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Emulsion-Based Encapsulation and Delivery Systems for Polyphenols

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23 **Abstract**

24 *Background*

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

32 *Scope and Approach*

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

36 *Key Findings and Conclusions*

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

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

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49 **Keywords:** polyphenol, encapsulation, delivery, emulsion

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65 Introduction

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

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

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

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

93 A wide range of technologies have been developed to encapsulate polyphenols, including
94 spray drying, coacervation, emulsions, liposomes, micelle, nanoparticles, freeze-drying,
95 cocrystallization and yeast encapsulation (Fang & Bhandari, 2010; Munin & Edwards-Levy,
96 2011). Each of these has its own specific strengths and weaknesses in encapsulation,
97 protection, delivery, cost, regulatory status, ease of use, biodegradability and biocompatibility.
98 Among these, emulsions are widely considered as one of the most popular encapsulation and
99 delivery systems for a wide range of lipophilic, hydrophilic and amphiphilic bioactive
100 molecules (McClements & Li, 2010), due to their high-efficiency encapsulation, maintenance
101 of chemical stability of encapsulated molecules (Klinkesorn, 2005) and controlled
102 release (Mao, Roos, & Miao, 2013). Furthermore, some emulsion-encapsulated polyphenols
103 presented even higher biological activities compared with pure free molecules (Wang et al,
104 2008).

105 An emulsion consist of two immiscible liquids, usually oil and water, with one of the
106 liquid being dispersed as the small spherical droplets in the other. Emulsions can be classified
107 according to the relative spatial distribution of the oil and aqueous phase (McClements, 2005).
108 A system that consists of oil droplets dispersed in an aqueous phase is called an oil-in-water
109 (O/W) emulsion, e.g., milk and soups; while a system that consists of water droplets
110 dispersed in an oil phase is called a water-in-oil (W/O) emulsion, e.g., butter. In the last two
111 decades, a variety of emulsions with desirable structures and properties have been
112 successfully developed for the protected encapsulation and delivery of many kinds of
113 bioactive nutrients with significant health benefits (McClements, 2010, 2012; Norton,
114 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°C (curcumin) to above
131 360°C (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

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

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

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

152

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

154 Conventional oil-in-water (O/W) emulsions consist of oil droplets dispersed in an aqueous
155 continuous phase, with the oil droplets being surrounded by a thin interfacial layer consisting
156 of emulsifier molecules (**Fig. 2**). The concentration and particle size distribution of the oil
157 droplets in emulsions can be controlled by oil phase proportion and preparation technologies.
158 The oil droplets typically have diameters between 0.1 and 100 μm while the interfacial layer
159 is generally between 1 nm and 10 nm thick for emulsifiers (McClements, Decker, & Weiss,
160 2007), e.g., surfactants, phospholipids, proteins, or polysaccharides (McClements, 2005). The
161 electrical charge on the droplets can be controlled by selecting an appropriately charged

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

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

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

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

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

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

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

211 Sonaje, et al., 2007), EGCG (Italia, Datta, Ankola, & Kumar, 2008; Onoue, Ochi, & Yamada,
212 2011; Siddiqui, et al., 2009), resveratrol (Shao, et al., 2009) and curcumin (Tsai, et al., 2011).
213 Encapsulated polyphenols obtained by this method are always solid nanoparticle powders.
214 Biopolymers, e.g., PLA and PLGA, are mainly used as matrixes to form nanoparticles that
215 entrap the polyphenols (**Table 2**). Encapsulation of polyphenols based on this method can
216 achieve high encapsulation efficiency, and encapsulated polyphenol compounds showed a
217 large increase in their solubility and physical or chemical stability. Furthermore, compared
218 with free compounds, functional evaluations in these studies confirmed strengthened
219 biological effects, such as antioxidant and anti-cancer activity, of encapsulated polyphenol
220 compounds *in vitro* and *in vivo*. Moreover, a significant improvement in *in vivo* intestinal
221 absorption efficiency of polyphenols was also observed by using emulsion encapsulation
222 technology based on emulsification-solvent removal methods (**Table 2**).

