1   # Microencapsulation of vitamin A: a review

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10   ## Abstract

11   **Background**

12   Vitamin A deficiency is recognized as a public health problem in a large number of

13   countries. It mainly affects young children and pregnant women in low-income

14   countries in Africa and South-East Asia regions (World Health Organization data).

15   Vitamin A is a fat-soluble vitamin and an essential nutrient provided to the human body

16   in form of carotenoids (provitamin A) and retinol or retinyl esters (preformed vitamin

17   A). The inadequate intake of this micronutrient through the diet may compromise a

18   large spectrum of biological functions, namely vision, growth and development,

19   immunological activity, reproduction and cellular growth and differentiation. The

20   preparation of functional food and enteral formulas arises as a solution to provide to the

21   individuals the partial or complete vitamin A nutritional requirements.

22

23   **Scope and Approach**

24   Due to the properties of vitamin A and other retinoids these compounds have been used

25   for several pharmaceutical and cosmetic formulations. However, the poor solubility in

26 water and chemical instability of vitamin A can lead to its degradation during

27 processing and storage. Microencapsulation may promote the stabilization of vitamin A

28 in certain conditions and may improve a controlled release.

29

30 **Key Findings and Conclusions**

31 The present work starts with a reference to several topics of vitamins A. General aspects

32 about microencapsulation are presented, as well as the reasons to apply this technology

33 to vitamin A. The main encapsulating methods (the principles and main considerations)

34 and encapsulating agents applied to this micronutrient are also discussed. The final

35 section focuses on vitamin A release studies and its kinetics.

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39 **Keywords: vitamin A, retinoids, food industry,**

40 **microencapsulation, controlled release.**

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1. Introduction

The importance of vitamin A for vision health dates back to ancient Egypt as early as 1500 BC. At that time and according to the papyrus Ebers, patients who suffered from vision reduction in semi darkness conditions (nyctalopia or night blindness disease) were cured by topical application of liver juice or ox liver extract (previously cooked) in the eye (Ebell, 1937; Wolf, 1978, 1996). About this procedure, Wolf (1978) suggested that the droplets of liver oil, which is rich in vitamin A (retinol), entered the lachrymal duct where they were absorbed into the blood circulation and finally reached the retina. Currently the role of vitamin A in the visual process is well known (J. Dowling & Wald, 1958; Wald, 1955, 1968) and it is directly related to the rod cells present in retina of the eye. These cells are light-receptors that are responsible to enable us to distinguish between light and dark and contain the visual pigment rhodopsin. Rhodopsin is composed by 11-cis-retinal, an isomer of retinal (an aldehyde obtained by oxidation of retinol), and by opsin, the light-sensitive receptor protein. The exposure of rod cells to the light leads to rhodopsin destruction by bleaching, occurring the conversion of light into an electrical signal that is sent to the brain, resulting in the vision. According to this process it is important to ensure the continuous replacement of vitamin A constituent of rhodopsin to prevent vision impairment. In fact, rhodopsin works under low-light promoting dark adaptation.

Night blindness can be the first sign of xerophthalmia, warning to a severe vitamin A deficiency (Sommer, 1998, 2001). The following stages of xerophthalmia include conjunctival xerosis (on the conjunctival surface, mainly adjacent to the temporal side of the cornea, dry patches of keratinized epithelium appear) (Sommer, 2001), Bitot's spots (appearance of foamy or cheesy white accumulations of keratinized squamous epithelium and observation of an overgrowth of gram negative rods) (Sommer, Green,

68   & Kenyon, 1981), corneal xerosis (the cornea loses its normal sheen and clarity due to

69   corneal epithelium keratinized) (Sommer, 1998, 2001), cornea ulceration

70   (keratomalacia) (Sommer & West, 1996), cornea necrosis, and blindness (Sommer &

71   West, 1996; Sommer, 2001). World Health Organization (2009) reports the results of

72   collaboration work between Micronutrient Initiative and UNICEF, and Tulane

73   University, which allowed them to estimate that in year 2000 about 7 million preschool-

74   age children had night blindness and Bitot's spots. Additionally, the West (2002) work

75   estimated for the same year that 19.8 million pregnant women had low vitamin A levels

76   (serum retinol or breast milk concentrations < 1.05 µmol·L^{-1}) and, from those, about 6.2

77   million suffered of gestational night blindness. The last referred estimation also enabled

78   to understand that approximately two-thirds of the world's night blindness women lived

79   in South and South-East Asia.

80   The importance of vitamin A goes beyond the vision health. This micronutrient, more

81   precisely retinoic acid (a carboxylic acid which results of further irreversible oxidation

82   of retinal) (J. E. Dowling & Wald, 1960) plays an important key role in reproduction,

83   embryonic development, cellular growth and differentiation, maintenance of epithelial

84   cellular integrity and immune function. As consequence, an insufficient ingestion of

85   vitamin A can lead to spermatogenesis commitment/anomalies' (Mason, 1933; Wolbach

86   & Howe, 1925) and reproduction failure before implantation (Evans, 1928), fetal

87   development commitment (malformation of tissues and organs) (Dickman, Thaller, &

88   Smith, 1997; Hale, 1933; Kaiser, Merrill, Stein, Breburda, & Clagett-Dame, 2003;

89   Warkany & Roth, 1948; Warkany & Schraffenberger, 1946; White, Highland, &

90   Clagett-Dame, 2000; White, Highland, Kaiser, & Clagett-Dame, 2000; White et al.,

91   1998; Wilson & Warkany, 1948), disturbed cellular differentiation (Sommer, 2001,

92   2008), slowed growth and development (Bloch, 1931), impaired immunological

93 function (Ross, 2012), anemia (Sommer & Davidson, 2002), infections (*i.e.* measles)

94 (Ellison, 1932; Green & Mellanby, 1928; Green, Pindar, Davis, & Mellanby, 1931), and

95 morbidity and mortality (Sommer, 2001).

96 Vitamin A deficiency is recognized as a public health problem in more than half of

97 world countries and mainly affects individuals from poor societies and developing

98 countries (WHO, 2009). The application of the adequate treatment can reduce the risk

99 of development of complications related to vitamin A deficiency. These may include

100 skin disorders (psoriasis and acne) (Sauvant, Cansell, Sassi, & Atgié, 2012), psychiatric

101 pathologies (schizophrenia and Alzheimer's disease) (C. R. Olson & Mello, 2010) and

102 certain cancers (C. R. Olson & Mello, 2010), among others (Sommer, 2001).

