapsulation of minerals and vitamins including form and concentration of core materials, concentration of inner water phase and lipophilic emulsifier, type and concentration of oil phase, type and concentration of hydrophilic emulsifier and stabilizer and the pH of the outer water phase have been reviewed in this article.

Keywords: water-in-oil-in-water emulsions, encapsulation, minerals, vitamins

1. Introduction

Minerals and vitamins are very important to sustain body functions and are essential for all parts of the body. Although minerals and vitamins are present in various foods from both animal and plant sources, many people still have mineral and vitamin deficiencies. This does not only occur with poor people in developing countries who do not have enough food to eat, but also with other people around the world. These mineral and vitamin deficiencies may appear in some groups of people such as the elderly, pregnant and lactating women, some people with limited consumption choices, such as vegetarians or vegans and may also come from changing food consumption behaviors to eating high processed food more than fresh whole foods (Bonnet *et al*., 2009; Guzun-Cojocaru *et al*., 2010; Kroner, 2011).

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The direct fortification of minerals and vitamins into food is not straightforward. Some micronutrients may cause adverse effects to the sensory quality of the food such as color changes, undesirable flavor, and a sandy texture, while some micronutrients may accelerate chemical reactions leading to decreased nutritional quality of the food and may also lead to toxicants. Furthermore, some micronutrients are very sensitive to high temperatures, exposure to light, changes in pH, high levels of oxygen, and moisture content, which can lead to degradation during preparation, processing, and storage (Ball, 2006; Mehansho *et al*., 2006; Jimenez-Alvarado *et al*., 2009; Liu, 2009; Lutz *et al*., 2009; Stevanovic and Uskokovic, 2009; Guzun-Cojocaru *et al*., 2010; Lakkis *et al*., 2011; Giroux *et al*., 2013).

The strategy to solve these problems is encapsulation technology, a technique that entraps sensitive material, e.g. minerals and vitamins, in the carrier or coating material before being added to food. Emulsion technology is one promising technology for the encapsulation of such materials. The advantage of an emulsion system is it allows nutrients to be quite stable and easily dispersed in the medium.

Usually, emulsion systems consist of two or more immiscible liquids, such as oil and water, where one of liquids is dispersed as small spherical droplets in the other. Emulsion systems are conventionally classified into two main groups; simple or single emulsions and multiple or double emulsions. Oil-in-water (O/W) emulsions consist of oil droplets dispersed in a continuous aqueous phase (Figure 1A), and water-in-oil (W/O) emulsions that consists of water droplets dispersed in a continuous oil phase (Figure 1B) are examples of a single emulsion. Whereas, water-in-oil-in-water (W/O/W) emulsions containing W/O droplets dispersed in a continuous aqueous phase (Figure 1C), and oil-in-water-in-oil (O/W/O) emulsions where O/W droplets are dispersed in a continuous oil phase (Figure 1D) are classified as multiple emulsions.

W/O emulsions and W/O/W emulsions are normally used to encapsulate water-soluble substances. The W/O emulsions are suitable used for nutrient fortification in oil-based foods, while W/O/W emulsions are properly used in water-based food products. O/W emulsions and O/W/O emulsions are mostly used for encapsulation of oil-soluble substances. The O/W emulsions encapsulate nutrients for fortification in water-based foods products, while O/W/O emulsions are used for fortification in oil-based foods. Furthermore, W/O/W emulsions and O/W/O emulsions can be used as a co-encapsulation system which is encapsulation together of both water-soluble and oil-soluble substances. For example, W/O/W can entrap water-soluble nutrients in the inner water phase and also entrap oil-soluble substances in the oil phase of the same system (Benichou *et al*., 2002; Benichou *et al*., 2007; Bonnet *et al*., 2009; Lutz *et al*., 2009; Bonnet *et al*., 2010a; Bonnet *et al*., 2010b; O'Regan *et al*., 2010; Li *et al*., 2012; Giroux *et al*., 2013).

The aim of this work is to offer an overview of the health effects of some minerals and vitamins including iron, calcium, magnesium, vitamin C (ascorbic acid), vitamin B_1 (thiamine), vitamin B_2 (riboflavin), and vitamin B_{12} (cobalamin). In addition, the use of W/O/W emulsions for encapsulation and the factors affecting the properties of W/O/W emulsions including form and concentration of core material, concentration of inner water phase and lipophilic emulsifier, type and concentration of oil phase, type and concentration of hydrophilic emulsifier and stabilizer, and pH of outer water phase are also highlighted.

