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Review

The impact of formulation design on the oral bioavailability of omega-3 polyunsaturated fatty acids

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ABSTRACT

Omega-3 polyunsaturated fatty acids (n-3 PUFA) are essential dietary supplements with widespread health benefits. However, achieving therapeutic n-3 PUFA levels in systemic circulation represents a significant dosing challenge, complicated by a complex interaction between different physiological and chemical factors. Recently, significant efforts have been directed towards creating "bio-accessible" n-3 PUFA formulations that overcome this dosing challenge by enabling increased oral absorption across the small intestine. However, the impact of varying physiochemical formulation properties on n-3 PUFA bioavailability remains poorly understood and requires further investigation. This review explores the impact of formulation design, including self-emulsifying systems, micro- and nano-emulsions, chewable gels, and microencapsulation, on n-3 PUFA pharmacokinetics, considering both clinical and preclinical investigations. Key challenges in developing highly bioavailable n-3 PUFA formulations and quantifying their absorption, biodistribution and metabolism are discussed. Finally, recent progress in developing next-generation n-3 PUFA formulations, including solid lipid nanoparticles and nanostructured lipid carriers, and their targeting through innovative lipid structuring approaches will be addressed. The oral bioavailability of n-3 PUFA is ultimately influenced by multiple design factors related to each formulation strategy, underscoring the need for a systematic formulation approach that involves comprehensive testing of candidate formulation under simulated gastrointestinal conditions.

1. Introduction

Omega-3 long-chain polyunsaturated fatty acids (n-3 PUFA), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have attracted significant interest as essential nutrients and functional foods owing to their positive functions in promoting neurological maturity and improving cardiovascular functions. Consequently, a broad spectrum of dietary intake recommendations has been established by various national and international health organizations, ranging from 250 mg up to 2 g of EPA and DHA per day (Ghasemifard, Turchini, & Sinclair, 2014; Joint, 2008). However, achieving such high doses is challenging as naturally occurring and unprocessed fish oil – the most common natural source of n-3 PUFA – only provides a limited concentration that does not exceed 30 % for both EPA and DHA.

Typically, n-3 PUFA are naturally present in fish oil as either triglyceride (TG) or phospholipid (PL) esters, but to overcome the dosing challenges, several chemical derivatives of n-3 PUFA are added to the dietary supplements, enabling an increased intake of EPA and DHA at precisely balanced ratios. Subsequently, n-3 PUFA supplements typically comprise a liquid dosage form containing one or a combination of three n-3 chemical forms: naturally occurring fish oil triglycerides (TG), n-3 ethyl ester (EE), or n-3 concentrate present as re-esterified fish oil TG (Xie et al., 2023).

Despite advancements in chemical synthesis, achieving optimal blood levels of n-3 PUFA remains a considerable dosing challenge, further complicated by the variability of the rate and extent to which n-3 fatty acids reach systemic circulation (i.e. pharmacokinetic variability). This variability is primarily governed by several factors, including their chemical binding form, the specific location of n-3 PUFA within the triglyceride molecule, and physiological factors such as pancreatic

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lipase activity (impacted by food intake state) (Na & Lee, 2020; Offman et al., 2013). This complex interplay collectively causes different n-3 PUFA derivatives to exhibit variable digestion and absorption behaviors in and across the gastrointestinal (GI) tract, leading to significant differences in their pharmacokinetic properties following oral administration. For instance, the absorption of n-3 ethyl esters is less efficient compared to n-3 triglycerides and phospholipids forms (Lapointe et al., 2019). This occurs due to the requirement for further lipolysis by the carboxyl ester lipase enzyme released into the gut following the ingestion of fatty meals. Unlike the n-3 ethyl ester (EE) form, free fatty acids exhibit enhanced bioavailability independent of meal fat (Offman et al., 2013).

An alternate approach for optimizing the pharmacokinetic properties of n-3 PUFA is the generation of bio-accessible formulations that overcome the absorption-dependent limitations of chemically synthesized n-3 derivatives. In recent years, various formulation strategies have been explored to maximize their oral bioavailability and biodistribution in targeted tissue by increasing the accessible surface area of n-3 PUFA formulations in the GIT. These include strategies, such as self-emulsifying formulations (Cortés, Califano, & Lorenzo, 2019; Hayashida et al., 2022; Zheng, Liu, Wang, & Baoyindugurong, 2011), nano-sized emulsions (Karthik & Anandharamakrishnan, 2016; Nunes et al., 2020), gelled emulions (Haug et al., 2011), microencapsulation (Joyce, Gustafsson, & Prestidge, 2018; Shen, Augustin, Sanguansri, & Cheng, 2010), liquid crystalline nanoparticles (Kang, Jeon, De, Hong, & Park, 2023), micellar matrix (Ibi et al., 2024), and Salt formation (Manusama et al., 2021), However, the impact of varying the physicochemical properties of these formulations on n-3 PUFA pharmacokinetics is poorly understood and requires further investigation. This review aims to investigate the impact of employing various n-3 PUFA formulation design strategies, such as self-emulsification, controlling droplet size, stabilization in a gel matrix, and microencapsulation on improving their bioavailability through exploring their impact on digestion rates, absorption, and pharmacokinetics in preclinical investigations and human clinical trials. Overall, this review seeks to create a better understanding of the key physicochemical formulation properties that dictate n-3 PUFA bioavailability, thereby guiding the future development of highly bioavailable formulations by applying functional pharmaceutical excipients such as emulsifiers, surfactants, co-surfactants, and gelling agents that drive absorption across the GI tract.

2. Factors influencing n-3 PUFA bioavailability

The oral bioavailability of n-3 PUFA is controlled by a complex interaction of different chemical and physiological factors. Among these physiological factors are the lipase enzymatic activity, co-administration of meals, and fat content in meals. On the other hand, chemical factors include n-3 fatty acid source, chemical binding form, and fatty acid distribution on triglyceride molecules. The following sections highlight the specific effects of these factors on the bioavailability of n-3 PUFA, including a summary of these factors, as described below in Fig. 1.

2.1. Physiological factors

2.1.1. Interfacial and colloidal processes of lipid digestion

After ingestion, lipids contained within the food are ground into small pieces and mixed with saliva by the mechanical action of the teeth in the oral cavity. Upon entering the stomach, peristaltic waves facilitate emulsification by the action of released bile salts and phospholipids.

