Accepted Manuscript

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PII: S0924-2244(15)00240-X

DOI: 10.1016/j.tifs.2015.10.015

Reference: TIFS 1722

To appear in: *Trends in Food Science & Technology*

Received Date: 22 May 2015

Revised Date: 13 October 2015

Accepted Date: 22 October 2015

Please cite this article as: Lu, W., Kelly, A.L., Miao, S., Emulsion-Based Encapsulation and Delivery Systems for Polyphenols, *Trends in Food Science & Technology* (2015), doi: 10.1016/j.tifs.2015.10.015.

Emulsion-Based Encapsulation and Delivery Systems for Polyphenols

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Abstract

Background

Instability and low bioavailability of polyphenols greatly limit their potential health benefits in preventing aging, cancer, inflammation and neurodegenerative diseases. Utilization of protected encapsulation and delivery system can improve the stability and bioavailability of polyphenols. A wide range of technologies have been developed to encapsulate polyphenols. Among these, emulsion-encapsulation is regarded as one of the most promising techniques for protection and delivery of polyphenols, due to its high-efficiency encapsulation, maintenance of chemical stability and controlled release.

Scope and Approach

In this review, preparation, applications and limitations of emulsion-based encapsulation and delivery systems for polyphenols, including single, multiple and nano-emulsions, are discussed.

Key Findings and Conclusions

Utilization of encapsulated polyphenols instead of free molecules improves both the stability and bioavailability of the molecules *in vitro* and *in vivo*. Many emulsion-based delivery systems for polyphenols have been well established, including single, multiple and nano-emulsions. However, variations in composition and preparation technologies result in the formation of a range of emulsions of new properties with great potential in delivery of polyphenols or other bioactive nutrients, e.g., using unsaturated fatty acids as the oil phase, which can achieve the delivery of multiple nutrients at the same time. Furthermore, very few studies have been done on the *in vivo* absorption, transportation and release of polyphenols incorporated emulsions, which are essential to their deeper and wider applications. Hence,

systematic and intensive investigation of metabolism and physiological effects of encapsulated polyphenols or other potential bioactive nutrients *in vivo* are required.

Keywords: polyphenol, encapsulation, delivery, emulsion

Introduction

Polyphenols are a structural class of mainly natural, but also synthetic or semisynthetic, organic chemicals, which are characterized by the presence of large multiples of phenol structural units; they widely exist in numerous natural plants and foods, e.g., herbs, fruits and vegetables **(Fig. 1)**. Although plant-derived products have been used in Asia for centuries, the term polyphenol has only been in use since 1894 (Prasad, 2014). Their biological effects and their values in human health have been demonstrated during the last two decades.

Polyphenols are widely regarded as a major groups of highly effective antioxidants, since they exhibit potent free radical scavenging capability and protections against oxidation of transition metals and lipid peroxidation (Zhou & Elias, 2013). However, biological effects of these phytochemicals have turned out to be more complex than originally expected. They can inhibit cancer cell proliferation and cholesterol uptake (Leifert & Abeywardena, 2008; Noratto, Porter, Byrne, & Cisneros-Zevallos, 2009), regulate transcription, expression and mode of action of different enzymes including telomerase (Naasani, et al., 2003), cyclooxygenase (Hussain, Gupta, Adhami, & Mukhtar, 2005; O'Leary, et al., 2004) and lipoxygenase (Rocio de la Puerta, Gutierrez, & Hoult, 1999; Sadik, Sies, & Schewe, 2003; Schewe, et al., 2001), participate in several signal transduction pathways (Kong, Yu, Chen, Mandlekar, & Primiano, 2000; Masella, et al., 2004; Rosenblat & Aviram, 2009; Spencer, Rice-Evans, & Williams, 2003; Wiseman, Mulder, & Rietveld, 2001), and modulate cell cycle and platelet functions (Murphy, et al., 2003). Polyphenols can also prevent endothelial dysfunctions (Carluccio, et al., 2003).

However, low bioavailability and instability of polyphenols in digestion and absorption process greatly limits their health benefits. In fact, only a small proportion of them taken orally are absorbed, because of insufficient gastric residence time, low permeability, and water-solubility (Wildman, 2006). In addition, they are sensitive to physical and chemical

conditions, such as light, heat and oxidation (Munin & Edwards-Levy, 2011). The delivery of these compounds therefore requires protection mechanisms that can maintain their chemical integrity and deliver them to the physiological target (Chen, Remondetto, & Subirade, 2006).

A wide range of technologies have been developed to encapsulate polyphenols, including spray drying, coacervation, emulsions, liposomes, micelle, nanoparticles, freeze-drying, cocrystallization and yeast encapsulation (Fang & Bhandari, 2010; Munin & Edwards-Levy, 2011). Each of these has its own specific strengths and weaknesses in encapsulation, protection, delivery, cost, regulatory status, ease of use, biodegradability and biocompatibility. Among these, emulsions are widely considered as one of the most popular encapsulation and delivery systems for a wide range of lipophilic, hydrophilic and amphiphilic bioactive molecules (McClements & Li, 2010), due to their high-efficiency encapsulation, maintenance of chemical stability of encapsulated molecules (Klinkesorn, 2005) and controlled release(Mao, Roos, & Miao, 2013). Furthermore, some emulsion-encapsulated polyphenols presented even higher biological activities compared with pure free molecules (Wang et al, 2008).

An emulsion consist of two immiscible liquids, usually oil and water, with one of the liquid being dispersed as the small spherical droplets in the other. Emulsions can be classified according to the relative spatial distribution of the oil and aqueous phase (McClements, 2005). A system that consists of oil droplets dispersed in an aqueous phase is called an oil-in-water (O/W) emulsion, e.g., milk and soups; while a system that consists of water droplets dispersed in an oil phase is called a water-in-oil (W/O) emulsion, e.g., butter. In the last two decades, a variety of emulsions with desirable structures and properties have been successfully developed for the protected encapsulation and delivery of many kinds of bioactive nutrients with significant health benefits (McClements, 2010, 2012; Norton, Espinosa, Watson, Spyropoulos, & Norton, 2015). Emulsions are thus essential encapsulation

115 systems in many particular applications, especially in the food industry. Formulation,
116 structure-functionality relationship and delivery behaviours of emulsions are also the focus of
117 current research.

118 This review summarizes the literature focusing on the preparation, applications and
119 limitations of emulsion-based systems for encapsulation and delivery of polyphenols, their
120 applications in the nutrition, health and pharmaceuticals areas, and the development of
121 emulsions as the delivery systems. Oil-in-water single emulsions, water-in-oil-in water
122 double emulsions and nanoemulsions are mainly discussed.

