



<b>Title</b>	Oral delivery strategies for nutraceuticals: Delivery vehicles and absorption enhancers
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<b>Publication date</b>	2016-07
<b>Publication information</b>	Gleeson, John P., Sinéad M. Ryan, and David James Brayden. "Oral Delivery Strategies for Nutraceuticals: Delivery Vehicles and Absorption Enhancers." Elsevier, July 2016. <a href="https://doi.org/10.1016/j.tifs.2016.05.007">https://doi.org/10.1016/j.tifs.2016.05.007</a> .
<b>Publisher</b>	Elsevier
<b>Item record/more information</b>	<a href="http://hdl.handle.net/10197/8777">http://hdl.handle.net/10197/8777</a>
<b>Publisher's statement</b>	This is the author's version of a work that was accepted for publication in Trends in Food Science and Technology. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in Trends in Food Science and Technology, 53 2016-07, pp.90-101. DOI: 10.1016/j.tifs.2016.05.007
<b>Publisher's version (DOI)</b>	10.1016/j.tifs.2016.05.007

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# 1 **Oral delivery strategies for nutraceuticals: delivery vehicles and absorption** 2 **enhancers**

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## 6 ***Background***

7 Lifestyle issues contribute to the development of obesity, type 2 diabetes, and cardiovascular  
8 disease. Together with appropriate diet and exercise, **nutraceuticals** may contribute to  
9 managing prevention at an early stage prior to therapeutic intervention. However, many  
10 useful **food-derived bioactive compounds** will not sufficiently permeate the small intestine  
11 to yield efficacy without appropriate oral delivery technology. The pharmaceutical industry  
12 uses commercialised approaches for **oral delivery** including solubilizing technologies for  
13 small molecules, which could be applied to selected nutraceuticals with solubility issues.  
14 Systems currently being studied for labile and poorly permeable hydrophilic peptides and  
15 macromolecules include **nanoparticles, intestinal permeation enhancers (PE)** and  
16 mucolytics. These may also have potential for application to nutraceuticals with similar sub-  
17 optimal physicochemical characteristics.

## 18 ***Scope and Approach***

19 We introduce factors which effect oral delivery of four types of nutraceuticals, namely fatty  
20 acids, bioactive peptides, micronutrients, and phytochemicals. Factors preventing oral  
21 absorption can arise from molecule physicochemical characteristics, which influence  
22 solubility, stability, and epithelial permeability in the gastrointestinal tract (GIT). We  
23 highlight the potential of selected delivery strategies to improve oral bioavailability of  
24 different types of nutraceuticals.

## 25 ***Key Findings and Conclusions***

26 There is an opportunity for the nutraceutical industry to leverage the pharmaceutical  
27 industry's progress in oral drug delivery. The use of delivery approaches using formulation  
28 with excipients or substances with a history of use in man has potential to improve solubility,  
29 stability, or permeability of nutraceuticals, leading to improved **oral bioavailability**.

30 Leveraging oral delivery formulation approaches across nutraceutical and pharmaceutical  
31 molecules will lead to synergies for both fields.

32 **Key words:** Nutraceuticals; food-derived bioactives; oral delivery; nanoparticles; intestinal  
33 permeation enhancers; oral bioavailability.

34

## 35 **Introduction**

36 With growing prevalence of lifestyle-associated diseases, including obesity, Type II diabetes  
37 and cardiovascular disease, there is a need to reduce risks of onset of these diseases (Menotti  
38 & Puddu, 2015). Nutraceuticals are defined as isolated food-derived bioactive molecules,  
39 which provide physiological benefits beyond basic nutrition (Pan, Lai, Dushenkov, & Ho,  
40 2009). Recently, research has focused on such bioactives with anti-oxidative, anti-  
41 inflammatory, anti-hyperlipidemic and anti-hypertensive activities. However, there are many  
42 hurdles to overcome for the oral delivery of nutraceuticals depending on the bioactive's  
43 physicochemical properties. The molecule may be prone to sub-optimal release and  
44 dispersion from the delivery dosage form and/or low solubility in small intestinal fluids  
45 (bioaccessibility), pH- and enzymatic degradation, biotransformation during gastrointestinal  
46 transit, poor diffusion across mucus and low intestinal epithelial permeability; all of which  
47 must be overcome prior to absorption into the bloodstream (Braithwaite, et al., 2014;  
48 McClements, Decker, Park, & Weiss, 2009). Without appropriate delivery systems, current  
49 nutraceuticals with such characteristics are unlikely to provide the intended physiological  
50 effect, despite marketing claims to the contrary.

51 The pharmaceutical industry has examined microbes and plants as sources of drug discovery  
52 molecules, examples being penicillin (*Penicillium* species), colchicine (autumn crocus),  
53 acetyl salicylic acid (willow tree bark), and paclitaxel (pacific yew tree) (Dias, Urban, &  
54 Roessner, 2012). There is now additional focus on food as a new source of bioactives. With  
55 the growing consumer market for nutraceuticals, there is scope for the nutraceutical industry  
56 to leverage innovative research from the pharmaceutical industry in delivering poorly soluble  
57 and poorly absorbed molecules. These particular nutraceuticals may assist with reducing the  
58 risks of certain diseases before pharmaceutical intervention is required, but without  
59 appropriate oral formulation they will have limited efficacy.

60 Innovative strategies are being attempted by the pharmaceutical industry for oral delivery of  
61 peptides including insulin, octreotide, salmon calcitonin (sCT) and parathyroid hormone  
62 (PTH). Approaches include entrapment in protective delivery vehicles, strategies for  
63 enhanced mucus penetration and epithelial permeation, as well as incorporation of excipients  
64 as protease enzyme inhibitors (Maher, Duffy, Ryan, & Brayden, 2014). Chemical  
65 modification by a prodrug approach has been successful in improving small molecule oral  
66 bioavailability. For example, the anti-viral prodrug, valacyclovir is converted to acyclovir *in*

67 *vivo* and improves oral bioavailability (Huttunen, Raunio, & Rautio, 2011). Pro-vitamins are  
68 similar to synthetically- designed prodrugs and can yield improved oral bioavailability of  
69 supplements: pantothenic acid (vitamin B<sub>5</sub>) is unstable, so a stable alcohol, panthenol (pro-  
70 vitamin B<sub>5</sub>), is the parent molecule that is subsequently oxidised to the bioactive form *in vivo*.

71 Here, we discuss factors which affect the oral delivery of different classes of *isolated*  
72 bioactive components (nutraceuticals) including fatty acids, bioactive peptides,  
73 micronutrients and phytochemicals, and we highlight strategies to improve their oral  
74 bioavailability (**Fig. 1**). **Another class of nutraceuticals, bioactive carbohydrates have shown**  
75 **beneficial effects *in vitro* and *in vivo*, which are discussed in detail elsewhere (Brown, et al.,**  
76 **2014; Liu, Willför, & Xu, 2015).** Discussion of factors impacting the delivery of bioactive  
77 components within functional food and whole food matrices has been discussed extensively  
78 in previous reviews with highly on bioaccessibility, absorption and transformation  
79 (McClements, 2013b; McClements, et al., 2009; McClements, Li, & Xiao, 2015;  
80 McClements & Xiao, 2014). We review the potential of approaches used in pharmaceutical  
81 oral delivery (use of mucolytic agents and intestinal permeation enhancers), as well as new  
82 strategies based on nanotechnology and assess whether these might be applied to food-  
83 derived bioactive compounds in order to overcome the hurdles in orally delivering  
84 nutraceuticals.

## 85 **Factors affecting oral delivery of nutraceuticals**

86 Physicochemical and physiological factors affect oral delivery of nutraceuticals. However,  
87 solubility, stability and intestinal permeability are the major factors which impede effective  
88 delivery of compounds including fatty acids (e.g. omega-3 fatty acids), bioactive peptides  
89 (e.g. Ile-Pro-Pro), micronutrients (e.g.  $\alpha$ -tocopherol) and phytochemicals (e.g. resveratrol)  
90 (**Fig. 1**). Delivery systems should be designed based on overcoming specific factors which  
91 can affect the particular loaded nutraceutical.

### 92 ***Nutraceutical compounds:***

#### 93 ***Fatty acids***

94 Long chain polyunsaturated fatty acids (LC-PUFA) are recognised for their role in brain  
95 development and potential to decrease risk of cardiovascular disease. Two fatty acids are  
96 essential for human health,  $\alpha$ -linolenic acid (ALA, an omega-3 fatty acid) and linoleic acid  
97 (LA, an omega-6 fatty acid). However, the process involved in converting ALA to

98 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the body is inefficient and  
99 supplementation is often required (Deckelbaum & Torrejon, 2012). Although cod liver oil has  
100 been an established source of EPA and DHA, there is interest in sustainable alternatives  
101 including krill oil, flax-seed and walnut oil (Adarme-Vega, Thomas-Hall, & Schenk, 2014).  
102 EPA and DHA enhance production of anti-inflammatory lipid mediators, decrease production  
103 of pro-inflammatory cytokines and decrease serum C-reactive protein, a clinical marker of  
104 inflammation (Skulas-Ray, 2015). Supplementation with omega-3 fatty acids has anti-  
105 hyperlipidemic activity, reducing LDL-cholesterol and triglycerides (Maki, Yurko-Mauro,  
106 Dicklin, Schild, & Geohas, 2014). Furthermore, Amarin Corporation's (Dublin, Ireland)  
107 Vascepa® icosapent ethyl (eicosapentaenoic acid ethyl ester) is an FDA-approved  
108 prescription medication for hypertriglyceridemia and there are plans to achieve a wider label  
109 for use in patients with moderately elevated triglyceride levels (Braeckman, Stirtan, & Soni,  
110 2015).