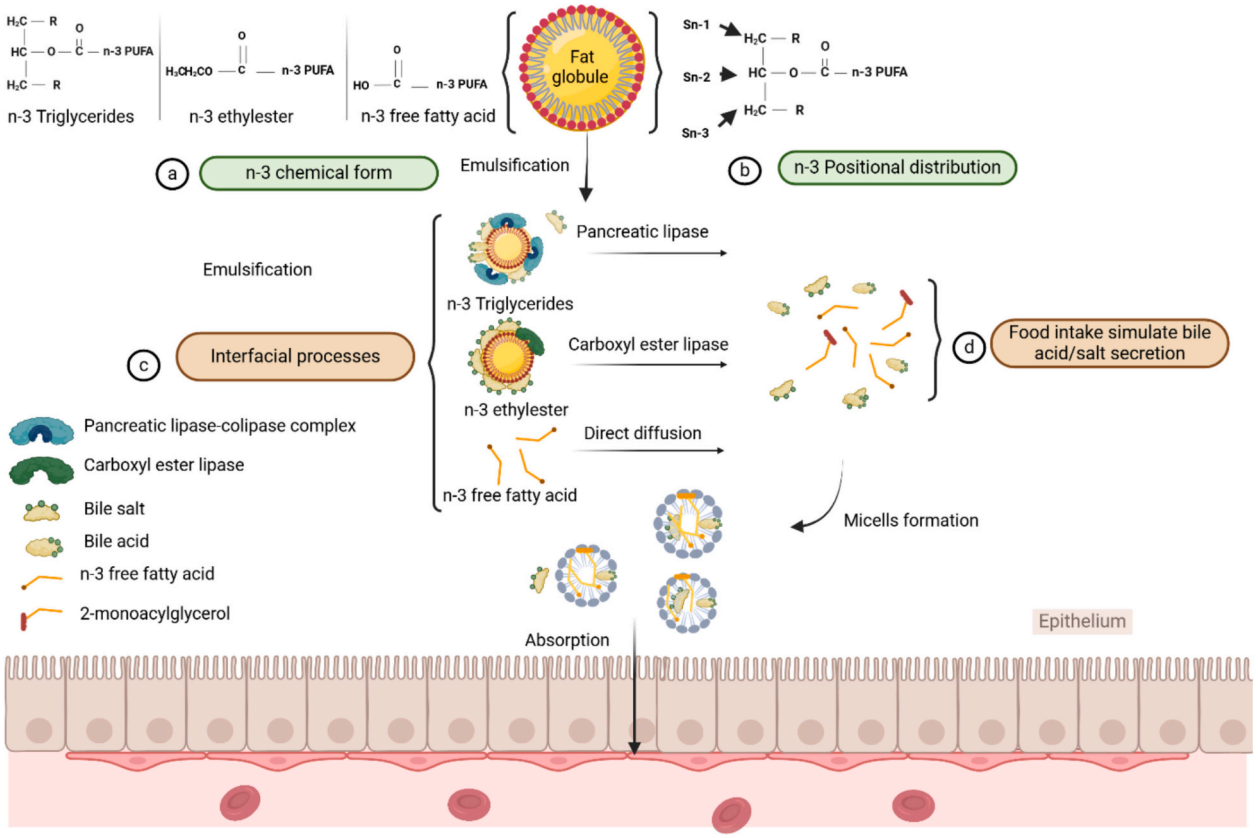

Fig. 1. The impact of physiological and chemical factors on the bioavailability of n-3 PUFA. Bioavailability can be influenced by chemical factors including a) n-3 chemical form (i.e., ester binding form), b) positional distribution of n-3 PUFA on triglyceride molecule, and physiological factors such as c) interfacial process of lipid digestion (i.e., lipid emulsification, lipase-colipase complex formation, lipolysis, micelles formation, and absorption), d) dietary factors affecting bile salt secretion. Created with BioRender.com

Gastric digestion is initiated when gastric lipase binds to the emulsified lipid oil/water (o/w) interface, hydrolyzing triglycerides (TAGs) at the sn-3 position forming sn-1,2-diacylglycerides (DAGs). Lipid digestion by gastric lipase accounts for 10–30 % of total lipolysis (Pafumi et al., 2002). In the small intestine, further hydrolysis is performed by pancreatic lipase at positions sn-1 and sn-3 forming absorbable 2-monoacylglycerols (2-MAGs) and free fatty acids. Pancreatic lipase has an optimum pH activity between 7.5 and 8.5 and relies on the function of colipase, a lipase co-factor, that initiates lipid digestion by binding to the (o/w) interface and stabilizing pancreatic lipase at the surface (Bezzine et al., 1999). Pancreatic lipase activity is significantly influenced by the presence of bile, which is secreted in response to food intake. Bile salts, phospholipids, and cholesterol enhance the efficiency of the lipolysis process by two main mechanisms. Firstly, by removing lipolysis by-products accumulated at the o/w interface limiting lipase accessibility. Secondly, by forming diverse colloidal phase structures such as mixed micelles which enhance the solubility of digested products and facilitate transport to the gut mucosal. The uptake of medium chain fatty acids (6–12 carbon) takes place via distinct pathways depending on their carbon chain length. Specifically, C6:0 (caproic acid) and C8:0 (caprylic acid) are primarily absorbed via the portal vein and delivered directly to the liver, whereas C12:0 (lauric acid) is predominantly absorbed via the lymphatic. In contrast, longer-chain fatty acids are re-esterified again into TAGs, integrated into chylomicrons followed by incorporation into the lymphatic pathway (Acevedo-Fani & Singh, 2022; Lemarié, Beauchamp, Legrand, & Rioux, 2016; McCarty & DiNicolantonio, 2016).

2.1.2. Dietary factors

Currently, the US Food and Drug Administration (FDA) has approved EPA and DHA for therapeutic use in the form of ethyl ester (EE) or free fatty acid (FFA) (Harris, Dayspring, & Moran, 2013). However, the bioavailability of both forms can be influenced by the co-administration of dietary fats. Generally, fats are responsible for stimulating the pancreas to secrete lipid hydrolyzing enzymes (including lipases and esterases), and the gallbladder to eject emulsifying bile that also activates pancreatic lipase (Qin, Nyheim, Haram, Moritz, & Hustvedt, 2017). The significance of dietary fat intake on n-3 PUFA absorption becomes more pronounced for n-3 ethyl esters. According to Offman et al. the bioavailability of EPA and DHA supplied as free fatty acids was four times higher compared to n-3 ethyl ester formulations under low-fat supply conditions (Offman et al., 2013). However, under conditions of high fat intake, the bioavailability of the ethyl ester form increased significantly by 5.4 fold, exceeding the 1.6-fold increase observed with free fatty acid in comparison to periods of low-fat intake. This can be explained by the fact that the ethyl ester form needs to be hydrolyzed by lipase enzymes, which are more abundantly released under fed-state conditions, converting ethyl ester form into free fatty acids for intestinal absorption (Offman et al., 2013). However, it's not always recommended to consume these supplements with a fatty meal, especially for those at high risk of heart disease. Therefore, several attempts have been made to enhance the fasting absorption of omega-3 fatty acids through the application of self-emulsifying delivery systems (Bremmell et al., 2020; Maki et al., 2018; Qin et al., 2017). According to Qin et al. (2017), a novel self-micro-emulsifying delivery system (SMEDS) formulation of n-3 ethyl ester improved fasting state absorption by achieving a 6.4- and 11.5-fold higher baseline corrected 24-h area under the concentration-time curve (AUC_{0-24}), compared to control after the administration of 630 mg and 1680 mg total dose of EPA + DHA, respectively (Qin et al., 2017).

2.2. Chemical factors

2.2.1. N-3 PUFA source and chemical form

Fish oil concentrate containing as much as 90 % EPA and DHA has been developed through the chemical processing of fish oil triglycerides. This can be achieved via an enzymatically driven transesterification reaction that hydrolyzes TG-bound fatty acids into free FA and glycerol, followed by a re-esterification reaction that attaches the free fatty acids back to the glycerol backbone (Haraldsson, Kristinsson, Sigurdardottir, Gudmundsson, & Breivik, 1997). Alternatively, EPA/DHA ethyl esters (EEs) can be derived from fish oil's TG by replacing the glycerol molecule with ethanol, while EPA/DHA free fatty acids (FFAs) can be synthesized by a hydrolysis reaction of EPA/DHA ethyl ester to yield free carboxylic acids.

A comprehensive investigation of n-3 PUFA bioavailability delivered by different forms (triglycerides, reconstituted triglycerides, ethyl esters, and free fatty acids forms) across published short-term designed human and animal studies (up to 72 h) revealed the superior bioavailability of EPA and DHA in the FFA form compared to triglycerides bound form (or re-esterified triglyceride), which in turn showed better bioavailability than EE form (Ghasemifard et al., 2014). The significant differences observed in the bioavailability can be explained by the variable rate of digestion reported for each ester form, which can ultimately affect the absorption rate. For example, the EPA/DHA ethyl ester bond is more resistant to pancreatic lipase hydrolysis compared to the naturally occurring EPA/DHA triglycerides ester bond. Since EPA/DHA FFAs require no digestion step before absorption, they exhibit a greater bioavailability over other ester forms (Martin, Nieto-Fuentes, Señoráns, Reglero, & Soler-Rivas, 2010). Interestingly, the administration of EPA and DHA in the form of predigested monoacylglycerols resulted in a 3 and 2.5-fold increase in maximum plasma concentration (C_{max}) of EPA and DHA, respectively, compared to their ethyl ester form (Cuenoud et al., 2020). This enhancement is attributed to the direct intestinal absorption of monoacylglycerols, which bypass the need for hydrolysis by pancreatic lipases.

2.2.2. Positional distribution of n-3 PUFA

The positional distribution of n-3 PUFA on three distinct locations (i.e., sn-1, sn2, and sn-3) on the triglyceride molecule has been shown to influence their lipolysis kinetics and potentially their absorption. For example, the equal distribution of n-3 PUFAs at *sn*-1/3 positions altered the lipolysis kinetics of microalgal oil - a DHA-rich oil - which contains 83.5 % and 65.8 % C20 fatty acid or higher at sn-2 and sn-1,3 respectively. In mice, microalgal oil exhibited a significantly ($p < 0.05$) lower in vitro digestion rate and apparent digestibility compared with standard fish oil, because of the steric hindrance effect on the lipase-mediated hydrolysis action (Na & Lee, 2020). Fish oil concentrate can be synthesized via chemical and enzymatic methods to produce an equal distribution of EPA and DHA on the $sn-1/3$ position. However, limited knowledge exists regarding the impact of this distribution on their bioavailability in humans, despite the significance of this factor in influencing the bioavailability of fatty acids in foods as previously reviewd (Michalski et al., 2013). A good example of this is palmitic acid (16:0) which is predominantly esterified to the sn-2 positions of triacylglycerol (TAG) in human milk and at sn-1/3 positions of TAG in infant formulas. Altering the distribution of fatty acids at the sn-2 position of triglyceride molecule influenced the amount of fat absorbed from infant-fed formula compared to human milk in infants (Nelson & Innis, 1999).

