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Engineering oral delivery of hydrophobic bioactives in real world scenarios

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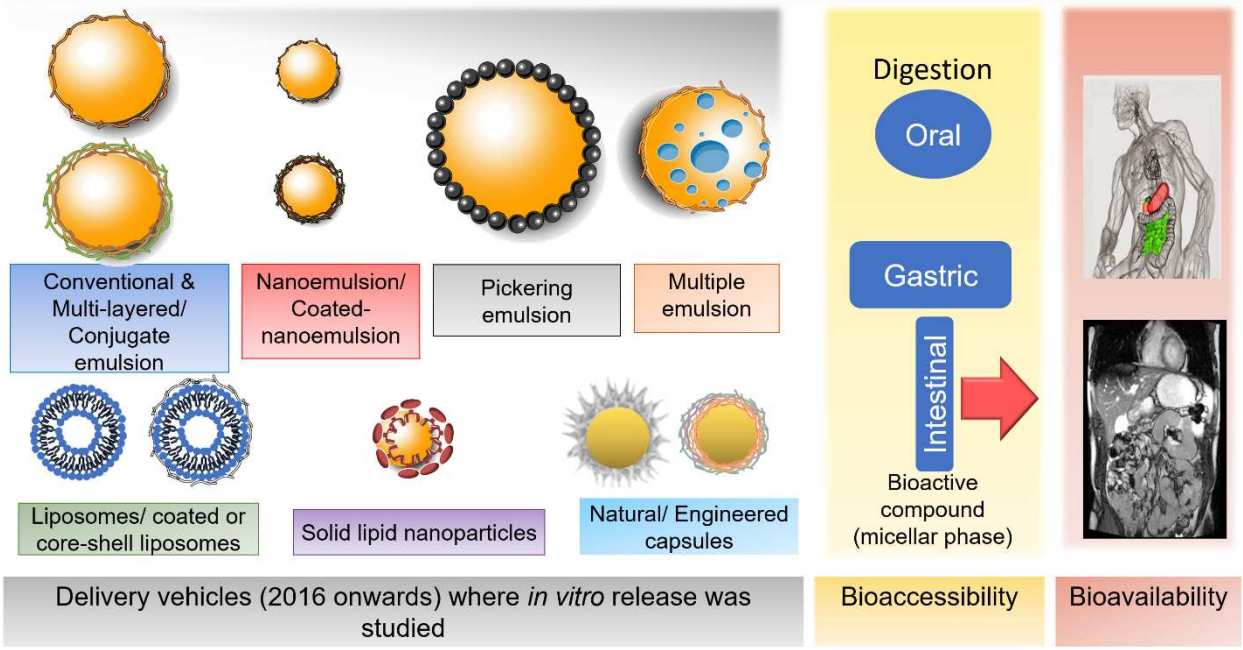
Abstract

Bioactive compounds, often hydrophobic in nature tend to degrade during processing outside or inside the body with rapid clearance rates, resulting in poor bioavailability. In this review, we survey recent scientific advances in lipid-based colloidal delivery systems (conventional/ nanoemulsions, Pickering emulsions, multi-layered and multiple emulsions, coated/ uncoated-liposomes, natural microcapsules *etc.*) that have been employed to improve the bioaccessibility and/or bioavailability of hydrophobic bioactive compounds. Specifically, we use a 'delivery to design' approach *i.e.* we discuss the desired release kinetics of the bioactive compounds first. This enables us to paint a more reasonable image of the optimal microstructure sought in the gastrointestinal tract, in order to lay out the design principles for fabricating the next generation of oral delivery carriers. Finally, we outline the challenges for translation of the oral delivery vehicles that show promises in bench-top experiments and how multidisciplinary approaches might help overcoming some of those challenges.

Keywords. Pickering emulsion; nanoemulsion; mathematical model; bioaccessibility; bioavailability; release

Graphical abstract



Highlights

- Colloidal systems continue to be created to deliver hydrophobic bioactive compounds
- Mathematical models are crucial to derive kinetic parameters of the bioactives
- Great emphasis has been given on *in vitro* release of bioactives and bioaccesibility
- Bioavailability studies of hydrophobic bioactive compounds are scarce in literature
- A ‘delivery to design’ can be employed for future tailoring of delivery systems

Introduction

Globally, functional foods, nutraceuticals and dietary supplements containing bioactive compounds that offer health-promoting properties are increasingly becoming a part of our daily diets. The global nutraceutical market comprised of functional foods and dietary supplements was worth approximately $469 billion in 2019, which is forecasted to reach around $671 billion by 2024 [1]. The growth of this sector is largely fueled by the increasing prevalence of food-linked chronic diseases such as obesity, cardiovascular diseases and cancers and the rise in health-conscious consumers seeking health benefits beyond the provision of basic nutrition from ingested foods.

Bioactive compounds include diverse classes of nature-engineered chemicals that are present in relatively small proportions in foods and show biological activity [2] with the ability to modulate one or more metabolic functions [3]. In other words, bioactive compounds are known to promote health by preventing, delaying the onset of or treating diseases. Often, the bioactive compounds of interests have poor aqueous solubility or tend to crystallize, posing severe challenges in absorption of these compounds to the intestinal enterocytes and finally into the lymphatic system [4]. For instance, curcumin, which is a potent bioactive used in literature has a C log *P* (calculated octanol-water partition coefficient [5]) value ranging between 2 and 3 [6] and consequently, has moderate lipophilic properties and poor aqueous solubility [7]. On the other hand, another extensively investigated group of bioactive compounds are the carotenoids, which have a high degree of lipophilicity (C log $P > 10$) [8] and require lipid-based carriers [7] to render them dispersible in aqueous media at the site of action. Also it is known from drug delivery studies that less polar and more lipophilic compounds with C log $P > 3.0$ pose risks for adverse toxicity effects in *in vivo* trials [9]. Other challenges that limit the use of these bioactive compounds as 'ideal therapeutics' in real-world scenarios include: limited chemical stability during incorporation into food systems or during gastrointestinal transit (pH, ions, binding to nutrients/ enzymes) post ingestion; high

metabolic conversion rate and/or rapid elimination / clearance from the body resulting in limited bioavailability and distribution in the relevant tissues [10-12].