103 Prevention of vitamin A deficiency has been carried out by food fortification (functional

104 food) (Dary & Mora, 2002) and enteral formulas prepared to provide complete or

105 supplemental nutritional support to individuals (Fávaro, Iha, Mazzi, Fávaro, & Bianchi,

106 2011). In developed countries the overconsumption of these products is often associated

107 to the toxicity of vitamin A (Dary & Mora, 2002). Therefore, the current market of

108 vitamin A covers the food and pharmaceutical industries. Moreover, a review about the

109 effect of this micronutrient on anti-aging treatment (Siddharth Mukherjee et al., 2006)

110 also shows the application of vitamin A in the cosmetic industry. However, vitamin A is

111 poorly water soluble and highly unstable in the presence of oxidants, light, heat,

112 temperature and moisture, among others (Gonnet, Lethuaut, & Boury, 2010; Teleki,

113 Hitzfeld, & Eggersdorfer, 2013). Microencapsulation has been explored in order to

114 overcome these limitations. In addition it is also an effective technique of controlled

115 release of vitamin A (Donhowe, Flores, Kerr, Wicker, & Kong, 2014).

116   This review highlights an overall discussion about structure and historical perspective of

117   sources, metabolism, microencapsulation (its importance, techniques and encapsulating

118   agents) and release studies of vitamin A, and its kinetics.

2. Structure and historical perspective of vitamin A

Vitamin A is a term used to designate retinol and its natural derivatives with the same biological activity, namely retinal and retinoic acid (Blomhoff & Blomhoff, 2006). Retinyl esters, the storage form of retinol, and carotenoids are also considered vitamin A (Chapman, 2012; Siddharth Mukherjee et al., 2006). Retinol (or all-trans-retinol) is a molecule with a cyclohexenyl ring linked to a side chain with four double bonds (all in trans configuration) and with an alcohol end group (Siddharth Mukherjee et al., 2006). The oxidation of alcohol end group results in the formation of retinal or all-trans retinaldehyde, which can be further oxidized to all-trans retinoic acid (Siddharth Mukherjee et al., 2006) (Figure 1).

Experiments of McCollum and Davis (1913) enabled the first description of vitamin A. They reported that rats fed for several months with purified rations composed of pure casein, carbohydrates (in some rations a part of the carbohydrates was replaced by lard) and salt mixtures could restore their growth when diet was supplemented by ether extract of egg or of butter. This essential compound that naturally occurs in this type of food was named "fat soluble A" (later called vitamin A (Drummond & Coward, 1920)), as opposed to other accessory dietary factors called "water soluble B" (McCollum & Davis, 1915). At the same time, similar experiments were performed by Osborne and Mendel (1913) who observed the rats growth when their diet was supplemented with evaporated whole-milk powder. Hence, Osborne and Mendel realized that milk contained something other than protein that was necessary for the growth of animals. Steenbock (1919) observed that "fat soluble A" obtained from butter, egg yolk and carrots presented the yellow color, probably due to the association of a yellow pigment (known today as β-carotene) (Steenbock & Gross, 1920). Furthermore, Steenbock speculated about the possibility of converting this factor into a non-colored compound

144 with biological activity (retinol) (Steenbock & Gross, 1920). This theory was later

145 proved by Moore (1930), when he fed rats with carotene to promote their growth and it

146 was able to observe the accumulation of that non-colored compound in the liver of these

147 animals. Hence, the carotene was suggested as the precursor or pro-vitamin of retinol.

148 The isolation and chemical structure of β-carotene and retinol was achieved in 1930 and

149 published one year later by Karrer *et al.* (1931; 1966). The first synthesis of retinol was

150 performed by Isler *et al.* (1947) and the first synthesis of β-carotene was reported by

151 Milas *et al.* (1950).

152 The term retinoid was first defined by Sporn *et al.* (1976) and from there several

153 definitions were presented until the currently accepted (Dixon, 1983; M. Sporn &

154 Roberts, 1985). The retinoid family includes vitamin A and synthetic derivates

155 (Siddharth Mukherjee et al., 2006). Retinoids are classified and grouped in four

156 generations: non-aromatics, mono-aromatics, poly-aromatics and pyranones,

157 respectively. Several synthetic retinoids have been developed with investigation about

158 their biological activities (Table 1). The activity of retinoids in the cellular processes is

159 performed by their interaction with specific receptors. In case of Retinoid X Receptors,

160 the new synthetic retinoids act as selective antagonists (Griffiths, 1998; Sauvant et al.,

161 2012).

162

163 ## 3. Sources and metabolism of vitamin A

164 The inability of human body to produce vitamin A forces us to have a balanced diet in

165 order to intake the recommend supply of this nutrient. Children aged between 1 and 8

166 years should receive 400-600 µg/d and with more than 8 years should receive about

167 600-800 µg/d of vitamin A. On the other hand, adult men and adult women should

168 intakes 900 μg/d and 800 μg/d, respectively. However, during pregnancy women should

169 only consume 700 μg/d due to potential teratogenic effects (Chapman, 2012).

170 Vitamin A can be provided in form of carotenoids (provitamin A) and retinol or retinyl

171 esters (preformed vitamin A).

172 Carotenoids are organic hydrocarbon-based pigments with yellow, orange, red or purple

173 colors and can be produced by plants, algae and some bacteria (Chapman, 2012; Fraser

174 & Bramley, 2004). β-carotene is probably the most known carotenoid and can be

175 obtained by carrots, sweet potatoes, pumpkin and green leafy plants, among others

176 (Fraser & Bramley, 2004). Some carotenoids (α-carotene, β-carotene, and

177 β-cryptoxanthin) can be directly absorbed by small intestine (Moore, 1930) and be

178 converted into retinal via central cleavage mechanism at the 15, 15' carbon double bond

179 performed by β, β-carotene-15, 15'-monooxygenase (Blomhoff & Blomhoff, 2006;

180 Goodman & Huang, 1965; J. A. Olson & Hayaishi, 1965). The formed retinal is

181 afterward reduced to retinol.