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

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

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

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

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

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

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

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

285 design emulsions with different rheological properties, which can meet some specific
286 applications in delivery of lipophilic polyphenols (Genovese, Lozano, & Rao, 2007).
287 Furthermore, O/W emulsions can either be used in wet state (Chen-yu, et al., 2012; Ru, et al.,
288 2010) or be dried to solid powders (Kumari, et al., 2011; Kumari, et al., 2010), which greatly
289 facilitates their processing, transportation, storage and thus the application in encapsulation
290 and delivery of polyphenols.

291 In spite of O/W emulsions have been widely employed as delivery system for a variety of
292 bioactive nutrients and show huge potentials as delivery system, there are still some
293 disadvantages. For example, O/W emulsions are usually sensitive to environmental stress,
294 such as heating, chilling, extreme pH and salt concentrations, all of which can lead to their
295 physical and chemical instability, e.g., creaming, flocculation, coalescence, breaking and
296 Ostwald ripening for common physical instability (**Fig. 5**) (Becher, 1996; Dickinson, 2010),
297 and oxidation and hydrolysis for their common chemical instability (McClements & Decker,
298 2000). All these instabilities can potentially cause damage or even break-down of emulsions,
299 and accordingly will decrease physical and chemical stability of encapsulated polyphenols
300 and thus their final beneficial effects. In addition, it is challenging to precisely control the
301 release of encapsulated polyphenols in O/W emulsions, because the simple oil-water interface
302 structure in O/W emulsion result in a very short time for diffusion of encapsulated
303 polyphenols from inside to the outside of the oil droplets (McClements, et al., 2007).
304 Therefore, emulsions with more sophisticated structures are required for some particular
305 applications.

306

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

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

310 2005) (**Fig. 6**). W/O/W emulsions can also be more clearly defined as $W_1/O/W_2$ emulsions,
311 where W_1 is the inner water phase while W_2 is the outer water phase. In principle, it is
312 possible to design properties of inner water phase and oil phase, e.g., droplets size and
313 distribution, surface charge, and interfaces between water and oil, such as surface charge, and
314 environmental response behaviours.

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

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

334 Compared with O/W emulsions, W/O/W emulsions are ideal protected encapsulation
335 systems for hydrophilic polyphenols. These compounds can be trapped in the internal water
336 phase, which is isolated from the outer water phase by the oil phase, preventing their
337 diffusion across the water-oil interface into the outer water phase (Benichou, Aserin, & Garti,
338 2004; McClements, 2015). Furthermore, release of polyphenols entrapped within the inner
339 water phase will be prolonged and can be controlled (Garti & Bisperink, 1998). Moreover,
340 W/O/W emulsions can be also designed to encapsulate both lipophilic and hydrophilic
341 bioactive polyphenols at the same time (Cournarie, et al., 2004), which will achieve multiple
342 targeted delivery of multiple bioactive compounds in one particular system. Another potential
343 advantage of W/O/W emulsions is that they can be structured to have the same dispersed
344 phase volume and droplets size as conventional O/W emulsions, but with lower fat content,
345 which facilitate the development of functional food products with encapsulated polyphenols ,
346 which have lower-fat content but the same properties as the full-fat products (McClements, et
347 al., 2007).

348 However, W/O/W emulsions, like conventional O/W emulsions, are also highly
349 susceptible to environmental stresses (thermal processing, freeze and dehydration), which can
350 induce instability, such as conventional flocculation, coalescence, and Ostwald ripening (**Fig.**
351 **5**), which potentially will influence the delivery of encapsulated polyphenols. Furthermore,
352 the diffusion of encapsulated hydrophilic polyphenols or water molecules from the inner to
353 the outer aqueous phase or expulsion of whole water droplets from oil droplets, induced by
354 limited solubility of encapsulated compounds in oil phase, can also lead to the instability of
355 W/O/W emulsions (Garti, 1997; McClements, 2015). Moreover, polyphenols encapsulated in
356 the inner water phase of W/O/W emulsions can gradually diffuse into the oil phase or even
357 outer water phase, due to their amphiphilic properties, which potentially will change their
358 release pattern and thus influence expected controlled-release and targeted-delivery.