123

124 **Properties of Polyphenols and their limitations in applications**

125 The molecular structure, physicochemical properties, and health benefits of various
126 polyphenols have been reported by many previous studies, and properties of some
127 representative polyphenols are shown in **Table 1**.

128 In general, all these compounds have a poor solubility in water but can be easily dissolved
129 in organic solvents, except EGCG, which is soluble in both. Their appearances are normally
130 coloured crystals or powders, with melting points ranging from 183℃ (curcumin) to above
131 360℃ (ellagic acid).

132 These polyphenols possess a variety of health benefits, e.g., antioxidant activity and
133 prevention of cancer, diabetes, inflammation, virus, thrombus, cardiovascular and Alzheimer's
134 diseases, as well as UV radiation protection and hepatoprotective activities. Among these,
135 antioxidant activity is one of the most clearly documented health benefits of polyphenols.
136 Indeed, polyphenols' protective effects against human diseases are mainly attributed to their
137 significant antioxidant activity, e.g., scavenging of reactive oxygen species (ROS), since high

levels of ROS are widely reported to be correlated with a number of human disease conditions, such as those listed in **Table 1**.

In spite of the notable therapeutic potentials of polyphenols which have been confirmed by both *in vitro* and *in vivo* studies, some limitations can also be clearly observed, as shown in **Table 1**. To sum up, there are four main problems in their applications: (i) poor water solubility inducing a low bioavailability, e.g., curcumin, resveratrol, quercetin and ellagic acid; (ii) instability under exposure to light and/or certain pH conditions, e.g., resveratrol, quercetin or ellagic acid; (iii) poor oral and gastrointestinal absorption (due to degradation, low permeability or rapid metabolism), e.g., EGCG, curcumin, resveratrol, quercetin or ellagic acid; and (iv) very short half-life and rapid elimination from the body, e.g., EGCG, resveratrol, or ellagic acid. All of these factors potentially will lead to a loss in bioavailability of these compounds and thus reduced potential health benefits.

Novel emulsion-based protection and delivery strategies to overcome these problems will be discussed in the following sections.

Oil-in-water single emulsions for protected encapsulation of polyphenols

Conventional oil-in-water (O/W) emulsions consist of oil droplets dispersed in an aqueous continuous phase, with the oil droplets being surrounded by a thin interfacial layer consisting of emulsifier molecules **(Fig. 2)**. The concentration and particle size distribution of the oil droplets in emulsions can be controlled by oil phase proportion and preparation technologies. The oil droplets typically have diameters between 0.1 and 100 μm while the interfacial layer is generally between 1 nm and 10 nm thick for emulsifiers (McClements, Decker, & Weiss, 2007), e.g., surfactants, phospholipids, proteins, or polysaccharides (McClements, 2005). The electrical charge on the droplets can be controlled by selecting an appropriately charged

emulsifier, which may be positive, un-charged, or negative (Dickinson, 1992; Friberg, Larsson, & Sjoblom, 2003).

A variety of methods can be used to prepare polyphenols encapsulated in O/W emulsions, including homogenization, homogenization-solvent removal, emulsion-cooling and interfacial polycondensation.

Homogenization is the process of converting two immiscible liquids into an emulsion, and the classical device designed to carry out this process is called a homogenizer (Walstra, 1993). The preparation of an emulsion system directly from two separate liquids will be considered as primary homogenization, whereas the reduction of droplet size in existing emulsions is defined as secondary homogenization; the preparation of emulsions always involves the use of one or both of these homogenization processes (Binks, 1998). Properties of emulsions prepared by homogenization are often largely affected by the homogenization conditions used, including temperature, pressure and cycles (Yuan, Gao, Zhao, & Mao, 2008). These properties mainly include droplet size, stability and viscosity. Hence, emulsions with desired properties can be achieved by controlling the homogenization conditions for targeted encapsulation and delivery of polyphenols.

A canola oil O/W emulsion stabilized by ι-carrageenan and β-lactoglobulin with a droplet size of about 400 nm, which incorporated epigallocatechin-3-gallate (EGCG), was successfully prepared by high-pressure homogenization (Ru, Yu, & Huang, 2010). The emulsion-encapsulated EGCG showed enhanced *in vitro* anticancer activity compared to the free EGCG **(Fig. 3)**. Another O/W system with even smaller droplet size (<200 nm) was successfully prepared for the encapsulation of resveratrol (Donsì, Sessa, Mediouni, Mgaidi, & Ferrari, 2011). This emulsion system remained stable for 4 weeks and protected resveratrol from oxidation while maintaining its antioxidant activity.

In addition to the functional evaluation of encapsulated polyphenols, researchers have also investigated the *in vitro* digestion behaviour of oil droplets in O/W emulsions prepared by high-pressure homogenization (Ahmed, Li, McClements, & Xiao, 2012). O/W emulsions were prepared with different lipids of long-, medium- and short-chain triacylglycerols (LCT, MCT and SCT, respectively) for encapsulation of curcumin. Under the simulated intestinal digestive environment, the length of the triacylglycerol chain can significantly influenced the initial digestion rate (SCT > MCT > LCT), final digestion extension of the lipid phase (MCT > SCT > LCT) and the bioaccessibility of encapsulated curcumin (MCT > LCT > SCT).

Homogenization-solvent removal methods have been well established for encapsulation of a variety of polyphenols and improve both their stability and bioavailability. These processes are based on evaporation or extraction **(Fig. 4)** of the internal phase of an emulsion, resulting in the precipitation of the coating polymer in the form of particles while trapping the active ingredients (Munin & Edwards-Levy, 2011). In the solvent evaporation method, the polymer used to trap the bioactive nutrients is first dissolved in a volatile organic solvent which has a very low miscibility with water. The active compound is dispersed in the polymer solution; then, water containing emulsifier is added and the mixture is homogenized to obtain an O/W emulsion. Evaporation of the volatile organic solvent is performed upon heating and/or under vacuum to form the bioactive ingredients encapsulated in nanoparticles. In the solvent extraction method, the solvent used to dissolve the polymer must be completely miscible with water and this polymer solution is injected under agitation into a continuous water phase containing a water-soluble emulsifier. The polymer, insoluble in the mixture of water and volatile solvent, precipitates to form nanoparticles, while entrapping the active ingredient.