182 Retinyl esters present in food of animal origin result from conversion of carotenoids

183 into retinol, later stored in esterified forms in liver and adipose tissues (Sauvant et al.,

184 2012). Retinyl esters are first hydrolyzed into retinol and then enter the small intestinal

185 lumen. Afterwards, retinol enters the enterocytes and bound to a specific binding

186 protein called cellular retinol-binding protein type II (CRBP II) which will promote re-

187 esterification of retinol by the enzyme lecithin retinol acyl transferase (LRAT) (Herr &

188 Ong, 1992). The roles of CRBP-II are: solubilize retinol, protect this compound against

189 degradation and direct retinol to the enzyme LRAT (Blomhoff & Blomhoff, 2006). The

190 higher quantity of retinyl esters formed is then incorporated into chylomicrons

191 (Blomhoff, Helgerud, Rasmussen, Berg, & Norum, 1982), which are aggregates of

192 triacylglycerol and phospholipids molecules packed with carotenoids, retinyl esters,

193   retinol (small quantity), cholesteryl esters and a few specific apolipoproteins (Blomhoff

194   & Blomhoff, 2006). Chylomicron are then secreted from enterocytes to the intestinal

195   lymphatic circulation (Blomhoff, Green, Berg, & Norum, 1990), representing the

196   highest amount of total retinol from enterocytes (Sauvant et al., 2012). However, a

197   significant amount of retinol is secreted into portal circulation as unesterified retinol

198   (Harrison, 2005). Afterwards, chylomicron follows to the general circulation, where

199   triacylglycerol hydrolysis and apolipoprotein exchange enable the formation of

200   chylomicron remnants (Blomhoff et al., 1990). Chylomicrons remnants are taken by the

201   liver and transferred to hepatocytes and then to hepatic stellate cells, where the released

202   retinol is stored under esterified form in characteristic lipid droplets essential for normal

203   liver physiology (Sauvant et al., 2012).

204   In blood, retinol circulates bound to retinol binding protein (RBP) and transthyretin

205   (TTR), occurring recognition of RBP by the membrane transporter STRA6 from target

206   tissues and the retinol is internalized (Bouillet et al., 1997; Kawaguchi et al., 2007;

207   Wolf, 2007).

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4. Microencapsulation of Vitamin A

4.1. General aspects about microencapsulation

Microencapsulation is a technology wherein small solid, liquid or gas particles are coated with or entrapped within a continuous film of polymeric material (Aguilera & Lillford, 2008; Bansode, Banarjee, Gaikwad, Jadhav, & Thorat, 2010). The coated material is called core material, actives, fill, internal phase or payload and can be encapsulated pure or in combination with other materials (Gibbs, Kermasha, Alli, & Mulligan, 1999). In turn, the coating material is called encapsulating agent, wall material, shell or carrier (Gibbs et al., 1999) and several times arises as a mixture of materials with different physical and chemical properties to overcome limitations that can happen when using only one material (Aghbashlo, Mobli, Madadlou, & Rafiee, 2012; Cano-Higuita, Vélez, & Telis, 2015; Rodea-González et al., 2012; Tontul & Topuz, 2013; Ying, Sun, Sanguansri, Weerakkody, & Augustin, 2012).

The final products of microencapsulation procedure are small particles (between few micrometers and few millimeters) which provide an effective protection of core material regarding the surrounding environment. The typical morphology of these microparticles may vary between simple or irregular shape, with one or more encapsulating agents (and, in the second case, with or without aggregates), mono or multi-core, and matrix (Figure 2).

Microencapsulation was first applied in the industry in 1950s, when National Cash Register Company developed "carbonless carbon paper" using the coacervation technique. Several years of research were performed to achieve this goal, which started in the late of 1930s (Aguilera & Lillford, 2008). Currently, the usage of microencapsulation has been extended to pharmaceutical (Shah, Bashir, Tariq, & Hafiz, 2015; Tu, Dehghani, & Foster, 2002), cosmetic (Patravale & Mandawgade, 2008),

235   textile (Nelson, 2002; Rodrigues et al., 2009; Sánchez, Sánchez-Fernandez, Romero,

236   Rodríguez, & Sánchez-Silva, 2010), agricultural (Tsuji, 2001) and food (Champagne &

237   Fustier, 2007; B. M. A. N. Estevinho, Rocha, Santos, & Alves, 2013; Berta N

238   Estevinho, Carlan, Blaga, & Rocha, 2016; Berta N Estevinho, Ramos, & Rocha, 2015)

239   sectors. In food industry, microencapsulation is used to: [1] decrease the transfer rate of

240   core material to the surrounding material (*e.g.,* loss of flavors is very common during

241   the processing or storage of foods, since they are very sensitive compounds with

242   volatile properties (Berta Nogueiro Estevinho, Rocha, Santos, & Alves, 2013;

243   Pothakamury & Barbosa-Cánovas, 1995)), [2] reduce the reactivity and incompatibility

244   of compounds with the outside, enhancing their stability in conditions of heat, light,

245   moisture, radiation, oxygen, among others, [3] decrease the loss of nutritional value, [4]

246   mask the undesirable taste of some compounds, [5] promote an easier handling of

247   materials by changing their original shape and volume, [6] dilute the core material in

248   order to decrease the quantity of compound when desirable, and [7] control the release

249   of core material to the outside (Bansode et al., 2010; de Azeredo, 2005; Desai & Park,

250   2005; M. N. Singh, Hemant, Ram, & Shivakumar, 2010).

251

252   *4.2.* **Interest of vitamin A microencapsulation**

253   Currently the number of publications related to "microencapsulation" reaches 8857 and,

254   among these, 137 are related simultaneously to "microencapsulation" and "vitamin A"

255   (source: www.scopus.pt, 5th of October of 2015). Microencapsulation of vitamin A has

256   been proposed as a solution for its chemical instability. Vitamin A is a hydrophobic

257   compound and, therefore, may easily become inactive or rapidly degrade in the presence

258   of aqueous systems (Semenova, Cooper, Wilson, & Converse, 2002). Moreover, due to

259   their low polarity, vitamin A is poorly soluble in aqueous solvents (Semenova et al.,

260   2002). At last, vitamin A is a very sensitive compound reacting with oxidants, light,

261   heat, temperature, trace metals and moisture, among others (Gonnet et al., 2010; Teleki

262   et al., 2013). The increase of stability and dispersibility of vitamin A can be achieved by

263   incorporation of vitamin A into carriers with advantageous physical and chemical

264   properties, using suitable encapsulation methods. This strategy may also benefit

265   vitamin A in controlled release experiments promoting the release of this compound at

266   the target site, and optimizing the absorption to prevent its ineffective use (Sauvant et

267   al., 2012). The final purpose is to ensure its higher bioavailability in the human body.

268

269   *4.3.* **The encapsulation methods applied to vitamin A: the principles, main**

270   **considerations and the encapsulating agents used**

271   The development of microparticles with a given size, structure and shape is dependent

272   of the core material(s), encapsulating agent(s) and the microencapsulation methods used

273   (Berta Nogueiro Estevinho et al., 2013; Fang & Bhandari, 2010). The selection of an

274   appropriate encapsulating agent is mandatory to obtain the desirable encapsulation

275   efficiency, microparticle stability and the required characteristics for the final product

276   according to its applicability. Hence, encapsulating agent must attend to specific

277   physical and chemical properties, interaction with the core material (encapsulating agent

278   must not react with core material), protection of the core material against outside

279   surrounding environment, toxicity, and costs, among others (de Azeredo, 2005;

280   Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). Regarding the

281   encapsulating methods, numerous techniques are currently implemented and selected

282   according to the physical and chemical properties of core material and encapsulating

283   agent, the size and shape of microparticle, the required profile of controlled release,

284   scale up of process, and costs (de Vos, Faas, Spasojevic, & Sikkema, 2010; Ghosh,

285   2006).