3. Commercial formulation strategies for improving n-3 PUFA pharmacokinetics

Current strategies for formulating n-3PUFA aim to create bioaccessible delivery systems that overcome the previously mentioned absorption-dependent limitations of chemically synthesized n-3 derivatives, thus optimizing their overall bioavailability. The term bioavailability refers to the rate and extent to which n-3 fatty acid reaches blood circulation. The rate of absorption can be established by determining peak plasma concentration (Cmax) and time to attain this concentration (Tmax). While the extent of absorption is represented by the area under the curve (AUC) of concentration versus time profile. This

section provides a detailed summary of each formulation strategy considering the most critical design factors and their potential impact on digestibility, absorption, and bioavailability of n-3 PUFA, as depicted in (Fig. 2). Furthermore, a summary of these pharmacokinetics studies in both humans (Table 1) and experimental animal studies (Table 2), for each investigated delivery system is also highlighted, with a detailed description of each formulation design summarized in (Fig. 3).

3.1. Emulsion systems

3.1.1. Macro and microemulsions

Emulsification is a process of mixing two immiscible liquids to create a stable emulsion. It is a commonly used approach to improve the oral bioavailability of lipid-soluble vitamins and nutraceuticals. This can be achieved by increasing the rate of lipid digestion by controlling the size of the emulsion droplets. This will potentially influence the surface area of emulsions exposed to digestive enzymes, increasing the absorption of digested lipid products. Insufficient emulsification of n-3 fatty acid-rich oils during the digestive process is linked to reduced enzymatic lipolysis, particularly for longer-chain PUFA, EPA, and DHA. As a result, these longer-chain fatty acids are released at a slower rate compared to other fatty acids from fish oil emulsions (Zhu, Ye, Verrier, & Singh, 2013). For example, the absorption of EPA and DHA following the administration of oil mixtures was only 33.6 % and 44.3 %, respectively, of that administrated emulsified oil mixtures. This was determined by fatty acid profile analysis of plasma postprandial TAG concentrations (Garaiova et al., 2007).

Fabrication of emulsions can be achieved using a surface-active agent mainly by two main methods, distinguished by the required energy input (i.e., high, and low-energy methods). In high-energy methods, a high energy source is used to disperse initial emulsion droplets into nano-sized droplets (10 nm to 100 nm), using mechanical devices known as homogenizers (Sole, Maestro, González, Solans, & Gutiérrez, 2006). Conversely, a low-energy method spontaneously forms a thermodynamically stable emulsion under certain compositions of surfactant, co-surfactant, and oil, leading to a change in the interfacial properties and usually the formation of micro-sized emulsions (100 nm to 100 μm). In recent years, investigators have exploited microemulsions as a delivery system of n-3 PUFA to enhance their uptake in the body. Typically, microemulsion systems are created spontaneously by incorporating a non-aqueous n-3 oil source, an aqueous phase, and a surfactant. The aggregation stability of engineered emulsions under different environmental conditions in the gastrointestinal tract is significantly influenced by their interfacial compositions and initial droplet size (Armand et al., 1992; Borel et al., 1994; Wickham, Garrood, Leney, Wilson, & Fillery-Travis, 1998). Ultimately, this affects their gastric and pancreatic lipolysis rates and subsequent absorption. For example, the process of flaxseed oil emulsification using soy lecithin has been shown to enhance the gastric lipolysis of the oil by 30 %. Conversely, both tween 80 and sodium caseinate inhibited the gastric lipolysis of flaxseed oil by 80 % and 40 %, respectively. This impact on digestion was correlated with improving rats' intestinal absorption of

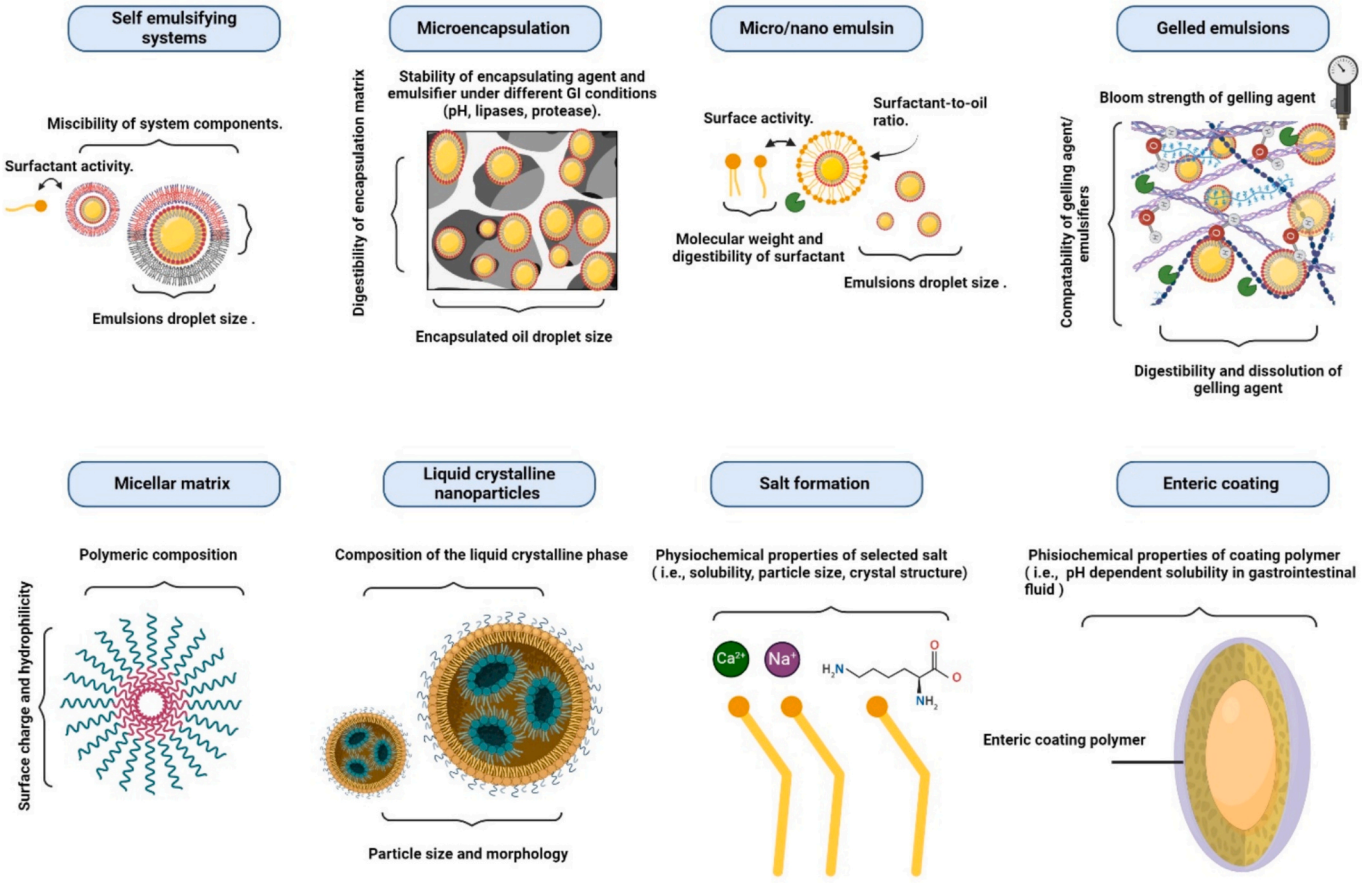

Fig. 2. The impact of n-3 PUFA formulation design on pharmacokinetics; formulation design of n-3 PUFA can potentially impacts their pharmacokinetics by affecting formulation stability under different GI conditions (i.e., degradation of emulsifier and/or encapsulating in the presence of digestive enzymes such as protease and lipase), altering n-3 PUFA digestion rates (i.e., droplet size of emulsions systems, molecular weight and surface activity of emulsifier(s),bloom strength of gelling agent and surfactant-to-oil ratio), affecting the long-term stability (i.e., achieving synergetic interaction between gelling agent and emulsifiers, optimizing the surface charge and size of n-3 PUFA polymeric micelles), altering dissolution rate and solubility in gastrointestinal fluid (i.e., modifying the composition and morphology of liquid crystalline nanoparticle, and selecting highly soluble salts of n-3 PUFA). Created with BioRender.com

Table 1
The impact of n-3 PUFA formulation design on overall bioavailability determined in human randomized, single dose clinical trials.