The homogenization-solvent removal method has been well developed for encapsulation of polyphenol compounds in last a few years, including quercetin (Kumari, et al., 2011; Kumari, Yadav, Pakade, Singh, & Yadav, 2010; Wu, et al., 2008), ellagic acid (Bala, et al., 2006;

Sonaje, et al., 2007), EGCG (Italia, Datta, Ankola, & Kumar, 2008; Onoue, Ochi, & Yamada, 2011; Siddiqui, et al., 2009), resveratrol (Shao, et al., 2009) and curcumin (Tsai, et al., 2011). Encapsulated polyphenols obtained by this method are always solid nanoparticle powders. Biopolymers, e.g., PLA and PLGA, are mainly used as matrixes to form nanoparticles that entrap the polyphenols (**Table 2**). Encapsulation of polyphenols based on this method can achieve high encapsulation efficiency, and encapsulated polyphenol compounds showed a large increase in their solubility and physical or chemical stability. Furthermore, compared with free compounds, functional evaluations in these studies confirmed strengthened biological effects, such as antioxidant and anti-cancer activity, of encapsulated polyphenol compounds *in vitro* and *in vivo*. Moreover, a significant improvement in *in vivo* intestinal absorption efficiency of polyphenols was also observed by using emulsion encapsulation technology based on emulsification-solvent removal methods (**Table 2**).

This method has also been successfully used to encapsulate polyphenol mixtures, including catechin (Taylor, Taylor, Belton, & Minnaar, 2009), tea polyphenol (Yaolan, Caihuan, Yingzhou, Shaoyu, & Shihai, 2000) and bayberry polyphenol (Zheng, Ding, Zhang, & Sun, 2011). The utilization of encapsulated polyphenol mixtures can significantly improve their storage stability while maintaining their antioxidant activity. In addition, these encapsulated polyphenol mixtures also showed a sustained or controlled release pattern, which is largely influenced by the environments (pH or enzymes).

Emulsion-cooling process consists of dissolving or dispersing the active compound in a lipid phase, which is then emulsified in a continuous aqueous phase (Vandamme, Poncelet, Subra-Paternault, & Benameur, 2007). The formation process of an emulsion is always maintained at a higher temperature than the melting point of the lipid phase and rapid cooling of the emulsion will generate lipid nanoparticles, in which bioactive ingredients are

encapsulated. The process allows the encapsulation of hydrophilic or lipophilic molecules if a continuous phase is chosen within which these molecules do not have sufficient solubility.

The emulsion-cooling method has been employed to prepare curcumin-encapsulated lipid nanoparticles; encapsulated curcumin was very stable when kept at 4 ℃ or 30℃ for 20 days (Donsì, et al., 2011). Preparation of EGCG-encapsulated lipid-nanocapsules (LNC) using this method has been reported, with a high encapsulation rate of 95% and stability of over 4 weeks in water, whereas free molecules in water showed 100% degradation within 4 h (Barras, et al., 2009). The method can also be used to encapsulate quercetin. Incorporation of quercetin into lipid-nanocapsules (LNC) dramatically increased its aqueous solubility (100-fold), improved physical instability (creaming or flocculation) and protected it from oxidation and light-induced decomposition (Barras, et al., 2009; Scalia & Mezzena, 2009). The most promising emulsion system was shown to be stable for at least 10 weeks. Furthermore, encapsulated quercetin showed a much higher transdermal absorption efficiency and enhanced antioxidant and anti-inflammation activity (Chen-yu, et al., 2012). All these results suggest that incorporation of quercetin into lipid-nanoparticles represents an effective strategy for enhancing its solubility, stability and bioavailability.

Interfacial polycondensation is a rapid, irreversible polymerization at the interface between aqueous solvent containing one reactant and an immiscible organic solvent containing a complementary reactant. It is based on the Schotten-Baumann reaction, in which acid chlorides are reacted with compounds containing active hydrogen atoms (-OH, -NH and -SH) (Wittbecker & Morgan, 1959). A large number of polymers (heat-sensitive and infusible as well as stable and meltable) can be prepared by this method. Interfacial polycondensation can also be used for the preparation of emulsion-based encapsulation systems, also known as emulsion diffusion methods (Janssen & Te Nijenhuis, 1992). This method for entrapment of bioactive ingredients is an attractive process for prohibiting light-induced oxidation with high

encapsulation efficiency (Choi, Soottitantawat, Nuchuchua, Min, & Ruktanonchai, 2009). Bouchemal et al (Bouchemal, et al., 2004) used an interfacial polycondensation combined with emulsification to encapsulate vitamin E, which is sensitive to light, heat and oxygen. The nanoencapsulation achieved by this method has many advantages, e.g., high encapsulation efficiency, better particle size control, and enhanced stability (Montasser, Briançon, & Fessi, 2007).

Interfacial polycondensation reactions have also been employed to encapsulate polyphenol compounds. Solid microparticles incorporating proanthocyanidin (GPO), a polyphenol extracted from grape seed, have been created using this method (Andry, Vezin, Dumistracel, Bernier, & Lévy, 1998). GPO-encapsulated polymers, formed by interfacial polycondensation, constituted the coating membrane of microparticles. GPO protected in this way showed an improved physical stability while maintaining its radical-scavenging activity. An optimized emulsion diffusion method was used to prepare polyurea and polyurethane nanoparticles for the encapsulation of curcumin, and more detailed investigation on the microstructure of nanoparticles has been done (Souguir, Salaün, Douillet, Vroman, & Chatterjee, 2013). Fourier transform infrared spectroscopy (FTIR) analysis confirmed the encapsulation of curcumin and differential scanning calorimetry (DSC) detection showed that the encapsulated molecule was found in an amorphous phase. Furthermore, the percentage of surfactant, organic solvent content, and hydrophilic monomer are the main factors that influenced the encapsulation efficiency, while the choice of monomer affected the particle size distribution mode as well as the mean diameter.

O/W emulsions show many potential advantages as encapsulation and delivery systems for lipophilic polyphenols. Firstly, physical and chemical stability of encapsulated polyphenols can be well protected by designing the oil-water interface or controlling the physical location of polyphenols (Mao, et al., 2013; Mao, Roos, & Miao, 2012). In addition, it is possible to

design emulsions with different rheological properties, which can meet some specific applications in delivery of lipophilic polyphenols (Genovese, Lozano, & Rao, 2007). Furthermore, O/W emulsions can either be used in wet state (Chen-yu, et al., 2012; Ru, et al., 2010) or be dried to solid powders (Kumari, et al., 2011; Kumari, et al., 2010), which greatly facilitates their processing, transportation, storage and thus the application in encapsulation and delivery of polyphenols.