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

363

364 **Nanoemulsions: advanced delivery system for polyphenols**

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

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

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

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

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

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

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

413

414 **Summary and future trends**

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

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

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

442

443

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447

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694 **Figure captions**

695

696 **Fig. 1** Classification of polyphenols

697

698 **Fig. 2** Preparation of single emulsions with homogenization technology

699

700 **Fig. 3** Cellular anticancer assay of free EGCG and submicrometer emulsion encapsulated EGCG on
701 HepG2 cells. Human hepatocellular carcinoma (HepG2) cell were cultured in MEM containing 10%
702 fetal bovine serum and antibiotics and were maintained at 37°C with 5% CO₂. *In vitro* anticancer
703 assay was performed using MTT assay. After treatment with free EGCG or EGCG-encapsulated
704 emulsion with the same concentration for 24 h, cells were treated with MTT and optical absorbance at
705 560 and 670 nm was recorded. Relative viability of cells was expressed as A_{560}/A_{670} . Data were
706 presented as mean (standard deviation with four repeats (n = 4) (Ru et al, 2010).

707

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

716

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

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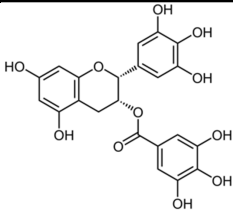
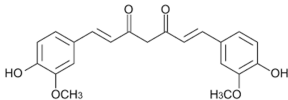
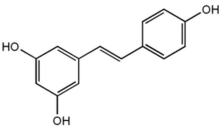
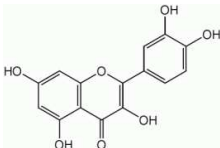
720 **Fig. 6.** Two steps of multiple water-in-oil-in-water (W₁/O/W₂) emulsion preparation

721

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

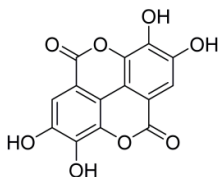
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Table 1 Molecular structure, physicochemical properties, and health benefits of mainly-reported polyphenols and their limitations in applications

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 °C; 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; Donsi, 2011; Souguir, 2013 Aditya, 2015; Anand, 2010
Resveratrol		A stilbenoid; colourless crystal with melting point between 261-263°C; 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	Donsi, 2011; Wenzel, 2005; Shao, 2009; Matos, 2014
Quercetin		Yellow crystalline powder with melting point at 316°C; 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 $>360^{\circ}\text{C}$ 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 <i>in vitro</i> 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 didodecyldimethylammonium 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 <i>in vitro</i> and <i>in vivo</i> ; 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 °C ; significant increase in plasma concentration of curcumin when intravenous (55%) or oral (21-fold) administered encapsulated curcumin to rats; highly improved <i>in vivo</i> bioavailability by using encapsulated curcumin	Tsai et al, 2011

