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MICROENCAPSULATION OF FUCOXANTHIN BY WATER-IN-OIL-IN-WATER (W/O/W) DOUBLE EMULSION SOLVENT EVAPORATION METHOD: A REVIEW

Mikroenkapsulasi Fukosantin dengan Metode Penguapan Pelarut Emulsi Ganda Air-dalam-Minyak-dalam-Air (A/W/A): Suatu ulasan

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ABSTRACT

Fucoxanthin is a major xanthophyll present in brown seaweeds such as *Sargassum binderi, S. duplicatum, Turbinaria turbinata, Padina australis, Undaria pinnatifida* and *Hijkia fusiformis*. This carotenoid has a unique structure including oxygenic functional group such as, two hydroxy, keto, epoxy (5,6-monoepoxide), and an allenic bond. Fucoxanthin has some anticancer activities such as inhibitory property on colon cancer cells and human hepatic carcinoma HepG2 cell line. This xanthophyll also induces apoptosis of human leukemia cancer HL-60 cells, human prostate cancer PC-3 cell, human lung cancer H1299 cell line *etc*. Unfortunately, the poor solubility of this carotenoid in water hinders it to be a drug candidate. Fucoxanthin is also a pigment that is sensitive to temperature and light. One of the possible ways to circumvent the problem with light and temperature is by microencapsulating it. Microencapsulation (ME) in biodegradable polymers, e.g. poly(D,L-lactic-co-glycolic acid) (PLGA) is a promising approach to protect any potential drug from rapid degradation. Solvent evaporation method is the most popular technique of preparing PLGA microsphere (MS) and this technique has been extensively studied in recent years for the preparation of MS. In the water-in-oil-in-water (w/o/w) double emulsion solvent evaporation method, stability of the primary emulsion (PE) is a critical factor. When the PE is unstable, encapsulation efficiency (EE) is low. Stability of PE can be enhanced by including emulsifying agent or stabilizers such as polyvinyl alcohol (PVA). The presence of a stabilizer/ emulsifier plays a significant role in influencing particle size (PS), external morphology of microsphere and colloidal stability.

Keywords: microencapsulation, fucoxanthin, double emulsion, PLGA, microsphere

ABSTRAK

Fukosantin adalah santofil utama yang terdapat dalam rumput laut coklat seperti *Sargassum binderi, S. duplicatum, Turbinaria turbinata, Padina australis, Undaria pinnatifida* dan *Hijkia fusiformis*. Karotenoid ini memiliki suatu struktur kimia yang unik mencakup gugus fungsional oksigenik seperti dua hidroksi, keto, epoksi (5,6-monoepoksida), dan ikatan alenik. Fukosantin memiliki beberapa aktivitas antikanker seperti, sifat penghambatan pada sel kanker kolon dan sel lestari HepG2 karsinoma hepatik manusia. Santofil ini juga menginduksi apoptosis pada sel HL-60 kanker leukemia, sel PC-3 kanker prostat, sel lestari H1299 kanker paru-paru manusia dan lain-lain. Sayangnya, kelarutan karotenoid ini dalam air adalah rendah sehingga menghambat untuk menjadikannya sebagai kandidat obat. Fukosantin juga merupakan pigmen yang sensitif terhadap suhu dan cahaya. Satu cara yang mungkin untuk mengatasi masalah dengan cahaya dan suhu ini adalah dengan mengenkapsulasinya. Mikroenkspsulasi (ME) dalam polimer *biodegradabel*, misalnya *poly(D,L-lactic-co-glycolic acid)* (PLGA) adalah suatu pendekatan yang menjanjikan untuk melindungi beberapa obat potensial dari degradasi yang cepat. Metode penguapan pelarut adalah teknik yang sangat populer untuk pembuatan mikrosfir PLGA dan teknik ini telah dipelajari secara intensif dalam tahun-tahun sekarang ini untuk penyiapan mikrosfir. Dalam metode penguapan emulsi ganda *w/o/w*, stabilitas emulsi primer adalah suatu faktor penentu. Ketika emulsi primer tidak stabil, maka efisiensi enkapsulasinya jadi rendah. Stabilitas emulsi primer dapat ditingkatkan dengan memasukkan zat pengemulsi atau stabilisator seperti polivinil alkohol (PVA). Keberadaan suatu penstabil dan pengemulsi adalah berperan sigifikan dalam mempengaruhi ukuran partikel, morfologi eksternal mikrosfir dan stabilitas koloidal.

Kata Kunci: mikroenkapsulasi, fukosantin, emulsi ganda, PLGA, mikrosfir

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1. Introduction

Fucoxanthin (3'-acetoxy-5,6-epoxy-3,5'-dihydroxy-6',7'-didehydro-5,6,7,8,5',6'-hexahydro-β,β-caroten-8-on) is a major xanthophyll present in brown seaweed (Beppu et al., 2012; Koji & Hosokawa, 2013). This compound contributes more than 10% of the estimated total production of carotenoids in nature (Matsuno, 2001; Miyashita, 2008), especially in the marine ecosystem (Dembitsky & Maoka, 2007; Moghadamtousi et al., 2014). It has a unique structure including oxygenic functional group, such as two hydroxy, keto, epoxy (5,6-monoepoxide), and an allenic bond in the molecule (Maeda et al., 2008; Miyashita, 2008; Riccioni et al., 2011; Kumar et al., 2013; Miyashita, 2014). Its structure differs from that of common carotenoids (Ishikawa et al., 2008) such as β-carotene and lycopene (Tsukui et al., 2007). The chemical structures of fucoxanthin and other epoxy xanthophylls are shown in Figure 1. The sources of fucoxanthin from brown seaweeds are summarized in Table 1.