286   The methods used for microencapsulation of vitamin A include spray-drying and spray-

287   cooling, coacervation (phase separation), emulsion system, liposomes, cochleates, solid

288   lipid nanoparticles and inclusion complexation. These are discussed and reviewed in the

289   following topics, as well as the respective encapsulating agents used. In fact, the

290   selection of encapsulation agents and microencapsulating techniques is an

291   interdependent choice (Desai & Park, 2005).

292

293   *Spray-drying*

294   Microencapsulation by spray-drying was developed in 1930s, being referred as one of

295   the oldest encapsulating methods (Shahidi & Han, 1993).

296   In the spray-drying technique (Figure 3), the compound to be encapsulated is

297   solubilized, dispersed or emulsified with the encapsulating agent in a solution,

298   suspension or emulsion, respectively. The homogenized system obtained is fed to the

299   spray-dryer and atomized by a hot gas (usually air), occurring the formation of the

300   droplet/air contact. After water evaporation, the dry power produced is separated by a

301   cyclone and can be further recovered (Berta Nogueiro Estevinho et al., 2013).

302   Several factors may influence the microencapsulation success, interfering with the

303   process efficiency or with the characteristics of microparticles. Regarding to the

304   mixtures to be atomized in the spray dryer, it is important to ensure about their

305   homogenization before use. Furthermore, in the case of emulsions, they must be stable,

306   with very small oil droplets to not interfere with the drying rate of powder, and low

307   viscosity to not affect the atomization process and prevent to obtain microparticles with

308   higher size (Drusch, Serfert, Heuvel, & Schwarz, 2006; Liu et al., 2001; Rosenberg,

14

309   Kopelman, & Talmon, 1990). About the operating conditions of spray-drying, the

310   increase of feed rate leads to microparticle size increase for the same amount of energy

311   provided from atomizer. For the same feed rate, the increase of energy from atomizer

312   promotes the decreases of size of microparticles formed (Gharsallaoui et al., 2007). The

313   feed, air inlet and air outlet temperatures are operating conditions to also pay attention.

314   The increase of feed temperature decreases the viscosity. In turn, low air inlet

315   temperature leads to low evaporation rate, which results in microparticles with high

316   density membranes, high water content, poor fluidity and with tendency to agglomerate

317   (Gharsallaoui et al., 2007). On the other hand, high air inlet temperature leads to an over

318   evaporation, whereby microparticles with fissures in the membranes are created which

319   compromises the process efficiency (Gharsallaoui et al., 2007). Regarding air outlet

320   temperature, it is dependent of air inlet temperature and of the properties of the solution

321   fed to the spray-dryer (Gharsallaoui et al., 2007). Despite the high air inlet/outlet

322   temperatures, this does not commit the most sensitive compounds, since the exposition

323   time is around of few milliseconds and the temperature inside the core material do not

324   exceed 100 ∘C (Desai & Park, 2005). In the final, the size of microparticles may change

325   between 10 µm and 50 µm or 2 mm and 3 mm (Gharsallaoui et al., 2007).

326   Since the final of 1950s, spray-drying has been used in the food industry. It is one of the

327   most common used methods due to its simplicity, low production costs, and easy scale-

328   up. Moreover, the process is rapid, continuous and reproducible, and particles with good

329   quality are obtained (de Vos et al., 2010; Desai & Park, 2005; Gharsallaoui et al., 2007;

330   Rattes & Oliveira, 2007; Schafroth, Arpagaus, Jadhav, Makne, & Douroumis, 2012).

331   However, microencapsulation by spray-drying is restricted to a limiting number of

332   encapsulating agents which must be soluble in water at an acceptable level (Desai &

333   Park, 2005).

334   Several studies about microencapsulation of vitamin A by spray-drying have been

335   reported, as summarized in Table 2. This strategy has been widely used to improve

336   stability, bioavailability and storage of carotenoids. Moreover, microencapsulation by

337   spray-drying was also investigated for protection of commercial produced vitamin A

338   formulations, being observed in the most stable formulation the retention of 77.73 % of

339   vitamin A after 2 months of storage at 40 °C, 60 % relative humidity, and a retention of

340   95 % of vitamin A at ambient conditions (Raileanu & Diosady, 2006). The

341   encapsulating agents assessed, alone or/and in combination, include carbohydrates such

342   as polysaccharides (*e.g.* arabic gum, mesquite gum, maltodextrin and starch) and sugars

343   (*e.g.* sucrose), and proteins (*e.g.* gelatin, soy protein isolate). Carbohydrates get

344   emphasis because their low viscosity at high solids contents and good solubility, despite

345   some of them lack the interfacial characteristics required for high microencapsulation

346   efficiency (*e.g.* maltodextrin present poor emulsifying properties). Hence, carbohydrates

347   are usually used combined with other carbohydrates (*e.g.* arabic gum, which enable to

348   obtain stable emulsions with several oils over a wide pH range) or proteins (which

349   provide excellent functional properties) (de Azeredo, 2005; Gharsallaoui et al., 2007).

350   Microencapsulation by spray-dryer is usually performed as an individual method, as

351   described above. However, the literature reports the combination of this technique with

352   others. For example, Moraes *et al.* (2013) investigated the production of proliposomes

353   incorporating β-carotene by spray-drying, and Rodríguez-Huego *et al.* (2004) studied

354   microencapsulation by spray-drying of multiple emulsions containing carotenoids.

355

356   *Spray-cooling*

357   Spray-cooling is a technology very similar to microencapsulation by spray-drying and

358   also applied to vitamin A. However, the solution, dispersion or emulsion is atomized by

359 cooled air, which enables to use it with damage sensitive ingredients. Therefore, the

360 water vaporization does not occur and the encapsulating agents (usually vegetable oil or

361 its derivatives with melting points between 45–122 ∘C) solidify around the core

362 material (Desai & Park, 2005).