111 Delivery of omega-3 fatty acids is difficult due to low aqueous solubility in the small  
112 intestine and oxidative instability. Unsaturated fatty acids are prone to lipid oxidation, which  
113 is accelerated by exposure to air, light and heat, resulting in a loss in functionality and leading  
114 to off-flavour (Arab-Tehrany, et al., 2012). Upon reaching the small intestine, the fatty acids  
115 need to be liberated from the delivery matrix, often an oil capsule, to allow incorporation into  
116 mixed micelles, which seem to permeate the mucus layer and intestinal epithelia (Walker,  
117 Decker, & McClements, 2015). Delivery platforms are required to reduce lipid oxidation,  
118 improve solubility and overcome poor mucus penetration.

### 119 ***Bioactive peptides***

120 Proteins from food undergo enzymatic hydrolysis by digestive enzymes thereby releasing  
121 smaller peptides, which have bioactive properties if they can be absorbed. Some peptides  
122 inhibit angiotensin-converting enzyme (ACE), which can help maintain normal blood  
123 pressure and prevent escalation of hypertension by subverting the renin-angiotensin-  
124 aldosterone system (Turpeinen, Jarvenpaa, Kautiainen, Korpela, & Vapaatalo, 2013). Two  
125 such tripeptides have been focussed on: Ile-Pro-Pro (IPP) and Val-Pro-Pro (VPP), both  
126 isolated from milk  $\beta$ -casein (**Fig. 1**) following fermentation by *Lactobacillus helveticus*  
127 (Nakamura, et al., 1995). Other derived antihypertensive peptides include Val-Tyr-Pro (VYP,  
128 rice protein) (Chen, et al., 2013), Gly-Leu-Pro (GLP, chum salmon skin) (Lee, Jeon, &  
129 Byun, 2014) and His-Leu-Phe-Gly-Pro-Pro-Gly-Lys-Lys-Asp-Pro-Val (HLFGPPGKKDPV,

130 fertilised hen egg) (Duan, et al., 2014). These peptides can reduce systolic blood pressure  
131 following oral gavage to the spontaneously hypertensive rat (SHR). VPY is present in soy  
132 protein hydrolysate, inhibits pro-inflammatory cytokine production and reduces histological  
133 scoring of lesions in a rodent colitis model (Kovacs-Nolan, et al., 2012). Food-derived  
134 proteins such as  $\alpha$ -lactalbumin may also have anti-inflammatory action, and this is also of  
135 interest for potential treatment of inflammatory bowel disease (IBD) (Chatterton, Nguyen,  
136 Bering, & Sangild, 2013).

137 Peptides are prone to pancreatic serine protease digestion by chymotrypsin, trypsin and  
138 elastase into small fragments and then further digestion to single amino acids by intracellular  
139 carboxypeptidases. Presence of Pro residues confers resistance to such enzymes (Gleeson,  
140 Heade, Ryan, & Brayden, 2015). Due to their hydrophilic nature and high molecular weight  
141 however, peptides more than three residues long typically have low mucus penetration and  
142 intestinal permeability, resulting in variable oral bioavailability (Renukuntla, Vadlapudi,  
143 Patel, Boddu, & Mitra, 2013). Delivery strategies therefore need to protect bioactive peptides  
144 from enzyme degradation and to enhance both mucus and intestinal permeability.

#### 145 ***Micronutrients***

146 Essential vitamins and minerals are required in small doses, with deficiencies leading to  
147 rickets (vitamin D), scurvy (vitamin C), neural tube defects (vitamin B<sub>9</sub>), hypothyroidism  
148 (iodine), hypokalaemia (potassium), and Keshan's disease (selenium). A nutritionally-  
149 balanced diet will provide the required micronutrients to a healthy individual, however, there  
150 are many conditions that can still benefit from micronutrient supplementation including  
151 calcium for osteoporosis and iron for iron-deficient anaemia (Wallace, et al., 2015). The  
152 physiological role of micronutrients includes roles as co-enzymes for metabolic processes,  
153 antioxidants to remove reactive oxygen species (ROS), modulation of gene transcription and  
154 structural components.

155 Delivery of micronutrients are also limited by individual physicochemical characteristics, as  
156 they may be susceptible to bioaccessibility, stability, solubility, and bioavailability issues.  
157 Vitamins C and E are prone to oxidation during processing and delivery, while fat soluble  
158 vitamins (A, D, E and K) may not be liberated from the delivery matrix due to excessive  
159 lipophilicity. Micronutrient bioavailability is effected by multiple processes, for example,  
160 vitamin E is easily oxidised and has poor solubility. Anti-nutrients are compounds that

161 interfere with the absorption of nutrients and limit their bioavailability. Calcium, iron and  
162 zinc can be chelated and cleared by dietary anti-nutrient phytate, hence the benefit of adding  
163 phytase to a micronutrient delivery system. Orally-delivered phytase can therefore improve  
164 oral calcium absorption in a pig model (Vigors, Sweeney, O'Shea, Browne, & O'Doherty,  
165 2014). Other dietary components act similarly by reducing mineral bioavailability, oxalic  
166 acid (spinach) binds calcium while glucosinolates (cruciferous vegetables) bind iodine.  
167 Therefore, oral delivery of minerals, somewhat ironically, may benefit from being taken in  
168 the *absence* of food.

### 169 ***Phytochemicals***

170 “Phytochemicals” are a large group of plant-derived compounds (**Fig. 1**), which have been  
171 studied for their potent antioxidant activity and potential anti-inflammatory and anti-  
172 hyperlipidemic activity. They include phytosterols (e.g. plant stanol esters); organosulfers  
173 (e.g. allicin from garlic); terpenoids (e.g. lycopene carotenoid from tomatoes) and  
174 sesquiterpenes. Polyphenols are the largest class containing stilbenes (e.g. resveratrol from  
175 grapes), isoflavones (e.g. genistein from soybean) and flavonoids-based agents (e.g.  
176 kaempferol and naringenin from spinach and grapefruit respectively) (Gonzalez-Castejon &  
177 Rodriguez-Casado, 2011). The anti-inflammatory mechanism of action of polyphenols  
178 involves modulation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-  
179  $\kappa$ B), inhibition of Mitogen-activated protein kinases (MAPK) cascade, activation of nuclear  
180 factor erythroid 2-related factor 2 (Nrf2), and reduction in pro-inflammatory cytokines, all of  
181 which are relevant in treatment of IBD (Martin & Bolling, 2015). Resveratrol improved  
182 expression of the cytoprotective NAD(P)H dehydrogenase, quinone 1 (NQO1), in cancer  
183 patients dosed with [ $^{14}$ C]-resveratrol (Cai, et al., 2015).

184 There are a multitude of factors affecting oral bioavailability of phytochemicals due to their  
185 unique physicochemical properties. For example, kaempferol has poor water solubility and  
186 favours alkaline pH conditions, resulting in low oral bioavailability (2%) in a rat model  
187 (Barve, et al., 2009). It is also prone to biotransformation by Phase I oxidative metabolism  
188 and Phase II glucuronidation in intestinal epithelia (Barve, et al., 2009). Resveratrol is readily  
189 soluble in ethanol, however, it has poor water solubility and is easily photo-isomerised and  
190 metabolised by glucuronidation (Patel, et al., 2011). Oral delivery approaches for  
191 phytochemicals need to overcome solubility issues, provide a dose sufficient to overcome  
192 partial metabolism, and boost epithelial permeability. It is important to note however, that

193 assumptions of straight-forward pharmacological dose-response concepts being applied to  
194 phytochemicals has been challenged by Cai *et al.*, who demonstrated a non-linear dose  
195 response for the chemoprotective effects of resveratrol in humans and mice (Cai, et al., 2015),  
196 with efficacy seen at the low doses found in food, but not at high doses.