2. Fucoxanthin and its Anticancer Activity

Fucoxanthin, a major carotenoid in brown seaweed (Das et al., 2008) exhibits inhibitory property on colon cancer cells, and this effect was associated with growth arrest (Das et al., 2008), inhibited the growth of human hepatic carcinoma HepG2 cell line (Das et al., 2008), and enhanced the antiproliferative effect of the peroxisome proliferator-activated receptor (PPAR) gamma ligand troglitazone on colon cancer cells (Hosokawa, 2004). Furthermore, fucoxanthin also induced apoptosis of human leukemia HL-60 cells (Kotake-Nara et al., 2005b), human prostate cancer PC-3 cell line (Kotake-Nara et al., 2005a), and human lung cancer H1299 cell line (Jaswir et al., 2011a Noviendri, 2014; Noviendri et al., 2014a) (Figure 2 and 3).

The activity of fucoxanthin as anticancer agent is shown in Table 2. In addition, fucoxanthin and its two metabolites, fucoxanthinol and halocynthiaxanthin have antioxidant activities. The proposed metabolic transformation of fucoxanthin to fucoxanthinol then finally converted to amarouciaxanthin A is shown in Figure 4. This metabolic conversion was observed in mice and in human hepatoma cell HepG2 (Asai et al., 2004; Hashimoto et al., 2009).

3. Definition and the Main Reasons for Microencapsulation (ME)

ME has been defined as the technology of packaging or coating of solid, gaseous and liquid materials with a thin protective layer or wall material (McNamee et al., 1998) or in small capsules that release their contents at controlled rates over prolonged periods of time (Campagne & Fustier, 2007). ME is a well-known technique that is used to delay and modify drug release from pharmaceutical dosage forms (Kim et al., 2002). ME in biodegradable polymers, e.g. poly-(D,L-lactic-co-glycolic acid) (PLGA), was found to be a promising approach to protect the potential compounds from rapid degradation (Wischke & Borchert, 2006). Moreover, a large number of ME techniques are available for the formation of sustained release (Wischke & Borchert, 2006) microparticulate drug delivery systems (Kim et al., 2002). Furthermore, microparticles (MPs) or

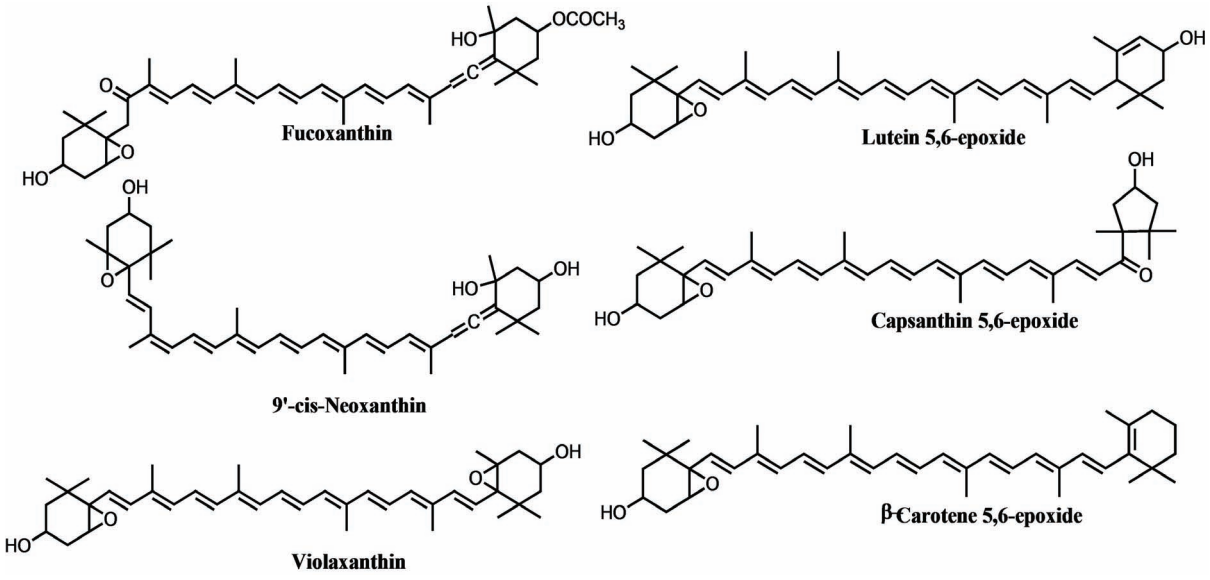

Figure 1. Chemical structures of various epoxy xanthophylls (Redrawn from Kotake-Nara and Nagao, 2011).