363 Microencapsulation by spray-cooling has been used to prepare stable and efficacious

364 microparticles with iodine, iron and vitamin A (WegmÜller, Zimmermann, BÜhr,

365 Windhab, & Hurrell, 2006; Zimmermann et al., 2004).

366

367 *Coacervation (phase separation)*

368 The principle of microencapsulation by coacervation is the phase separation of one

369 (simple coacervation) or many (complex coacervation) hydrocolloids (encapsulating

370 agent) from an initial solution. Further, the new phase appear as liquid drops which

371 deposit and harden around the core material suspended or emulsified in the same

372 reaction media, forming the coacervate (de Azeredo, 2005; Gouin, 2004). The process

373 may be conducted by chemical or physical changes (pH, ionic strength, temperature,

374 molecular weight and concentration of polymers) in the solution, reducing the solubility

375 of hydrocolloids (de Azeredo, 2005). The steps involved in coacervation involve the

376 formation of three phases (1 – with core material, 2 – with encapsulating agent, 3-

377 connection phase with phases 1 and 2), formation of core material due to deposition of

378 encapsulating agent around the coating material, and stabilization and hardening of

379 encapsulating agent to form self-sustaining microparticles (de Azeredo, 2005; Lazko,

380 Popineau, & Legrand, 2004). The main disadvantageous of microencapsulation by

381 coacervation is the cost, whereby it is little used in the food industry despite its high

382 efficiency (Dziezak, 1988).

383   The most used and understood coacervation system is probably the gelatine/arabic gum

384   system (Gouin, 2004). In fact, this system was used for the microencapsulation of

385   vitamin A palmitate, being evaluated the colloid mixing ratio, core-to-wall ratio,

386   hardening agent, concentration of core solution, and drying method on

387   the coacervation process and the properties of the microparticles (Junyaprasert,

388   Mitrevej, Sinchaipanid, Boonme, & Wurster, 2001). Furthermore, Albertini *et al.*

389   (2010) stabilized vitamin A palmitate for animal supplementation with butylated

390   hydroxytoluene in double layer microparticles constituted by a core of chitosan, Tween

391   20, calcium chloride and EDTA surrounded by a first chitosan-alginate membrane and

392   an outer membrane of calcium-alginate. The results revealed high drug loading (42%

393   w/w) and high encapsulation efficiency (94%). Among the encapsulating agents used,

394   both alginate and chitosan are polysaccharides and, therefore, they are natural products

395   (Berta Nogueiro Estevinho et al., 2013). Additionally, chitosan is also non-toxic,

396   biocompatible, degradable, it does not cause allergies or irritant reactions, permeability

397   increases with decrease of pH, it has ability to adhere to the gastric mucosa and presents

398   good results in release experiments (Berta Nogueiro Estevinho et al., 2013).

399

400   *Emulsion system*

401   Emulsions are a mixture of at least two immiscible fluids (oil(s) and water), wherein the

402   particles of one phase (the dispersed phase) are dispersed as small spherical droplets

403   within the other (the dispersant phase) (Fang & Bhandari, 2010). According to the

404   spatial organization of oil and water phases, emulsions can be water-in-oil (W/O,

405   droplets water are dispersed in the oil) or oil-in-water (O/W, droplets of oil are

406   dispersed in water) combinations (Aveyard, Binks, & Clint, 2003). The thermodynamic

407   stabilization of emulsions can be achieved by using several surfactants, ethoxylated

408   mono- and diacylglycerides and phospholipids being the most used in the food industry

409   (Loveday & Singh, 2008). More complex multiple emulsions can be prepared for

410   microencapsulation, namely oil-in-water-in-oil (O/W/O), water-in-oil-in-water

411   (W/O/W), water-in-oil-in-oil (W/O/O) and water-in-oil-in-oil-in-water (W/O/O/W)

412   (Gao, Wang, Liu, Chen, & Tong, 2010; J.-H. Lee, Park, & Choi, 2000; Zheng, 2009).

413   Microencapsulation of vitamin A into emulsion systems may consider the selection of

414   the oil to be used, in order to ensure the oxidative stability of core material. This

415   parameter is influenced by chemical and physical characteristics of droplet

416   (McClements, Decker, & Weiss, 2007). Yoshida *et al.* (1999) studied O/W, W/O and

417   O/W/O emulsions, observing a decrease of retinol stability from O/W/O to W/O and

418   from W/O to O/W emulsion. The remaining retinol percentage after storage during 4

419   weeks at 50 ∘C was 56.9, 45.7, and 32.3, respectively. Other studies show the

420   importance of emulsion system for application in cosmetic formulations. For example,

421   Yanaki (2001) prepared a O/W/O emulsion for encapsulation of retinol (in the inner oil

422   phase). The method was effective for stabilization of core compound (emulsion

423   maintained stable at 50 ∘C for at least 1 month) and the addition of antioxidants

424   improved that stability. On the other hand, Lee *et al.* (2004) encapsulated vitamin A into

425   poly(methylmethacrylate)-g-polyethylenimine (PMMA-g-PEI) microspheres by using

426   an O/W emulsion. Chemical stability of encapsulated vitamin A was improved by the

427   presence of PEI, maintaining 91% of their initial activity after 30 days of incubation at

428   40 ∘C. Also, Semenzato *et al.* (1994) explored the stability of vitamin A palmitate in

429   O/W cosmetic emulsions, having observed that chemical stability of this compound is

430   strictly dependent of physical stability of the formulation. At last, Moyano and Segall

431   (2011) performed a similar investigation, aiming to understand the effect of the

432   presence of vitamin E and other antioxidants on the stability of vitamin A.

433

434 *Liposomes*

435 Liposomes consist of bilayer lipid systems which are concentric around an aqueous

436 space (Fang & Bhandari, 2010). It is the result of hydrophilic and hydrophobic

437 interaction between phospholipids and water molecules. Several methods for liposomes

438 formation are described in the literature and the main advantages about these structures

439 is the capability of control release rate at the target site and at the desirable time (Fang

440 & Bhandari, 2010).

441 Microencapsulation of vitamin A in liposomes has been used in pharmaceutical,

442 medical and cosmetic applications, several studies being described in the literature.

443 Arsić *et al.* (1999) reported encapsulation of vitamin A-palmitate in liposomes made

444 from the purified phospholipid fraction with 90% phosphatidylcholine, in order to

445 increase stability against the oxidation process caused by UV radiation. On the other

446 hand, Singh and Das (1998) showed that retinol has greater affinity to bind with

447 liposomes compared to retinol palmitate.

448

449 *Cochleates*

450 Cochleates are nanoparticules obtained by introduction of polyvalent cations into

451 suspensions of anionic liposomes, occurring the fusion of liposomes (Loveday & Singh,

452 2008). The general aspect of this structure consists in stacking of phospholipid bilayers

453 in a rolled and spiral configuration and with aqueous solutions of multivalent cations

454 between each sheet (Loveday & Singh, 2008). Several patents about the incorporation

455 of vitamin A in nanocochleates to enhance vitamin A stability have already been

456 presented (Loveday & Singh, 2008).