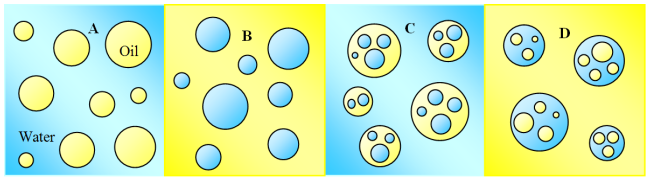

Figure 1. Schematic diagram of oil-in-water (O/W) emulsions (A), water-in-oil (W/O) emulsions (B), water-in-oil-in-water (W/O/W) emulsions (C), and oil-in-water-in-oil (O/W/O) emulsions (D) (not to scale).

2. Health Benefits, Deficiency and Fortification of Minerals and Vitamins

Minerals and vitamins have different health benefits and they are important for normal functions. Minerals and vitamins are essential for all parts of the body. Deficiency of minerals and vitamins may cause dysfunction and lead to ill-health. Thus, we gathered the health benefit, deficiency problem, and food source of some minerals and vitamins that have the important health effect on our body including iron, calcium, magnesium, ascorbic acid, thiamine, riboflavin, and cobalami as shown in Table 1.

Fortification of minerals and vitamins in foods may solve the problem of mineral and vitamin deficiency. However, direct fortification of some minerals and vitamins into foods is not straightforward because it may not be stable or may cause adverse effects on the food products (Table 2). In addition, bioavailability of the nutrients is also an important point and should be considered. Bioavailability is the amount of nutrients that can be absorbed and utilized by our body. There are three main factors that affect bioavailability, including personal factors, nutrient stability, and dissolution or release of nutrients. The personal factors include age, sex and physiologic state (e.g. pregnancy) which affect the gastric residence time and permeability through the gastrointestinal tract. Formulation (form of nutrients, pH and presence of other nutrients), food processing, and storage conditions (temperature, light and oxygen) are factors that affect nutrient stability.

The factors that influence dissolution or release of nutrients are the encapsulation process and formulation such as coatings or fillers excipient and surfactants. Thus, the design and utilizing the proper encapsulation technique may be a promising way to enhance the fortification and bioavailability of nutrients in food products by protecting them until the time of consumption and delivery them to the target site within the human body (Yetley, 2007; Fang and Bhandari, 2010).

3. Encapsulation Technology

Generally, encapsulation technology is a technique that entraps sensitive materials, solid, liquid, or gas state, in wall material in small sealed capsules to protect them from adverse environmental conditions such as high temperatures, high or low pH, exposure to UV light, and high levels of dissolved oxygen. Moreover, the freeing of this substance from the encapsulated capsule can be controlled to release at certain rates and specific conditions. The size of the encapsulated particle may cover a range from sub-micron to millimeters. Encapsulation technology can be also used to reduce the evaporation of aromatic compounds, mask undesirable flavors and tastes of some substances or improve the dispersion ability of some substances by making them disperse more uniformly throughout the medium (Fang and Bhandari, 2010; Abbas *et al*., 2012).

Table 1. Summary of food sources, health benefit, and deficiency problem of some minerals and vitamins.

Nutrients	Food sources	Health benefit	Deficiency problem	References
Iron	Meats, poultry, fish, shellfish, and some plants	Essential for blood circulatory system, transports oxygen throughout the body	Anemia, high risk of heart attack, slow development in children, and complications of pregnancy	Kroner, 2011
Calcium	Milk, cereals, and their products.	Essential for bone and teeth, muscle contraction, digestion, and neurotransmitter secretion	Calcium release from bones, high risk of osteoporosis, and fracture of bones	Theobald *et al*., 2005; Saeidy *et al*., 2013
Magnesium	Vegetables, fruits, and pulses	Essential for protein synthesis, enzymatic reactions, and muscular contraction	Cardiovascular diseases, hypertension, muscular weakness, and diarrhea	Bonnet *et al*., 2009; Kroner, 2011
Ascorbic acid (vitamin C)	Fruits and vegetables such as citrus fruits, guava, potatoes, and tomatoes	Degenerative chronic diseases, involved in synthesis of collagen and epinephrine, amino acid and cholesterol metabolism, against oxidative damage and cancers	Dry and splitting hair, rough, dry and hemorrhage skin, decreased wound healing rate, weakened tooth enamel, scurvy, gingivitis, anemia, and high risk of infections	Liu, 2009; Lutz *et al*., 2009; Stevanovic and Uskokovic, 2009; Kroner, 2011
Thiamine (vitamin B_1)	Meat, whole grain cereals, nuts, and beans	Role in cellular energy production, and helps neuron to be normal actives	Symptom to two systems including cardiovascular system and nervous system	Bates, 2007; Osiezagha *et al*., 2013
Riboflavin (vitamin B_2)	Dairy products, cereals, offal, fish, and dark-green vegetables	Protecting against cancers, cardiovascular diseases, and plays a role in thyroxine metabolism	Night blindness, high risk of cancer & cardiovascular disease, peripheral neuropathy, neurodegeneration, and mental illness	Powers, 2003
Cobalamin (vitamin B_{12})	Found in animal tissues	Essential for production of blood cells and DNA, and necessary for normal neurologic function	Anemia, irritability, memory loss, weakness, trembling and unstable movements, and psychosis	Kroner, 2011