To address these delivery challenges, myriad lipid-based colloidal delivery vehicles such as nanoemulsions [13], liposomes [14], cubosomes, [15], micelles [16], oleogels [17], hydrogel particles [18], nanoparticles [19] *etc.* have transformed delivery vehicle research into a mature and rapidly expanding domain. Attempts to design novel colloidal carriers for using bench-top experiments date back over three decades and continue to offer significant promise for further exploration. In particular, we recommend previous articles of importance that have reviewed literature in emulsion-based delivery vehicles and excipient emulsions [20-22] and also drug delivery-inspired approaches for designing effective delivery systems for food applications [23]. Nevertheless, the central importance of bioaccessibility and bioavailability of these encapsulated bioactive compounds have only recently been emphasized and relatively few studies are devoted to address the complexity of this topic. Bioaccessibility refers to the fraction of a bioactive compound that is released from its parent colloidal carrier within the gastrointestinal tract to the micellar phase, typically based on *in vitro* procedures [24]. Bioavailability refers to the fraction of the ingested bioactive that is actually available at the site of action e.g. organs, tissue, cells *etc*, and is determined through *in vivo* assays and clinical trials. In pharmacological terms, bioavailability refers to a series of closely integrated processes; specifically, liberation, absorption, distribution, metabolism and excretion (LADME) [25] and bioaccessibility is a vital factor in bioavailability. Analytical methods for measuring bioaccessibility and bioavailability are described elsewhere [26].

With these definitions in mind, the aim of this review is to critically examine only the recent advances (*i.e.* in the last five years) in colloidal delivery vehicles focusing on the release, bioaccessibility and bioavailability of the bioactive compounds; the systems covered are summarized in **Figure 1**. Firstly, we discuss the delivery vehicles that have successfully demonstrated the release of the encapsulated bioactives *in vitro* into

gastrointestinal media or physiologically-relevant buffers and/or effective plasma concentration of these compounds in pre-clinical settings. This has enabled to set the scene for the latest advances in colloidal systems for delivering hydrophobic compounds. Unlike previous reviews in the field dealing with 'design to delivery' approach, we have then used a 'delivery to design' approach to first think of the delivery environment in order to design the optimized vehicle and detail our opinion for future tailoring of delivery systems with high efficacy, safety and stability. In other words, we discuss the downstream features *i.e.* release profiles in systematic circulation and associated kinetic models in order to identify the key microstructural features needed in the gastrointestinal tract to achieve those desired release profiles. This methodology of delivery to design' from the desired release kinetics to identify the upstream optimal features of the colloidal carrier can provide powerful insights into the design principles for developing the next generation of orally administered delivery vehicles.

Since this review focuses on bioactive compounds with functional foods/ dietary supplements as target application areas, we only focus on carriers that are of colloidal length scale before ingestion. Using literature on nanoemulsions or solid-lipid nanoparticles in this review can instill a sense of selection bias. We note that many of the nanoemulsions used for bioactive delivery in literature in the past half-decade show mean droplet sizes/ particle sizes just below a micron, which suggests that they are not true 'nanomaterials'. Therefore, based on European Commission's 2011 Recommendation, the Novel Food Regulation, and the Biocide Regulation [27], we have excluded articles dealing with nanoemulsions or liposomes that have mean size of < 100 nm. Additionally, non-lipidic, biopolymer-based carriers such as microgels, complexes, micro- / nano-capsules that valorize binding aspects of specific proteins such as zein, lactoferrin *etc.* rather than solubilizing the bioactive compounds are beyond the scope of this review as these have been adequately covered in the previous reviews [18, 28, 29]. Delivery challenges of specific bioactive compounds such as polyphenols, curcumin [30] and resveratrol [31] and carotenoids such as lutein [32] are

also described elsewhere.

Setting the scene: Review of colloidal delivery vehicles

Scientific interests in the food colloid community have essentially opted for a few exemplar bioactive compounds, namely, curcumin, carotenoids (chiefly *β*-catotene and others, such as fucoxanthin, lycopene), poorly water-soluble vitamins (vitamins D2, D3 and E), and *ω*-3 fatty acids. **Figure 1** shows the landscape of colloidal delivery vehicles that have surfaced in the past five years to encapsulate and examine the release profiles of these afore-mentioned bioactive compounds.