Formulation design	Intervention	n	Duration	Key physiochemical properties	AUC_{0-t} (ratio)	C_{max} (ratio)	T_{max}(h)	Ref
Gelled emulsion	5 g of EPA and DHA in fish oil TG[a] delivered in soft gelatin capsules, or dispersed in gelatin matrix equivalent	17	26 h	D50[b]: 1.81 ± 0.02 μm	1.45 (EPA), CI {1.13–1.86} 1.46 (DHA), CI {0.87–2.46} 1.43 (EPA + DHA), CI {1.05–1.96};	2.00 (EPA) CI (1.27–3.16). 2.16 (DHA) CI (1.14–4.09). 2.06 {EPA + DHA} CI (1.21–3.49).	2 (EPA + DHA) gelled emulsion 6 (EPA + DHA) Soft gel capsule	(Haug et al., 2011)
Micro-emulsification	546 mg DHA and 396 mg EPA in emulsified cod liver oil formulation, versus a non-emulsified cod liver oil.	50	24 h	NA	1.64 (EPA) $p < 0.01$ 1.78, (DHA) $p < 0.01$ 1.66 (DHA + EPA) $p < 0.01$	1.12 (EPA) 1.09 (DHA) 1.09 (DHA + EPA)	5.8 (EPA + DHA) emulsion 7.7 (EPA + DHA) Non-emulsified formulation	(Conus, Burgher-Kennedy, van den Berg, & Kaur Datta, 2019)
Microencapsulation	1 g of EPA and DHA encapsulated within: milk protein mixture (F1), protein–sugar– starch mixture(F2), or in soft gelatin capsule.	48	48 h	D50[c]: 0.35 μm	AUC (0–48) for EPA, DHA, and EPA + DHA showed no significant differences.	Cmax EPA, DHA, EPA + DHA showed no significant differences.	(EPA + DHA): 8.5 (F1), 18.8 (F2), 21.6 (Fish oil)	(Sanguansri et al., 2015)
Self-emulsifying delivery system (SMEDS)	a low dose of SMEDS equivalent to 630 mg of EPA + DHA, high dose of SEDDS equivalent to 1680 mg EPA + DHA, or equivalent dose of unformulated EPA + DHA.	59	48 h	NA	6.4 (EPA + DHA Low dose study) 11.5 (EPA + DHA high dose study) 23.8 (EPA low dose study) 25.7 (EPA high dose study) 3.6 (DHA low dose study) 5.6 (DHA high dose study)	6.7 (EPA low dose study) 9.2 {CI 7.1–11.9} (EPA-high dose study) 2.68 {CI 2.09–3.44} (DHA-low dose study 4.29 {CI 3.31–5.55} (DHA-high dose study	NA	(Qin et al., 2017)
Self-emulsifying delivery system (SMEDS)	600 mg of EPA and DHA (rTG)[d] delivered by SMEDS, or via soft gelatine capsule.	59	24 h	D50: 30–45 μm	1.70 (DHA + EPA) 1.67 (DHA) 2.11 (EPA)	1.61 (DHA + EPA) 1.64 (DHA) 1.38 (EPA)	Tmax of EPA and DHA show no significant difference	(Sin et al., 2023)
Self-emulsifying delivery system	476 mg of EPA + DHA EE[e] administered via self-emulsifying formulation, or via soft gelatin capsule.	61	24 h	D50: 43 ± 2.58 μm. Zeta potential: − 27 mV.	5.08 (DHA + EPA) 6.88 (DHA) 5.08 (EPA)	2.79 (DHA + EPA) 3.46 (DHA) 2.79 (EPA)	Tmax OF EPA, DHA, EPA + DHA show no significant difference	(Bremmell et al., 2020)
Salt formation	1400 mg of EPA/DHA administered as L-lysine salts of free fatty acids (AvailOm®), or EE form.	8	12 h	Complete dissociation of the salt was achieved under both gastric (0.1 N HCL) and simulated fed-state intestinal. Conditions (pH 5).	5.54 (DHA + EPA) 5.83 (DHA) 5.73 (EPA)	6.07 (DHA + EPA) 5.22 (DHA) 7.84 (EPA)	3 (EPA + DH L-lysine salts) 5 (EPA + DHA EE)	(Manusama et al., 2021)
Liquid crystalline nanoparticles	Four capsules of both (IMD-Omega®, 580 mg n-3 acid EEs) or the reference drug (Omacor®, 1000 mg EEs),	24	72 h	Nanoparticles exhibited a non-lamellar cubic phase	109.5 % (EPA) 134.3 % (DHA)	122.9 % (EPA) 151.5 % (DHA)	Tmax OF EPA and DHA show no significant difference	(Kang et al., 2023)
Micellar soft gel capsules	374 mg of EPA/DHA delivered via micellar formulation (LMF), or 600 mg of EPA/DHA administered via standard soft gel (STD) or enteric coated capsules (ENT).		24 h	NA	Up to 4-fold higher (EPA + DHA) compared to other treatments, ~ 5-fold and 6-fold higher (EPA), relative to ENT and STD groups, respectively. DHA show no significant differences	Cmax of total n-3 fatty acids show no significant between treatments.	10 ± 2.1 (LMF) 4.7 ± 2.1 (ENT) 3.9 ± 1.1 (STD)	(Ibi et al., 2024)

(*continued on next page*)

Table 1 (*continued*)

Formulation design	Intervention	n	Duration	Key physiochemical properties	AUC_{0-t} (ratio)	C_{max} (ratio)	T_{max}(h)	Ref
Enteric coated capsules	1680 mg EPA + DHA as triacylglycerols in uncoated or coated fish oil capsules	12	72 h	Gastric resistance was confirmed by first incubating capsules at 37 °C in a 0.1 mol/L HCL for at least 1 h, followed by complete dissolution in phosphate buffer solution (pH 6.8), within 15 min.	No significant differences were detected for EPA, DHA and total EPA + DHA fatty acids	Cmax show no significant difference between coated and uncoated formulations	Tmax show no significant difference between coated and uncoated formulations	(Schneider, Schuchardt, Meyer, & Hahn, 2011)

[a] TG: triglycerides.
[b] D_{50}: mean volume diameter.
[c] Determined for emulsions before encapsulation.
[d] rTG: re-esterified fish oil triglycerides.
[e] EE: ethyl esters.

Table 2
The impact n-3 PUFA formulation design on overall bioavailability determined in preclinical animal randomized, single dose studies using rats as the animal model.

Formulation design	Intervention	Duration	Key physiochemical properties	Main findings	Ref
Nanoemulsion	Perilla oil nanoemulsions, prepared at different homogenization pressures (0, 40, 80, and 120 MPa).	21 days	Average particle size of emulsions was significantly reduced with the increase in homogenization pressure.	The highest EPA level measured in plasma was for nanoemulsion prepared at 120 Mpa.	(Hu, Xie, Zhang, Qi, & Li, 2021)
Microemulsion	1 mL of fish oil equivalent to (14 mg of EPA + 10 mg of DHA), or groundnut oil (control)	30 days	Average droplet size of emulsion using different surfactants: Chitosan (293–1296 nm) Acacia (1095–1099 nm) Whey protein (702–708 nm) Lipoid (203–209 nm)	The DHA level in serum lipids was significantly higher in lipoid-based emulsions (56 μg/mL compared to non-encapsulated fish oil (22 μg/mL).	(Sugasini & Lokesh, 2013)
Self-emulsifying delivery system (SEDDS)	1 g of rTG SEDDS, versus a control group received rTG only.	48 h	Particle size, 324.3 ± 37.2, Zeta potential −79.9 ± 0.6.	The mean AUC $_{(0-48)}$ of EPA and DHA formulations were 1.27 and 1.29-fold higher, respectively, compared to the control group ($P < 0.05$). Cmax of DHA and EPA were 1.38 and 1.40-fold higher for optimized SED formulation compared to the control group ($P < 0.01$).	(Kim et al., 2022)
Self-nano emulsifying delivery system (SNEDDS)	300 mg/kg/day of marketed formulation ($n = 6$), DHA-SNEDDS formulation (n = 6), or normal saline (n = 6).	28 days	Optimized SNEDDS formulation showed an emulsification time of 27 ± 4.7 s, droplet size of 17.6 ± 3.5 nm, and zeta potential of 37.6 ± 0.5 mV.	Intestinal absorption study depicted 18.3 %, 21.5 %, 41.5 %, 98.7 % absorption of DHA with SNEDDS-based formulation in comparison to 8.2 %, 15.1 %, 28.8 %, 46.1 % absorption of DHA with oil-based marketed formulation after 0.5, 1, 2 and 4 h.	(Puri et al., 2016)
Self-nanoemulsifying drug delivery system (SNEDDS)	100 mg/kg of standard DHA aqueous dispersion, or DHA-containing SNEDDS	24 h	Droplet size, size distribution, and zeta potential were found to be 111.5 + 4.2 nm, 0.269 + 0.05 nm, and À23.53 + 2.9 mV, respectively.	The relative bioavailability of DHA from the SNEDDS formulation was found to be 332 + 32 % relative to the bioavailability of DHA from an aqueous dispersion.	(Alhakamy et al., 2020)

alpha-linolenic acid using soya lecithin (C_{max} = 24 mg/mL) compared to sodium caseinate (C_{max} = 7 mg/mL) and non-emulsified flaxseed oil (C_{max} = 16 mg/mL) (Couëdelo et al., 2015).