In spite of O/W emulsions have been widely employed as delivery system for a variety of bioactive nutrients and show huge potentials as delivery system, there are still some disadvantages. For example, O/W emulsions are usually sensitive to environmental stress, such as heating, chilling, extreme pH and salt concentrations, all of which can lead to their physical and chemical instability, e.g., creaming, flocculation, coalescence, breaking and Ostwald ripening for common physical instability **(Fig. 5)** (Becher, 1996; Dickinson, 2010), and oxidation and hydrolysis for their common chemical instability (McClements & Decker, 2000). All these instabilities can potentially cause damage or even break-down of emulsions, and accordingly will decrease physical and chemical stability of encapsulated polyphenols and thus their final beneficial effects. In addition, it is challenging to precisely control the release of encapsulated polyphenols in O/W emulsions, because the simple oil-water interface structure in O/W emulsion result in a very short time for diffusion of encapsulated polyphenols from inside to the outside of the oil droplets (McClements, et al., 2007). Therefore, emulsions with more sophisticated structures are required for some particular applications.

Water-in-oil-in-water double emulsions for the encapsulation of polyphenols

Water-in-oil-in-water (W/O/W) double emulsions consist of small water droplets contained within larger oil droplets that are dispersed in an aqueous continuous phase (McClements,

2005) **(Fig. 6)**. W/O/W emulsions can also be more clearly defined as $W_1/O/W_2$ emulsions, where W_1 is the inner water phase while W_2 is the outer water phase. In principle, it is possible to design properties of inner water phase and oil phase, e.g., droplets size and distribution, surface charge, and interfaces between water and oil, such as surface charge, and environmental response behaviours.

Polymer capsules formed by the solvent evaporation of a W/O/W emulsion have been developed, and shown to control the release of encapsulated riboflavin-5′-phosphate (R5-P) (Koo, et al., 2008), a light-sensitive polyphenol molecule, which acts as a prosthetic group for various oxidoreductases, as well as a cofactor in biological blue-light photo receptors. The heterogeneous wall formed efficiently blocked the sun-light and hence stabilizes photo-sensitive R5-P. This encapsulation technology potentially can be utilized to stabilize a wide variety of photo-sensitive, water-soluble molecules, which may lead to practical applications in many fields. Other technologies, such as mechanical agitation and membrane emulsification, have been developed to prepare stable W/O/W double emulsion with polyphenol-encapsulation capability. High initial encapsulation efficiency (EE) of resveratrol in a W/O/W emulsion is achieved by employing proper inner or external phase emulsifier or their combination with an external continuous water phase solution, which may result in a synergetic effect and thus a higher initial EE (Matos, Gutiérrez, Coca, & Pazos, 2014).

W/O/W double emulsion systems can also be employed to co-encapsulate both hydrophilic catechin and hydrophobic curcumin simultaneously by using a two-step emulsification method (Aditya, et al., 2015). This fabricated system showed a synergistic effect between the components; encapsulation of curcumin and catechin increased their stability and bioavailability, and the presence of catechin and curcumin helped to reduce the droplet size of the emulsion.

Compared with O/W emulsions, W/O/W emulsions are ideal protected encapsulation systems for hydrophilic polyphenols. These compounds can be trapped in the internal water phase, which is isolated from the outer water phase by the oil phase, preventing their diffusion across the water-oil interface into the outer water phase (Benichou, Aserin, & Garti, 2004; McClements, 2015). Furthermore, release of polyphenols entrapped within the inner water phase will be prolonged and can be controlled (Garti & Bisperink, 1998). Moreover, W/O/W emulsions can be also designed to encapsulate both lipophilic and hydrophilic bioactive polyphenols at the same time (Cournarie, et al., 2004), which will achieve multiple targeted delivery of multiple bioactive compounds in one particular system. Another potential advantage of W/O/W emulsions is that they can be structured to have the same dispersed phase volume and droplets size as conventional O/W emulsions, but with lower fat content, which facilitate the development of functional food products with encapsulated polyphenols , which have lower-fat content but the same properties as the full-fat products (McClements, et al., 2007).

However, W/O/W emulsions, like conventional O/W emulsions, are also highly susceptible to environmental stresses (thermal processing, freeze and dehydration), which can induce instability, such as conventional flocculation, coalescence, and Ostwald ripening (**Fig. 5**), which potentially will influence the delivery of encapsulated polyphenols. Furthermore, the diffusion of encapsulated hydrophilic polyphenols or water molecules from the inner to the outer aqueous phase or expulsion of whole water droplets from oil droplets, induced by limited solubility of encapsulated compounds in oil phase, can also lead to the instability of W/O/W emulsions (Garti, 1997; McClements, 2015). Moreover, polyphenols encapsulated in the inner water phase of W/O/W emulsions can gradually diffuse into the oil phase or even outer water phase, due to their amphiphilic properties, which potentially will change their release pattern and thus influence expected controlled-release and targeted-delivery.

Many strategies have been developed to overcome these problems, including use pf combinations of oil- and water-soluble emulsifiers, incorporation of biopolymers into the outer water phase, and osmotic balancing of the inner and outer water phases to prevent water diffusion (Garti & Benichou, 2004).

Nanoemulsions: advanced delivery system for polyphenols

Nanoemulsions are metastable dispersions of sub-100-nm droplets of one liquid in a different immiscible liquid(Mason, Wilking, Meleson, Chang, & Graves, 2006). A nanoemulsion can be considered to be a conventional emulsion that contains very small droplets. A wide range of technologies can be used to prepare both O/W or W/O nanoemulsions. High-flow homogenization provides a simple route to forming nanoscale droplets, wherein externally applied shear and/ or elongational flow overcome the interfacial and internal viscous stress to rupture bigger droplets into smaller droplets; examples of this include high-pressure microfluidic homogenization (Meleson, Graves, & Mason, 2004) or ultrasonic emulsification (Leong, Wooster, Kentish, & Ashokkumar, 2009).

Compared with conventional emulsions, nanoemulsions represent a more stable physical system to gravitational separation and aggregation than conventional emulsions, due to their smaller droplets size and higher liquid droplet interface area (Fryd & Mason, 2012). Nanoemulsions can be nearly transparent, because their relatively small droplet size ($r<\lambda/4$) results in less scattering of visible spectrum (Mason, et al., 2006); nanoemulsion exhibits very different rheological properties, e.g., viscosity, elasticity, and response to shear (Russel, Saville, & Schowalter, 1992), which make it possible to modify or design the texture of food products (McClements, 2011). Nanoemulsion is reported to show a wide applications in food and nutrition, biology and pharmacology areas, especially in the high-efficiency

encapsulation and targeted delivery of bioactive ingredients (Ezhilarasi, Karthik, Chhanwal, & Anandharamakrishnan, 2012).