### 197 ***Bioaccessibility and solubility in GIT***

198 Solubility is one of the first hurdles which must be overcome in oral delivery of bioactive  
199 molecules. The Food and Drug Administration (FDA) adopted the Biopharmaceutics  
200 Classification System (BCS) in 1995. It aims to predict *in vivo* performance of small drug  
201 molecules from immediate release solid oral dosage form based on *in vitro* measurements of  
202 solubility and permeability (Larregieu & Benet, 2014). Permeability is typically determined  
203 across Caco-2 human *in vitro* intestinal epithelial monolayers grown on filters and solubility  
204 is determined in 250 ml of aqueous media of simulated gastric fluid and simulated intestinal  
205 fluid over pH ranges 1.0-6.8 over the course of 24 hours. The BCS is also used as a guide for  
206 oral drug delivery formulation strategy, for example BCS Class III drugs are often formulated  
207 to improve intestinal permeability, while Class II drugs are formulated to improve solubility  
208 and cater for food effects (Buckley, Frank, Fricker, & Brandl, 2013). Recently, McClements  
209 *et al* developed the Nutraceutical Bioavailability Classification System (NuBACS) (**Fig. 2**),  
210 factoring in major issues affecting the oral bioavailability of nutraceuticals (McClements, et  
211 al., 2015).

212 NuBACS introduces the concept of “bioaccessibility”, the ability of the bioactive compound  
213 to be accessible to the body for absorption from the delivery matrix. Bioaccessibility,  
214 absorption and transformation of nutraceuticals are features of the NuBACS, and this is  
215 relevant for functional foods. In contrast, oral delivery of pharmaceutical agents is achieved  
216 by capsules, tablets or suspensions and is governed by the BCS specifically in terms of  
217 solubility and permeability. Although some nutraceuticals may require liberation from a  
218 functional food or whole food, the focus here is on delivery strategies for isolated  
219 nutraceutical bioactives, therefore, liberation refers to release from a delivery vehicle or  
220 formulation prior to solubilisation. Solubility is however, a major hurdle for certain  
221 nutraceuticals, particularly fatty acids and phytochemicals.

## 222 ***Degradation and Metabolism***

223 Degradation and metabolism are hurdles which an oral delivery system needs to overcome  
224 after solubilisation has been achieved. The recently developed biopharmaceutics drug  
225 disposition classification system (BDDCS) factors in drug metabolism by Phase I and II  
226 processes and is useful in predicting drug-drug interactions that may occur in the intestine  
227 and liver (Larregieu & Benet, 2014). Enzymatic metabolism is particularly relevant to fatty  
228 acids and peptides which are targeted by lipases and peptidases respectively. Lingual and  
229 gastric lipases account for a small amount of lipid hydrolysis, whereas pancreatic lipases act  
230 on bile-derived emulsified lipids resulting in 90% lipid digestion (Aarak, et al., 2013).  
231 However, EPA and DHA are resistant to pancreatic lipase hydrolysis due to the location of  
232 bond conjugation in the carbon chain (Akanbi, Sinclair, & Barrow, 2014). Bioactive peptides  
233 are prone to peptidases which access specific labile amino acids, although certain peptides are  
234 stable due to a lack of target amino acids for peptidases.

235 On the other hand, metabolism can assist in absorption of some phytochemicals: the  
236 flavonoid quercetin, is commonly found as a glycoside of either glucose or rutinose, but the  
237 capacity to metabolise the sugar moiety effects quercetin's bioavailability. Quercetin-4'-*O*-  
238 glucoside is absorbed intact in the small intestinal lumen by sodium-glucose transporter 1  
239 (SGLT1) and then hydrolysed by intracellular  $\beta$ -glucosidases, thereby cleaving the sugar  
240 moiety, which then passively diffuses across the basolateral membrane (Lotito, Zhang, Yang,  
241 Crozier, & Frei, 2011). On the other hand quercetin-3'-*O*-rutinoside does not permeate the  
242 small intestine, instead caecally-located bacterial  $\alpha$ -rhamnosidases convert it to the quercetin,  
243 aglycone, which in turn is absorbed from the colon.

## 244 ***Intestinal permeation***

245 Nutraceuticals may have limited capacity to permeate the gut wall. Prior to reaching the  
246 epithelia, bioactives must traverse intestinal mucus. Mucus is a complex hydrogel consisting  
247 of a mixture of glycoproteins, lipids, and sloughed epithelial cells. Interaction with mucus  
248 reduces permeability of mucoadhesive lipophilic molecules or large molecules due to steric  
249 blocking (Sigurdsson, Kirch, & Lehr, 2013). Lipophilic bioactives can be transported through  
250 the mucus layer by mixed micelles formed from bile salts, phospholipids and free fatty acids.  
251 Therefore, the lipophilic molecules, EPA, DHA, resveratrol, and kaempferol may pass  
252 through mucus in association with luminal-derived moieties. Nanoparticles coated with small

253 hydrophilic polymers including low molecular weight high density polyethylene glycol  
254 (PEG) and polysialic acid, can slip through the mesh of mucus potentially allowing for  
255 release of nutraceutical at intestinal epithelia or uptake of nutraceutical-entrapped  
256 nanoparticles (Ensign, et al., 2013).

257 Upon diffusion through the mucus, there are several routes which a bioactive agent may  
258 permeate the intestinal epithelia. Transport via the paracellular route requires movement  
259 through tight junctions. A molecular radius between 10-50 Å and molecular weight <500 Da  
260 is required and the bioactive must be hydrophilic in nature (Larregieu & Benet, 2014).  
261 Paracellular transport reduces risk of intracellular metabolism, which is relevant for  
262 phytochemicals and bioactive peptides. Transcellular transport involves molecules passing  
263 across the apical membrane by passive diffusion, receptor mediation or endocytosis.  
264 Hydrophobic molecules can pass across the phospholipid bilayer by passive diffusion.  
265 According to Fick's law of diffusion molecules with a relatively high oil-water partition  
266 coefficient ( $K_{ow}$ ) or greater hydrophobicity ( $\log P$ ) can pass the cell membrane more  
267 efficiently (e.g.  $\beta$ -carotene,  $\log P=15.2$ ) compared to molecules with lower values (e.g. IPP,  
268  $\log P= 1.07$ ; vitamin C,  $\log P= 2.77$ ) (McClements, et al., 2015). There is a balance required,  
269 as the greater the  $\log P$  the less the solubility: a  $\log P$  of < 2.5 may be optimal, however, this  
270 depends on the formulation or presence of bile salts and surfactants, which may assist in  
271 solubilising lipophilic nutraceuticals and presenting them as components of mixed micelles.

272 The epithelium of the small and large intestine has a multitude of transporters localised on the  
273 apical membrane which have roles in uptake of nutrients and absorption of drugs. These  
274 membrane bound proteins are relevant to the uptake of many nutraceuticals. Fatty acids are  
275 transported by intestinal fatty acid-binding proteins (I-FABP), and bioactive di- and  
276 tripeptides are carried by the proton coupled peptide transporter (PEPT1). Calcium uptake is  
277 mediated by the vitamin D receptor, while vitamin C is carried on the sodium-vitamin C co-  
278 transporter (SVCT) (Lin, Yee, Kim, & Giacomini, 2015). In the case of IPP at least two  
279 uptake pathways are likely to play a role: the paracellular route due to its low molecular  
280 weight (MW) and the transcellular route due to its interaction with the PEPT1 carrier.  
281 Appropriate exploitation of one or both of these intestinal permeation routes may enhance  
282 absorption of these types of molecules.

## 283 **Food-based strategies for improving oral delivery of nutraceuticals**

### 284 ***Delivery vehicles***

285 A delivery vehicle can control delivery and release of the nutraceutical. The use of delivery  
286 vehicles in the pharmaceutical industry has been investigated for oral delivery of antibiotics,  
287 vaccines, cancer therapeutics and biopharmaceuticals (Choonara, et al., 2014; Ryan, et al.,  
288 2013). Due to the hurdles which must be overcome to orally deliver a therapeutically effect  
289 dose of a nutraceutical, delivery vehicles are of increasing interest. In particular the utilisation  
290 of food grade ingredients with GRAS (generally regarded as safe) status to create the delivery  
291 vehicle is a promising area of current research. Furthermore, nutraceutical loaded in  
292 pharmaceutical grade delivery vehicle formulations has also emerged in recent years.

### 293 ***Lipid and surfactant based systems***

294 *Liposomes* or *nanoliposomes* are formed when phospholipids self-assemble into a lipid  
295 bilayer due to hydrophobic interactions with the fatty acid chain. *Niosomes* are formed when  
296 non-ionic surfactants assemble into similar structures (**Fig. 3**). Cholesterol is often added to  
297 the formulation as it increases rigidity strength of the membrane and confers steric stability.  
298 Egg yolk- and soy-derived phosphatidylcholines are commonly used to form liposomes,  
299 whereas Tween® 80, Span® 80 and sucrose laurate have been used to form niosomes (Nui, et  
300 al., 2012; Pando, Gutiérrez, Coca, & Pazos, 2013; Shin, Chung, Kim, Joung, & Park, 2013).  
301 There are some characteristic differences between liposomes and niosomes, particularly the  
302 oxidative stability of the particles due to phospholipid oxidative degradation. They are both  
303 suitable for loading of lipophilic nutraceuticals in the inner core of the bilayer membrane, as  
304 well as hydrophilic compounds in the aqueous core.