Table 1. Fucoxanthin in brown seaweeds

Species of brown seaweeds	References
Alaria crassifolia	Terasaki et al., 2009; Airanthi et al., 2011a.
Analipus japonicus	Terasaki et al., 2009.
Cladoshipon okamuranus	Mise et al., 2011; Tafuku et al., 2012.
Cystoseira hakodatensis	Terasaki et al., 2009; Airanthi et al., 2011a.
C. barbata	Ryabushko et al., 2014.
Eisenia bicyclis	Airanthi et al., 2011a.
Fucus distichus	Terasaki et al., 2009.
F. evanescences	Tatiana et al., 2013
F. vesiculosus	Zaragozá et al., 2008.
Hijkia fusiformis	Maeda et al., 2007; Maeda et al., 2006; Urikura et al., 2011.
Himanthalia elongate	Rajauria and Abu-Ghannam, 2013.
Hincksia mitchellae	Wu et al., 2014.
Hormophysa triquetra	Nursid et al., 2013.
Ishige okamurae	Kim et al., 2010.
Kjellmanielly crassifolia	Airanthi et al., 2011a.
Laminaria japonica	Das et al., 2008; Zhang et al., 2008; Miyata et al., 2009.
L. ochtotensis	Miyata et al., 2009.
Laminaria sp	Das et al., 2005; Das et al., 2008.
Leathesia diiformis	Terasaki et al., 2009.
Melanosiphon intestinalis	Terasaki et al., 2009.
Myagiopsis myagroide	Heo et al., 2010.
Padina australis	Zailani & Purnomo, 2011; Jaswir et al., 2011a; Nursid et al., 2013; Noviendri, 2014.
P. tetrastromatica	Sangeetha et al., 2009; Sangeetha et al., 2010.
Saccharina sculpera	Terasaki et al., 2009.
Sargassum confusum	Terasaki et al., 2009.
S. binderi	Noviendri et al., 2010; Noviendri et al., 2011a and 2011b; Jaswir et al., 2011b; Jaswir et al.,2012; Nursid et al., 2013; Noviendri, 2014; Yip et al., 2014.
S. duplicatum	Noviendri et al., 2010; Noviendri et al., 2011a and 2011b; Jaswir et al., 2011b; Jaswir et al., 2012; Noviendri, 2014.
S. echinocarpu	Zailani & Purnomo, 2011.
S. horneri	Terasaki et al., 2009; Airanthi et al., 2011a.
S. ilicifolium	Nursid et al., 2013
S. filipendula	Zailani & Purnomo, 2011; Zailanie & Sukosa, 2014.
S. fulvellum	Maeda et al., 2007; Terasaki et al., 2009; Urikura et al., 2011;
S. fusiforme	Terasaki et al., 2009
S. plagyophillum	Jaswir et al., 2011b; Noviendri et al., 2011b; Jaswir et al., 2013; Noviendri, 2014
S. siliquastrum	Soo-Jin et al., 2008; Soo-Jin & You-Jin, 2009; Heo et al., 2010.
S. thunbergii	Terasaki et al., 2009.
Silvetia babingtonii	Terasaki et al., 2009.
Turbinaria conoides	Zailani & Purnomo, 2011.
T. decurens	Nursid et al., 2013.
T. turbinata	Jaswir et al., 2011b; Noviendri et al., 2011b; Jaswir et al., 2013; Noviendri, 2014.
Undaria pinnatifida	Hosokawa, 2004; Maeda et al., 2006; Maeda et al., 2009; Sachindra et al, 2007; Nakazawa et al., 2009; Airanthi et al., 2011b; Liu et al., 2011; Urikura et al., 2011. Fung, 2012; Fung et al., 2013; Kanda et al., 2014; Wang et al., 2014.

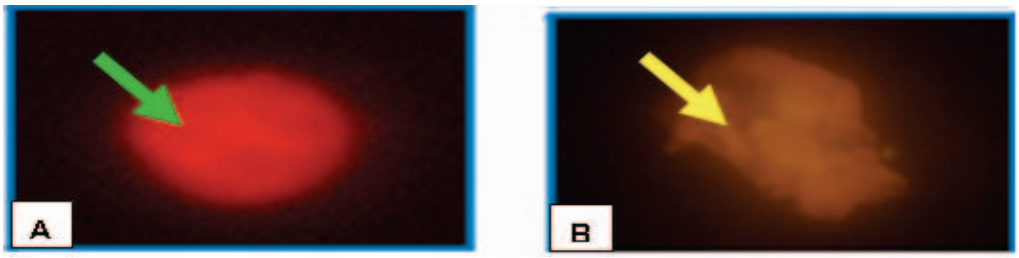

Figure 2. Fluorescence microscopy images of nuclei morphology of H1299 cells. Nuclear morphology of control cells (untreated cell) (A), cells treated with fucoxanthin (IC_{50} = 163.40 µg/ml) (B). Live and healthy cells appearing red color (green arrow) and apoptotic cells show yellow-green (yellow arrow). The cells were viewed under an Olympus inverted fluorescent microscope and photographed as described under Materials and Methods (Noviendri, 2014; Noviendri *et al*., 2014a).

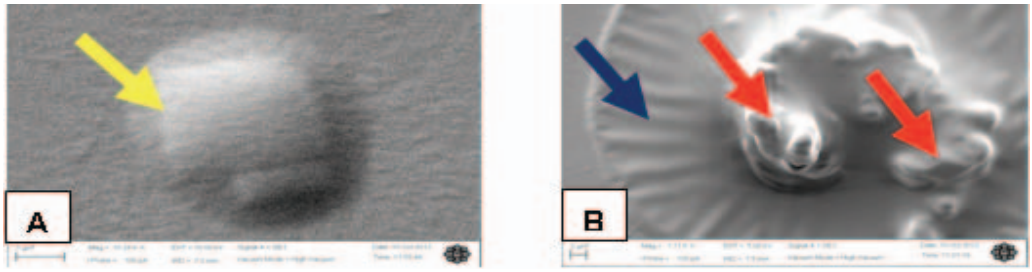

Figure 3. Scanning electron micrographs of surface ultrastructural characteristics of H1299 cells. Cells untreated (control cells) (A), cells treated with fucoxanthin (IC_{50} = 163.40 µg/ml) (B). Cells were viewed under a scanning electron microscope (SEM) (Carl Zeiss Evo® 50, Germany). Live and healthy cells appeared a normal membrane with smooth surface (yellow arrow), and features of apoptotic cells such as membrane blebbing (blue arrow) and formation of apoptotic bodies (red arrows) (Noviendri, 2014; Noviendri et al., 2014a).