The growing recognition of amphiphilic phospholipid-based emulsifiers in the literature highlights their significance in enhancing the stability and bioavailability of various formulations, particularly in food and pharmaceutical applications (Chang & McClements, 2016; Li, Pedersen, Anankanbil, & Guo, 2018; Yin, Chen, Matsuoka, Xi, & Wang, 2022). One key advantage of using phospholipid-based emulsifiers is their ability to improve absorption of bioactive compounds, such as n-3 PUFA. For instance, a previous study investigated the potential impact of using several types of food-grade emulsifiers on EPA and DHA absorption using the everted intestinal sac model (Sugasini & Lokesh, 2013). The findings revealed that the use of chitosan, gum acacia, whey protein, or phospholipid-based emulsifier (lipoid S75–3), increased the bioavailability of EPA and DHA by 7 %, 9 %, 23 %, and 68 %, respectively, when compared to the non-emulsified oil. Moreover, DHA levels determined in the heart and brain of rats were enhanced by 77 and 41 % respectively, with phospholipid-based emulsion, compared to control. Interfacial saturation is a critical design factor in emulsions design, as it influence the lipolytic activity of lipases acting on emulsion interface. This can be achieved by optimizing the interfacial surface coverage for each specific emulsifier, thus modulating lipase activity as discussed previously by Joyce et al. (Joyce, Whitby, & Prestidge, 2016) However,

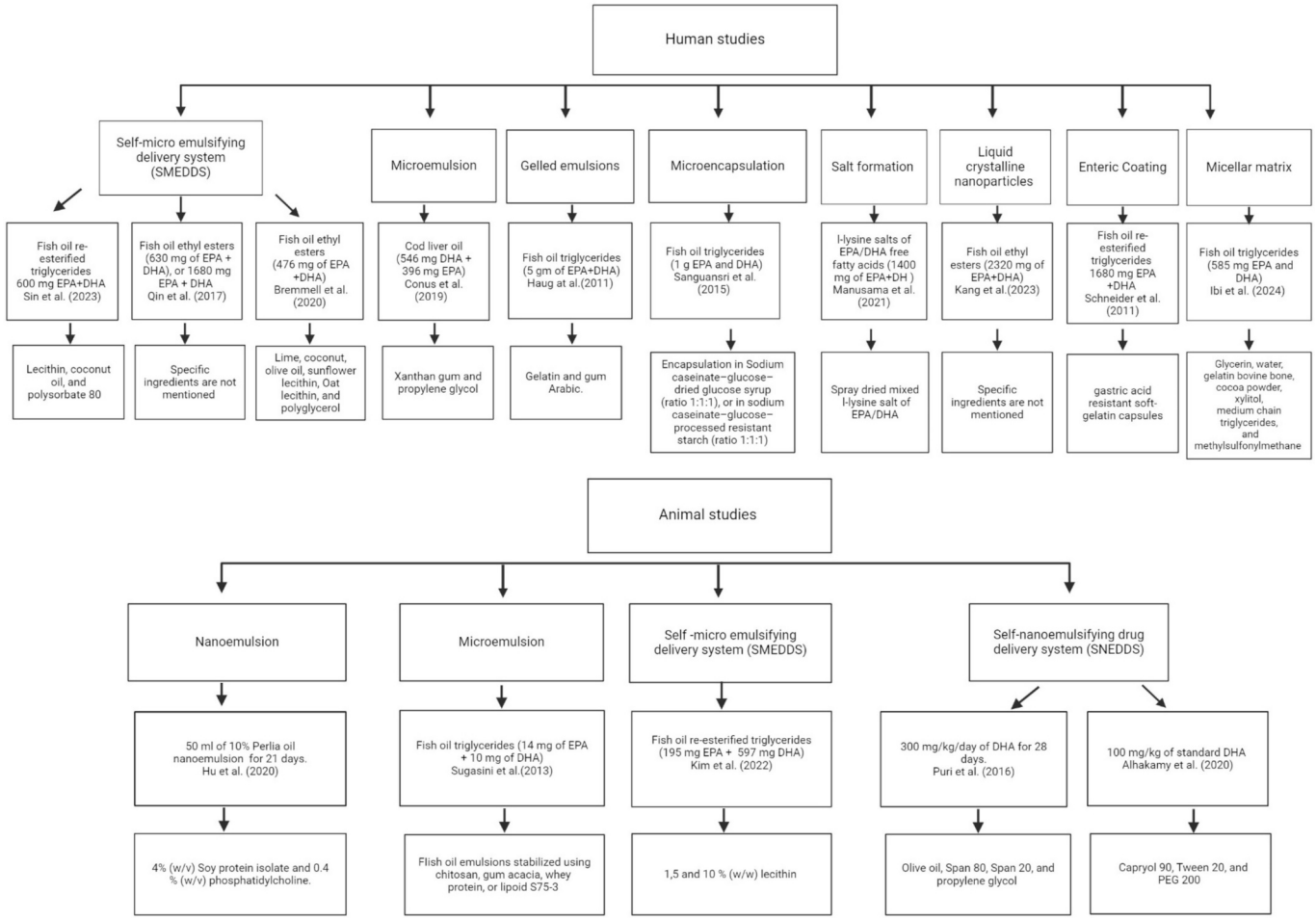

Fig. 3. Detailed description of n-3 PUFA formulations used in both human and animal studies. Created with BioRender.com.

the optimal concentration of phospholipid-based emulsifiers that modulate lipase activity for n-3 PUFA emulsion systems remains unclear, suggesting further studies to investigate this factor.

Multiple human studies have consistently demonstrated a significant enhancement in the absorption rate of emulsified fish oil compared to non-emulsified oil mixtures (Harris & Williams, 1989). (Garaiova et al., 2007; Haug et al., 2011; Hussey et al., 2012) In a randomized crossover study, authors investigated the effect of emulsification on plasma triacylglycerol levels and individual fatty acid absorption following administration of micron-sized emulsion consisting of 43 % concentrated fish oil, 31 % borage, and 26 % flaxseed oil, with a median droplet size of 1.3 μm (Garaiova et al., 2007). The study found that emulsification substantially enhanced the rate and extent of plasma triacylglycerol absorption compared to non-emulsified mixtures. The Area under the curve (AUC) between the oil and emulsion groups was 904.20 h. μmol/L, with an AUC ratio (oil/emulsion) of 60.4 % ($P = 0.0182$; 95 % CI of 169.9 to 1638.5).

Furthermore, emulsification of cod liver using Xanthan gum and propylene glycol resulted in significantly higher incremental $AUC_{(0–24h)}$ values for DHA + EPA, DHA, and EPA in comparison to non-emulsified formulation ($p \leq 0.01$). (Conus et al., 2019) The adjusted mean $iAUC_{(0–24h)}$ values for DHA + EPA, DHA, and EPA were 1.66, 1.78, and 1.64 times higher, respectively, for the emulsion in comparison to standard oil (all $p \leq 0.0068$).The time to reach maximum absorption (T_{max}) of DHA + EPA and DHA was also significantly reduced by 2.0 h, 95 % CI (−3.0, 0.0) for DHA + EPA ($p = 0.0063$) and by 1.0 h, 95 % CI (−3.0, 0.0) for DHA ($p = 0.0004$). However, no significant difference in (T_{max}) was observed for EPA ($p = 0.9490$).

3.1.2. Nanoemulsions

Nanoemulsions based delivery systems have attracted significant interest due to their nanoscale size, remarkable stability, and increased bioavailability. Owing to their nanosize and high surface-to-volume ratio, nanoemulsions can improve lipid absorption in the gastrointestinal tract (McClements, 2013). In contrast to microemulsions, the fabrication of nanoemulsions needs a reduced quantity of surfactants. However, their preparation necessitates external high-energy treatment. Nanoemulsions can be stabilized using various types of food-grade surfactants such as non-ionic synthetic surfactants (e.g., ethoxylated sorbitan esters such as Spans and Tweens), phospholipids (e.g., soy lecithin), proteins (e.g., milk caseinate) and polysaccharides (e.g., gum Arabic) (Salvia-Trujillo, McClements, & Martín-Belloso, 2021). A key factor in producing a stable emulsion is the rapid creation of a continuous monomolecular layer encircling emulsified oil droplets. Therefore, a sufficient concentration of emulsifier is necessary to cover the entire generated interface. Moreover, the adsorption process of the emulsifier at the interface is affected by its molecular weight. For example, small monomeric surfactants such as Tween 20 and sodium dodecyl sulphate adsorbs rapidly and effectively producing nanoemulsions compared to caseinate and β-lactoglobulin under the same homogenization parameters (Bouyer, Mekhloufi, Rosilio, Grossiord, & Agnely, 2012). Nevertheless, it's also important to optimize the surfactant-to-oil ratio (SOR) to avoid the existence of increasing amounts of unbound surfactant within the continuous phase. Free surfactants will engage in competition and displace lipases from the oil–water interface. This occurs, particularly for highly surface-active surfactants that present in sufficiently high concentrations (McClements & Xiao, 2012). According to Salvia-

Trujillo et al. Tween 80 stabilized nanoemulsions with a SOR of 2 exhibited a significantly reduced digestion rate compared to micro-emulsions produced with a SOR of 0.1 (Salvia-Trujillo et al., 2019).