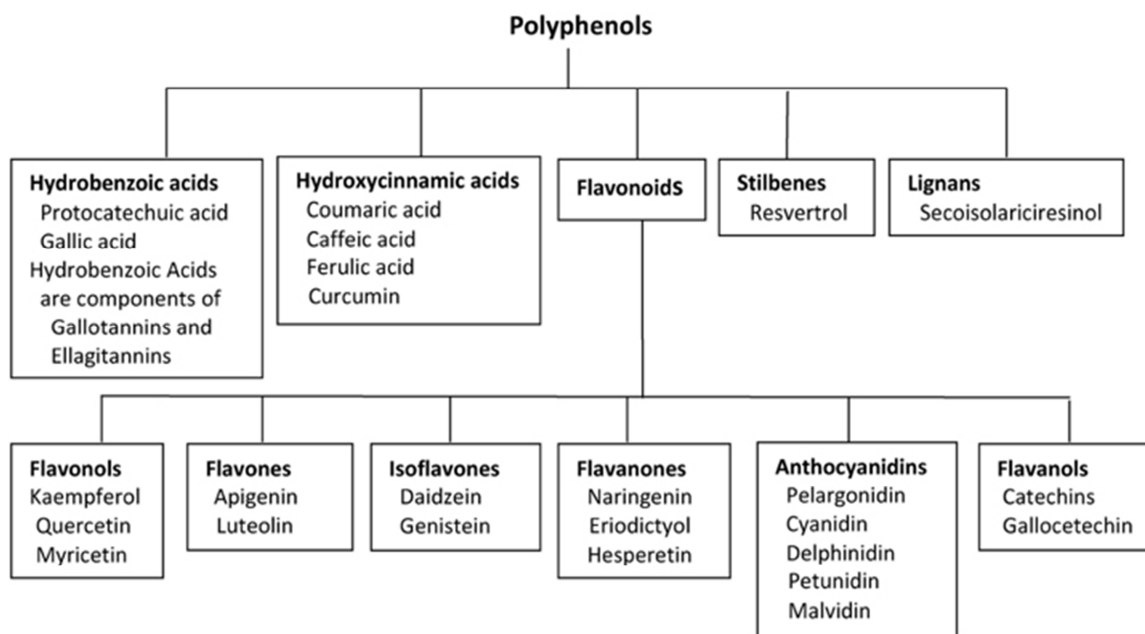


Figure 1

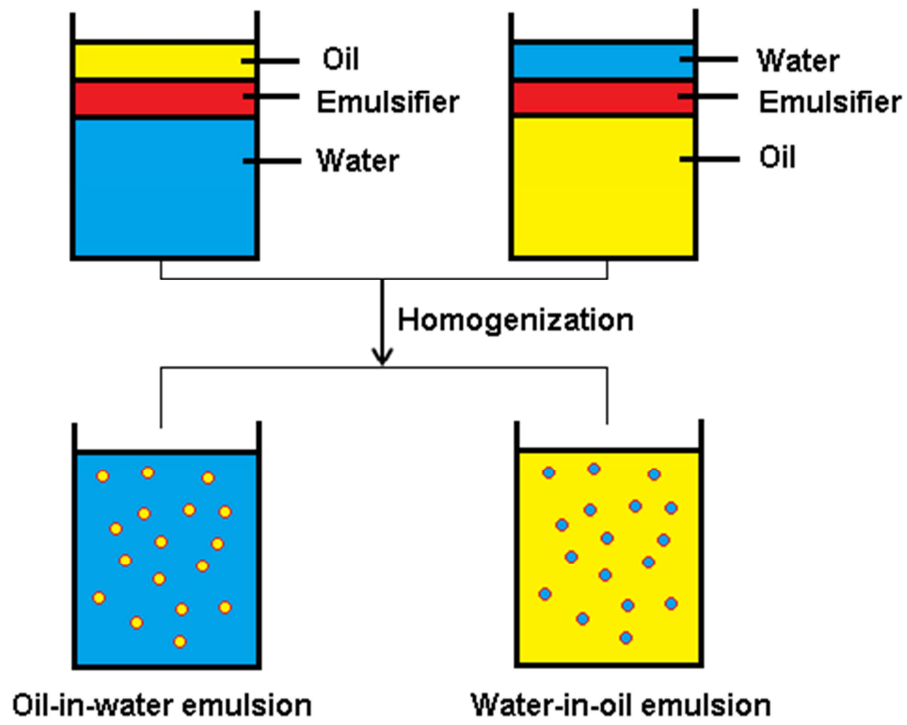


Figure 2

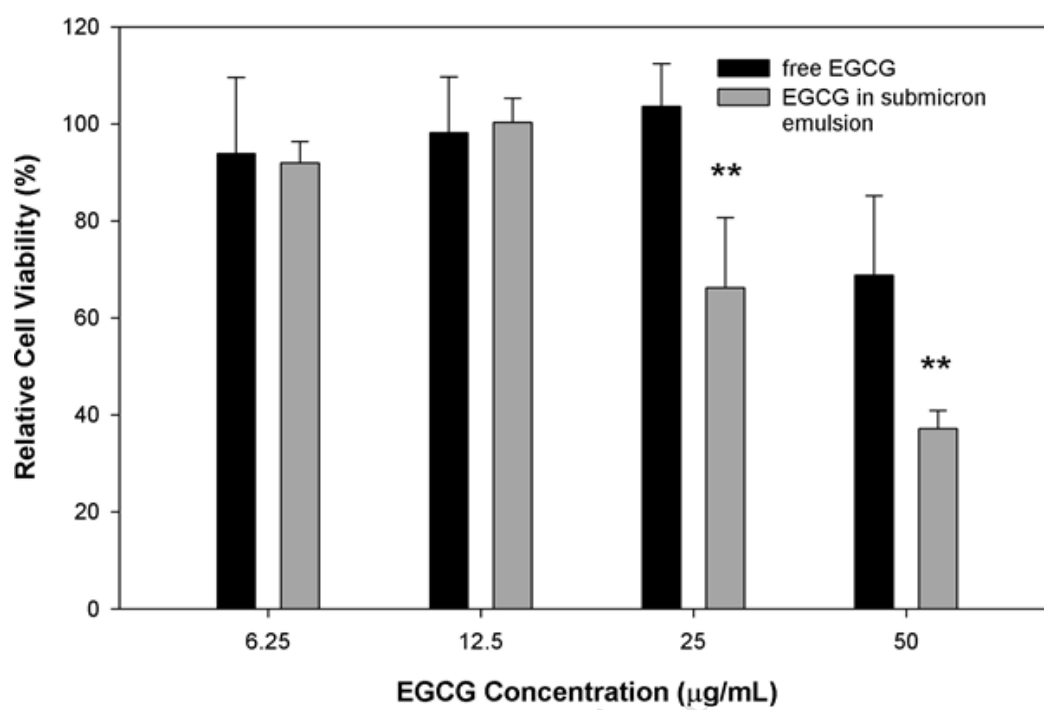