Table 2. Fucoxanthin and its anticancer activity

Anticancer activity of fucoxanthin	References
Bladder cancer	Zhang et al., 2008.
Breast adenocarcinoma cancer	Wang et al., 2014.
Cervix squamous carcinoma cancer	Wang et al., 2014.
Colon carcinoma cancer	Hosokawa et al., 2004; Das et al., 2005; Eid et al., 2012; Wang et al., 2014.
Hepatoma cancer	Yoshiko & Hoyoku, 2007; Das et al., 2008; Liu et al., 2009; Satomi & Nishino, 2009; Liu et al., 2011; Wang et al., 2014.
Leukemia cancer	Yamamoto et al., 2011; Kotake-Nara et al., 2005b; Kim et al., 2010., Liu et al., 2013
Lung cancer	Jaswir et al., 2011a; Noviendri, 2014; Noviendri et al., 2014a; Wang et al., 2014.
Neuroblastoma cancer	Wang et al., 2014.
Osteosarcoma cancer	Rokkaku et al., 2013
Prostate cancer	Asai et al., 2004; Kotake-Nara et al., 2005a; Satomi & Nishino, 2009; Satomi, 2012.

microspheres (MSs) have some of interesting advantages as summarized in Table 3.

4. Techniques of ME

ME techniques are grouped into two basic categories, namely chemical and physical techniques, with the latter being further subdivided into physico-mechanical and physico-chemical techniques (Table 4) (Ghosh, 2006). Furthermore, biodegradable PLGA MSs or MPs can be prepared by several methods, but the most widely used techniques are solvent evaporation, phase separation (coacervation), and

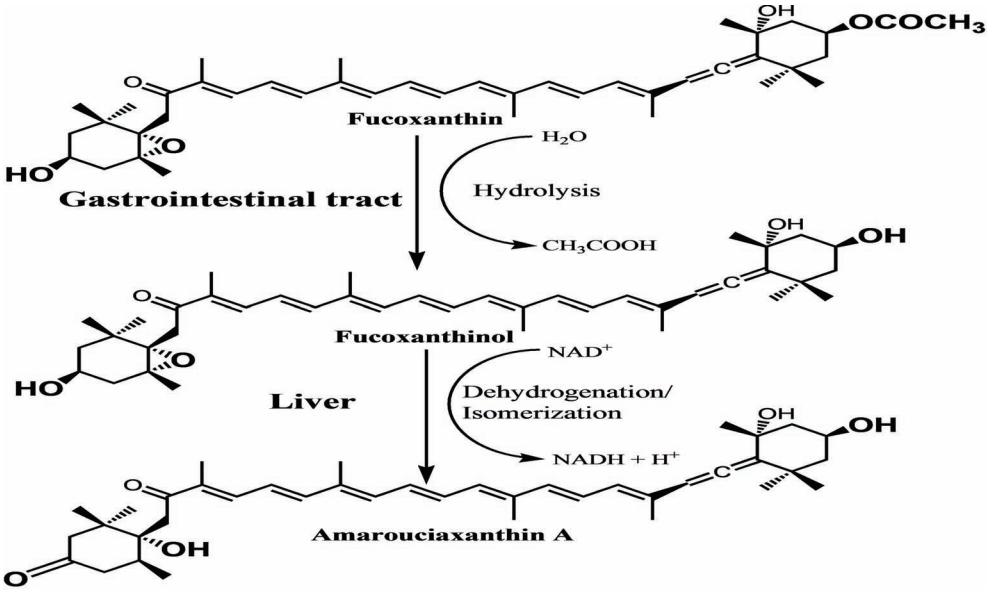

Figure 4. Proposed metabolic transformation of dietary fucoxanthin in mammals (Redrawn from: Sugawara et al., 2002; Asai et al., 2004; Maoka, 2011; Kotake-Nara & Nagao, 2011; Niwano & Beppu, 2012; Miyashita, 2014; Moghadamtousi et al., 2014).

Table 3. The advantages of ME technique

Reason for ME technique	References
To protect the sensitive compounds from the external environment factors such as moisture, oxygen, heat or light.	Jyothi et al., 2010; Shekhar et al., 2010; Singh et al., 2010; Ahmad et al., 2011.
To mask the organoleptic properties like odor, taste and colour of the compounds.	Dubey et al., 2009; Yoshizawa, 2004; Jyothi et al., 2010; Shekhar et al., 2010; Ahmad et al. 2011.
To obtain controlled release of the substance of drug.	Jyothi et al., 2010.
For sustained or prolonged drug release.	Dubey et al., 2009; Jyothi et al., 2010; Shekhar et al., 2010.
To modify and retard drug release.	Naha et al., 2008
For safe handling of the toxic materials.	Carrasquillo et al., 2001; Jyothi et al., 2010.
To get targeted release of the drug.	Dubey et al., 2009; Jyothi et al., 2010.
To minimise negative effects like gastric irritation of the drug.	Jyothi et al., 2010; Shekhar et al., 2010.