The hydrophilic-lipophilic balance (HLB) value of surfactants can similarly affect the digestion rate of lipids under a simulated lipolysis model. Speranza et al. demonstrated that Increasing the HLB value using different nonionic and anionic surfactants resulted in a negative impact on the digestion rate of trioctanoyl glycerol emulsions (Speranza et al., 2013). In contrast, Zheng et al. found that increasing HLB value of sucrose stearates stabilized emulsions leads to a better digestion under simulated gastrointestinal conditions (Zheng et al., 2024).

The impact of nanoemulsions droplet size on n-3 PUFA absorption was investigated by Hu et al. Several Perilla oil nano-emulsions containing 52.58 to 61.98 % ALA were fabricated at variable homogenization pressures. Overall, the highest plasma levels of fatty acids (Palmic acid, stearic acid, oleic acid, and EPA) were achieved when the emulsion had the lowest droplet size of 293.87 ± 6.55 nm. Similarly, Dey et al. investigated the impact of nano-sizing on the ex vivo bioavailability of EPA and DHA using Sprague Dawley rat everted intestinal sac model (Dey, Koley, Ghosh, Dey, & Dhar, 2019). The droplet size of conventional (1528.5 ± 187.29 nm) and nano-sized (89.7 ± 27.7 nm) emulsions was controlled using Tween 20 and Span 80. According to the authors, it was observed that the efficiency of lipid uptake across the everted sac from the conventional emulsion reached a state of stagnation at 45 min, while a significant increase ($p > 0.05$) was achieved in n-3 PUFA uptake from nanoemulsion beyond the 45-min incubation period, in all the three intestinal segments. Moreover, extending the incubation period from 30 to 90 min was associated with a significantly ($p > 0.05$) higher trans-epithelial lipid absorption for nano-emulsion compared to conventional emulsions. These findings show that nano-sizing can have a significant impact on n-3 PUFA absorption, however, further studies are necessary to recognize the impact of nanosizing on various pharmacokinetic properties of n-3 PUFA.

3.1.3. Self-emulsifying systems

Self-emulsifying drug delivery systems (SEDDS) are isotropic mixtures of oil(s), surfactant(s) cosurfactant(s), and possibly cosolvents, which form a fine emulsion under mild agitation encountered in the gastrointestinal tract (Pouton, 1997). Improved bioavailability of lipophilic drugs can be attained using SEDDS by various mechanisms, such as increasing drug solubility, permeability, and uptake across the intestinal lymphatic system. (Ahmad, Kohli, Mir, & Amin, 2012; Alwadei, Kazi, & Alanazi, 2019)

Droplet size is a critical design factor of SEDDS, as it impacts the lipolysis process of n-3 fatty acids after dispersion in the GI. This can be optimized by choosing suitable surfactant(s) and optimizing their concentration. For instance, Alhakamy et al. (2020), successfully prepared DHA SEDDS using Capryol 90, Tween 20, and polyethylene glycol 200 as oil, surfactant, and cosurfactant, respectively (Alhakamy et al., 2020). Authors found that increasing the surfactant/cosurfactant ratio from 1:1 to 1:3 resulted in decreasing droplet size of DHA SEDDS from 118.2 + 1.9 nm to 101.3 + 3.1 nm due to adsorption and stabilization effects of surfactant molecules at the oil–water interface. However, the droplet size of SEDDS was increased again when a 4:1 ratio was used resulting in breaking down the established interfacial tension and loss of droplet stabilization. Among the various formulation strategies explored in this review, self-emulsifying drug delivery systems (SEDDS) stand out as particularly promising. These systems have demonstrated superior enhancement of n-3 PUFA pharmacokinetics compared to other formulation approaches. Recently, a Self-emulsifying technology was developed to improve the pharmacokinetics of EPA and DHA ethyl ester concentrate. The optimized formulation comprising 15 % (*w/v*) of SEDDS excipients (lime oil, coconut oil fractionated, lecithin (sunflower), lecithin (oat), olive oil, polyglycerol polyricinoleate) and 85 % (w/v) EPA + DHA ethyl esters, which exhibited rapid emulsification in water with a D50 of 43 ± 2.58 μm and zeta potential of −27 mV. Furthermore, the pharmacokinetics of EPA and DHA were investigated in a randomized, double-blind design for 62 healthy adults over 24 h. Each participant received 680 mg of oil (corresponding to 272 mg of EPA EE and 204 mg of DHA EE) of formulated and non-formulated oil. The Self-emulsification process significantly increased the maximum concentration $C_{(max)}$ of EPA, DHA, and EPA + DHA, by 2.8, 3.5, and 3.2-fold, respectively, compared to unformulated oil. Additionally, SEDDS formulation achieved a 6.1-fold increase in total EPA and DHA absorption demonstrating a greater $AUC_{(0–24h)}$ of SEDDS compared to the control. For EPA, DHA, and EPA + DHA, both groups displayed a median $T_{(max)}$ range from 6 to 9 h, and no significant difference was detected between the two groups. (Bremmell et al., 2020)

The pharmacokinetics of re-esterified omega-3 triglyceride (rTG) delivered by a self-emulsification system was also explored using coconut oil, polysorbate 80, and lecithin (Sin et al., 2023). Candidate formulations were selected based on phase-diagram construction. All formulations were inspected, and the optimal formulation was determined based on droplet size distributions, oil floating area, and turbidity. The selected formulation showed a unimodal particle distribution (D_{50} 30–45 μm) and minimal turbidity changes within 24 h. The pharmacokinetics of EPA and DHA were examined in a double-blind, single-dose, crossover study. Randomly assigned participants ($n = 44$) received either 600 mg of SEDDS formulation or a raw omega 3 rTG (control group). The $AUC_{(0–24)}$ for DHA, EPA, and total DHA and EPA were 1.67, 2.11, and 1.70 times higher, correspondingly, compared to the control group. Furthermore, the $C_{(max)}$ values for DHA, EPA, and DHA+ EPA were 1.64, 1.38, and 1.61 times higher, compared to the group that received control treatment.

These findings highlight the superior performance of SEDDS in enhancing the absorption and systemic exposure of n-3 PUFA. This improvement could potentially lead to more effective therapeutic outcomes and optimized dosing regimens for theses essential fatty acids.

3.1.4. Gelled emulsions

Gelled emulsions are soft-solid colloidal materials consisting of a pre-emulsified oil dispersed throughout a gelling matrix (Dickinson, 2012). Typically, gelled emulsions are made by incorporating an omega-3 oil phase into the aqueous phase of a gelling agent above the melting temperature. Subsequently, emulsions are cooled down so the gelling agent can polymerize/solidify under refrigeration (Alejandre, Poyato, Ansorena, & Astiasarán, 2016). Commonly used gelling agents include carrageenan, alginate, and gelatin. Among these, gelatin is the most widely used gelling agent due to its superior surface activity and emulsifying properties compared to other polysaccharides (Baydin, Arntsen, Hattrem, & Draget, 2022). Emulsion stabilization is a critical step that should be optimized throughout the continuous gel matrix to maximize the available surface area for lipase enzyme to act upon ingestion and release of fine oil droplets in the GI (Dille, Hattrem, & Draget, 2018). For this purpose, gelatin can act as an emulsifier to maintain the stability of dispersions at elevated temperatures during the manufacturing process. Optimizing the concentration of stabilizer is a key factor determining the availability of excess stabilizer forming multiple layers on emulsified oil droplets which can act to sterically hinder the lipase lipolysis action. As a result, optimizing droplet size diameter while maintaining stabilizer concentration would provide less steric hindrance and increase the hydrolysis rate. (Yi, Li, Zhong, & Yokoyama, 2014) Furthermore, gelatin type can significantly impact the physiochemical properties of chewable gels and the subsequent release of entrapped oil droplets. For example, dissolution studies have shown that type A gelatin with large molecular weight can produce a gel with higher transition temperature and slower dissolution kinetics compared to type B at body temperature (Hattrem, Molnes, & Draget, 2014).

Droplet size distribution of gelled emulsions can also be influenced by the stabilization behavior of selected gelling agents and emulsifiers through synergistic or competitive adsorption at the oil/water interfaces of designed emulsions (Zhang et al., 2020). This can affect the long-term

stability of gelled emulsions by influencing their creaming rate, thus altering the emulsion droplet's size distribution,which leads to significant alterations in the surface area of oil available for digestion, negatively affecting both fatty acid release, and uptake in the GI.