Figure 3

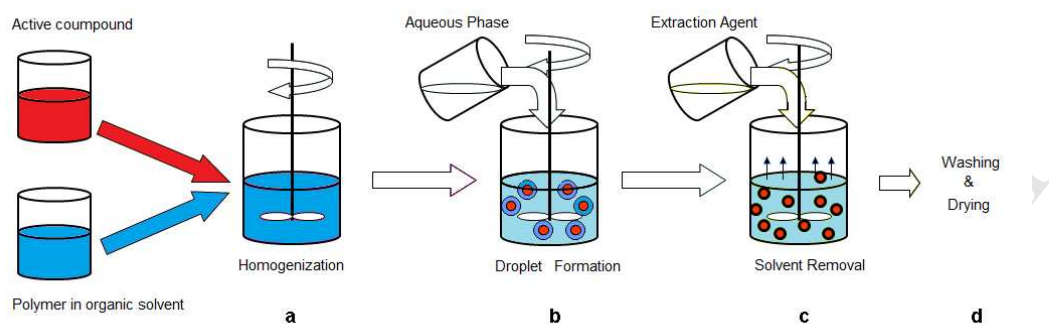


Figure 4

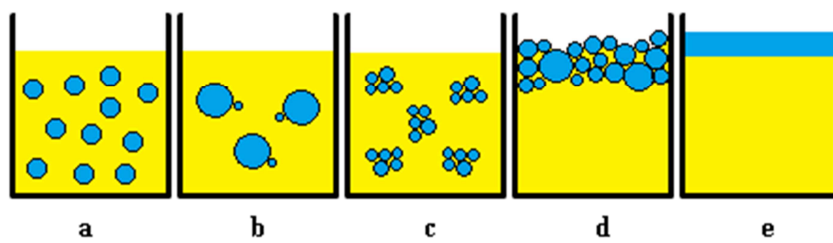


Figure 5