Table 4. Two basic groups of ME techniques

Chemical process	Physical process	
	Physico-mechanical	Physico-chemical
Suspension, dispersion and emulsion polymerization	Spray drying	Supercritical CO_2-assisted microencapsulation
Polycondensation	Centrifugal technique	Layer-by–layer assembly
	Multiple nozzle-sprying	Sol-gel encapsulation
	Vacuum encapsulation	
	Fluid-bed coating	

Source: Ghosh (2006).

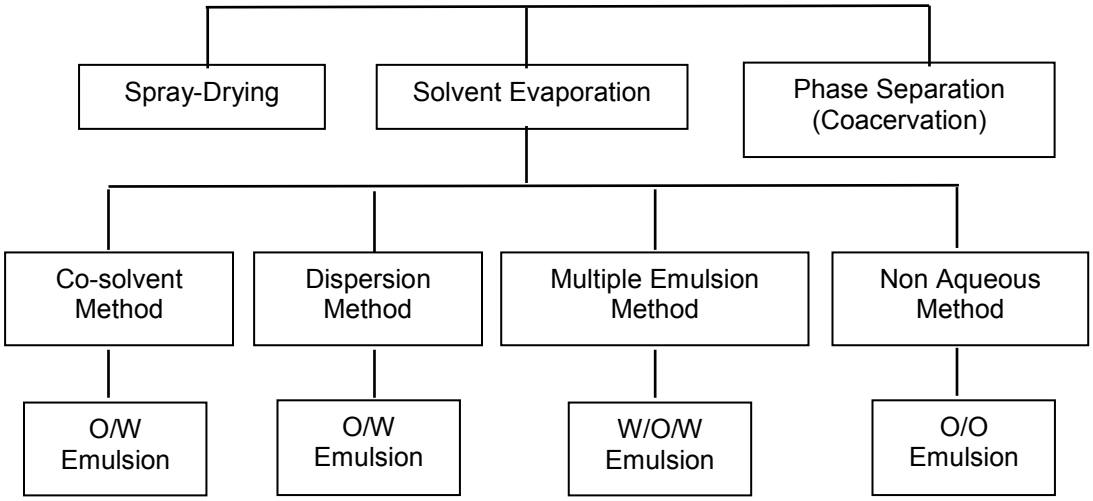

Figure 5. Several methods for preparation of PLGA MSs (Manca, 2009).

spray drying (Figure 5) (Manca, 2009). However, Mainardes and Evangelista (2005) reported the most commonly used ME technique is based on the concept of solvent and employs methylene chloride or dichloromethane (DCM) (Ansary et al., 2014) and water as disperse and continuous phase, respectively, in an emulsion type system.

5. Water-in-oil-in-water (w/o/w) Double Emulsion Solvent Evaporation Technique

Jaraswekin et al., (2007), and Sahoo et al., (2011) have reported that solvent evaporation method is the most popular technique of preparing PLGA MSs or MPs. This method does not require phase separation inducing agents or elevated temperatures (Freitas et al., 2005; Emami, 2009), and ME could also be easily scaled up to produce sterile microcapsules (Emami, 2009). Depending on the state of drug in the dispersion medium and the polymer solution, this method can be further classified into w/o/w double emulsion, water-in-oil (w/o) and oil-in-water (o/w) methods (Manca, 2009). Then, from several available methods, the w/o/w double emulsion solvent evaporation is the most widely used method for preparation of MSs (Chaudari et al., 2010).

The technique of ME by emulsion solvent evaporation removal technique has been applied extensively in pharmaceutical industries to obtain the controlled release of drug (Li et al., 2008), protection of the drugs from degradation, and protection of the body from the negative effects of the drugs (Obeidat, 2009). This method has been widely used to prepare MSs loaded with various drugs, especially hydrophobic drugs (Yeo et al., 2001). Moreover, because of their

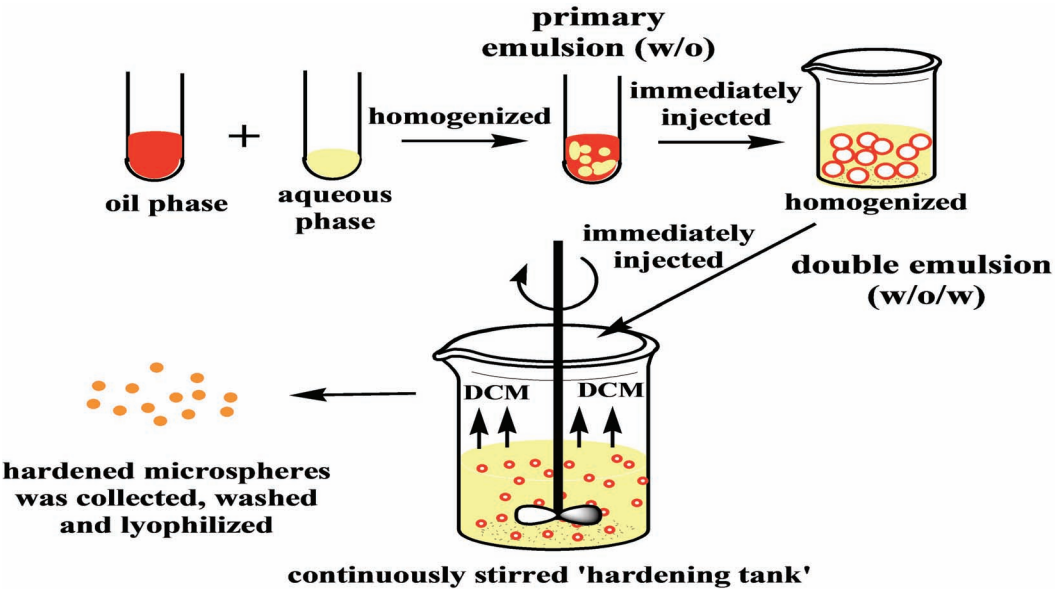

Figure 6. Schematic diagram of w/o/w double emulsion solvent evaporation method of microsphere synthesis (Redrawn from Mohamed, 2008).