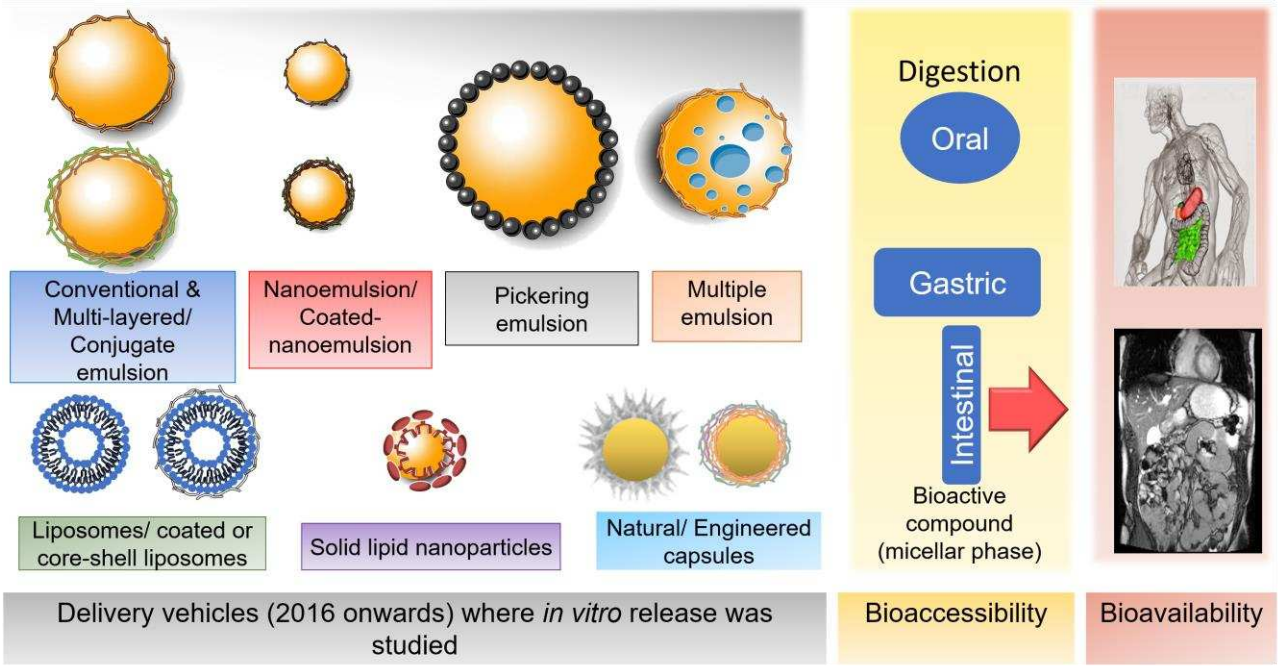


Figure 1| **Library of colloidal delivery vehicles that surfaced since 2016.** Most articles focussed on design of colloidal delivery vehicles including conventional, multi-layered or conjugate emulsions [33-41], nanoemulsions and biopolymer-coated nanoemulsions [36, 42-47], Pickering emulsions [48-54], multiple emulsions [55, 56], liposomes, niosomes and coated liposomes [57-61], solid lipid nanoparticles [62], natural or engineered capsules [63, 64], which have been assessed for *in vitro* release of the bioactive compounds in relevant buffer or free fatty acids (FFA) release during *in vitro* gastrointestinal digestion. Not all of these have performed *in vitro* bioaccessibility to check the quantity or release kinetics of the bioactive compound in the micellar phase. Bioaccessibility studies were performed using delivery vehicles, such as conventional/ multi-layered emulsions [33, 34, 36-40], nanoemulsions/ biopolymer-coated nanoemulsions [36, 42-46], Pickering emulsions [48, 51-54], multiple emulsions [55, 56], liposomes [60], and solid lipid nanoparticles [62] in the past five years. Limited bioavailability studies using *in vivo* or *ex vivo* mice trials to check the level of bioactive compound in the plasma or distribution of the bioactives in relevant organs have been performed by only administering conventional or conjugate emulsions [40, 65] and nanoemulsions [47, 66, 67]. The bioactive compounds encapsulated by these delivery vehicles mainly included carotenoids (*β*-carotene), curcumin, vitamins (D2, D3 and E) and others. Any delivery vehicles that have been in literature having mean hydrodynamic diameter below < 100 nm are excluded. In addition, delivery vehicles tested using administration routes other than oral are excluded.

Bioactive delivery vehicles of different geometries, hydrophobicities, surface properties and other biophysical features have evolved, ranging from conventional and nanoemulsions without or with additional coating materials, multiple emulsions, liposomes with or without biopolymeric coatings, niosomes, solid-lipid nanoparticles to Pickering emulsions (particle-stabilized emulsions) and natural microcapsules such as plant spores. Many of these delivery vehicles in **Figure 1** have investigated bioaccessibility of the encapsulated compounds [33, 34, 36-40, 42-46, 48, 51-56, 60, 62].

Harmonized *in vitro* static [68, 69] and semi-dynamic [70] protocols on simulating gastrointestinal fluids are proving to be invaluable for understanding the release of bioactive compounds from the delivery vehicles in the micellar phase that would otherwise be time-consuming to allow comparison of release between different vehicles. In addition, food colloid researchers are now reporting bioaccessibility kinetics of the compound from the delivery vehicles such as emulsions, nanoemulsions and solid lipid nanoparticles [33, 36, 37, 62] using first-order model *i.e.* assuming a time independent rate constant (see **Table 1** for the mathematical models used to derive relevant kinetic parameters [71, 72]).

On the other hand, literature from the drug delivery field dealing with bioactive compounds loaded in liposomes, core-shell liposomes, niosomes with or without being encapsulated within biopolymeric hydrogels [59, 73, 74] determine release profiles using dialysis methods in physiologically relevant buffers rather than *in vitro* digestion models. Ideally, one should consider combining the *in vitro* digestion with dialysis methods, which is rarely done. In this way, the quantification of the micellar phase post digestion would provide bioaccessibility information and dialysis experiments using appropriate molecular cut offs would provide some indirect indication of the transport phenomena. In comparison to bioactive delivery, the drug delivery studies also fit the release profiles with more sophistical mathematical models taking into consideration the release mechanism. As can be seen from **Table 1**, the Higuchi model involving a Fickian diffusion-based release, and/or the

Korsmeyer-Peppas model that also contains a non-Fickian mass transport based release component and a geometric parameter describing the delivery vehicle have been commonly used.

Table 1| **Mathematical models for fitting release data.** Brief description of the mathematical models that can be used to fit observed release of the bioactive compound from a delivery vehicle into a physiological/ *in vitro* gastrointestinal fluid based on drug delivery studies.

Zeroth-order model	**First-order model**	**Higuchi model**
The plasma concentration has a linear relationship with time during the elimination phase *i.e.* a constant amount of the nutraceutical is eliminated per unit time.	This model suits more to water-soluble nutraceuticals encapsulated in a porous delivery vehicle, such that the amount of nutraceutical released decreases per unit time.	Higuuchi model is relevant to study the release of water soluble and lower soluble nutraceuticals that are incorporated in semi-solid and/or solid delivery vehicle and involves a diffusion process based on Fick's law.
$M_t=M_0+k_0t$	$logC_t=logC_0-K_t/2.303$	$M_t=K_Ht^{1/2}$
M_t: amount of nutraceutical released in time t M_0: initial amount of nutraceutical in the solution k_0: zero-order release constant t: time	C_t: concentration of nutraceutical dissolved in time t C_0: initial concentration of nutraceutical K: first order rate constant t: time	K_H: Higuchi dissolution constant From Higuichi model, **Baker-Lonsdale model** can be derived that is only relevant for spherical delivery vehicle: $f_t=\frac{3}{2}\left[1-(1-\frac{M_t}{M_\infty})^{\frac{2}{3}}\right]-\frac{M_t}{M_\infty}=kt$ f_t: fraction of nutraceutical released in time t M_∞: amount of nutraceutical released at infinite time k: release rate constant
Hixson-Cromwell model	**Korsemeyer-peppas model/ Power law model**	**Weibull model**
This model takes the surface-volume relation of the delivery vehicle and assumes that the release rate is limited by the dissolution rate of the nutraceutical particles and not by the diffusion that might occur through the delivery vehicle in which the nutraceutical is encapsulated	This is a semi-empirical model relating the release of nutraceutical exponentially to time.	This is a general empirical equation adapted to the dissolution/ release process and does not include any parameter on the intrinsic dissolution rate of the nutraceutical.
$M_0^{1/3}-M_t^{1/3}=\kappa t$	$\frac{M_t}{M_\infty}=at^n+b$	$M_t=M_0[1-e^{-k(t-T_i)^b a}]$
κ: constant incorporating the surface-volume relation	b: burst effect, if there is no burst release, it is a Power law model n: release exponent, $n=0.5$ for Fick diffusion and higher values of n for mass transfer following a non-Fickian model a: constant incorporating geometric feature of the delivery vehicle	T: lag time before the release process begins a: time scale parameter b: shape parameter; exponential (b=1), sigmoid with upward curvature followed by a turning point (b>1), or parabolic, with a higher initial slope and after that consistent with the exponential (b<1)
Hopfenberg model		
In this model, the release of nutraceutical is based on surface erosion of the delivery vehicle. $\frac{M_t}{M_\infty}=1-\left[1-\frac{k_0t}{C_0a_0}\right]^n$ k: erosion rate constant a_0: initial radius of the delivery vehicle, a_0 is 1, 2 and 3 for a slab, cylinder and sphere, respectively.		