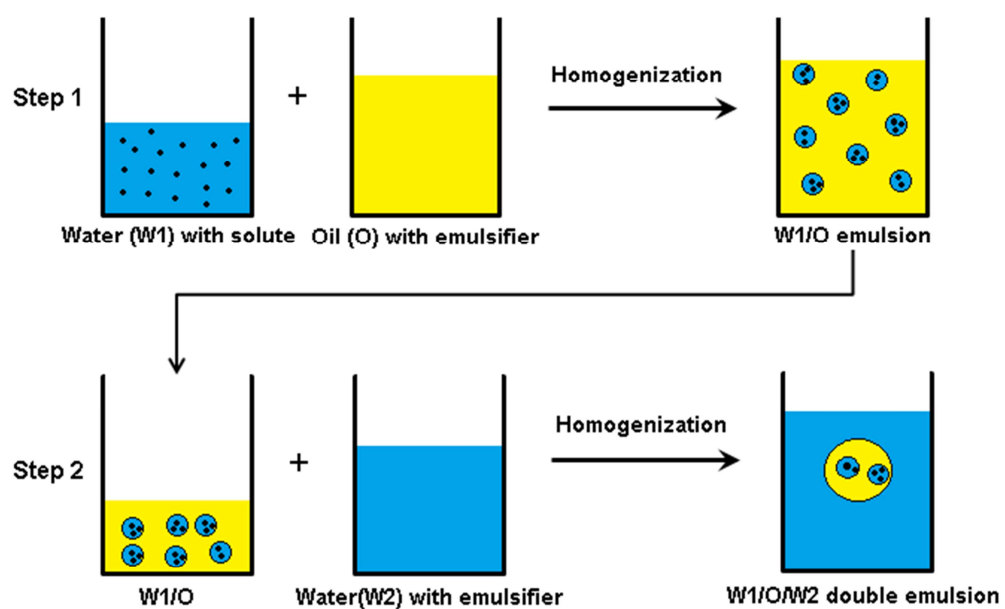


Figure 6

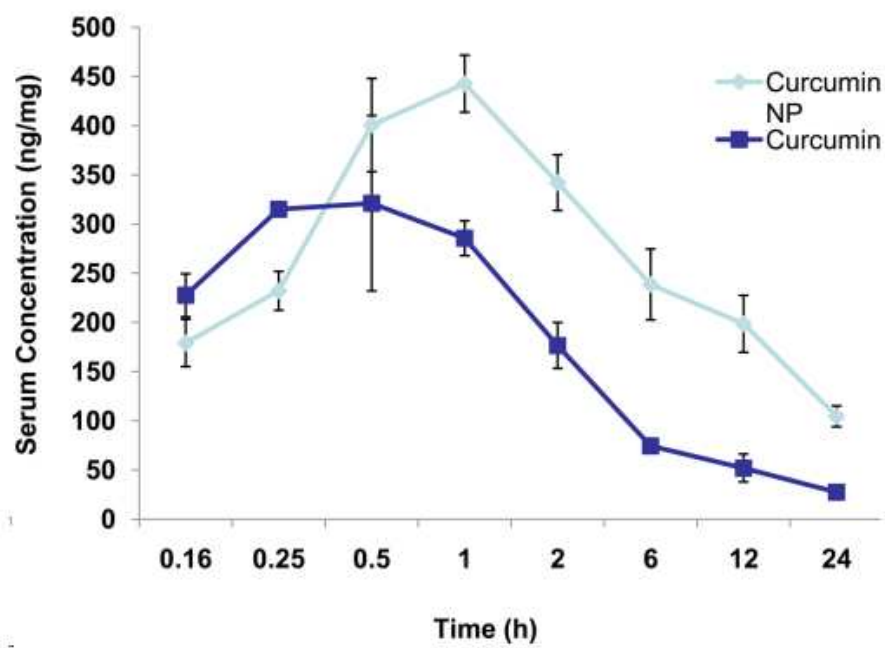


Figure 7

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**