305 The carotenoid class of phytochemicals show strong anti-oxidative potential, however, they  
306 are highly hydrophobic ( $\log P > 13$ ), which makes them suitable candidates for liposome  
307 formulation. Lutein was found to be most easily incorporated from a series of carotenoids  
308 with the rank order lutein >  $\beta$ -carotene > lycopene > canthaxanthin (Xia, et al., 2015). *In vitro*  
309 release showed lycopene and canthaxanthin exhibited a burst release from liposomes whereas  
310 lutein and  $\beta$ -carotene displayed a sustained release (Tan, et al., 2014). Curcumin is another  
311 lipophilic phytochemical with anticancer and antimalarial activity, which can be incorporated  
312 into liposomes (Shin, et al., 2013). Curcumin was soluble upon *in vitro* lipolysis, and  
313 permeation across Caco-2 monolayers was enhanced compared to free curcumin (Memvanga,

314 Coco, & Pr at, 2013). When delivered in combination with  $\beta$ -arteether (an antimalarial drug),  
315 curcumin loaded liposomes increased survival rate in rodents compared to  $\beta$ -arteether or  
316 curcumin alone, thereby showing potential of the liposome formulation.

317 Due to their structure, liposomes and niosomes have the potential for co-encapsulation. One  
318 example is to have curcumin-loaded cyclodextrin in the core along with a curcumin-loaded  
319 bilayer membrane, a formulation which induced apoptosis in the osteosarcoma xenograft  
320 mouse model (Dhule, et al., 2012). Niosomes have co-encapsulated antioxidant  
321 nutraceuticals, two examples of which are gallic acid (hydrophilic core) with curcumin, and  
322 ascorbic acid (hydrophilic core) with quercetin. Co-encapsulation of two antioxidants  
323 resulted in an improved antioxidant scavenging effect *in vitro* compared to individual  
324 molecules (Tavano, Muzzalupa, Picci, & de Cindo, 2014).

325 *Nanoemulsions* are colloidal dispersions formed from emulsified oils in water (O/W) with a  
326 core-shell structure (**Fig. 3**). Emulsions are commonly found in food and examples are  
327 mayonnaise (O/W) emulsion stabilised by egg yolk lecithin, or butter (W/O) emulsion  
328 stabilised by milk proteins. Nanoemulsions differ from traditional emulsions in a number of  
329 ways: <100 nm in droplet size, high optical clarity and increased stability against flocculation  
330 and coalescence. Nanoemulsions can be fabricated by low-energy (spontaneous formation  
331 due to high concentrations of surfactants) or high-energy (mechanical disruption of oil phase  
332 resulting in nano-sized droplets). They are suitable for loading of lipophilic nutraceuticals,  
333 which are solubilised in the oil phase prior to addition of surfactant and/or mechanical  
334 disruption, resulting in an entrapped bioactive (McClements, 2013a).

335 The loading of phytochemicals into nanoemulsions such as curcumin, genistein, and the  
336 citrus flavonoid, 5-demethyltangeretin (5DT), greatly improved solubility in simulated  
337 intestinal fluid from ~10% to 80% (Aditya, et al., 2013). Formation of emulsions from  
338 essential oils is of particular interest, as it only requires addition of an emulsifier to a  
339 bioactive oil. Many of these oils exhibit antimicrobial and antioxidant activity and have been  
340 investigated to prevent food spoilage (Xue & Zhong, 2014). Lipid oxidation may be a  
341 limitation of nanoemulsions, although addition of an antioxidant like ascorbic acid was found  
342 to reduce lipid hydroperoxide production of soybean oil emulsions (Uluata, McClements, &  
343 Decker, 2015). The size of the lipid droplet effects epithelial cellular uptake of flavonoid  
344 loaded nanoemulsions in HCT116 cells, with 67 nm and 125 nm showing 4-fold higher  
345 uptake compared to 203 nm (Zheng, et al., 2014). Coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) is a powerful

346 antioxidant which is highly hydrophobic and is also required for healthy mitochondrial  
347 function. CoQ<sub>10</sub> formulated into a salmon oil-salmon lecithin nanoemulsion had a 10-fold  
348 increase in plasma concentration compared to water vehicle after oral gavage in Wistar rat  
349 (**Fig. 4**) (Belhaj, et al., 2012). Tangeretin, a citrus flavone, has shown potential as an  
350 anticancer agent when formulated into a nanoemulsion it improved *in vitro* tumour  
351 suppression and reduced incidence of colonic adenomas compared to control in the  
352 azoxymethan/dextran sodium sulphate (AOM/DSS)-induced colitis mouse model (Ting,  
353 Chiou, Pan, Ho, & Huang, 2015).

354 *Solid lipid nanoparticles (SLNs)* are O/W emulsions in which the internal lipid core has been  
355 fully or semi-solidified (**Fig. 3**). SLNs are prepared as a 'hot' nanoemulsions at a temperature  
356 above the melting point of the particular lipid, and temperature is rapidly decreased inducing  
357 lipid crystallisation. SLNs have shown promise as a pharmaceutical oral delivery system  
358 since the early 90's as they combine the advantages of polymeric particles, liposomes, and  
359 emulsions.  $\beta$ -carotene is prone to oxidation and degradation over time and during GIT transit.  
360 When formulated into a SLN (stearic acid emulsified with lecithin), degradation was  
361 prevented for up to 20 days incubation at room temperature (Helgason, et al., 2009).  
362 Curcumin formulated into a SLN showed improved permeability across co-cultured  
363 monolayers of HT29-MTX and Caco-2 cells compared to curcumin formulated in a  
364 nanoemulsion *in vitro*, although only 1% of loaded curcumin permeated (Guri, Gulseren, &  
365 Corredig, 2013). The serum area under the curve (AUC) concentration of a 50mg/kg of either  
366 free curcumin or curcumin-loaded SLNs in a rat model showed that the latter increased the  
367 AUC to 41  $\mu\text{g/mL}$  compared to 1  $\mu\text{g/mL}$  (Kakkar, Singh, Singla, & Kaur, 2011). Resveratrol  
368 is sensitive to light, however, resveratrol-loaded SLNs improved the photostability of the  
369 bioactive and improved its oral bioavailability 8-fold compared to resveratrol solution in a rat  
370 model (Pandita, Kumar, Poonia, & Lather, 2014). SLN also improved oral bioavailability  
371 with other bioactives in rat studies: candesartan cilexetil (a treatment for hypertension) and  $\beta$ -  
372 arteether (second line treatment for malaria) (Dwivedi, et al., 2014; Zhang, Gao, Bu, Xiao, &  
373 Li, 2012).

#### 374 ***Biopolymer based systems***

375 *Polyelectrolyte complexes (PECs)* are formed by electrostatic interaction between oppositely  
376 charged biopolymers e.g. iota carrageenan and protamine (**Fig. 3**). Entrapped PECs are  
377 formed by solubilising nutraceuticals in either the positively or negatively charged

378 biopolymer, and then the opposite charged biopolymer is mixed in. PECs formed between  
379 cationic gelatin and gum Arabic swelled and aggregated at pH 4.5, whereas they were stable  
380 between pH 5.5 – 7.5 and had diameters of 110 – 160 nm (Sarika, Pavithran, & James, 2015).  
381 These PECs may be promising carriers for nutraceuticals, however the swelling at lower pH  
382 poses issues at gastric pH values.

383 GRAS food biopolymers are an abundant source for polyelectrolyte complexation e.g.  
384 amylose, starch, pectin, carrageenan and chitosan. Resveratrol complexed in a gelatin PEC  
385 showed improved anti-proliferative efficacy than free resveratrol and improved  
386 bioavailability in mice compared to free resveratrol solution after intravenous injection  
387 (Karthikeyan, Rajendra Prasad, Ganamani, & Balamurugan, 2013). PECs are a class of  
388 nanoparticles which are not well exploited for nutraceuticals to date with limited *in vivo* data;  
389 on the other hand they have shown promise for therapeutic peptides. For example, insulin and  
390 sCT display improved stability when complexed in PECs (Lu, et al., 2012; Ryan, et al.,  
391 2013).

392 *Hydrogels* are a 3D polymer network with an extremely high abundance of water, which  
393 when appropriately cross-linked can form hydrogel particles in the nano-sized range (**Fig. 3**).  
394 These can be formed from protein gelation via physical, chemical or biochemical methods  
395 which self-crosslink between denatured proteins, whereas, carbohydrate based hydrogels  
396 generally require addition of an ionic cross-linker. They may be composed of GRAS  
397 biopolymers including pectin, alginate, carrageenans, agar, chitosan, gelatin, whey protein,  
398 caseins, soy protein. Hydrogel particles can also contain dispersed oil droplets for carrying  
399 lipophilic molecules. When  $\beta$ -carotene was formulated into each of a conventional emulsion,  
400 a hydrogel, and an oil dispersion-“filled” hydrogel, the latter had an improved release of the  
401 bioactive compared to both formulations due to solubilising effect and increased lipid surface  
402 area (Mun, Kim, & McClements, 2015).