Table 2. Summary of fortification form, recommended daily dosages and direct fortification problem of some minerals and vitamins.

Nutrients	Form for fortification	Recommended daily dosages *	Direct fortification problem
Iron	Ferrous sulphate, ferrous gluconate, ferric ammonium citrate, sodium iron EDTA, ferrous bisglycinate, ferrous fumarate, ferric saccharate, ferrous citrate, ferric citrate, carbonyl iron, and ferric pyrophosphate (Lynch, 2005; Kroner, 2011)	1-3 years 7 mg, 4-8 years 10 mg, 9-13 years 8 mg, girls 14-18 years 15 mg, boys 14-18 years 11 mg, women 19-50 years 18 mg. Pregnancy 27 mg, lactation 9 mg, after 51 years 8 mg	Sensitive to reaction, color change, lipid oxidation, precipitation (Guzun-Cojocaru *et al*., 2010; Zimmermann and Windhab, 2010)
Calcium	Calcium carbonate, calcium triphosphate, and calcium malate (Theobald, 2005; Kressel *et al*., 2010; Kroner, 2011)	0-6 months 210 mg, 6-12 months 270 mg, 1-3 years 500 mg, 4-8 years 800 mg, 9-18 years 1,300 mg, 19-50 years 1,000 mg, 50 years and older 1,200 mg, pregnancy and lactation: 18 years or younger 1,300 mg, 19-50 years 1,000 mg	Flocculation, sandy texture, precipitation (Lakkis *et al*., 2011)
Magnesium	Magnesium phosphate, magnesium pyrophosphate, and magnesium potassium phosphate (Skelcey *et al*., 1974)	0-6 months 30 mg, 7-12 months 75 mg, 1-3 years 80 mg, 4-8 years 130 mg, 9-13 years 240 mg, men: 14-18 years 410 mg, 19-30 years 400 mg, more than 30 years 420 mg, women: 14-18 years 360 mg, 19-30 years 310 mg, over 30 years 320 mg, pregnancy: 14-18 years 400 mg, 19 to 30 years 350 mg, over 31 years 360 mg, lactation:14-18 years 360 mg, 19 to 30 years 310 mg, over 31 years 320 mg	Protein aggregation, nutrients degradation, unfavorable taste (Bonnet *et al*., 2009)
Ascorbic acid (vitamin C)	Ascorbic acid, ascorbate salt, and esterified ascorbate (Liu, 2009; Kroner, 2011)	Men 90 mg and women 75 mg	Very unstable to high pH, high temperatures, UV light, dissolved oxygen (Ball, 2006)
Thiamine (vitamin B_1)	Thiamine hydrochloride (Benichou *et al*., 2002)	9-13 years 0.9 mg, boys 14-18 years 1.2 mg, girls 14-18 years 1.0 mg, men 1.2 mg, women 1.1 mg, pregnancy & lactation 1.4 mg	Unstable to high pH, high temperatures, high moisture content (Ball *et al*., 2006)
Riboflavin (vitamin B_2)	Riboflavin, and riboflavine-5'-monophosphate (Owusu *et al*., 1992)	1-3 years 0.5 mg, 4-8 years 0.6 mg, 9-13 years 0.9 mg, boys 14-18 years 1.3 mg, girls 14-18 years 1.0 mg, men 1.3 mg, women 1.1 mg, pregnancy 1.4 mg	Unstable to light and high pH (Ball *et al*., 2006)
Cobalamin (vitamin B_{12})	Cobalamin and adenosylcobalamin (Collins, 2003)	1-3 years 0.9 µg, 4-8 years 1.2 µg, 9-13 years 1.8 µg, 14-18 years 2.4 µg, men & women 2.4 µg, pregnancy 2.6 µg, lactation 2.8 µg	Unstable to High pH (Ball *et al*., 2006)

*(Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline and Subcommittee on Upper Reference Levels of Nutrients Food and Nutrition Board Institute of Medicine, 1998; Kroner, 2011; Osiezagha et al., 2013)

Many methods and techniques can be applied for encapsulation of nutrients including spray drying, fluid bed coating, spray chilling or cooling, melt extrusion, coacervation, liposome, inclusion encapsulation, cocrystallization, nanoencapsulation, freeze drying, yeast encapsulation and emulsions (Stevanovic and Uskokovic, 2009; Fang and Bhandari, 2010; Zuidam and Shimoni, 2010; Abbas *et al*., 2012).