encapsulation and protection efficiency, w/o/w double emulsions solvent evaporation are potentially suitable materials for applications in various domains such as cosmetics, pharmaceutics, and foods (Bonnet et al., 2010).

In the w/o/w double emulsion solvent evaporation method (Figure 6), stability of the primary emulsion (PE) is a critical factor for efficient internalization of the active ingredient (Maa & Hsu, 1997). When the PE is unstable, encapsulation efficiency (EE) is low. Stability of PE can be enhanced by including emulsifying agent or stabilizers such as polyvinyl alcohol (PVA) (Yang et al., 2001), Tween-80, or Span-80 (Li et al., 2008). The presence of a stabilizer/ emulsifier (Mu & Feng, 2003) plays a significant role in influencing particle size (PS), external morphology of microsphere (Noviendri, 2014) (Figure 7), and colloidal stability (Karata et al., 2009).

6. Biodegradable polymers for Microspheres (MSs)

Polymers used in ME are of two types (Alagusundaram et al., 2009), namely synthetic polymers and natural polymers. The synthetic polymers are divided into two types: (a) non-biodegradable polymers e.g. epoxy polymers, acrolein and glycidyl methacrylate, (b) biodegradable polymers e.g. lactides, glycolides and their copolymer poly anhydrides, poly-(ortho esters) and polyesters such as PLGA, and others. Natural polymers are obtained from different sources like, carbohydrate, chemically modified carbohydrate and chemically modified protein, such as hyaluronic acid, chitosan and alginic acid (Figure 8).

Among the different classes of biodegradable polymers, poly(lactide) (PLA) and PLGA are the most commonly used as drug carrier due to their biodegradability, mechanical strength (Athanasiou, 1996), biocompatibility, and versatility in terms of physical, chemical and biological properties (Mohamed, 2008). Furthermore, some of the requirements of biodegradable polymers can be summarized as follows (Llyod, 2002):

1. The substance should have suitable processibility and permeability for the intended application.
2. The degradation time of the substance should match the healing.
3. The substance should not evoke a toxic response upon implantation in the body.
4. The substance should have acceptable shelf life. The substance should have appropriate mechanical properties for the indicated application. The degradation products should be non-toxic, and able to get metabolized and cleared from the body.

7. Poly-(D, L-lactic-co-glycolic acid) (PLGA)

PLGA polymer is synthesized from homopolymer lactide and glycolide, respectively (Bouissou & Walle, 2006; Ansary et al., 2014) (Figure 9). PLGA polymer is most popular among the various synthetic

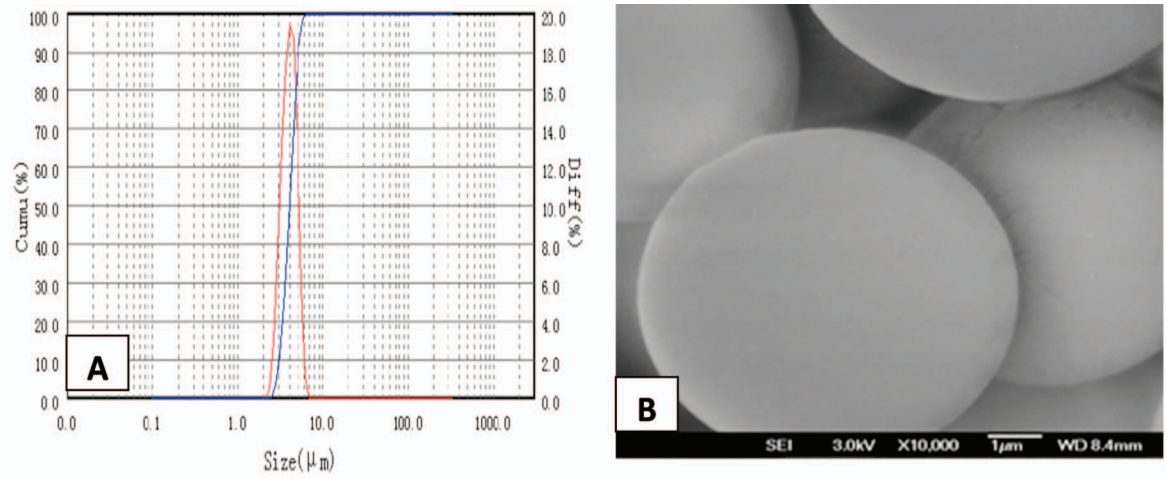

Figure 7. A representative of particle size (PS) distribution of fucoxanthin-loaded microsphere (F-LM) by using a laser particle size analyser (LPSA) (A), and a representative of morphology of F-LM by using field emission-scanning electron microscope (FE-SEM) with magnification of 10,000X (B) (Noviendri, 2014; Noviendri et al., 2014b).