Nanoemulsion-encapsulation of EGCG significantly improved its *in vitro* neuronal α-secretase enhancing activity and *in vivo* bioavailability (Smith, et al., 2010), which was doubled compared with free EGCG. The study demonstrated the ability of nanoparticles to increase the systemic absorption of EGCG taken orally; it is likely that the small diameter of these particles will also lead to improved blood-brain barrier penetration. Nanoemulsion encapsulation can also be used to promote the bioavailability of curcumin while maintaining its biological activities (Anand, et al., 2010). Encapsulated curcumin (EC), compared with free compounds, exhibited very rapid and more efficient *in vitro* cellular uptake; EC was more bioavailable and had a longer half-life than free curcumin *in vivo* **(Fig. 7)**. Furthermore, EC was also more active in inhibiting TNF-induced NF-κB activation and thus showed effects in regulating cell proliferation, invasion and angiogenesis.

A number of studies have shown that the bioavailability of lipophilic components encapsulated in lipid droplets increased when the droplets size decreased (Acosta, 2009). There are several possible reasons for this increase. Firstly, Nanoemulsion always shows a very rapid release of encapsulated compounds ($t_{1/2}$<1 ms) (McClements, 2005), due to their small droplets; a large surface area of small droplets leads to their quick digestion so that encapsulated molecules are released easily. Secondly, small droplets are more easily to be absorbed into lymphatic vessels through the mucous layer that coats the epithelium cells within the small intestine (Jenkinsa, 1994). Thirdly, small particles can be directly transported across the epithelia mucus *via* paracellular, endocytosis and mucosa-associated lymphoid tissues (MALT) mechanisms (Lu, et al., 2012).

Compared with conventional emulsions with droplet size ranging from 100 nm-100 μm, nanoemulsions showed better stability to gravitational separation, flocculation and

coalescence (McClements, 2011), but worse stability to Ostwald ripening (Taylor, 1998), a process of net migration of dispersed-phase molecules from smaller droplets into larger droplets. However, nanoemulsions are more susceptible to chemical degradation due to their large specific surface area of oil-water interface and transparency caused by small droplet size.

Summary and future trends

Studies on encapsulation and delivery of polyphenols by emulsion-based delivery systems have been reviewed. It is clear that utilization of encapsulated polyphenols instead of free molecules improves both the stability and bioavailability of the molecules *in vitro* and *in vivo*. Many emulsion-based encapsulation and delivery systems for polyphenols have been well established, including single, multiple, and nano-emulsions. However, variations in composition and preparation technologies result in the formation of a range of emulsions with novel properties, which may show even greater potentials in delivery of polyphenols. Studies on these emulsions will contribute to the establishments of high-performance delivery systems and extend the application of both polyphenols and emulsions, e.g., using unsaturated fatty acids as the oil phase of polyphenol-encapsulated emulsion, which can achieve the delivery of multiple nutrients (unsaturated fatty acids and polyphenols) at the same time. Furthermore, very few preliminary studies have evaluated the *in vivo* absorption, transportation and targeted release of polyphenol incorporated emulsions, which are essential to their deeper and wider applications. Hence, systematic and intensive investigation of *in vivo* metabolic mechanism and physiological effects of encapsulated polyphenols or any other bioactive nutrients are urgently required.

Actually, at present, the applications of free polyphenols or encapsulated compounds are mainly used as functional foods or nutraceutical due to the fact that there are still limited

evidence justifying the use of polyphenols in prevention and treatment of human diseases. However, it can be predicted that, with a better understanding of molecular structure and function mechanisms of polyphenols, emulsion-based delivery systems with high-performance in protected encapsulation, controlled release, and potential site-specific targeted delivery will play an important role in increasing the efficiency of encapsulated polyphenols in biology or even pharmaceuticals. There is no doubt that the progress of encapsulation technology will also contribute to a faster and better development of bioactive phytochemicals, not only as food additives or nutritional supplements, but also as active biological products or even as drugs, all of which will potentially benefit human health.

Acknowledgement

This work is supported by Food Institutional Research Measure (FIRM) Project 11/F/001 titled “Formulation and Design for Food Structure and Stability”

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Figure captions

Fig. 1 Classification of polyphenols

Fig. 2 Preparation of single emulsions with homogenization technology

Fig. 3 Cellular anticancer assay of free EGCG and submicrometer emulsion encapsulated EGCG on HepG2 cells. Human hepatocellular caricinoma (HepG2) cell were cultured in MEM containing 10% fetal bovine serum and antibiotics and were maintained at 37℃ with 5% CO_2. *In vitro* anticancer assay was performed using MTT assay. After treatment with free EGCG or EGCG-encapsulated emulsion with the same concentration for 24 h, cells were treated with MTT and optical absorbance at 560 and 670 nm was recorded. Relative viability of cells was expressed as A_{560}-A_{670}. Data were presented as mean (standard deviation with four repeats (n = 4) (Ru et al, 2010).

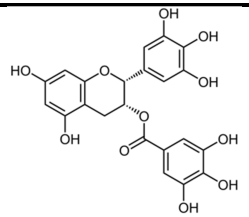
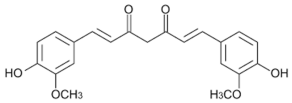
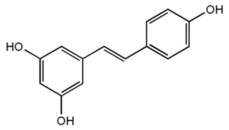
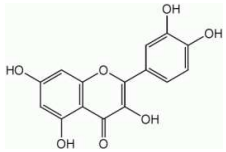
Fig. 4 Solvent removal technology for preparation of bioactive nutrients encapsulated emulsion. (a) All non-water soluble ingredients such as polymers and the bioactive ingredient to be encapsulated are first dissolved in an organic solvent. (b) This solution is mixed with a water phase, which includes an emulsifier, and stirred in to form an emulsion. (c) The organic solvent is removed from the droplets by an extraction process, adding additional amount of water. During this stage, the polymers immigrate to the interface of the capsule and the water, forming a solid wall around the active compound. (d) The microcapsules are then washed and filtered and dried using a lyophilization process.

Fig. 5. Typical physical instability of emulsion. (a) Stable emulsion. (b) Coalescence. (c) Flocculation. (d) Creaming. (e) Breaking.