403 Comprehensive preclinical *in vivo* studies of oral delivery of hydrogel particles with  
404 nutraceutical bioactives are lacking similar to PECs, providing an area of under-exploited  
405 delivery vehicles. However, one rodent study suggested that Nile Red-loaded conventional  
406 emulsions had superior oral bioavailability compared to lipid-entrapped hydrogels (Li, Kim,  
407 Park, & McClements, 2012). Caveats were that the diameters of particles were not  
408 comparable (0.36  $\mu\text{m}$  vs. 510  $\mu\text{m}$  respectively) and the loading of a nutraceutical may yield  
409 different results compared to Nile Red. Insulin has been delivered orally *in vivo* in hydrogels

410 in rodents, but there are major issue in how to translate such formulations from rodent models  
411 to clinical trials (Déat-Lainé, et al., 2013). Hydrogel particles formulated from whey protein  
412 and alginate were loaded with insulin and yielded ~2.4% relative bioavailability after intra-  
413 duodenal instillation in a rat model. Polyacrylic acid-derived hydrogels were cross-linked  
414 with poly(L-glutamic acid) and then loaded with insulin (60 IU/kg); this formulation resulted  
415 in a 33% reduction of plasma glucose levels in the streptozotocin (STZ)-induced rat Type 1  
416 diabetes model (Gao, He, Xiao, Zhuang, & Chen, 2013). Finally, insulin was also loaded into  
417 lectin-functionalized and ionically-gelated carboxymethylated *kappa*-carrageenan particulates  
418 and induced 14% relative bioavailability by the oral route compared to subcutaneous  
419 injection in rats (Leong, et al., 2011).

420 *Protein-carbohydrates (self-assembly structures)* are formed from interaction between  
421 anionic polysaccharides and cationic protein surface groups, similar to PECs. Alternatively,  
422 they may be formed by thermal denaturation or aggregation of a globular protein followed by  
423 addition of an ionic polysaccharide, while still relying in part on electrostatic charge (**Fig. 3**).  
424 Vitamin D<sub>2</sub> was bound to β-lactoglobulin and complexed with anionic pectin, resulting in  
425 stable nanoparticles 50-70 nm, which improved the shelf-life stability of the bioactive  
426 compared to storage in water and uncomplexed β-lactoglobulin (Ron, Zimet, Bargarum, &  
427 Livney, 2010). Nanoencapsulation of anthocyanins in a complexation of whey protein and  
428 pectin also resulted in improved protection against thermal degradation (Arroyo-Maya &  
429 McClements, 2015). Due to the amphiphilic nature of proteins, it is possible to load  
430 hydrophilic or lipophilic nutraceuticals inside the self-assembly structured particulates.

431 Curcumin was complexed into chitosan-zein particulates, improved thermal and UV stability  
432 and anti-oxidative scavenging capacity was retained (Liang, et al., 2015). Similarly, when  
433 curcumin was complexed in a carboxymethyl chitosan- kafirin (a prolamin protein from  
434 sorghum) particulates, it again improved UV stability and improved cellular uptake in Caco-2  
435 (Xiao, Nian, & Huang, 2015). EGCG is an abundant polyphenol from green tea and a potent  
436 antioxidant; it had a burst release profile and retained cytotoxicity against cancer cell lines *in*  
437 *vitro* when complexed in a chitosan-caseinophosphopeptide particulate (Hu, Xie, Zhang, &  
438 Zeng, 2014). Furthermore, EGCG complexed in ovalbumin-dextran, saw a small increase in  
439 permeability across Caco-2 monolayers compared to free EGCG (Li & Gu, 2014).  
440 Resveratrol complexed in a zein-based nanoparticle improved oral bioavailability 19-fold

441 compared to resveratrol solution (**Error! Reference source not found.**) and reduced serum  
442 TNF- $\alpha$  (15%) against control in a mouse model of endotoxic shock (Penalva, et al., 2015).

### 443 *Intestinal Absorption Improvements*

444 Although delivery vehicles increase permeability *in vitro* and *in vivo* animal models, there is  
445 still potential to further increase the intestinal permeability. Intestinal permeation enhancers  
446 (PEs) have been researched for oral delivery of hydrophilic peptide drugs in the last two  
447 decades (Choonara, et al., 2014). Improving nutraceutical absorption can be achieved in two  
448 ways (**Fig. 6**); improve mucodiffusion of lipophilic agents (e.g. omega-3 fatty acids and  
449 phytochemicals) using mucolytics; improve paracellular and transcellular permeability of  
450 bioactive peptides, micronutrients and hydrophilic phytochemicals using PEs.

### 451 *Mucolytics*

452 Mucus diffusion enhancers such as *N*-acetylcysteine (NAC), bromelain, and papain hold  
453 potential for nutraceuticals affected by inability to penetrate the small intestinal mucus layer.  
454 Papain is a mucolytic protease found in papaya; when decorated on nanoparticles, it  
455 improved permeation and reduced mucus viscosity *in vitro* (Müller, et al., 2012). Bromelain,  
456 a pineapple stem mucolytic enzyme, was formulated on the surface of nanoparticles and  
457 compared against papain for *in vitro* mucus permeation resulting in enhanced penetration:  
458 bromelain > papain > conventional nanoparticles (Pereira de Sousa, et al., 2015). Papain  
459 decorated nanoparticles were also shown to penetrate into deeper mucus layers, when  
460 delivered by oral gavage in a rat model, with higher retention within the jejunum (Müller,  
461 Perera, König, & Bernkop-Schnürch, 2014). This is of particular interest, as the jejunum is  
462 the main target for nutraceutical bioactive absorption.

463 NAC is an antioxidant nutritional supplement and it is also used as a mucolytic agent by  
464 breaking disulphide bonds (Yuan, et al., 2015). When an intestinal PE, tetradecyl maltoside  
465 (TDM) was tested on Caco-2- and mucus-producing HT29-MTX-E12 monolayers, it was  
466 shown that NAC-pre-treatment on E12 monolayers resulted in comparable apparent  
467 permeability ( $P_{app}$ ) values of salmon calcitonin across Caco-2 and E12 (Petersen, Nielsen,  
468 Rahbek, Guldbandt, & Brayden, 2013). The blood serum levels of fluorescein  
469 isothiocyanate–dextran MW 4000 (FD-4, a fluorescent marker molecule for the paracellular  
470 route) was improved 2.8-fold upon intra-jejunal administration of NAC (5% w/v) in rats, and

471 showed a mucolytic effect up to 60 minutes (Takatsuka, Kitazawa, Morita, Horikiri, &  
472 Yoshino, 2006).

473 The application of mucolytic agents also holds promise for lipophilic nutraceuticals, which  
474 interact with glycoproteins and lipids in mucus (Sigurdsson, et al., 2013). This interaction  
475 reduces the likelihood of epithelial permeation as mucus is continuously turned over and  
476 would result in the bioactive being washed away. Whereas mucolytics reduce this risk of this  
477 occurring by enhancing mucus penetration. Mucolytics are most often investigated in the  
478 context of airway mucus in cystic fibrosis, where NAC is used at high concentrations.  
479 Recently a synthetic thiol-carbohydrate (methyl 6-thio-6deoxy- $\alpha$ -D-galactopyranoside) was  
480 found to be a more potent mucolytic (Yuan, et al., 2015). Co-administration of lipophilic  
481 nutraceuticals and mucolytics in the context of an enteric coated oral dosage form may  
482 therefore control release in the small intestine, improve mucus penetration and improve  
483 absorption.

#### 484 ***Intestinal Permeation Enhancers (PEs)***

485 PEs can increase oral bioavailability assuming that the nutraceutical can also survive liver  
486 first pass metabolism. Of these, the medium chain fatty acid (MCFA) sodium caprate (C<sub>10</sub>) is  
487 well established as a food additive and was a component of an antibiotic suppository once  
488 marketed in Sweden and Japan (Maher, et al., 2014). Ideally, PEs should be  
489 pharmacologically inert, have excipient or Generally-Regarded-As-Safe (GRAS) status, and  
490 have a history of use in man. PEs are often used for peptide oral delivery with candidates  
491 including sCT, insulin, glucagon-like Peptide 1 (GLP-1) analogues, and octreotide. For  
492 example, The technology of Enteris Biopharma (New Jersey, USA) is currently in Phase II  
493 with a PE (an acyl carnitine), a peptidase inhibitor (citric acid) and parathyroid hormone  
494 (Stern, Mehta, & Carl, 2013). The technology of Chiasma (Jerusalem, Israel) recently  
495 completed Phase III for oral octreotide and it comprises a PE (caprylic acid) in a water-in-oil  
496 suspension (Tuvia, et al., 2012). Merrion Pharmaceuticals (Dublin, Ireland) uses a  
497 gastrointestinal permeation enhancement technology (GIPET™) built around the PE (C<sub>10</sub>) in  
498 matrix tablets and it completed an oral Phase I study with GLP-1 (Karsdal, et al., 2015).  
499 Finally, the technology of Oramed (Jerusalem, Israel) is has reached Phase IIb for oral insulin  
500 and it comprises a PE (EDTA) and soy-bean trypsin inhibitor (Lewis & Richard, 2015).