For instance, the Korsmeyer-Peppas and Weibull models predicted anomalous transport (rather than typical Fickian diffusion) of resveratrol suggesting specific interactions between

176 niosomes and hydrogel matrices [74]. Therefore, fitting *in vitro* release or bioaccessibility
177 kinetics data to relevant mathematical models (**Table 1**) is providing useful quantitative
178 comparison between different delivery vehicles in order to predict *in vivo* release kinetics
179 and the release mechanisms.

180 One of the remarkable commonalities in research using conventional emulsions,
181 nanoemulsions and liposomes in the past few years has been the use of positively-charged
182 species such as chitosan of different molecular weights as a coating. This has been done
183 with an ultimate goal to improve mucoadhesion of the delivery vehicles to the epithelial cells.
184 The mucus and glycocalyx are inherently negatively charged owing to the phosphate and
185 sialic acid groups [34, 43, 44, 46, 57, 75]. Such a coating can improve the biophysical
186 stability of the delivery vehicles [46] (see the chitosan coated nanoemulsions in **Figure 2a**).
187 It can also slow the release kinetics of the bioactive (see chitosan coated-liposome in **Figure**
188 **2b** for loading curcumin in this specific case) [57]. For example, the bioaccessibility of
189 curcumin was reduced with increasing molecular weight of chitosan [43, 46]. Such
190 decreased bioaccessibility in the presence of chitosan as a coating has also been seen in
191 conventional emulsions loaded with carotenoids [34].

192 One can hypothesize that such decreased bioaccessibility is associated with
193 bioactive compounds being somehow trapped or cross-linked within the large chitosan
194 aggregates and not released into the micellar phase post *in vitro* lipid digestion. However,
195 this was contradicted by another study showing better bioaccessibility of curcumin in
196 nanoemulsions with chitosan coating particularly in the *in vitro* ileum [44]. A higher uptake
197 of these chitosan-coated nanoemulsions by Caco-2 cells was also noted, validating the
198 afore-mentioned hypothesis of synergistic binding between negatively-charged cells and
199 cationic chitosans resulting in higher antioxidant capacity at the cellular level. Also, chitosan
200 decreasing the transepithelial electrical resistance (TEER) suggested that chitosan-coated
201 nanoemulsions were able to directly diffuse through the Caco-2 cell tight junctions and

consequently, enhanced the paracellular transport [44]. Overall, the muco-adhesive benefits of vehicles with chitosans or disruption of the tight junctions can be only realized using optimized molecular weight, degree of deactylation and modification of chitosans or any other positively charged species.

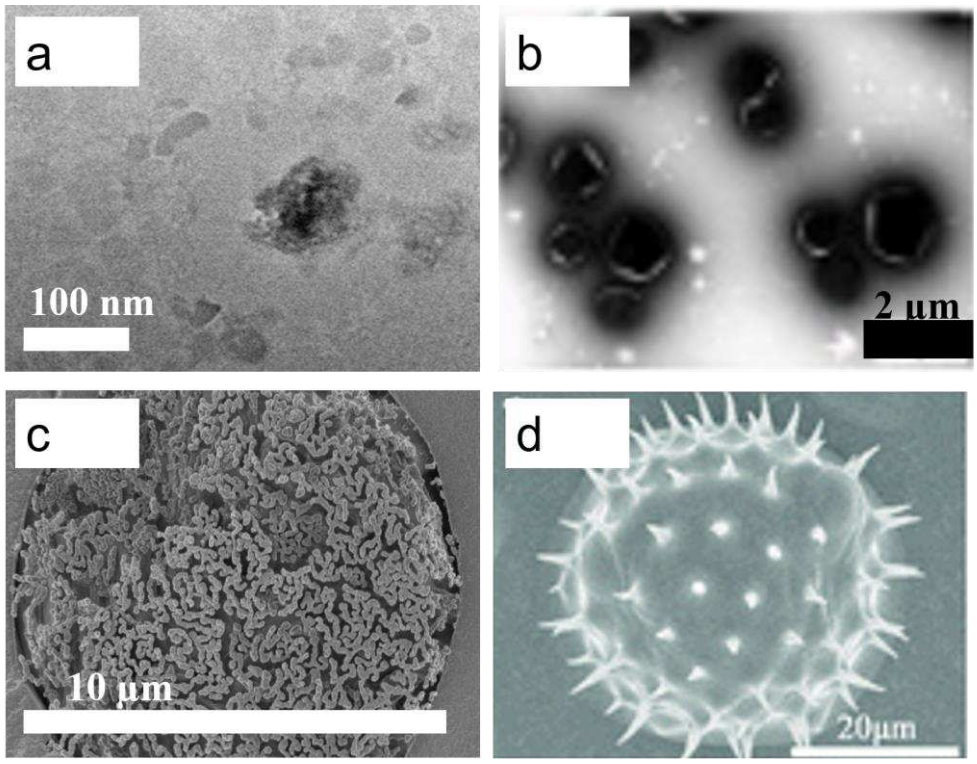


Figure 2| **Electron micrographs of delivery vehicles. a**| Chitosan-coated nanoemulsions (mean hydrodynamic dimeter D_H, ~ 125.8 nm, created using soy lecithin and Tween 80, and high molecular weight chitosan (Mw= 310 kDa, deacetylation degree ~ 85%)) [46], **b**| chitosan-coated liposomes (mean hydrodynamic dimeter D_H, ~ 1729 nm, created using soy lecithin and cholesterol, and high molecular weight chitosan (Mw= 310–375 kDa, deacetylation degree > 75%)) [57], **c**| Pickering emulsion (mean droplet size, d_{43} ~10 µm stabilized by 83 nm-sized whey protein nanogel particles) [49], and **d**| Natural sunflower pollen grains (d_{43} ~37 µm) [64]. Reproduced with permissions from Elsevier Inc. [46, 49, 57] and RSC [64].

The use of Pickering emulsions for encapsulation and investigating the release of bioactive compounds has been a rather recent endeavor [48, 49, 51, 54] and this field is gaining significant momentum (**Figure 1**) with the advent of laboratory-synthesized biocompatible particles for stabilization of oil against coalescence. The high desorption energies of the colloidal Pickering particles adsorbed at the oil-water interface enable these particles to be resilient to displacement by biosurfactants (bile salts) during intestinal lipid digestion resulting in slower release of the free fatty acids (FFAs) and mono- and diacylglycerols (MAGs and DAGs / the micellar phase) [76]. This is also expected to delay

223 the release kinetics of the bioactive compound that are generally associated with the
224 micellarphase. The most striking study in this domain was release of curcumin using
225 Pickering emulsions stabilized by chitosan-tripolyphosphate nanoparticles [50]. A slow and
226 sustained release of the encapsulated curcumin for 4 days (96 h) was reported in an *in vitro*
227 release media at pH 7.4 with eventually 74% release of curcumin, giving some preliminary
228 evidence of Pickering emulsions as a suitable delivery vehicle. Clearly the 4 day timescale
229 may not be physiologically relevant.