Fig. 6. Two steps of multiple water-in-oil-in-water ($W_1/O/W_2$) emulsion preparation

Fig. 7. Bioavailability of free curcumin and encapsulated-curcumin (NP). Mice were divided into two groups (6 mice in each group), group one was given free curcumin and group two was given encapsulated-curcumin (NP). Free curcumin and NP were administered intravenously (2.5 mg/kg) and the blood was collected at different time intervals. Serum was separated and the concentration of curcumin was determined by HPLC analysis. (Anand et al, 2010)

Table 1 Molecular structure, physicochemical properties, and health benefits of mainly-reported polyphenols and their limitations in applications

Polyphenol	Molecular structure	Physicochemical properties	Reported health benefits	Limitations in application	References
EGCG		the ester of epigallocatechin and gallic acid, a type of catechin; soluble in water and organic solvents	Antioxidant activity, and UV radiation protection, as well as preventing thrombus, cancer, diabetes, and cardiovascular diseases	Low bioavailability due to its containing many hydrogen bond donors or acceptors; poor stability in gastrointestinal tract (GI); low intestinal permeability and short plasma half life	Nagle, 2006; Kumari, 2011; Lipinski, 2012; Italia, 2008; Onoue, 2011; Siddiqui, 2009 Barras, 2009
Curcumin		A diarylheptanoid; bright yellow-orange powder with melting point of 183 ℃; insoluble in water and soluble in organic solvents and alkaline solutions	Antioxidant and antiinflammation, as well as preventing cancers, major depressed disorder, myelodysplastic syndromes, and Alzheimer's disease	Extremely insoluble in water and low bioavailability; poorly absorbed in gut and metabolism fast in liver; degradation in alkaline pH conditions and under exposure to light	Ahmed, 2012; Tsai, 2011; Donsì, 2011; Souguir, 2013 Aditya, 2015; Anand, 2010
Resveratrol		A stilbenoid; colourless crystal with melting point between 261-263℃; slightly soluble in water and easily soluble in organic solvents	Antioxidant, chemoprevention and cardioprotection; anti-inflammation and anticancer	Poor water solubility; easily oxidized and photosensitive; short biological half-life very limited oral absorption due to rapid and metabolism and elimination	Donsì, 2011; Wenzel, 2005; Shao, 2009; Matos, 2014
Quercetin		Yellow crystalline powder with melting point at 316℃; insoluble in water and soluble in organic solvents and alkaline solutions	Antioxidant, antiinflammation, antitumor, antiviral activities, and as well as antiradical and hepatoprotective activities	Extreme water insolubility; degradation under exposure to light; low permeability and rapid metabolism before reaching systematic circulation	Kumari, 2011; Kumari, 2010; Wu, 2008; Barras, 2009; Scalia, 2009;

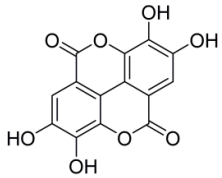
					Chen-yu, 2012
Ellagic acid		Dilactone of hexahydroxydiphenic acid; yellow acicular crystal with melting point>360℃ and slightly soluble in water	antioxidant,antimutagenic,anti cancer,anti-diabetes,anti-inflammatory, and apoptosis inducing and preventing hypertension activity	Poor water solubility, permeability and stability under physiological pH; rapid metabolism in gastrointestinal tract and rapid elimination from the body; first pass effect and irreversible binding to cellular DNA and proteins	Bala, 2006; Sonaje, 2007

Table 2 Homogenization-solvent removal method for encapsulation of polyphenols

Polyphenols	Encapsulation material	Observations	References
Quercetin	Poly-D,L-lactide (PLA) with polyvinyl alcohol (PVA) as emulsifier	PLA formed nanoparticles with higher encapsulation efficiency and *in vitro* initial burst release followed by the sustained release; less fluorescence quenching of encapsulated compound than free ones, suggesting controlled release	Kamuri et al, 2011 & Kamuri et al, 2010
Quercetin	Aminoalkyl methacrylate Copolymers with PVA as emulsifier	Droplet size depended on the weight ratio of EE:PVA; high encapsulation efficiency (over 99%); intermolecular hydrogen binding of quercetin with nanoparticle; higher release rate and antioxidant activity of encapsulated quercetin than free compound	Wu et al, 2008
Ellagic acid	Poly lactic-co-glycolic acid (PLGA) and polycaprolactone (PCL)with didodecyldimethy-lammomium bromide (DMAB) and PVA, alone and in combination with chitosan as emulsifier or stabilizer	Different particle size, encapsulation efficiency and release rate were observed due to utilization of different stabilizer or emulsifier; higher intestinal uptake efficiency of encapsulated ellagic acid than free drugs; prevention of Cyclosporine A-Induced nephrotoxicity at three times lower dose suggesting improved oral bioavailability	Bala et al, 2006 & Sonaje et al, 2007
EGCG	polylactic acid (PLA)–polyethylene glycol (PEG) with PVA as emulsifier	Encapsulated EGCG showed significant improved human prostate cancer inhibition activity both *in vitro* and *in vivo*; over 10-fold advantage in proapoptotic and angiogenesis inhibitory effects; enhanced bioavailability and limited unwanted toxicity of chemopreventive agents	Siddiqui et al, 2009
EGCG	PLGA with DMAB as stabilizer	EGCG was incorporated into PLGA nanoparticles with DMAB as stabilizer; encapsulated EGCG was found to be equally efficacious as intraperitoneal administered in ameliorating Cyclosporine A-Induced renal damage at three times reduced dose	Italia et al, 2008

EGCG	Eudragit S100 as oil phase with PVA as emulsifier	pH-dependent controlled release with limited initial burst release; moderated bioadhesive property in isolated small intestine of rats; significant improvement in chemical and metabolic stability of EGCG was observed in the EGCG/MS, possibly due to the controlled release and/or bioadhesion	Onoue et al, 2011
Resveratrol	mPEG-PCL (methoxy poly(ethylene glycol)-poly(caprolactone) with	Higher glioma cell death induced by resveratrol-loaded nanoparticles at lower concentration compared with free compound; significantly lower intracellular ROS levels in free resveratrol treated cells than encapsulated-resveratrol treated cells.	Shao et al, 2009
Curcumin	PLGA with PVA and sucrose as emulsifier	Kept stable for one month at 4 ℃; significant increase in plasma concentration of curcumin when intravenous (55%) or oral (21-fold) administered encapsulated curcumin to rats; highly improved *in vivo* bioavailability by using encapsulated curcumin	Tsai et al, 2011