457

458 *Solid lipid nanoparticles*

459   Solid lipid nanoparticles have been proposed as an alternative to microencapsulation,

460   instead of the use of an emulsion system, liposomes and polymeric nanoparticles. It is

461   explained by the possibility to incorporate target compounds into nanostructures and,

462   therefore, improve the approaches used to obtain controlled release and in the desired

463   site (S Mukherjee, Ray, & Thakur, 2009). In addition, they enable to obtain high core

464   material contentare biocompatible, less expensive, increase the stability of core

465   compound and enable an easy scale-up (Mehnert & Mäder, 2001; S Mukherjee et al.,

466   2009). However, solid lipid nanoparticles may have a relatively high water content of

467   the dispersions and can lead to core material expulsion after polymeric transition during

468   storage (S Mukherjee et al., 2009). A wide range of methods are reviewed in the

469   literature to describe the production of solid lipid nanoparticles (Üner & Yener, 2007).

470   In the final, three types of these particles have been described: imperfect, amorphous

471   and multiple.

472   Jenning and Gohla (2000) compare wax and glyceride solid lipid nanoparticles for

473   stabilization of retinol. Additionally, Sapino *et al.* (2005) evaluated the protective effect

474   of different solid lipid nanoparticles on the photodegradation and thermal degradation of

475   retinyl palmitate introduced in hydroxyethylcellulose gel.

476   Similar to what was observed for spray-drying method, the use of solid lipid

477   nanoparticles can arise combined with other techniques. Carlotti *et al.* (2005) report the

478   use of cetyl palmitate, glyceryl behenate, and palmitic acid solid lipid nanoparticles, all

479   loaded with vitamin A-palmitate, which were prepared and introduced in an O/W

480   emulsion. The final aim was the protection of retinyl palmitate from the photo

481   degradation induced by UVA and UVB radiation.

482

483   *Inclusion complexation*

484 Microencapsulation in inclusion complexation is usually performed by the use of

485 cyclodextrins. Cyclodextrins are a family of compounds with six, seven or eight glucose

486 residues linked by α (1-4) glycosidic bonds, forming respectively α-, β-, and γ-

487 cyclodextrins (Pagington, 1986). The interior part of cyclodextrins is hydrophobic and

488 is suitable to receive slightly polar compounds, while the exterior part is hydrophilic

489 (Fang & Bhandari, 2010; Szejtli, 1998). However, it is important to consider the

490 moderate and limited loading capacity regarding to the cyclodextrins (Sauvant et al.,

491 2012). Inclusion of retinyl palmitate into β-cyclodextrin was performed by

492 Vilanova and Solans (2015) in order to increase its water solubility and stability against

493 external factors. Also, Koeda *et al.* (2014) combined β-cyclodextrin with maltodextrin

494 to stabilize retinyl palmitate.

495

496 ## 5. Vitamin A controlled release and kinetics

497 The quality of microparticles produced by a certain encapsulating method and by the

498 usage of specific conditions is evaluated according to retention of core material and the

499 stability of the system over the time. Afterwards it is also important to evaluate the

500 release systems. They must certify that release of core material occurs at the target site

501 and at the desirable rate and time. The final objectives are the decrease of the loss of

502 target compound during the process and storage, and the optimization of absorption and

503 the increase of effective use, as mentioned previously.

504 Numerous release mechanisms are described and they vary with the encapsulating

505 technique applied, with the encapsulating agents used and the conditions selected for the

506 release experiments(Berta Nogueiro Estevinho et al., 2013). The classification of release

507 mechanisms is based on the physicochemical phenomena that promote the release of

508 core material. They may act individually or combined and include diffusion-controlled,

509   barrier-controlled, pressure-activated, solvent-activated, osmotically-controlled, pH-

510   controlled, temperature-sensitive, melting-activated and combined systems (Berta

511   Nogueiro Estevinho et al., 2013).

512   Estevinho *et al.* (2013) report the main mathematical models for controlled release of

513   compounds: a kinetics of zero order shows a constant release rate and is observed when

514   the core material is a pure compound and its release from the microparticle also occurs

515   as a pure compound; a half order kinetics occurs with matrix particles; a kinetics of first

516   order occurs as the result of a core material that is in a solution. In practice, the release

517   rate of the active compound may be slightly different from zero, half or first order

518   kinetics, , and because of that, more complex mathematical models attempt to describe

519   the phenomena. Higuchi and Korsmeyer-Peppas equations express the kinetics of

520   controlled release of substances, Hixson-Crowell equation is applied to release of

521   compounds in form of pharmaceutical doses and Kopcha empirical equation is used to

522   fit released data of optimized batches.

523   These mathematical models are important in order to develop a system with specific

524   characteristics and simulate the effectiveness of certain parameters on the resulting

525   release kinetics.

526   The number of studies developed to analyze *in vitro* and, more important, *in vivo* release

527   of vitamin A are still few. Some of them are discussed in this section.

528   Jenning *et al.* (2000) investigated microencapsulation of vitamin A in glyceryl behenate

529   solid lipid nanoparticles aiming to understand the potentialities of their application in

530   the cosmetic industry. Release was covered for 24 h and revealed interesting results.

531   Within the first 6 h, a controlled release of retinol from nanoparticles was observed.

532   Afterwards, between 12 h and 24 h the release rate increased and even exceeded the

533   release rate of comparable nanoemulsions. Also, Jenning *et al.* (2000) researched the

534 dermatologic and cosmetic application of glyceryl behenate solid lipid nanoparticles

535 loaded with vitamin A (retinol and retinyl palmitate). The best results were obtained

536 with retinol-loaded solid lipid nanoparticles incorporated in the O/W cream, with

537 observation of drug release retarding onto porcine skin.

538 Oh *et al.* (2006) evaluated *in vitro* permeation of retinol in Tween 20-based deformable

539 liposomes in human skin and keratinocyte models. Arayachukeat *et al.* (2011) studied

540 the encapsulation of retinyl acetate into two different single polymers: ethyl cellulose

541 (EC) and poly (ethylene glycol)-4-methoxycinnamoylphthaloylchitosan (PCPLC). The

542 stability of retinyl acetate in an aqueous solution and UVA radiation registered a

543 significant improvement when PCPLC was used. Free and encapsulated retinyl acetate

544 into PCPLC were applied on the surface of freshly excised skin of a baby mouse and the

545 results indicated for the encapsulated retinyl acetate a significantly slower skin

546 absorption rate and a total retention of retinyl acetate after 24 hours of contact with the

547 skin tissue.