501 MCFA-based PEs act by re-organising proteins at the epithelial tight junction (**Fig. 6**), (e.g.  
502 tricellulin and claudin 5), and by mild detergent fluidizing effect on the plasma membrane  
503 (Brayden, Gleeson, & Walsh, 2014; Krug, et al., 2013). This allows for poorly permeable  
504 molecules to either transiently permeate across tight junctions, or possibly to be entrapped in  
505 mixed micelles with capacity to cross lipid bilayers. PEs generally cause a reduction of  
506 transepithelial electrical resistance (TEER) using *in vitro* and *ex vivo* intestinal epithelial  
507 models. This reduction suggests an opening of tight junctions or perturbation of the epithelia.  
508 They have shown significant increase in apparent permeability of [<sup>14</sup>C]-mannitol (a marker  
509 for paracellular transport) and FD-4 across isolated intestinal mucosa on the Ussing chamber  
510 model. For example, C<sub>10</sub> showed an increase in FD-4 permeability in Caco-2 monolayers, an  
511 8-fold increase across isolated colonic mucosa and a 2-fold increase in colonic instillations *in*  
512 *vivo* (Brayden & Walsh, 2014). This effect is also associated with a temporary perturbation of  
513 the intestinal epithelia. However this mild damage induced by MCFA such as C<sub>10</sub> is quickly  
514 repaired, which was shown after *in situ* intestinal injections in rats (Wang, Maher, &  
515 Brayden, 2010). The continuing progress of C<sub>10</sub> and other PEs in clinical trials for oral  
516 peptides is also addressing safety aspects that may be associated with increased oral  
517 bioavailability.

518 Many of these PEs are commonly used in food processing with GRAS status or are of food  
519 origin. Candidates PEs include coco-glucosides (CG), chitosan derivatives, bromelain,  
520 EDTA, oleic acid, alkyl maltosides, medium chain fatty acids (MCFA) and sucrose esters  
521 (Aguirre, et al., 2014; Szűts & Szabó-Révész, 2012). Furthermore, many isolated food  
522 components can modulate tight junction integrity *in vitro* by enhancing permeability by  
523 opening tight junctions. Although many of these PEs work especially well in the colon, the  
524 target site for absorption of nutraceuticals is predominantly the jejunum. The capacity for  
525 enhancement was tested in different regions of the rat intestine with C<sub>10</sub> and insulin using an  
526 *in situ* loop model, which showed a rank order of plasma glucose reduction: colon > ileum >  
527 jejunum > duodenum (Morishita, Morishita, Takayama, Machida, & Nagai, 1993). The apical  
528 membrane of the small intestine is often exposed to bile salts and fatty acids resulting in  
529 resistance to surfactant perturbation compared to colon. TEER decreased in isolated rat  
530 jejunum and ileum using TDM and CG, however, there was no increase in the permeability  
531 of FD-4. This lack of effect from these PEs may be due to the marker MW of 4kDa, because  
532 when HT-29/B6 monolayers were treated with C<sub>10</sub>, a 3-fold increase in fluorescein (330Da)  
533 was detected (Krug, et al., 2013). Isolated rat jejunum was treated with C<sub>10</sub> and sodium salt of

534 10-undecylenic acid (uC<sub>11</sub>, an antifungal agent), a 1.4-3.6-fold increase was shown for FITC-  
535 labelled IPP (714 Da) and LKP (745 Da) (Brayden & Walsh, 2014; Gleeson, et al., 2015).  
536 Therefore, using the appropriate PE in the jejunum holds the potential to improve the  
537 permeability of nutraceuticals and potentially improve oral bioavailability. On the other hand,  
538 kaempferol, curcumin and daidzein may have potential for useful application in inflammatory  
539 bowel disease (Kosińska & Andlauer, 2013), where they can repair membranes and reduce  
540 abnormally high epithelial permeability.

## 541 **Conclusions**

542 Nutraceuticals offer the opportunity to prevent onset and escalation of lifestyle-associated  
543 diseases due to their range anti-oxidative, anti-inflammatory, anti-hyperlipidemic and  
544 antihypertensive activities. Progress has been made in adopting Pharma oral delivery  
545 strategies to improve solubility, stability and permeability of nutraceutical bioactives. In  
546 particular, solubilisation technologies can overcome issues associated with the delivery of  
547 hydrophobic compounds (e.g. resveratrol and curcumin) using lipid-based systems. There has  
548 often been too much emphasis put on the *in vitro* assays suggesting anti-oxidative, anti-  
549 inflammatory and anti-hypertensive actions of nutraceuticals. One of the main issues is that  
550 many nutraceuticals are not tested in *in vivo* preclinical studies, it is therefore impossible to  
551 assess whether they are predictive of efficacy. However, at least some nutraceuticals can be  
552 efficiently formulated and show promising data in rodent models (Dhule, et al., 2012;  
553 Kakkar, et al., 2011; Karthikeyan, et al., 2013; Memvanga, et al., 2013; Pandita, et al., 2014;  
554 Penalva, et al., 2015). In relation to clinical trials of nutraceuticals, these are costly and rare,  
555 difficult to design, and display conflicting results. For example, opposing effects have been  
556 detected in man for antihypertensive tripeptide, IPP, although a meta-analysis concluded that  
557 it has a hypotensive effect in pre-hypertensive subjects (Xu, Qin, Wang, Li, & Chang, 2008).  
558 A renewed emphasis on clinical data is required to establish a relationship between  
559 nutraceuticals in delivery systems and possible health benefits. **However, to obtain a health  
560 claim, different countries have different regulations. For example, the US Food and Drug  
561 Administration (FDA) has granted category ‘A’ status to soy with the health claim “reduction  
562 of the risk of heart disease”. Yet in Europe, the European Food and Safety Authority (EFSA)  
563 rejected a similar application due to lack of confirmatory data establishing a reduction in  
564 blood LDL-cholesterol due to the intake of isolated soy protein (Girgih, Myrie, Aluko, &  
565 Jones, 2013; Mannarino, Ministrini, & Pirro, 2014).**

566 Absorption enhancement is an area yet to be used for improving oral nutraceutical delivery.  
567 Mucolytics and PEs hold potential to improve absorption of both lipophilic and hydrophilic  
568 nutraceuticals, particularly as many of these are food-grade and/or food additives. However,  
569 there is a question regarding the safety of PEs due to perturbation of the intestinal epithelia  
570 through mild detergent-based surfactant effects, even for agents with GRAS status or with a  
571 history of use in man (Chassaing, et al., 2015). Although toxicity of various PEs under acute  
572 dosing regimens has not been found in clinical trials for oral peptides to date (Melmed, et al.,  
573 2015; Tuvia, et al., 2014), PEs would not be suitable for administration to patients with  
574 inflammatory bowel- or coeliac disease (Laukoetter, Nava, & Nusrat, 2008) and chronic  
575 dosing studies are yet to be investigated for most PE examples (McCartney, Gleeson, &  
576 Brayden, 2016). There is therefore potential to harness strategies in oral drug delivery to  
577 nutraceutical delivery using established excipient and GRAS-listed reagents. This will result  
578 in an overall improved knowledge of delivery systems allowing for development of oral  
579 nutraceutical systems for important candidate molecules.

## 580 **Acknowledgements**

581 This study was supported by an Irish Department of Agriculture Food Institutional Research  
582 Measure (FIRM) grant ‘NUTRADEL,’ grant number 11F042.

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937 **Figure Captions**

938 **Fig. 1** – Overview of food-derived bioactive compounds being investigated as nutraceuticals;  
939 Fatty acids (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)), bioactive  
940 peptides (Ile-Pro-Pro (IPP), and Leu-Lys-Pro, (LKP)), micronutrients (Vitamins B6, C and  
941 D3) and phytochemicals (the remainder). Adapted with permission (Pan, et al., 2009).

942 **Fig. 1** - Comparison of the Biopharmaceutics Classification System (BCS) and the recently  
943 proposed Nutraceutical Bioavailability Classification System (NuBACS) (Larregieu & Benet,  
944 2014; McClements, et al., 2015).

945 **Fig. 2** – Examples of food-based delivery systems currently being investigated for delivery of  
946 nutraceuticals. Lipid and surfactant-based vehicles including liposomes, niosomes,  
947 nanoemulsions and solid lipid nanoparticles (**Error! Reference source not found.**) are  
948 suitable for loading lipophilic bioactives (curcumin and resveratrol). Biopolymer-based  
949 vehicles including polyelectrolyte complexes, hydrogel particles and protein-polysaccharide  
950 structures are suitable for loading hydrophilic bioactives such (EGCG and ascorbic acid).