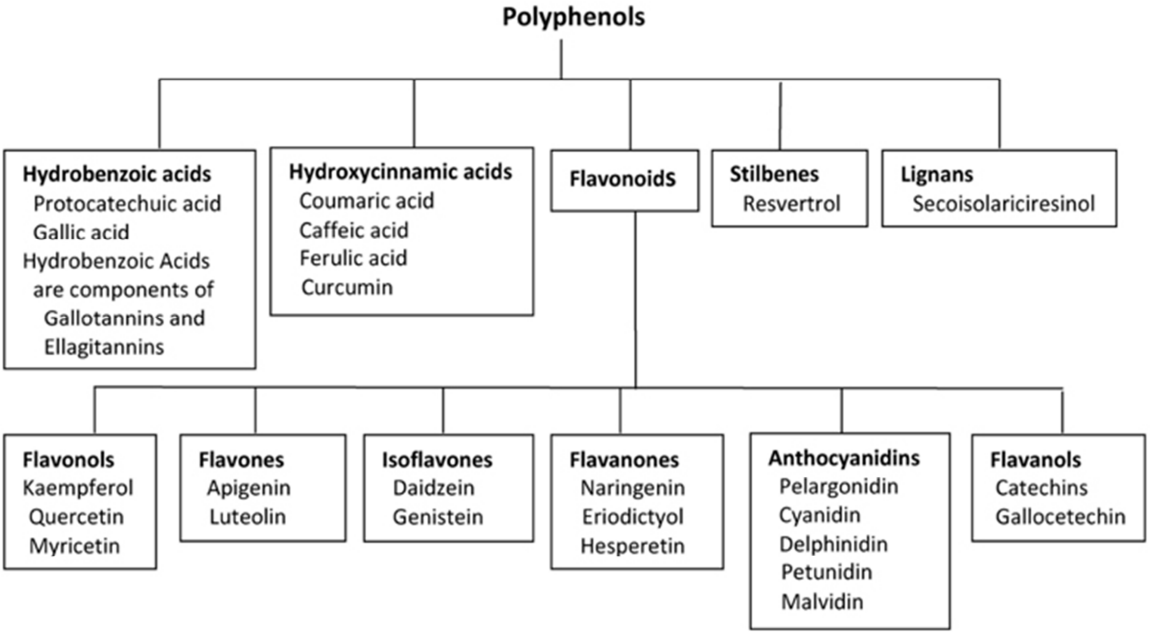


Figure 1

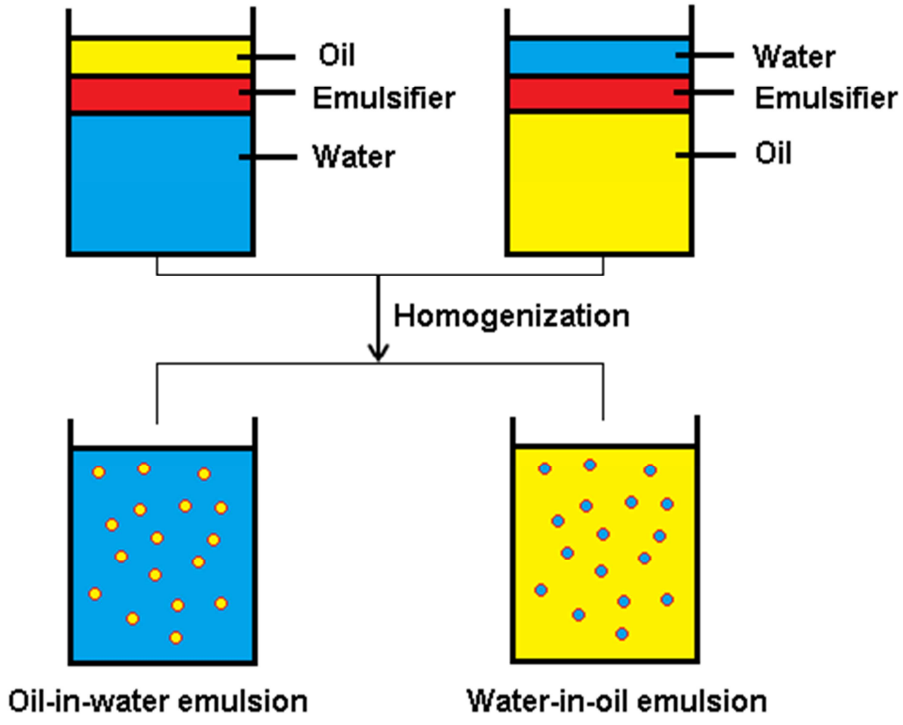


Figure 2

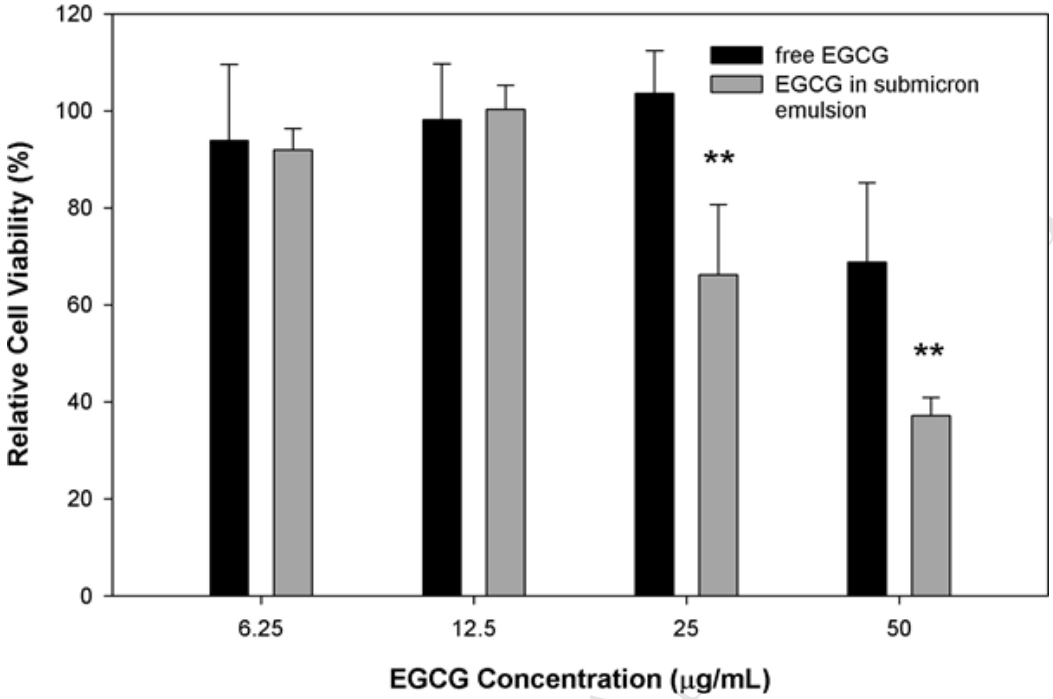
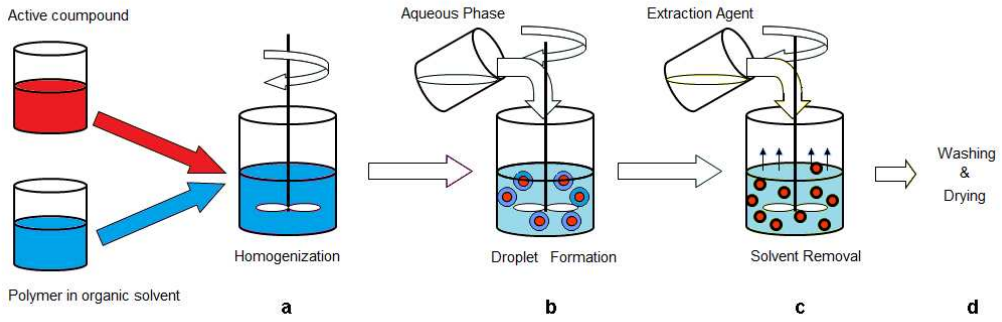