230 Lu and co-authors [51] demonstrated the protective effects conferred by milled
231 starch-based Pickering emulsions to oil droplets that eventually improved the
232 bioaccessibility of the encapsulated curcumin. In addition, such Pickering emulsions
233 showed that the cellular uptake of curcumin was improved, as shown qualitatively using
234 imaging techniques. Stability of curcumin within the oil phase was further demonstrated in
235 our laboratory recently [49] using biocompatible whey protein nanogel-stabilized Pickering
236 emulsions (**Figure 2c**), where some part of the retention of curcumin within the delivery
237 vehicle was linked to the binding of curcumin to the interfacial proteinaceous particles, which
238 might result have lower bioaccessibility consequences.

239 Although most studies showed improved bioaccessibility of the bioactive compound
240 using Pickering emulsions, such data should be taken more cautiously. This is because
241 most of these particle-stabilized emulsion studies compared the bioaccessibility data with
242 respect to free curcumin in oil *i.e.* in non-emulslified lipids [53]. Therefore, one may debate
243 that the bioaccessibility was improved just due to the increased droplet surface area versus
244 non-emulsified droplets and increase lipase binding sites during *in vitro* digestion in the
245 former and consequently generation of more FFAs and larger fractions of micelles where
246 hydrophobic bioactive compound was solubilized. Therefore, appropriate controls should be
247 used for bioaccessibility studies by comparing Pickering emulsions with a surfactant- or
248 biopolymer-stabilized emulsions with similar droplet size range. Since particles in general

249 are much larger in size (100 nm to several μm) compared to the size of surfactants or
250 proteins, the latter being only a few nm, the droplet size for Pickering emulsions are
251 generally 10-100 times larger than conventional/ nanoemulsions. From a surface area
252 perspective, due to the larger size of Pickering emulsion droplets versus conventional or
253 nanoemulsions, it is obvious that the FFA release will decrease in the former, which can
254 also have an impact on release of the bioactive compounds. However, the barrier effects
255 provided by the particles at the interface can be beneficial in protecting the bioactives
256 against degradation during physiological transit or preventing bile salt-mediated rapid
257 metabolism and clearance, which definitely demands future investigation.

258 Besides designing novel vehicles, an elegant new approach recently investigated in
259 delivery of hydrophobic bioactive compounds has been the use of nature-engineered
260 microcapsules (**Figure 1**). Plant spores are excellent bio-derived microcapsules that have a
261 number of advantages which colloid scientists can only envy. The outer wall (the exine) of
262 pollen and spores are primarily constructed of the biopolymer 'sporopollenin' [77], which is
263 uniquely resistant to temperature, pressure, most chemicals and degradation by enzymes
264 [78]. This can be advantageous over human enzyme-responsive protein- or starch-based
265 delivery vehicles [76]. Wu and co-authors [64] recently employed natural sunflower pollen
266 grains (**Figure 2d**) and *Lycopodiastrum casuarinoides* spore exine capsules of 35-41 μm
267 size to examine the encapsulation and release of nobiletin (a hydrophobic flavonoid from
268 citrus peel) as a model bioactive compound by passive loading technique. Although the
269 bioactive molecules leaked easily owing to the surface pores on these exine microcapsules,
270 a biopolymeric coating with alginate enabled the pores on the pollen surfaces to be closed,
271 acting as a transient barrier to the nutraceutical release.

272 Although designing elegant delivery vehicles and assessing the bioaccessibility of
273 the encapsulated compounds has achieved remarkable progress, not to our surprise, only
274 a few of these studies have taken these vehicles forward to assess the bioavailability [40,

47, 65-67] (**Figure 1**). In context of pre-clinical trials, an elegant study on bioaccessibility and bioavailability was recently conducted by Salvia-Trujillo and co-workers [65], where emulsions of different droplet sizes (d_{43} of 0.11 μm (small), 0.53 μm (medium) and 14.5 μm (large) were compared for the delivery of vitamin D2. As one might expect, the rate of release of FFA from the small droplet-sized emulsion was higher than the large droplet-sized emulsions during *in vitro* digestion. The former having higher surface area per unit of the emulsified lipids and thus the small droplet-sized emulsion also had significantly higher bioaccessibility of vitamin D2. However, such behavior was not observed *in vivo* where the rat serum showed higher concentration of vitamin D2 in the large-sized emulsions highlighting a conflict between *in vitro* bioaccessibility and *in vivo* bioavailability data.

Kadappan and coworkers [67] from the same laboratory, however, showed some beneficial effects of nanoemuslions in an *in vivo* study. Comparing coarse emulsion and nanoemulsion stabilized by saponins for delivery of vitamin D3, where the coarse emulsions were nearly ≈20-folds higher in mean droplet size as compared to the nanoemulsions, demonstrated that the FFA release and bioaccessibility was higher in the nanoemulsions. Of more importance was that the supplementation of vitamin D3 via nanoemulsion route significantly increased the serum concentration of the vitamin as compared to non-emulsified systems by nearly 4-fold [67]. Although the serum concentration was not significantly improved in nanoemulsions versus the coarse emulsions, nanoemulsification resulted in much lower coefficient of variation of (11.8%) in the plasma concentration of vitamin D3 as compared to the coarse counterparts (35.2%). Testing of delivery vehicles in clinical settings is very rare to date, which is of prime importance for substantiation of health-claims in real world application, highlighting a clear opportunity space in this area, particularly with biocompatible delivery vehicles.

Delivery to design approach: Starting from understanding release

kinetics mechanisms

Unless the bioactive compound is introduced into the systemic circulation, it is difficult to understand the efficacy of the delivery vehicle in protecting and/or delivering the actives in sufficient quantify to accumulate in appropriate tissues. Although hydrophobic fluorescent probe loaded in delivery vehicles can be used to investigate the tissue distributions of the loaded compound [40], the gold-standard technique to quantify bioavailability is to measure the blood plasma concentration of that compound over a period of time after ingestion. This allows us to understand the absorption and eventually elimination of the bioactive from the circulation. **Figure 3** displays the different release kinetic profiles based on single-dose oral administration of the bioactive compound, drawing inspirations from pharmacokinetics [79-81]. Here, we restrict our discussion only to single dosing of the bioactive-loaded delivery vehicle.