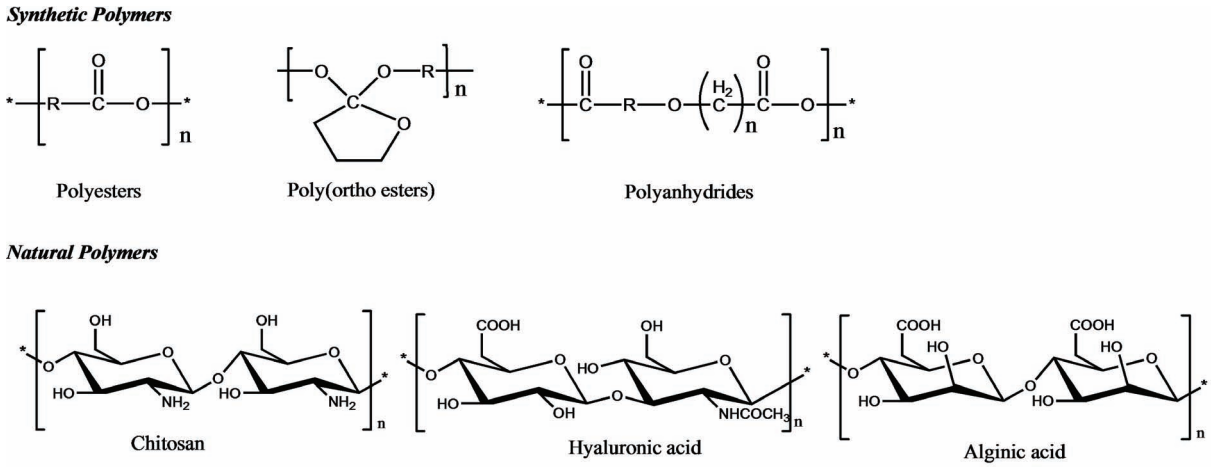

Figure 8. Chemical structures of several biodegradable polymers (Park et al., 2005).

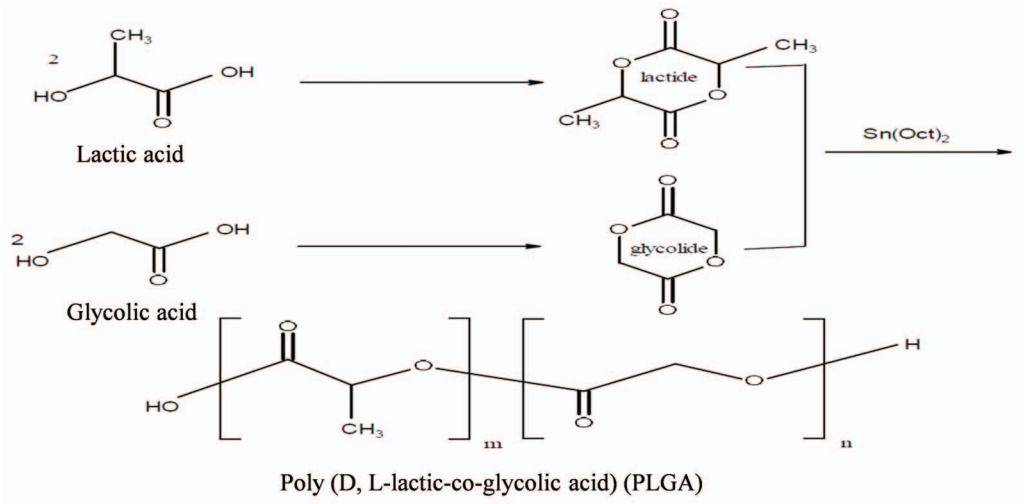

Figure 9. Synthetic scheme of poly-(D, L-lactic-co-glycolic acid) (PLGA)(Ansary et al., 2014).

biodegradable polymers (Varde & Pack, 2004) for pharmaceutical (Çirpanli et al., 2009) and biomedical applications (Emami et al., 2009), because it has been approved by the United State Food and Drug Administration (US FDA) for clinical usage (Makadia & Siegel, 2011; Wang, 2012), and biocompatibility (Cai et al., 2009; Çirpanli et al., 2009; Chaudhari et al., 2010).

The use of biodegradable MSs or MPs as drug delivery system offers various advantages, such as: (i) the improvement of bioavailability (Barakat & Ahmad, 2008), (ii) no need of (surgical) removal, (iii) easy administration (Siepmann et al., 2004), (iv) the possibility to accurately control the release rate of an incorporated drug, (v) enhancement of stability and targeting a drug to specific sites (Barakat & Ahmad, 2008), (vi) desire, pre-programmed drug release profile can be provided which matchs the therapeutic needs of the patients (Singh et al., 2010), (vii) localized drug delivery and, (viii) high local drug concentration (Berkland et al., 2003).

8. Biodegradation of PLGA

Among the families of synthetic polymers, PLGA have been attractive for pharmaceutical and medical applications because of their ease of degradation by hydrolysis of ester linkage (Gunatillake & Adhikari, 2003; Makadia & Siegel, 2011). In addition, PLGA can be degraded into oligomeric and finally monomeric acids (Tsai, 2003; Siepmann et al., 2004; Makadia & Siegel, 2011) (Figure 10) including lactic acid and glycolic acid that are nontoxic to the human body (Torres-Lugo & Peppas, 2000), and can be completely biodegraded into CO_2 and H_2O (Yamaguchi et al., 2002). Furthermore, in the human body, polyglycolides are broken down into glycine (amino acid) which can be excreted in the urine or converted into CO_2 and H_2O via the citric acid cycle (Torres-Lugo & Peppas, 2000; Maurus & Kaeding, 2004; Mohamed, 2008).