548 He *et al.* (2013) performed *in vitro* and *in vivo* studies with the aim to prolong ocular

549 retention time and improve bioavailability of vitamin A palmitate. Vitamin A palmitate-

550 loaded cationic liposomes coated by N-trimethyl chitosan were prepared and dispersed

551 in thermo-sensitive in *situ* gels with poloxamer 407 as the base. *In vitro* and *in vivo*

552 corneal retention time of N-trimethyl chitosan coated vitamin A palmitate in *situ* gels

553 were notably extended. N-trimethyl chitosan coated vitamin A palmitate in *situ* gels

554 revealed a delayed drug release when compared with uncoated vitamin A palmitate in

555 *situ* gels and commercial oculotect gels. The release of vitamin A palmitate gels

556 exhibited the characteristics of zero-order kinetics.

557

558 **6. Conclusion**

559   This review aims to discuss the main methods used for microencapsulation of

560   vitamin A, arising as a strategy to improve stability and bioavailability of this

561   compound, as well to enable a controlled release. Vitamin A is a constituent of

562   functional food and integrates several pharmaceutical and cosmetic formulations,

563   whereby it is important to protect this compound against less suitable conditions

564   (moisture, oxidants, light, heat and temperature, among others), and improve shelf life

565   of final product. Moreover, according to vitamin A metabolism and function, it is

566   important to ensure the release of this compound in the desirable place (small intestine)

567   and release rate.

568   The success of microencapsulation depends on the selection of an appropriate

569   encapsulating agent and microencapsulating method. The encapsulating agent will

570   determine the encapsulation efficiency, microparticle stability and the characteristics of

571   final product, while the microencapsulating method must be selected according to the

572   physical and chemical properties of core material and encapsulating agent, the desirable

573   size and shape of microparticle, and the required profile of controlled release.

574   Spray-drying technology is the most popular technique for the microencapsulation of

575   carotenoids, several works were reported where numerous biopolymers are used as

576   encapsulating agents. Among these, the most promising encapsulating agent seems to be

577   arabic gum due to reviewed capability to form stable emulsions, adequate solubility and

578   low viscosity.

579   The total number of studies performed about microencapsulation of vitamin A is still

580   very low, whereby it is expected that more *in vitro* and *in vivo* protocols may be

581   developed to explore no reported target areas. Therefore, in the future,

582   microencapsulation might become a real option in the treatment of a large spectrum of

583   disorders.

584

Acknowledgments

596

597

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1046 **Figure Captions**

1047 Figure 1 – Interconversion between vitamin A structures (adapted from Clagett-Dame &

1048 Knutson (2011), Heller and Shiggman (1985) and Mukherjee *et al.* (2006)).Figure 2 –

1049 Microparticles morphology: (A) Simple, (B) Irregular, (C) Multiwall, (D) Multi-core, (E)

1050 Aggregate and (F) Matrix (adapted from Estevinho *et al.* (2013)).

1051 Figure 3 - Schematic representation of the spray-drying procedure (adapted from

1052 Estevinho *et al.* (2013)).

Table Captions

Table 1 – Structure of some synthetic retinoids (adapted from Heller and Shiffman (1985)

and Mukherjee *et al.* (2006)).

Table 2 – Studies of microencapsulation of vitamin A by spray-drying.

Retinol

CH₂OH → CH_2OH

Retinal

CHO

Retinoic acid

COOH
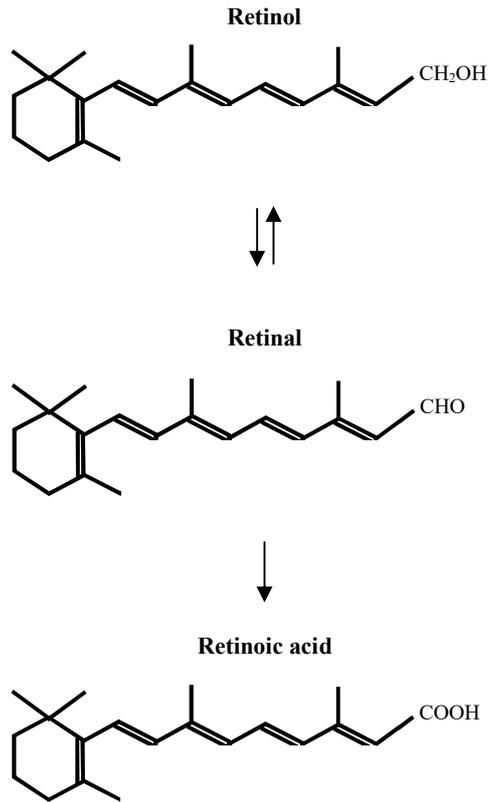
Figure 1 – Interconversion between vitamin A structures (adapted from Clagett-Dame & Knutson (2011), Heller and Shiffman (1985) and Mukherjee *et al.* (2006)).

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1100

1101

1102

        A   **B**   **C**

1103

1104

1105

1106   **D**   **E**   **F**
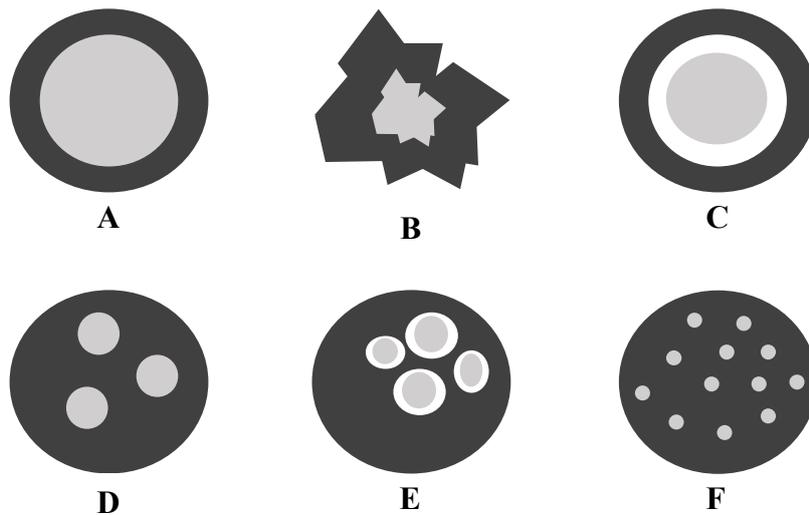
1107 **Figure 2 –** Microparticles morphology: (A) Simple, (B) Irregular, (C) Multiwall, (D) Multi-core, (E) Aggregate and

1108 (F) Matrix (adapted from Estevinho *et al.* (2013)).