Hung et al. (Haug et al., 2011) investigated the pharmacokinetics of EPA and DHA after single dose administration of both chewable gels consisting of pre-emulsified fish dispersed into a gel matrix, and soft gelatin capsule in healthy subjects. Specifically, the absorption of DHA and EPA triglycerides delivered by gelled emulsion was significantly increased by 44.9 and 43.3 %, over 26 h, respectively, compared to soft gel capsule. However, the bioavailability of DHA from the gelled emulsion was not significantly increased compared to the soft gel capsule treatment ($p < 0.177$). The maximum blood concentration $C_{(max)}$ of EPA and EPA + DHA was significantly increased by 100.4 and 105.6 %, respectively, compared to soft gel capsules. The time until the maximum concentration of EPA, EPA, and DHA was significantly reduced for chewable gels. However, no significant difference in $T_{(max)}$ was observed for DHA between both treatments.

3.2. Liquid crystalline nanoparticles

Nanoparticles have emerged as an effective solution in drug delivery systems due to their ability to enhance drug solubility and target specific tissues (Shepherd, Issadore, & Mitchell, 2021). Among these, liquid crystalline nanoparticles (LCNPs) have garnered significant interest over the past few decades. LCNPs exist in a state intermediate between the crystalline solids and the amorphous liquids. It may flow like liquids, but its molecular orientation may be like solid crystals. In particular, the class of lyotropic liquid crystals (LLC) is formed by amphiphilic molecules and solvents (usually water) that assemble into molecular aggregates under certain conditions of temperature, pressure, and relative concentration. When amphiphilic lipids such as glyceryl monooleate and lecithin are added to water, their polar head groups interact with the surrounding water, while the nonpolar tails move away from the water, into the air or a nonpolar liquid. This interaction disrupts the cohesive energy at the interface, leading to microphase separation and the formation of micellar aggregates or other small, closed interfaces. LLC offer several advantages as drug delivery systems, including thermodynamic stability in excess water, highly ordered nanostructures and the ability to deliver drugs in a sustained manner, enhancing both safety and efficacy (Lombardo, Kiselev, Magazù, & Calandra, 2015).

Based on their internal structures, lyotropic LCNPs are classified into three types: lamellar, cubic, and hexagonal phase. Lamellar phases have the highest concentration of surfactant compared to the other phases. They have high fluidity; because of which lamellae can easily slide over one another. In contracts, both cubic (cubosomes) and hexagonal (hexosomes) phases are widely used in oral administration because they have the ability to form highly ordered structures internally, resulting in the formation of a sustained release matrix for drugs of various polarities and sizes (Chavda et al., 2023).

The preparation of dispersed self-assembled LLC mesophases is typically achieved through two main approaches: a top-down method and a bottom-up method. In the top-down approach, initially a high energy is applied to shear the amphiphile, which has been hydrated and contains an excess of aqueous phase along with stabilizers. Techniques such as ultrasonication, centrifugation, vortex homogenization, high-pressure homogenization, high-speed shearing, and high-pressure extrusion are ideal for preparing these dispersions (Mulet, Boyd, & Drummond, 2013). Conversely, the bottom-up approach, involves using a hydrotrope (such as ethanol, chloroform, propylene glycol, or polyethylene glycol 400) to create a single-phase molecular solution. This solution is then diluted, leading to the spontaneous formation of LCNPs.

Recent studies have demonstrated that temperature, fatty acid composition, and pH directly affect LCNP formation and stability. Specifically, liquid crystalline nanoparticle dispersions of monoolein with various cis-unsaturated fatty acids have been prepared, and their partial temperature-composition phase diagrams and structures have been established using high-throughput Small Angle X-ray Scattering (SAXS) and cryogenic transmission electron microscopy (cryo-TEM). These studies reveal that low energy inverse micellar cubic to emulsion phase transformations can occur at physiological temperatures in systems containing monoolein with oleic acid and vaccenic acid (Fong, Zhai, Drummond, & Tran, 2020).

Further research has aimed at optimizing self-assembled liquid crystalline nanoparticles composed of n-3 ethyl ester, phospholipids and oleic acid, using a ternary phase diagram to identify optimal formulation (Jeon, Jin, & Park, 2022). The findings revealed that a cubic structure significantly enhanced the dissolution and permeability of n-3 EE compared to those with hexagonal, lamellar, or non-liquid crystalline structures. Notably, cubic liquid crystals increased oral bioavailability by 2.5-fold for EPA and 3.1-fold for DHA in studies conducted with male beagle dogs. This enhancement is due to the cubic structure's increased surface area and internal volume, which facilitate better solubilization of candidate formulation.

Recently, a novel LCNP-based formulation, known as IMD-Omega soft capsule, was evaluated to enhance the pharmacokinetics of EPA and DHA compared to the commercially available n-3 ethyl ester Omacor® (Kang et al., 2023). In a randomized, open-label, single-dose, crossover trial with healthy adults, the LCNP formulation demonstrated a 110 % increase in bioavailability for EPA and a 134 % increase for DHA relative Omacor® soft gel capsules over a 72- h period. Notably, the crossover design of this study allow each participant to serve as their own control, thereby reducing the impact of inter-participant variability, which is crucial for accurately assessing products bioequivalence. The enhanced bioavailability of LCNP formulation, was achieved through the spontaneous formation of liquid crystalline nanoparticles composed of phospholipids and oleic acids. This formulation enables effective therapeutic blood levels of ω-3 acid ethyl esters at lower dosages (less than 580 mg). The self-assembled liquid crystal, once formed, is further solubilized, and degraded by bile salts and lipolytic enzymes in the small intestine. Consequently, omega-3 fatty acid absorption is significantly increased compared to the commercialized Omacor® formulation.

Scaling up the production of liquid crystalline nanoparticles (LCNPs) from the laboratory to an industrial scale presents significant challenges due to their complex phase behavior and viscous properties. Manufacturing LCNPs on a large scale is complicated by factors such as particle size, size distribution, and stability of liquid crystalline mesophase which can be influenced by various production parameters including temperature, homogenization method, and homogenization cycles (Hua, De Matos, Metselaar, & Storm, 2018; Madheswaran, Kandasamy, Bose, & Karuppagounder, 2019). Addressing these challenges requires a thorough understanding of the physicochemical properties of LCNPs and precise control over the production conditions to ensure scalability while maintaining product quality and efficacy.

3.3. Salt formation

Salt formation is a valuable technique for enhancing the effectiveness of n-3 PUFA, primarily by addressing their solubility challenges. The poor water solubility of these fatty, can often hinder their absorption and efficacy when taken orally. By converting these fatty acids into their salt forms, their solubility is significantly improved. Initially, in the acidic environment of the stomach, these salts dissociate into their constituent ions—releasing the free fatty acids. (Manusama et al., 2021)

Currently, there is a prescription formulation on the market, Epanova®, which contains EPA and DHA as free carboxylic acids within a coated delivery system. Research has demonstrated that administering DHA and/or EPA as free fatty acids (FFA) results in significantly higher plasma peak levels and bioavailability within the first 12 to 24 h compared to administration in the form of ethyl esters (EE) or triglycerides (TAG). (Davidson, Johnson, Rooney, Kyle, & Kling, 2012;

Hansen, Grimsgaard, Nilsen, Nordøy, & Bønaa, 1998) however the widespread of oral preparations containing n-3 FFA is limited due to issues such as a strong taste, gastrointestinal irritation, and susceptibility to oxidation (Ismail, Bannenberg, Rice, Schutt, & MacKay, 2016).

To address these issues, extensive research has focused on salt formation has enhance the absorption the of n-3 PUFA. Various salts, including sodium and calcium (Harel, Ozkizilcik, Lund, Behrens, & Place, 1999; Leduc, Gervais, & Chouinard, 2017), have been explored, and basic amino acids such as lysine and arginine have also been reported as effective options for improving bioavailability (Bruzzese, 1998; Manusama et al., 2021). Recently, a mixed L-lysine salt of the carboxylic form of EPA and DHA has been developed by combining equimolar amounts of the fatty acids dissolved in ethanol with an aqueous solution of L-lysine, followed by spray-drying (Manusama et al., 2021). The Pharmacokinetics of this formulation were evaluated in a randomized, two-way cross-over design, comparing the oral administration of Lys-FFA (500 mg EPA plus 302 mg DHA) against n-3 EE (504 mg EPA plus 378 mg DHA) over a 48-h period in eight female volunteers. The results demonstrated a significant enhancement in the bioavailability of the L-lysine salt. Specifically, For EPA, the median $AUC_{(0-12)}$ ratio was 5.45, while the median C_{max} ratio was 6.75. For DHA, the median $AUC_{(0-12)}$ ratio was 2.92, while the median ratio C_{max} ratio was 4.1. Additionally, the median Tmax for EPA + DHA was 3 h, compared to 5 h for the n-3 EE formulation. These results indicate that L-lysine salt formulation significantly improved both the rate and extent of absorption for n-3 PUFA compared to EE form by achieving higher peak concentrations more rapidly and maintain higher levels over time. This suggests the potential benefit of investigating other amnio acids that could further enhance the pharmacokinetic properties of n-3 PUFA.