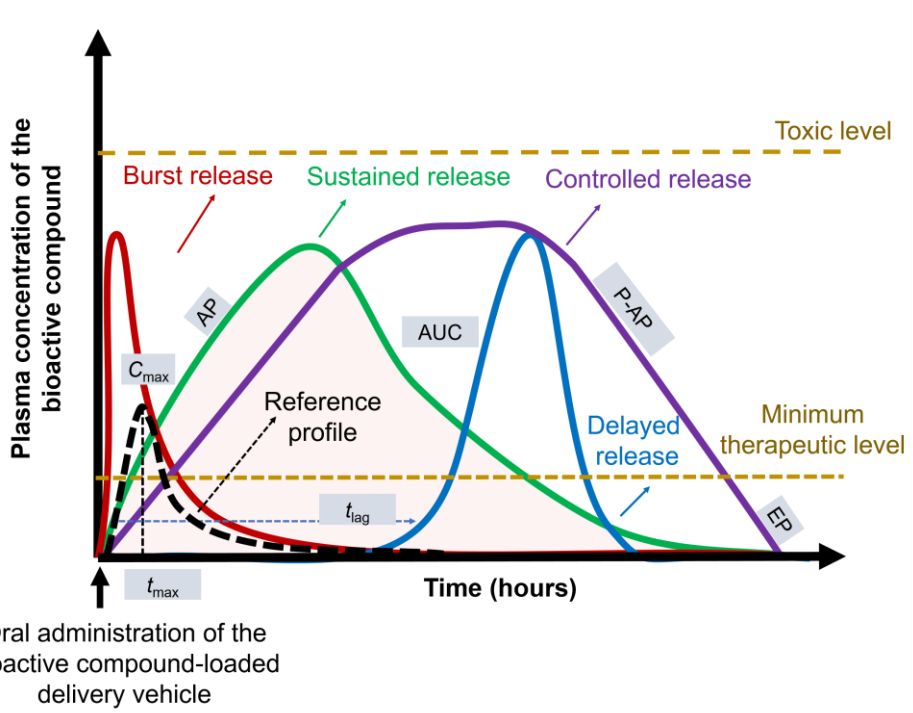


Figure 3| **Release kinetics.** Kinetic plots of plasma concentration versus time for a single dose of the delivery vehicle containing the bioactive compound introduced through oral administration route. C_{max} is the maximum plasma concentration, the time when it occurs is the peak time (t_{max}) and AUC is area under the curve. The lag time (t_{lag}) is used for characterizing the release behaviour of delayed-release carriers. AP, P-AP and EP refer to absorption phase, post-absorption phase and elimination phase, respectively.

Under extremely unusual circumstances, a bioactive compound can have an immediate (or

burst) release if it is introduced intravenously reaching high maximum plasma concentration (C_{max}) within very short time scales (t_{max}). For oral administration route, however, the encapsulated bioactive compounds reaches C_{max} within few hours (t_{max}) of ingestion (shown by the reference profile in **Figure 3**) and then is rapidly eliminated *via* a zeroth- or first-order rate kinetics (**Table 1**). In other words, at time $t < t_{max}$, the rate of absorption is greater than the rate of clearance of the bioactive compound [82]. The area under the curve (AUC) (**Figure 3**) provides a useful measure overall blood-plasma exposure of the bioactive.

The C_{max} is very low in case of most delivery systems investigated so far as compared to the concentration of bioactive compound administered and also the elimination rate is rapid resulting in low AUC (**Figure 3**). For instance, using commercial curcumin formulations containing lecithin or cyclodextrins as emulsifiers, the plasma concentration of curcumin was demonstrated to peak within the first two hours of oral administration (0.5-73.2 ng/ mL) in healthy human subjects. This was followed by a fairly rapid decline in plasma levels to below the minimum therapeutic levels within 12 h of dosing with $AUC_{12\ h}$ of 3.9-327.7 ng.h/mL [83]. In a laboratory setting, curcumin-loaded emulsions stabilized by Maillard conjugates of bovine serum albumin (BSA) and dextran (M_w= 10 kDa) [40] demonstrated almost 3-fold higher C_{max} (270 ng/ mL) and $AUC_{12\ h}$ of 1511 ng.h/mL of curcumin as compared to the afore-mentioned study with commercial curcumin, highlighting the beneficial effects of using sophisticated delivery vehicles. Although this BSA-dextran conjugate showed 4.8-fold increase in bioavailability versus a Tween-stabilized counterparts ($AUC_{12\ h}$ of 317 ng.h/mL) in mice, still, there was no accumulation of curcumin in the heart, liver, spleen, lung and kidney [40].

For an ideal case scenario, the bioactive compound should have a sustained, controlled or delayed release depending upon the biological function desired. Sustained or extended release specifically suggests that the rate of administration into the plasma can be sustained over a period of time ($AUC_{sustained}>>>AUC_{burst}$). This is in contrast to controlled

release where the plasma bioactive concentration is maintained at a constant therapeutic level for a prolonged period of time increasing the AUC further ($AUC_{controlled} > AUC_{sustained}$) and this maximizes the chances of accumulation of the compounds in relevant tissues [84] (**Figure 3**). For targeting delayed-release, comparison of lag times (t_{lag}) between delivery systems is important.

In order to engineer the design of the delivery vehicles, we propose a 'delivery to design' strategy such that we identify the ideal delivery carrier in order to increase the AUC. Although an *in vitro–in vivo* correlation (IVIVC) tool plays a key role in pharmaceutical development [85], such tools are currently not available and/or not validated for bioactive delivery due to limited *in vivo* trials. Therefore, we will provide our opinion on the 'delivery to design' cycle largely based on *in vitro* studies and well-established colloidal principles. From a reverse order, bioavailability (*BA*) can be mathematically expressed as a function of cellular uptake (*U*) of the bioactive including transport across mucus membranes, permeability into the cell membranes, bioaccessibility (BC), and molecular transformation (MT) that might have occurred during physiological transit and may affect biological function:

$$BA = f(U, BC, MT) \quad (1)$$

We will discuss this with respect to two conditions *i.e.* 'fasted state' and 'fed state'.