1109
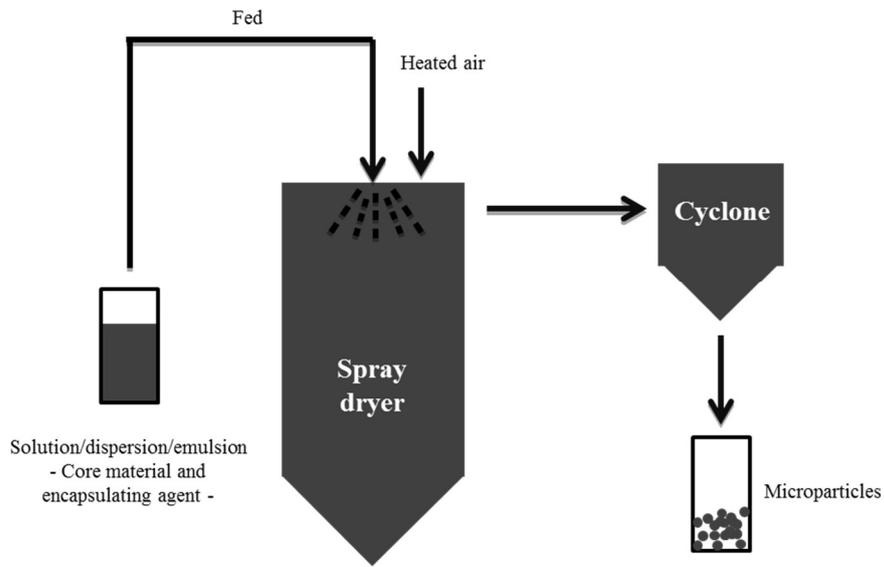
1110

1111   **Figure 3 -** Schematic representation of the spray-drying procedure (adapted from Estevinho *et al* (2013)).

1112

1113 **Table 1** – Structure of some synthetic retinoids (adapted from Heller and Shiffman (1985) and Mukherjee *et al.*
1114 (2006)).

Generation	Synthetic retinoid	Structure
First (non-aromatics)	Isotretinoin	
Second (mono-aromatics)	Etretinate	
Third (poly-aromatics)	Adapalene	
Fourth (pyranones)	Seletinoid G	
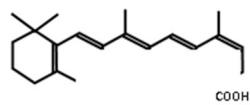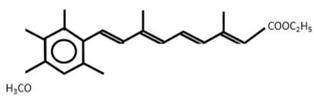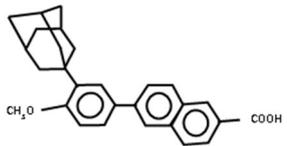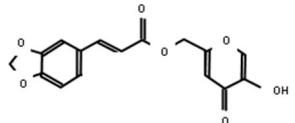
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Table 2 – Studies of microencapsulation of vitamin A by spray-drying.

Encapsulating agents	Compound	Inlet temperature (°C)	Outlet temperature (°C)	Aspiration rate	Feed rate	Pressure (bar)	Reference
Modified starch	Pequi pulp (rich in carotenoids)	140-200	82-115	10000 mL/min	4.6 mL/min	-	(Audirene A Santana, de Oliveira, Kurozawa, & Park, 2014)
Arabic gum	Pequi pulp (rich in carotenoids)	140-200	-	10000 mL/min	3.3 mL/min	-	(Audirene Amorim Santana, Kurozawa, de Oliveira, & Park, 2013)
Arabic gum and whey protein alone or in combination with maltodextrin or inulin	Carotenoid astaxanthin	120	70	100 %	-	39.2	(Bustos-Garza, Yáñez-Fernández, & Barragán-Huerta, 2013)
Soy protein isolate and octenylsuccinic anhydride-modified starch, alone or in combination	β-Carotene	160	≈ 85	-	10 mL/min	-	(Deng, Chen, Huang, Fu, & Tang, 2014)
25 Dextrose equivalent maltodextrin	β-Carotene	170 ± 5	95 ± 5	-	-	-	(Desobry, Netto, & Labuza, 1997)
Maltodextrin	β-Carotene	170 ± 5	95 ± 5	100 %	7.5 mL/min	-	(Donhowe et al., 2014)
Arabic gum or maltodextrin 20 dextrose equivalent	Carotenoids	170	110	30 mL/min	-	4.9	(Faria, Mignone, Montenegro, Mercadante, & Borsarelli, 2010)
Whey protein and arabic gum	Gail oil (with β-carotene and lycopene)	150 ± 3	95 ± 3	-	6.7 mL/min	-	(Kha, Nguyen, Roach, & Stathopoulos, 2014)
Acid-modified tapioca starch, native tapioca starch and maltodextrin	β-Carotene	170 ± 5	95 ± 5	-	-	-	(Loksuwan, 2007)
Modified starch (Capsul®)	Paprika oleoresin (rich in carotenoids)	180 ± 5	100 ± 5	-	-	-	(M P Rascón et al., 2015)
Arabic gum and soy protein isolate	Paprika oleoresin (rich in carotenoids)	160, 180 and 200 ± 5	110 ± 5	-	-	-	(Martha Paola Rascón, Beristain, García, & Salgado, 2011)
Maltodextrin	Watermelon juice (rich in β-carotene and lycopene)	145, 155, 165 and 175	-	60 %	10000 mL/min	4.5	(Quek, Chok, & Swedlund, 2007)
Modified starch (Capsul®)	Lycopene	180 ± 2	98 ± 2	-	10 mL/min	-	(Rocha, Fávaro-Trindade, & Grosso, 2012)
Mixture of biopolymers (Arabic gum, mesquite gum and maltodextrin)	Multiple emulsions containing carotenoids	170 ± 5	80 ± 3	-	20 mL/min	2.8	(Rodriguez-Huezo et al., 2004)
Arabic gum	Red pepper extract (rich in β-carotenoids)	185	103	90 %	10 %	-	(Romo-Hualde, Yetano-Cunchillos, González-

							Ferrero, Sáiz-Abajo, & González-Navarro, 2012)
Dextrose equivalent hydrolyzed starches	Carotenoids	200 ± 5	100 ± 5	-	-	-	(Wagner & Warthesen, 1995)
Modified starch and sucrose; and gelatin and sucrose	β-Carotene	-	-	-	-	-	(Xinde, Shanjing, Ning, & Bin, 2007)
Gelatin-sucrose and gelatin peach gum-sucrose	Vitamin A	180	80	-	-	-	(Y.-L. Xie, Zhou, & Qian, 2006)
Gelatin-sucrose, gelatin-peach-gum-sucrose and HI-CAP 100 (starch octenylsucciniate, OSA-starch)	Vitamin A	180	80	-	-	-	(Y. Xie, Wang, Lu, & Hui, 2010)
HI-CAP 100 (starch octenylsucciniate)	Vitamin A acetate	182	82	-	100 mL/min	-	(Y.-L. Xie, Zhou, & Zhang, 2007)
HI-CAP 100 (starch octenylsucciniate, OSA-starch)	Vitamin A acetate	182	82	-	-	-	(Y.-L. Xie, Zhou, Liang, He, & Han, 2010)

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