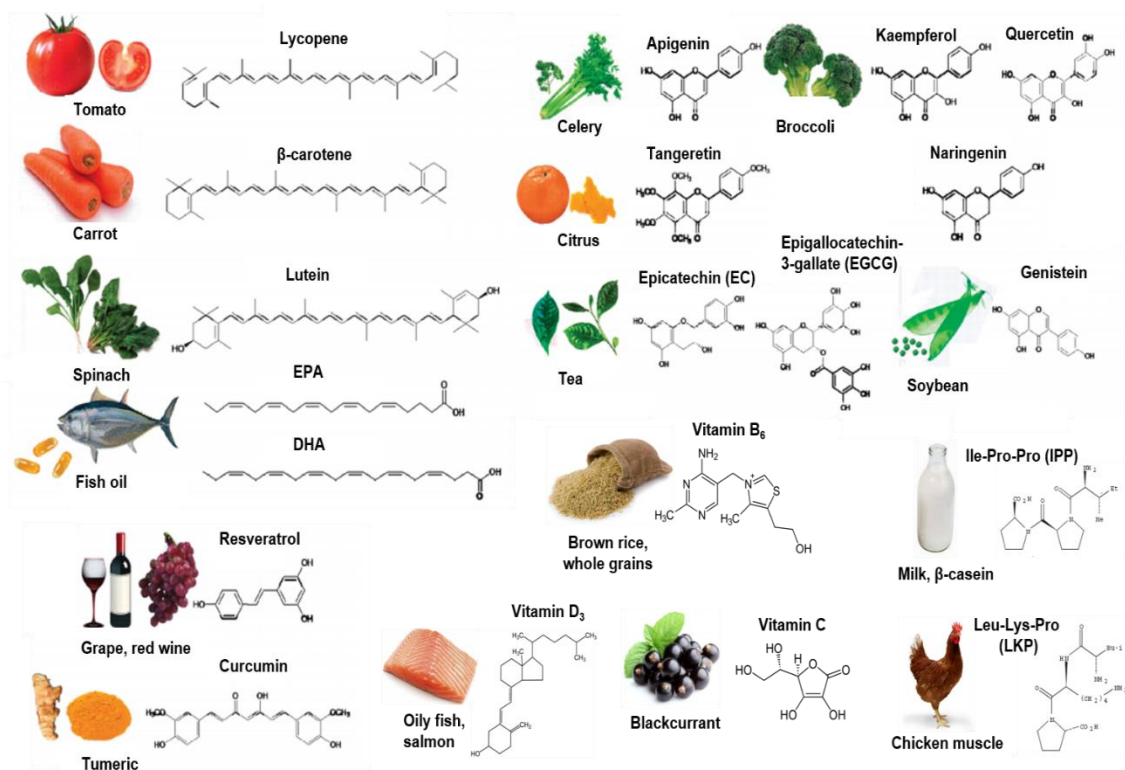
951 **Fig. 3.** – CoQ<sub>10</sub> plasma concentration after oral delivery in a nanoemulsion in rats. CoQ<sub>10</sub>  
952 plasma AUC 26.14 (CoQ<sub>10</sub> nanoemulsion) > 15.38 (commercial oil mixture) > 12.79 (oily  
953 mixture + CoQ<sub>10</sub>) > 2.32 (water and oily mixture). The commercial oil mixture consisted of

954 soybean oil and 6% CoQ<sub>10</sub>. Oily mixture consisted of same constituents of nanoemulsion  
 955 without water sonication. Reproduced with permission (Belhaj, et al., 2012).

956 **Fig. 4.** – Resveratrol plasma concentration significantly improved after oral delivery of  
 957 resveratrol loaded zein-based nanoparticle (■) compared to resveratrol solution (●) and  
 958 resveratrol suspension (▲) in an endotoxic shock mouse model. The dose was 15 mg/kg and  
 959 resveratrol plasma AUC was 5.17 > 0.60 > ND respectively. Adapted with permission  
 960 (Penalva, et al., 2015).

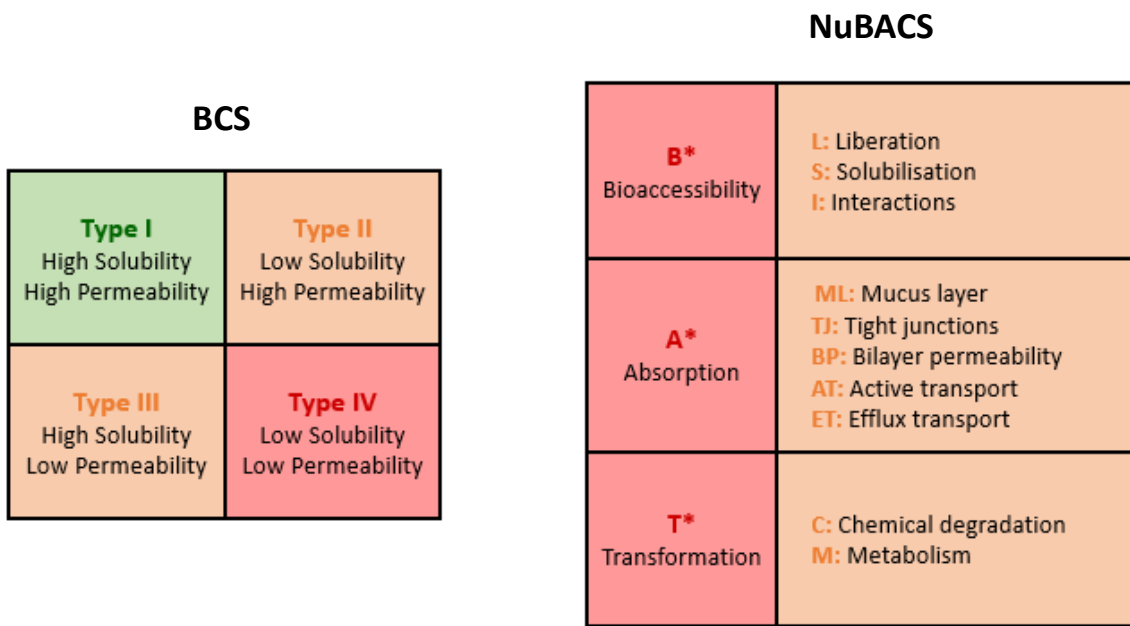
961 **Fig. 5** – The effect of PEs on nutraceutical compounds by improving mucodiffusion by  
 962 mucolytic agents and improving permeability. Adapted with permission (Gleeson, et al.,  
 963 2015).

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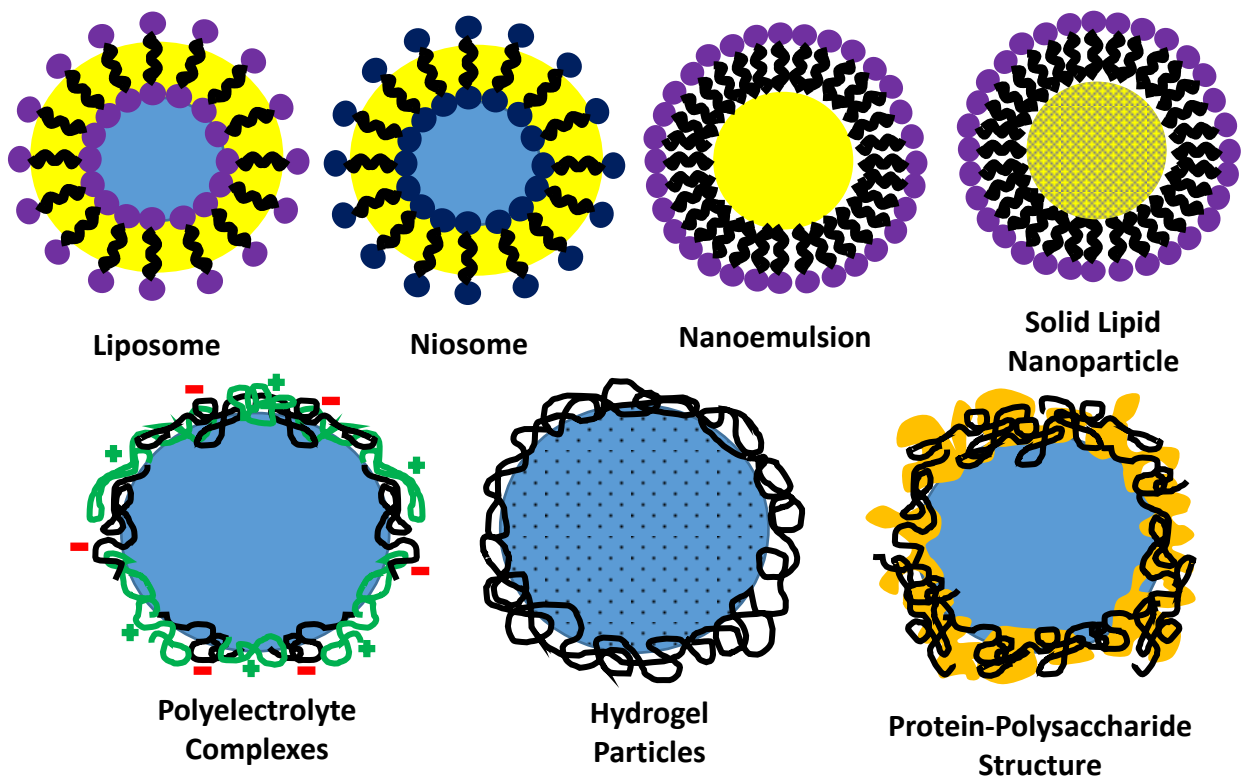
965