Fasted state. Fasted state implies a fairly artificial condition such that no other food is ingested alongside the bioactive-loaded delivery vehicle. In order to achieve high AUC (**Figure 3**), it is important to increase C_{max} such that the bioactive compound is still below the toxic levels but remains in the circulation. In a few cases, the bioactive compounds might be absorbed into intestinal enterocytes along with the MAGs and FFAs created during intestinal digestion process and enter the portal vein. However, these compounds associated with the FFAs will be normally incorporated into chylomicrons that are formed in

the endoplasmic reticulum, followed by exocytosis through the basolateral membrane of the enterocytes into the lymphatic system. Hence, to achieve desired delivery in terms of uptake, bioaccessibility and molecular transformation, following design strategies can be employed:

a. *Increase 'uptake':* Delivery strategies should consider interactions of the delivery vehicles with relevant components of plasma membrane of the cells to enhance uptake of the bioactive compounds [86]. The inherent negative charge of the cell membranes due to the fatty acids, lipoproteins and glycocalyx of the intestinal cells should be exploited. As discussed previously, use of positively-charged biopolymeric coating, such as chitosans or lactoferrin can be useful to allow effective adsorption of the delivery vehicles to the cells. The hydrophobic tail regions of the lipid bilayer encapsulating the cells can also offer effective anchoring points. Hence, bioactive compounds associated with FFAs (lipid digestion products) can act as suitable anchors to these hydrophobic domains and promote the cellular internalization of the bioactive chemicals. If human cancer cells are being targeted for a therapeutic effect of the administered bioactive, one strategy is to target folate receptors that are overexpressed in 40% of the carcinoma cells [87]. If such folate receptors are targeted, conjugation with folic acid can be an elegant strategy to bring the delivery vehicles into the vicinity of those receptors to allow cellular permeability [88].

b. *Improve 'bioaccessibility'*. The bioaccessibility kinetics as well as extent of a bioactive compund has a close correlation with FFA release and extent, respectively [33]. It is now evident from the discussion, that small size and consequent larger surface area is definitely advantageous for increasing bioaccessibility, highlighting clear benefits of using nanoemulsions over conventional or relatively larger sized Pickering emulsion droplets (**Figure 4**). However, in order to aim for a sustained concentrations of the bioactive compound in the blood and higher AUC (**Figure 3**), delayed release of FFA while reaching the maximum extent of release of FFA can be highly advantageous

(**Figure 4**). Delayed lipolysis has been shown in case of Pickering emulsions where the interfacial layers of particles have been fused by heat treatment of modified starch granules [89] or protein-based microgel particles [90]. However, such heat treatment after encapsulation of the bioactive compound might increase the degree of molecular transformation, which is not desirable. Hence, other fusing techniques such as enzymatic crosslinking at the interface can be employed to create a diffusive barrier to lipase during digestion, delaying the rate of lipolysis and eventually the release of the bioactive species into the micellar phase (**Figure 4**).

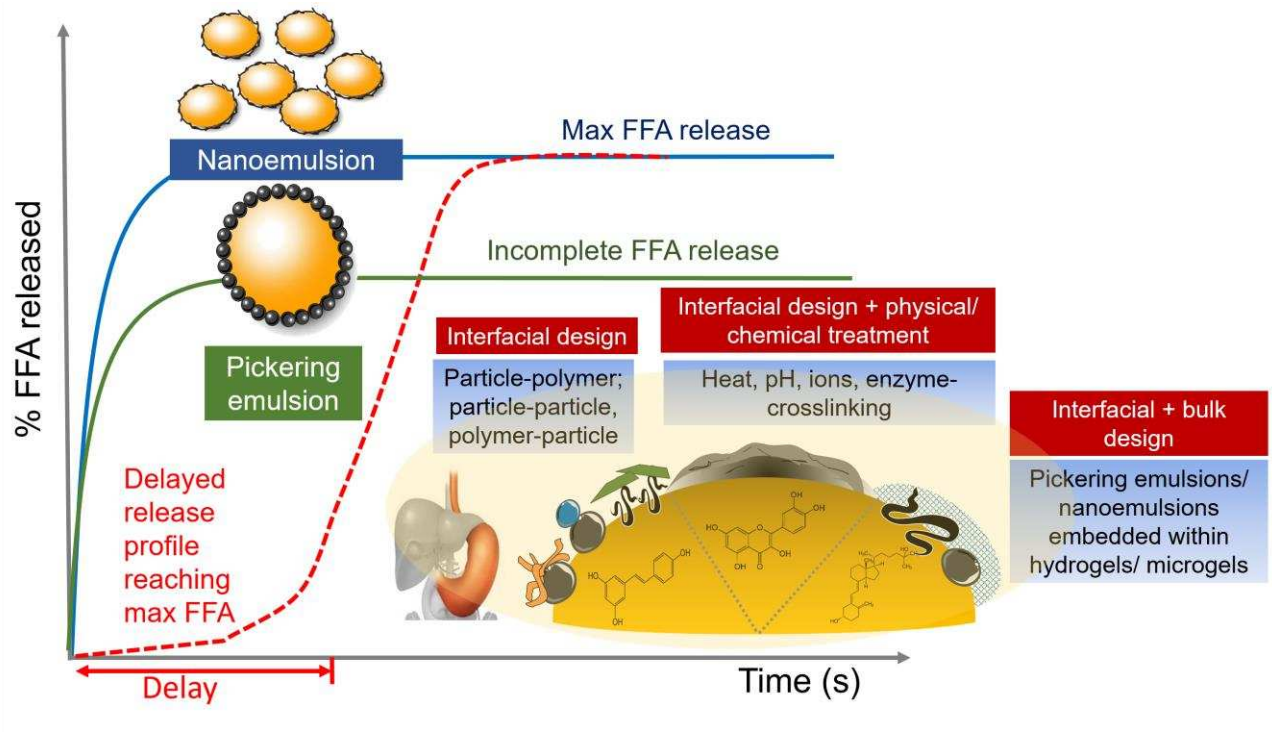


Figure 4| **Free fatty acid (FFA) release kinetics of emulsions loaded with bioactive compounds and microstructural design of vehicles offering tuned physiological fate.** Nanoemulsions provide higher extent and rate of FFA release versus Pickering emulsions due to increased droplet surface for lipase action in the former. Delayed release is desirable without compromising the maximum FFA release. Thus, design strategies that may offer delayed gastric emptying include 1) interfacial engineering using particle-biopolymer (*e.g.* whey protein nanogel particle + dextran sulphate [91]), particle-particle (*e.g.* lactoferrin nanogel particles + inulin nanoparticles [92]) or biopolymer-particle complexation (*e.g.* whey protein + cellulose nanocrystals [93]), 2) treatments particularly in case of Pickering stabilization to fuse particles using physical means (*e.g.* use of heat in starch granules [89] or whey protein microgel particles at interface [94]) or chemical means and 3) embedding emulsions within hydrogel/ microgels providing gatric stability and/or creating tortuous path to enzymes (*e.g.* whey protein emulsions embedded in gelatin matrix [95], whey protein-stabilized emulsion microgel particles [90]) The authors of the afore-mentioned references designed these delivery vehicles but have not tested encapsulation and delivery of bioactive compounds yet.