The rate of the PLGA biodegradation are dependent on the molecular weight (MW) of the polymer and the molar ratio of the glycolic and lactic acids in the polymer chain (Park, 1995), the degree of crystallinity (Tracy et al., 1999), and the glass transition temperature (*Tg*) of the polymer (Makadia & Siegel, 2011). The *Tg* of the MSs increase with increasing MW of PLGA. However, the MS preparation was not found to affect the *Tg* of the polymer (Tsai, 2003). The degradation rate of PLGA is inversely correlated to the crystallinity of the polymer since water does not penetrate easily into the crystalline regions (Tracy et al., 1999; Tsai, 2003). PLGA, which is amorphous, will degrade faster than either of its semi-crystalline homo-polymers (DeLuca et al., 1993).

For example, the biodegradability analysis of fucoxanthin-loaded microsphere (F-LM) was based on the change in morphology of microspheres (MSs). The change in morphology of F-LM with time following incubation in 0.1M phosphate buffer saline (PBS) (pH 7.4) at 37°C in static condition is shown in Figure 11.

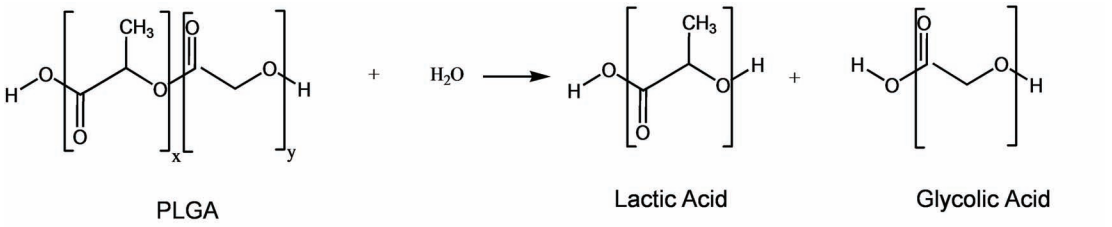

Figure 10. Hydrolysis of PLGA (Redrawn from Makadia and Siegel, 2011).

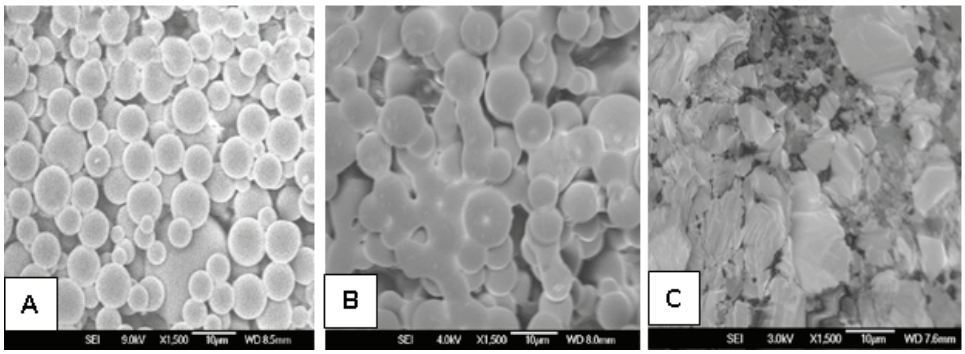

Figure 11. Morphology change of Fucoxanthin-Loaded Microsphere (F-LM): control (A), after 1 month (B) and after 2 months (C) by using FE-SEM with magnification of 1500X (Noviendri, 2014; Jaswir et al., 2014).

Noviendri (2014) and Jaswir et al., (2014) have reported that electron micrograph of F-LM before incubation (control) showed spherical, discrete MSs with smooth surface (Figure 11A). The major changes in F-LM morphology were observed within 1 month, the F-LM had deformed, aggregated, fused and the F-LM morphologies had turned into an unstructured mass (Figure 11B). Finally, the F-LM were totally collapsed and disintegrated into irregular particles, and no intact spheres were observed (Figure 11C). The F-LM fabricated from PLGA (50/50) as coating needs 2 months for their degradation (Noviendri, 2014; Jaswir et al., 2014). Wang (2012) reported that PLGA (50/50) polymer degraded in approximately 1 – 2 months, PLGA 75/25 and PLGA 85/15 are degraded 4 – 5 months and 5 – 6 months, respectively.

9. Conclusion

Microencapsulation of fucoxanthin in biodegradable polymers, e.g. poly(D,L-lactic-co-glycolic acid) (PLGA) is a promising approach to protect fucoxanthin from the light, temperature, rapid degradation and to increase fucoxanthin solubility in the water. Solvent evaporation method is the most popular technique of preparing PLGA microsphere (MS) and this technique has been extensively studied in recent years for the preparation of fucoxanthin-loaded microsphere (F-LM). Because of their encapsulation and protection efficiency, w/o/w double emulsions solvent evaporation are potentially suitable materials for applications in various domains such as cosmetics, pharmaceutics, and foods. In the w/o/w double emulsion solvent evaporation method, stability of the primary emulsion (PE) is a critical factor for efficient internalization of the active ingredient. The F-LM fabricated from PLGA (50/50) as coating needs 2 months for their degradation. The F-LMs were totally collapsed and disintegrated into irregular particles, and no intact spheres were observed under field emission-scanning electron microscope (FE-SEM).

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