An emulsion encapsulation technique can be used to encapsulate both water-soluble and oil-soluble liquid substances. The size of the liquid droplets in emulsion is usually in the range from 0.1-5,000 µm. Preparation of multilayer around the droplets in emulsions may obtain a more stable and effective encapsulated core. The liquid droplets in emulsion, especially oil-soluble substances, can be produced in a dry powder form by spraying or freeze drying their emulsion. The advantage of this technique is that it is flexible because it can be used to encapsulate both hydrophilic and lipophilic substances (Fang and Bhandari, 2010; Zuidam and Shimoni, 2010; Abbas *et al*., 2012).

Abbas *et al*. (2012) mentioned that there are many methods that can be applied for encapsulation, but there are no techniques that are effective for all food systems. Each technique has different advantages and limitations. However, W/O/W emulsions are the one encapsulation technique that is suitable to fortify nutrients in beverages and water-based foods because this technique can keep the nutrients to be quite stable and easily dispersed in an aqueous phase. Moreover, it is easily to be scaled up to industrial size (Krasaekoopt *et al*., 2003).

4. Encapsulation of Minerals and Vitamins in Water-in-oil-In-water Emulsions

The water-in-oil-in-water or W/O/W emulsion system (Figure 1C) consists of small internal water droplets embedded in larger oil droplets that are also dispersed in another outer water continuous phase. This can be referred to as emulsions of emulsions that is to say water-in-oil-in-water emulsions consisting of water-in-oil emulsions (Figure 1B) dispersed in a continuous water phase. The W/O/W emulsions have two types of interfaces; these are inner water-oil interface and oil-outer water interface, so it has to use oil-soluble emulsifiers to stabilize the inner water-oil interface and water-soluble emulsifiers to stabilize the oil-outer water interface (Benichou *et al*., 2002; Dickinson, 2011; McClements, 2012).

For fortification of minerals and vitamins into food products that have water as a continuous phase, W/O/W emulsions are mostly used with different components as summarized in Table 3. Moreover, W/O/W emulsions may also be used for co-encapsulation, which is encapsulation for both water-soluble substances and oil-soluble substances together by an encapsulating water-soluble substance in the inner water phase and encapsulating an oil-soluble substance in the oil phase of the same W/O/W emulsion system. For example, Li *et al*. (2012) produced W/O/W emulsions for use as an encapsulation system consisting of riboflavin in the inner water phase and α-tocopherol in the oil phase.

4.1 Preparation of W/O/W emulsions

W/O/W emulsions are usually produced using a two-step emulsification method (Figure 2). The first step is the production of W/O emulsions by homogenizing an oil phase and the inner water phase together in the presence of a water-soluble core material at high pressure or high shear rate. In this step, a lipophilic emulsifier or oil-soluble emulsifier was used to stabilize the water-oil interface. The second step is the production of W/O/W emulsions by homogenizing the former W/O emulsion and an outer water phase at a pressure or shear rate lower than that used in the first stage to avoid rupture or expulsion of inner droplets. To stabilize the oil-water interface, a hydrophilic emulsifier or water-soluble emulsifier was used in this step (Van der Graaf *et al*. 2005; McClements, 2012).

4.2 Factors affecting the properties of W/O/W emulsions for encapsulation

There are many factors that can affect the properties of W/O/W emulsions. In this review, we categorize them into five groups: form and concentration of core material, concentration of inner water phase and lipophilic emulsifier, type and concentration of oil phase, type and concentration of hydrophilic emulsifier and stabilizer and pH of outer water phase.