Figure 3



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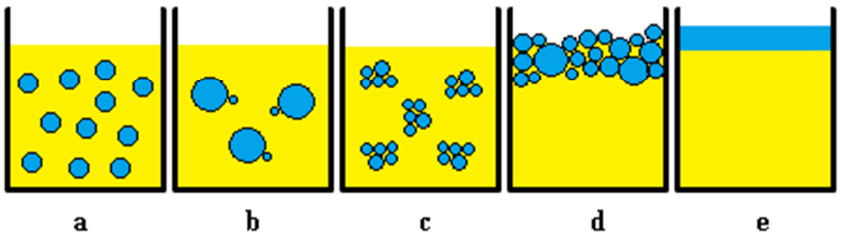

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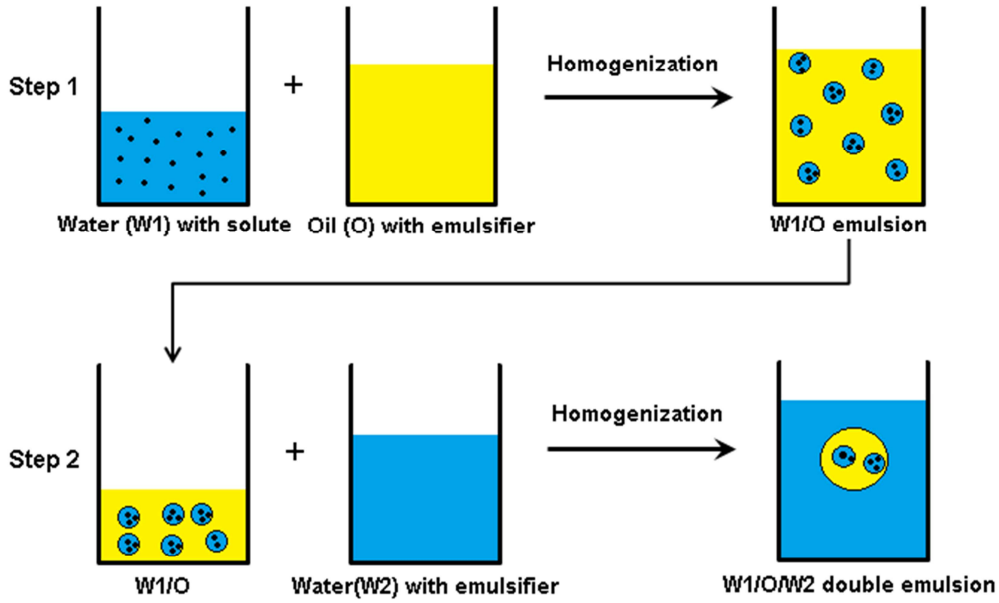

Figure 6

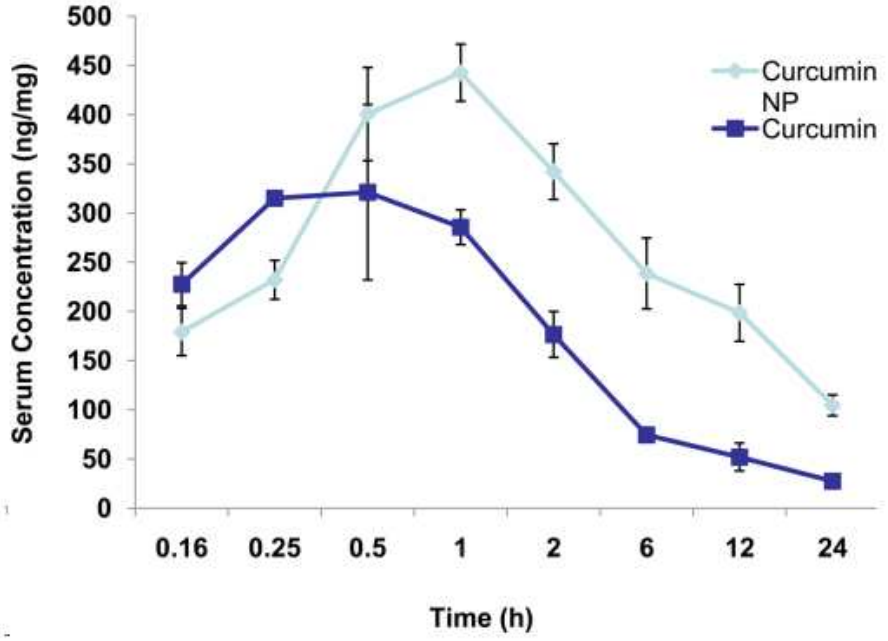

Figure 7

Highlights:

- **Health benefits of polyphenols are dramatically limited by their instability**
- **Emulsions are ideal protection and delivery system for polyphenols**
- **Emulsion delivery system greatly improve stability and bioavailability of polyphenols**
- **Emulsion-entrapped polyphenols showed controlled release and enhanced *in vivo* effects**