The other strategy to delay lipolysis is to improve gastric stability of the delivery vehicles

and eventually delay the gastric emptying time. Elegant interfacial strategies have been used to complex or conjugate Pickering particle-particle [92], Pickering particle-biopolymer [91], protein-biopolymer [40] or protein-particle [93, 96] where either the particle or the biopolymer is used as a coating to a create a rigid steric barrier to render improved gastric stability (**Figure 4**). Other strategies of combining interfacial and bulk properties such as encapsulating the emulsion droplets within a hydrogel or a microgel particle can provide stability in the gastric phase. Such systems provide a tortuous yet biodegradable network [90, 95] for lipases to reach the vicinity of droplets and eventually provide delay in the release of FFAs and consequently a sustained release of the bioactive compounds. Nevertheless, the design strategy should not comprise the maximum FFA release as this will determine the degree of bioaccesisbility, which can be quite often an issue with Pickering emulsions [94] and also with emulsions undergoing irreversible gastric flocculation, coalescence and/or partial coalescence (in case of presence of some solid fat in the latter) [97, 98]. Therefore, a combination of gastric stability, larger droplet surface area, and transient barrier to lipase access are the key features to deliver highest degree yet sustained bioaccessibility.

c. *Decrease 'molecular transformation' of the bioactive compound.* The key challenges with most bioactive compounds such as curcumin, *ω*-3 fatty acids, *β*-carotene, resveratrol *etc.* are that they undergo chemical degradation, conjugation or metabolic reactions in the gastrointestinal tract due to exposure to the complex milieu of pH, ions, enzymes and bile salts [22, 99]. Also, fermentation reactions in large intestines by gut microflora may generate chemical modification of the released bioactive. Hence, a key feature of the bioactive delivery vehicle is to protect the encapsulated species from high exposure to gastrointestinal transformation. The afore-mentioned colloidal principles of imparting gastrointestinal stability is important here. In addition, keeping the bioactive compound highly solubilized in the hydrophobic oil phase can be useful to limit contact with

physiological aqueous environment that can cause crystallization of the bioactive or chemical degradation. Hence, the polarity and the degree of saturation of oil used is an important design feature when creating the delivery vehicle [30].

Fed state. Fed-state refers to a more realistic condition where the colloidal delivery vehicle encapsulating the bioactive species is ingested along with a meal. This might then have confounding effects on the release of the bioactive compounds. Although the above strategies of fasted-state is still highly relevant, additional precautions should be taken when dealing with fed state:

a. *Predicting binding to nutrients.* There is an increased body of evidence on reduced bioavailability of bioactive compounds such as Vitamin D and curcumin due to binding to dietary fibre [100] and proteins [49], respectively. In addition, the long chain FFAs may bind to ingested calcium ions resulting in the formation of insoluble calcium salts [101], thus reducing the bioaccessibility of the bioactive compounds associated with those FFAs in the intestines. Ideally, a database is needed to have a clear picture of the type, concentration, binding affinities and biophysical features of hydrophilic nutrients that may limit the bioaccessibility of the bioactive compounds when co-ingested, and thus, it is crucial to consider the role of food matrix components on bioaccessibility.

b. *Using excipient emulsions.* An alternative strategy to deliver bioactive compounds using delivery vehicles is using excipient lipidic emulsions to improve the bioaccesisbility of hydrophobic bioactive compounds when co-ingested with it [20]. In other words, an excipient nanoemulsion might not have any health benefits itself, but it promotes the biofunctionality of the bioactive compounds consumed with it and consequently is hypothesized to increase their oral bioavailability via enhancing bioaccessibility, retarding molecular transformation, or increasing uptake. Interestingly, use of excipient nanoemulsions with curcumin powder has shown significant improvement in bioaccessibility (*BC* ~ 75%) of curcumin as compared to a curcumin-loaded

476 nanoemulsions (*BC* ~ 62%) [102]. Beneficial effects have also been observed in
477 bioaccessibility of lycopene in tomato juice when consumed with excipient
478 nanoemulsions (BC ~ 12.5%) versus without the excipient emulsions (BC ~ 7.5%).
479 However, the effects were subtle owing to crystalline nature of these carotenoids that
480 prevented enough leaching out into the nanoemulsion droplets. However, proving the
481 efficacy of excipient emulsions can be particularly challenging as other foods ingested
482 might have confounding effects on the bioaccessibility and bioavailability of the
483 compounds co-ingested. Therefore, long-term studies with well-controlled diets are
484 needed as well as comparative *in vitro* and *in vivo* trials are needed including excipient
485 emulsions + bioactive compound and emulsions loaded with bioactive compounds.
486

487 ## Conclusions

488 In this article, we have reviewed the recent advances in colloidal delivery vehicles that have
489 surfaced in past half-decade to improve the bioaccessibility of bioactive compounds and in
490 rare cases assessed the bioavailability of such compounds using *in vivo* trials. We identified
491 the desired release profiles of the bioactive compounds from the delivery vehicles with
492 relevant kinetic parameters in order to increase the residence time of the bioactives in
493 systemic circulation to improve the chances for their accumulation in tissues and
494 consequently provide positive health outcomes. Even using the 'design to delivery' approach
495 running from improving 'uptake', increasing 'bioaccessibility' and decreasing 'molecular
496 transformation' of the bioactive compound, fabricating the ideal oral delivery vehicle appears
497 to be not straightforward. Interactions with ingested dietary components present further
498 hurdles. Interestingly, the field of delivery of hydrophobic bioactive compounds is radically
499 shifting from testing *in vitro* digestion kinetics alone, to more pharmacokinetic modeling,
500 dialysis-based release experiments as well as Caco-2 cell monolayer-based permeation
501 studies. These represent an extremely versatile toolbox with fascinating fundamental

implications but also significance for generating rapid and predictive data for bioaccessibility and uptake. Finally, interdisciplinary research involving scientists from nutrition and medicine can be highly beneficial to test the vast realm of sophisticated delivery vehicles designed by colloid scientists in pre-clinical and clinical settings once the safety is ensured to prepare a rich dataset in order to design the first (IVIVC tool for bioactive compounds. This will help to accelerate the translation of bench-top success to real world functional foods, nutraceutical and effective bioactive-enriched supplements with approved health claims.

Conflicts of interests

The authors declare no competing financial interest.

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Special interest (•) or Outstanding interest (••)

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832

Conflict of Interests

'Declarations of interest: none