4.2.1 Form and concentration of core material

The form and concentration of the core material affects the properties of W/O/W emulsions. For the form of the core material, different forms of core material lead to differences in properties such as size, binding capacity, and hydrophilic property that all affect W/O/W emulsions characteristics. If the core material has the form of a bigger molecule, the

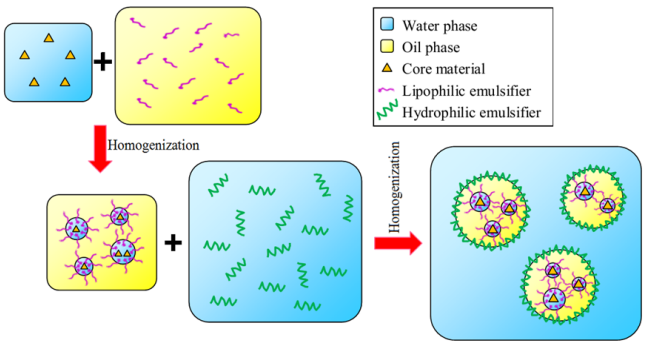

Figure 2. Schematic diagram of two-step procedure for preparation of water-in-oil-in-water (W/O/W) emulsions (not to scale).

Table 3. Examples of minerals and vitamins encapsulated in water-in-oil-in-water emulsions.

Nutrients	Hydrophilic emulsifier and stabilizer in inner water phase	Oil phase;lipophilic emulsifier	Hydrophilic emulsifier and stabilizer in outer water phase	References
Iron	- Whey protein isolate (WPI) - Panodan SDK (esters of monoglycerides and diglycerides of diacetyl tartaric acid)	- Corn oil; polyglycerol polyricinoleate ester (PGPR) - Mineral oil; PGPR	- Polyoxyethylene Sorbitan monolaurate (Tween20) - Whey protein concentrate (WPC) and gum arabic or mesquite gum or low methoxyl pectin (LMP)	Choi *et al.*, 2009 Jimenez-Alvarado *et al.*, 2009
Calcium	-	- Sunflower oil; PGPR	- Xanthan gum	Marquez and Wagner, 2010
	-	- Canola oil; glycerol monostearate (GMS)	- Gelatin and agar	Saeidy *et al.*, 2013
Magnesium	-	- Olein, miglyol, olive oil or rapeseed oil; PGPR	- Sodium caseinate	Bonnet *et al.*, 2009
	-	- Olive oil; PGPR	- Sodium caseinate	Bonnet *et al.*, 2010a
Ascorbic acid (vitamin C)	-	- Medium chain triglyceride or limonene; PGPR	- WPI and modified pectin	Lutz *et al.*, 2009
	- Panodan SDK andgellan gum	- Chia essential oil; PGPR	- Mesquite gum, maltodextrin and WPC	Carrillo-Navas *et al.*, 2012
Thiamine (vitamin B_1)	-	- Medium chain triglyceride; PGPR and monoglyceride oleate	- WPI and xanthan gum	Benichou *et al.*, 2002
Riboflavin (vitamin B_2)	-	- Soy oil; PGPR	- WPI and LMP or ê-carrageenan	Li *et al.*, 2012
Cobalamin (vitamin B_{12})	- Gelatin	- Medium chain triglyceride; PGPR	- Sodium caseinate or sodium caseinate-maltodextrin conjugate	O'Regan and Mulvihill, 2010
	-	- Butter oil; PGPR	- Skim milk or sodium caseinate	Giroux *et al.*, 2013

encapsulation efficiency will be higher than for smaller forms. If the core material attaches to the molecule that has a higher binding capacity, the encapsulation efficiency will be higher, because it will occur with a lower free form of the core material, so it is harder to release. Moreover, the higher hydrophilic property of the core material also leads to lower release, because higher hydrophilicity molecules are harder to diffuse through the oil phase.

Marquez and Wagner (2010) encapsulated different calcium salts in the inner water phase of W/O/W emulsions using sunflower oil as the oil phase, PGPR as a lipophilic emulsifier, soybean milk as the outer water phase, and xanthan gum as the stabilizer. They found that emulsions that used calcium lactate as the core material had lower G′ and G″ and higher tan δ than emulsions that used calcium chloride as the core material. The lower G′ and G″ and the higher tan δ values mean that the W/O/W emulsions have a weaker structure. This may due to the calcium lactate, which is a bigger molecule, and has a lower release value than calcium chloride, which occurs with a lower crosslink to the soybean proteins and therefore has a weaker structure.

In the same way, Bonnet *et al*. (2010b) encapsulated different magnesium salts in the inner aqueous phase of W/O/W emulsions using olive oil as the oil phase, PGPR as lipophilic emulsifier, and sodium caseinate as the hydrophilic emulsifier. They found that the encapsulated $MgCl_2$ had the highest release rate and completed release in a 15 day-storage, since small molecules of magnesium can easily transfer through the oil phase. The encapsulated gluconic acid hemimagnesium salt was found to have a lower release value than $MgCl_2$, but a higher release value than phosvitin with $MgCl_2$. This may due to the fact that gluconic acid has a lower binding capacity than phosvitin and is quite a small molecule, so it can easily pass through the oil phase. Whereas, encapsulated phosvitin with $MgCl_2$ was found to have the lowest release value because the hydrophilicity and high molecular weight of phosvitin cause it to be unable to pass though the oil phase.