966 **Fig 1**



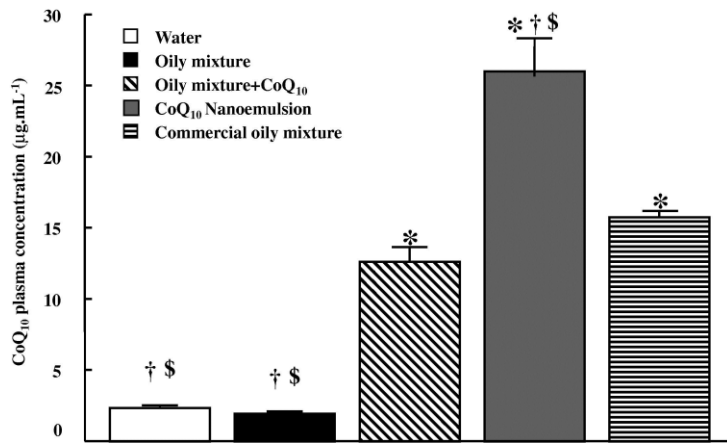
967

968 **Fig 2**



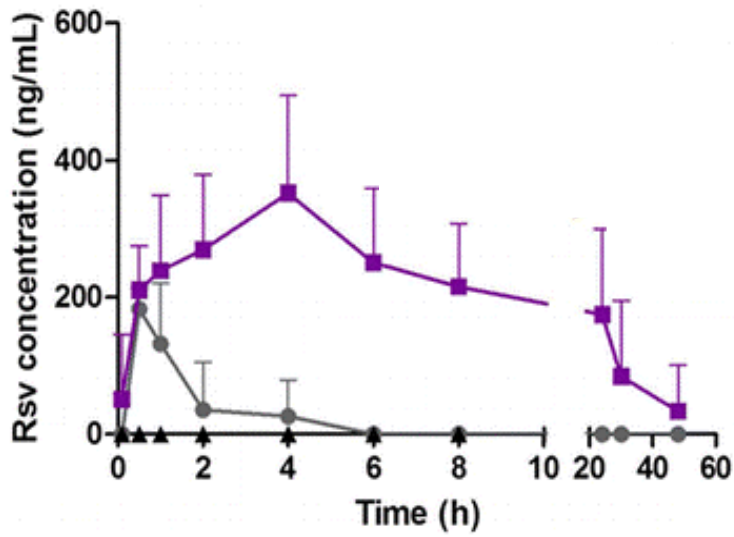
969

970 **Fig 3**



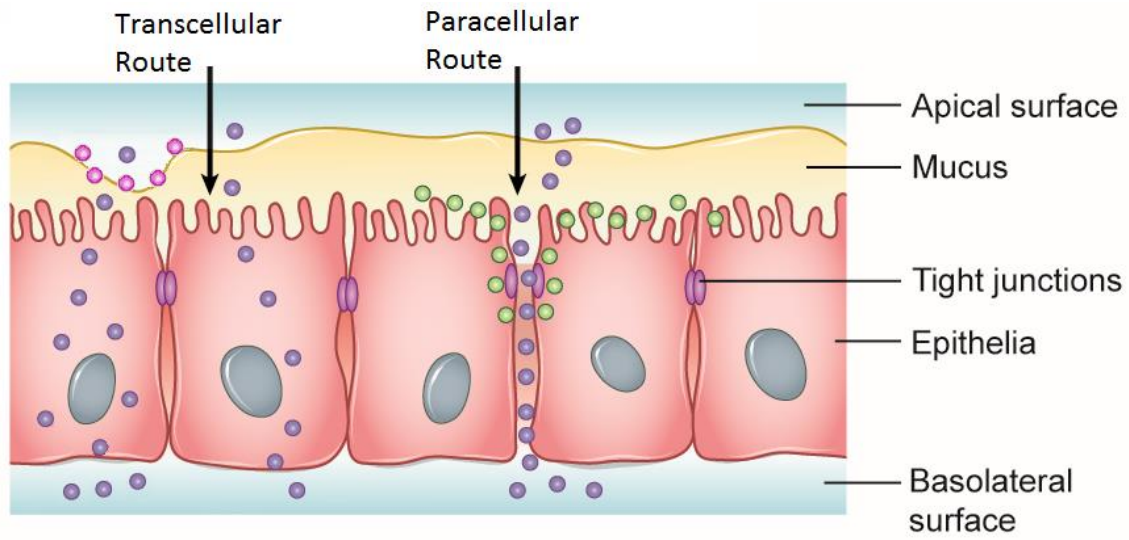
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972 **Fig 4**



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974 **Fig 5**



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976 **Fig 6**

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981 **Table 1** – Overview of nano-sized delivery vehicles created from food-based ingredients  
 982 which have been formulated containing nutraceutical bioactives.

Type	Primary constituent	Food-based vehicle (nanoparticle material)	Nutraceutical (loaded in nanoparticle)
Liposomes and Niosomes	Phospholipid or Non-ionic surfactant	Egg yolk phosphatidylcholine (Memvanga, et al., 2013; Tan, et al., 2014; Xia, et al., 2015); Sucrose laurate (Pando, et al., 2013; Tavano, et al., 2014)	Carotenoids (Tan, et al., 2014); Curcumin (Dhule, et al., 2012; Memvanga, et al., 2013; Nui, et al., 2012; Shin, et al., 2013);
Nanoemulsions	Surfactant and oil	Essential oils (Gulotta, Saberi,	Essential oils (Gulotta, et al.,

		Nicoli, & McClements, 2014; Xue & Zhong, 2014); <b>Medium chain tryglyceride</b> (Gulotta, et al., 2014; Zheng, et al., 2014); <b>Soybean lecithin</b> (Aditya, et al., 2013); $\beta$ -Lactoglobulin (Zheng, et al., 2014)	2014; Xue & Zhong, 2014); <b>5-DT</b> (Zheng, et al., 2014); <b>Curcumin</b> (Aditya, et al., 2013); <b>Genistein</b> (Aditya, et al., 2013); <b>omega-3 FAs</b> (Gulotta, et al., 2014);
<b>Solid Lipid Nanoparticles (SLN)</b>	Semi- or fully solidified lipid	Soy lecithin (Guri, et al., 2013; Kakkar, et al., 2011; Pandita, et al., 2014); <b>palmitic acid</b> (Kakkar, et al., 2011); <b>stearic acid</b> (Pandita, et al., 2014);	Curcumin (Guri, et al., 2013); $\beta$ -Carotene (Helgason, et al., 2009); Resveratrol (Pandita, et al., 2014)
<b>Polyelectrolyte complexes (PEC)</b>	Oppositely charged biopolymers	$\beta$ -Lactoglobulin (Hosseini, Emam-Djomeh, Sabatino, & Van der Meeren, 2015); <b>sodium alginate</b> (Hosseini, et al., 2015); <b>gelatin</b> (Karthikeyan, et al., 2013; Sarika, et al., 2015); <b>Arabic gum</b> (Sarika, et al., 2015)	Curcumin (Hosseini, et al., 2015); $\beta$ -carotene (Hosseini, et al., 2015); Resveratrol (Karthikeyan, et al., 2013)
<b>Hydrogels</b>	Denatured proteins or ionically crosslinked polysaccharides	Rice starch (Mun, et al., 2015); <b>caseinophosphopeptide</b> (Hu, et al., 2014); <b>chitosan</b> (Hu, et al., 2014); <b>Whey protein isolate</b> (Sung, Xiao, Decker, & McClements, 2015); $\beta$ -Lactoglobulin (Li, et al., 2012)	$\beta$ -Carotene (Mun, et al., 2015); <b>Epigallocatechin gallate (EGCG)</b> (Hu, et al., 2014);
<b>Protein-carbohydrate (self-assembly structures)</b>	Globular proteins and ionic polysaccharides	$\beta$ -Lactoglobulin (Ron, et al., 2010); <b>pectin</b> (Ron, et al., 2010); <b>zein</b> (Liang, et al., 2015); <b>kafirin</b> (Xiao, et al., 2015); <b>ovalbumin</b> (Li & Gu, 2014)	<b>Vitamin D<sub>2</sub></b> (Ron, et al., 2010); Curcumin (Liang, et al., 2015; Xiao, et al., 2015); EGCG (Hu, et al., 2014; Li & Gu, 2014)