For the effect of concentration of core material on encapsulation efficiency of W/O/W emulsions, there was report that higher concentrations of the core material can lead to higher encapsulation efficiency. For example, Benichou *et al*. (2002) entrapped thiamine hydrochloride in the W/O/W emulsions that used medium chain fatty acid triglycerides as the oil phase, PGPR as the surfactant, monoglyceride oleate for droplet size reduction and better stability, and whey protein isolate and xanthan gum as hydrophilic emulsifiers. They found that increasing vitamin B_1 concentrations caused a decrease in vitamin B_1 release. This may due to the fact that thiamine hydrochloride has an amphiphilic structure, so it has surface properties to reduce interfacial tension.

In a similar way, Lutz *et al*. (2009) encapsulated sodium ascorbate in the W/O/W emulsions using PGPR as emulsifier, and WPI and modified pectin as a hydrophilic emulsifier. They found that fresh emulsions had a reduced release rate with increasing sodium ascorbate concentration, but after 28 days there was no significant difference. The difference in release rate of fresh emulsions containing different core concentrations was not discussed by the authors. However, in our opinion, this may due to the effect of the osmotic gradient. The higher core material concentration led to the higher osmotic gradient, resulting in emulsion droplet swelling. This occurs from diffusion of the water phase from the outer water phase to the inner water phase to balance the osmotic gradient (Dickinson, 2011). The intensity of water diffused from the outer phase to the inner phase was higher than the diffusion of water from the inner phase to the outer phase resulting in a low release of sodium ascorbate in fresh emulsions. However, for a longer time storage, the diffusion of water from the outer phase to the inner phase may cause some droplets broken and release core material resulting in a high release of sodium ascorbate from emulsion droplets after 28 days.

4.2.2 Concentration of inner water phase and lipophilic emulsifier

The concentration of the inner water phase and lipophilic emulsifier affects the properties of W/O/W emulsions. Higher concentrations of the inner water phase lead to lower release rates of the core material. In the same way, higher concentrations of lipophilic emulsifier lead to lower release rates of the core material. An example for the effect of dispersed aqueous phase content was shown by Marquez and Wagner (2010). They found that higher dispersed aqueous phase content led to the formation of lower G′ and G″ and higher tan δ value emulsions. The lower G′ and G″ and the higher tan δ meant that the W/O/W emulsions were thinner. This can explain why higher dispersed aqueous phase content reduced the calcium concentration leading to reduced osmotic gradient and calcium release, so there was less crosslink with soybean proteins producing thinner emulsions. For the effect of lipophilic emulsifier concentration, Marquez and Wagner (2010) found that higher PGPR concentrations produced lower G′ and G″ and higher tan δ value emulsions. This may be due to increases in the PGPR concentration which reduced the water droplet size of the W/O droplets, so reduced calcium release led to less crosslink and less elastic behavior of the emulsion.

4.2.3 Type and concentration of oil phase

The type and concentration of the oil phase affects the properties of W/O/W emulsions. Different types of oil phase lead to variations in properties such as viscosity, and saturated fatty acid content affecting the encapsulation efficiency of W/O/W emulsions. An oil phase with higher viscosity values lead to higher encapsulation efficiency because the viscous oil phase retards diffusion and expulsion of the inner water phase with the core material inside. Moreover, oil types with higher saturated fatty acid levels yield higher hydrophobicity or lower compatibility with water

causing a faster expulsion of the inner water phase that has the core material inside.

For example, Bonnet *et al*. (2009) encapsulated $MgCl_2$ in W/O/W emulsions using olein, miglyol, olive oil or rapeseed oil as the oil phase, PGPR as a lipophilic emulsifier, sodium caseinate as a hydrophilic emulsifier, and lactose to match the osmotic pressure between the inner and outer water phase to avoid water transfer. They found that double emulsions produced with miglyol had significantly faster magnesium release than other oils. This can be explained since miglyol has the lowest viscosity and the highest saturated fatty acid of these four oils, so the lower viscosity leads to easier material transfer and release, and higher saturated fatty acid leads to higher hydrophobicity and lower compatibility with the water causing faster magnesium release. In contrast, double emulsion produced with rapeseed oil, which has a quite high viscosity and the lowest saturated fatty acid content, had the highest magnesium retention.

In the same way, Lutz *et al*. (2009) found that double emulsions produced from medium chain triglycerides had a lower sodium ascorbate release rate than produced from R(+)-limonene. This can be explained since R(+)-limonene has a lower viscosity which leads to easier release of the sodium ascorbate from the inner water phase to the outer water phase.

For the effect of lipid phase content on property of W/O/W emulsions, there was a report that higher lipid phase content lead to get more viscous W/O/W emulsions. Marquez and Wagner (2010) showed that increased lipid phase content led to the formation of higher G′ and G″ and lower tan δ value emulsions. The higher G′ and G″ and the lower tan δ values mean that the W/O/W emulsions were more viscous. This can be explained in a way that the higher lipid phase content produced in the more water-in-oil droplets led to tighter packing and a viscous systems.

4.2.4 Type and concentration of hydrophilic emulsifier and stabilizer

The type and concentration of the hydrophilic emulsifiers and stabilizers affect the properties of W/O/W emulsions. Some hydrophilic emulsifiers and stabilizers have a synergistic effect on the stabilization of emulsion when used together. Different types of hydrophilic emulsifiers and stabilizers, which have different properties such as molecular weight and charge, result in different effects on the properties of the W/O/W emulsions. Using two hydrophilic emulsifiers together or hydrophilic emulsifiers with stabilizers may increase the encapsulation efficiency more than using either alone. For example, O'Regan and Mulvihill (2010) encapsulated vitamin B_{12} in W/O/W emulsions with gelatin to solidify the inner water phase with medium chain triglyceride oil as the oil phase, PGPR as the lipophilic emulsifier, sodium caseinate or sodium caseinate-maltodextrin conjugate as the hydrophilic emulsifier. They found that the encapsulation efficiency of double emulsion stabilized with sodium caseinate-maltodextrin conjugate was significantly higher than that of sodium caseinate, especially in the case of maltodextrin with a dextrose equivalent (DE) of about 10. This was because the protein-polysaccharide conjugate formed a more bulky polymeric layer at the interface and some parts of the polysaccharide protruding toward the continuous phase caused better steric stabilization against droplet flocculation and coalescence.

The greater molecular weight of polysaccharides lead to more effective protection of the core material than lower ones, for example, Jimenez-Alvarado *et al*. (2009) entrapped ferrous bisglycinate in the W/O/W emulsions using mineral oil as the oil phase, and protein-polysaccharide complexes (whey protein concentrate (WPC) with gum arabic (GA) or mesquite gum (MG) or low methoxyl pectin (LMP)) as water-soluble surfactants in outer water phase. They found that the encapsulation yield and ferrous ion content of W/O/W emulsions stabilized with 5 wt% total concentration of WPC: MG and 0.7 wt% total concentration of WPC:LMP were significantly highest at the initial time. This may be due to formation of a thick layer at the interface of the droplet, but only ferrous ion content of water-in-oil-in-water emulsion stabilized with 5 wt% total concentration of WPC:MG were significantly higher at the end of storage time which may be due to the molecular weight of the polysaccharides. Mesquite gum has a greater molecular weight than gum Arabic and low methoxyl pectin, respectively. Since mesquite gum has a high molecular weight and can form a thick layer at the interface, therefore it was the most effective polysaccharide to protect against ferrous bisglycinate oxidation in between these polysaccharides.

The higher charge of polysaccharides has both positive and negative effects on the properties of W/O/W emulsions. For the positive effect, Lutz *et al*. (2009) encapsulated sodium ascorbate in W/O/W emulsions that used different modified pectin types U63 and C63, the modified pectin types U63 and C63 are 63% degree of esterification, but U63 has a more negative zeta potential and more intermolecular interactions than C63. They found that double emulsion stabilized with the WPI/U63 complex had lower sodium ascorbate release rates than found in a double emulsion stabilized with the WPI/C63 complex. They explained that U63 pectin have more charges which leads to more interaction and causes more elastic and a greater stiffness complex with WPI. Thus, the WPI/U63 complex was more effective to prevent the release of sodium ascorbate than the WPI/C63 complex.

In contrast, Li *et al*. (2012) encapsulated riboflavin in the W/O/W emulsions with hydrophilic emulsifiers and stabilizers of whey protein isolate and low methoxyl pectin (LMP) or κ-carrageenan (KCG), which has a higher minus charge than low methoxyl pectin. They found that the encapsulation efficiency of the WPI-LMP complex stabilized emulsion was higher than that of the WPI-KCG complex when the WPI: PS ratio was 5:0.5. They explained that the low charge polysaccharide chain has a flexible worm-like form that easily to

curvature and interact with protein molecule, while the high charge polysaccharide chain has a rigid stick-like form, so low methoxyl pectin, which has lower charge, has a more flexible chain, and can more easier bind with proteins to create a dense and seal interface leading to more encapsulation efficiency than that of the WPI-KCG complex.

For the effect of hydrophilic emulsifier or stabilizer concentration, the higher hydrophilic emulsifier or stabilizer concentration should have higher encapsulation efficiency because increased hydrophilic emulsifier levels lead to stronger interfacial films against core material release. For example, Li *et al*. (2012) found that increasing the polysaccharide concentration caused the encapsulation efficiency to increase. In the same way, Benichou *et al*. (2002) found that increases in the xanthan gum ratio reduced the vitamin B_1 release, because more xanthan gum content led to the production of a more synergistic and rigid film around the droplet.

However, increasing the concentration of some hydrophilic emulsifiers can lead to decreases in encapsulation efficiency. For example, Bonnet *et al*. (2010a) encapsulated magnesium in the W/O/W emulsions by using olive oil as an oil phase, PGPR as a lipophilic emulsifier, and sodium caseinate as a hydrophilic emulsifier. Surprisingly, the higher sodium caseinate concentrations led to higher magnesium release. This may due to increased levels of sodium caseinate meaning more sodium ions leading to more osmotic pressure in the external water phase. When the external osmotic pressure is higher than the internal osmotic pressure, the water may migrate from the internal to the external water phase.

4.2.5 pH of outer water phase

When two ionic hydrophilic emulsifiers or stabilizers are used together, the pH of outer water phase will affect the encapsulation efficiency of W/O/W emulsions. The pH that should be used is one that allows two ionic hydrophilic emulsifiers or stabilizers to have different charges in order to let them exhibit electrostatic attraction. For example, when protein and anionic polysaccharide are used as a hydrophilic emulsifier and stabilizer, respectively, the pH of the system should be lower than pI (isoelectric point) of protein in order to obtain the cationic structure of protein that can exhibit electrostatic attraction with an anionic polysaccharide structure (Figure 3A). The larger difference in charge intensity between the ionic hydrophilic emulsifiers or stabilizers lead to increased electrostatic attraction and provides a thicker and denser interfacial layer.

Benichou *et al*. (2007) entrapped thiamine hydrochloride in W/O/W emulsions by using medium chain fatty acid triglyceride as the oil phase, PGPR as the lipophilic emulsifier, monoglyceride oleate and glycerol for droplet size reduction and better stability. Whey protein isolate and xanthan gum was used as hydrophilic emulsifier and stabilizer, respectively. They found that lower pH levels led to lower vitamin B_1 release, but at a pH of 3.5 vitamin B_1 was released less than at pH 2 because at pH values more than about 4.5 (above the isoelectric point of the whey protein isolate) this caused the whey protein isolate to have a minus zeta potential as xanthan gum, so the whey protein isolate and xanthan gum did not interact (Figure 3B) which led to a less sealed interface and higher vitamin B_1 release. On the other hand, at pH 2 xanthan gum had less minus zeta potential and a reduced different of zeta potential between whey protein isolate and xanthan gum, which led to an interaction between them being less than at pH 3.5. This had a greater difference in zeta potential between the whey protein isolate and xanthan gum, so the complex interface at pH 3.5 was more sealed and had lower vitamin B_1 release.

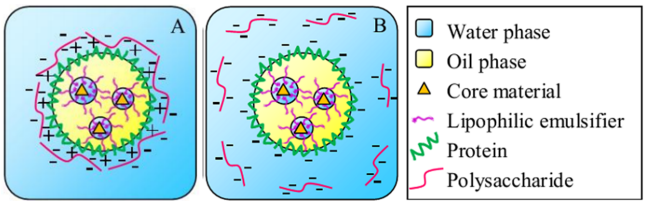

Figure 3. Schematic diagram of emulsion droplet stabilized by protein and anionic polysaccharide at pH << pI (A), and pH >> pI (B) (not to scale).

5. Conclusions

W/O/W emulsions can be used as an entrapping and delivering system for minerals and vitamins. However, there are many factors that affect the stability and efficiency of these emulsion systems that have to be considered such as volume and properties of oil and inner or outer water phase, concentration and form of core material, type and concentration of emulsifiers and stabilizers, and also process conditions. For encapsulation of minerals and vitamins, W/O/W emulsions should have both high encapsulation efficiency and physicochemical stability, including being stable to environmental stress and chemical reactions. Moreover, it should still be stable when it is applied to food products and should not decrease the quality of those products.

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