3.4. Enteric-coated capsules

Omega-3 supplements formulated with delayed-release technologies can help create a gastro-resistant barrier, potentially reducing the fishy aftertaste or smell often associated with these supplements (Sanguansri & Augustin, 2006). This barrier, is usually made from synthetic polymers or natural ingredients, ensures that the active compounds are released in the more neutral or alkaline environment of the small intestine (Maderuelo, Lanao, & Zarzuelo, 2019).

The growing trend of using enteric coating on fish oil capsules seems to have no effect on their bioavailability. For instance, Schneider et al. reported no significant difference in the bioavailability of EPA- and DHA delivered by enteric-coated and standard capsules. In this double-blind crossover study, plasma phospholipid fatty acid compositions were measured over 72 h post-ingestion as an indicator of bioavailability, and no differences in absolute absorption, as reflected by $AUC_{(0-72)}$ values, were observed. Similarly, there were no significant differences in maximum serum concentrations or the time to reach peak concentrations between coated and uncoated capsules. The lack of significant differences in bioavailability in this study may be due to the digestive process of lipids, which begins with the hydrolysis of triglycerides (TAG) into 2-monoacylglycerols and free fatty acids by gastric and sublingual lipases. However, the majority of lipid digestion occurs in the upper jejunum by pancreatic lipase and colipase (Iqbal & Hussain, 2009). The extended residence time of lipids in this segment allows sufficient time for pancreatic lipases to hydrolyze EPA and DHA TAG from both types of capsules equally, thus explaining the observed absence of significant differences in absorption. These findings suggest that the protective coating may not provide any added advantages in terms of bioavailability. This underscores the importance of understanding the digestive pathways and their pathways implications, particularly for n-3 PUFA.

3.5. Micellar matrix

Polymeric micelles are created through the self-assembly of amphiphilic monomers, typically forming spherical structures with a hydrophobic core and a hydrophilic shell (Gonçalves, Martins, Duarte, Vicente, & Pinheiro, 2018). Below a certain concentration, known as the critical micellar concentration (CMC), these polymers exist in solution as single molecules. However, once the CMC is exceeded, they self-assemble into micelles with a lipophilic core and a hydrophilic shell. Due to their straightforward self-assemble process in solution, micelles offer easier preparation and greater scalability compared to other nanocarriers like liquid crystalline nanoparticles, which requires more complex, time-consuming and costly manufacturing methods as discussed earlier.

Common methods for preparing micelles include dialysis, filtration and solvent evaporation (Goktas et al., 2020). additionally, the oil-in-water emulsion technique is frequently used to create casein micelles loaded with DHA or EPA. Active ingredient, such as n-3 PUFA can be encapsulated in the micelles during the formation of micelles or in a subsequent step. The choice of preparation method is influenced by the characteristics of both the polymer and the active ingredient (Aliabadi & Lavasanifar, 2006; Gaucher et al., 2005). Notably, casein micelles are commonly employed for encapsulation of DHA and EPA are casein micelles due to their natural self-assembly properties. Previous studies on casein micelles loaded with DHA have shown that these micelles can spontaneously bind DHA and EPA molecules through hydrophobic interactions, providing excellent protection of DHA, and maintaining its biological activity. This system demonstrated significant protection against DHA oxidation and exhibited good colloidal stability and bioactive retention throughout shelf life at 4 °C (Zimet, Rosenberg, & Livney, 2011).

LipoMicel® is a patent-pending micellar formulation of n-3 polyunsaturated fatty acids (PUFAs) that has demonstrated improved absorption of total n-3 fatty acids in a recent human clinical trial (Kang et al., 2023). In this trial, blood samples were collected from 12 healthy participants after administering LipoMicel® formulation, which delivered a total of 374 mg of n-3 fatty acids. This was compared to standard softgels and enteric-coated capsules (ENT), both providing 600 mg of n-3 PUFAs.

The results demonstrated that the micellar formulation produced the highest area under the curve (AUC_{0-24}), reflecting a total blood concentration of n-3 PUFA that was up to four times greater than the other treatments. Additionally, EPA levels were approximately five times higher in the LipoMicel® group compared to the ENT group and six times higher than those found in the softgel capsules. Although the AUC_{0-24} for DHA was nearly 1.5 times greater in LipoMicel® compared to the other formulations, this difference was not statistically significant. Additionally, DHA's overall concentration across all three formulations was significantly lower than that of EPA. According to authors, this reduced absorption of DHA in the gastrointestinal tract may be linked to its higher susceptibility to oxidation compared to DHA, however, further studies are needed to confirm this effect on the bioavailability of n-3 PUFA.

3.6. Microencapsulation technologies

The high susceptibility of n-3 PUFA to oxidation can lead to the formation of various degradation products. This process is responsible for fishy aftertaste development, especially after the administration of fish oil in large doses, leading consumers to avoiding omega-3 supplements. (Ismail et al., 2016) Microencapsulation can be employed to prevent fishy taste by protecting unsaturated fatty acid from oxidation before incorporation into food products. This can be achieved by entrapping sensitive ingredients within the core of another protective wall material by various encapsulation technologies, including spray, freeze, and fluidized bed drying (Assadpour & Jafari, 2019; Pourashouri et al., 2014). By far, spray drying is the most popular technique used for encapsulating n-3 PUFA due to its simplicity, low cost, reproducibility, and capacity to be easily scaled up (Encina, Vergara, Giménez, Oyarzún-Ampuero, & Robert, 2016). It is employed to entrap sensitive n-3 fatty

acids within the core of another protective wall material. This process typically consists of 3 stages. In the first stage, fish oil emulsion is blended with an aqueous solution of suitable wall material, followed by dispersion and homogenization. Secondly, a feed of emulsion is introduced to a drying chamber at a constant feeding rate and atomized under pressure into fine liquid droplets. Finally, the droplets are dehydrated by hot drying air which temperature is also controlled as inlet temperature and dry powders are separated and collected. Microencapsulation has been shown to have a positive impact on digestion rates of encapsulated n-3 PUFA thus improving their pharmacokinetics. The choice of encapsulating agent, emulsifier, and encapsulation method can influence the bioaccessibility of encapsulated n-3 PUFA. For example, a recent study has shown that carbohydrate-based encapsulation of fish oil using glucose syrup or maltodextrin had no impact on lipid digestion. However, the emulsifier used for stabilizing feed emulsion exerted a significant influence on bioaccessibility. Specifically, whey protein concentrates hydrolysate significantly improved lipid digestion compared to Tween 80 (Rahmani-Manglano, Tirado-Delgado, García-Moreno, Guadix, & Guadix, 2022). In another investigation, fish oil encapsulation within porous silica nanostructure enhanced n-3 PUFA bioaccessibility by 1.4-fold, compared to unprocessed fish oil formula, which was mainly due to the significant increase in the surface area available for the lipase-mediated action upon encapsulation (Joyce et al., 2018). The bioaccessibility of encapsulated n-3 PUFA can also be influenced by the selected encapsulation method. According to El-Messery et al. the encapsulation of 8 % (*w/v*) krill oil nanoemulsion by spray drying led to a higher bioaccessibility of 83.0 % compared to a bioaccessibility of 72.4 % for freeze-dried nanoemulsion (El-Messery, Altuntas, Altin, & Özçelik, 2020).

The bioequivalence of two n-3 PUFA microencapsulated powders was assessed relative to fish oil delivered by gel capsules in healthy participants (Sanguansri et al., 2015). Encapsulated powders were fabricated via spray drying within milk protein–sugar (F1) and milk protein–sugar–starch (F2) as wall materials. The pharmacokinetics parameters were assessed after ingestion of 1 g EPA + DHA, followed by measuring total plasma fatty acids over 48 h (acute study), and erythrocyte FA composition (known as omega-3 index) over 4 weeks. Over the 48 h period, F1 increased plasma EPA and total *n*-3 FA levels at 2 and 4 h and compared with fish oil capsules. The time to reach maximum plasma values of total EPA+ DHA was significantly decreased for F1 (8.5 h) than for fish oil (21.6 h). However, the AUC value for total EPA + DHA uptake showed no significant differences. Moreover, a similar omega-3 index (5·8–6·3 %) was achieved for all treatments in the long-term uptake study (Sanguansri et al., 2015).

4. Next generation n-3 PUFA formulations

Dietary supplements of n-3 PUFA fatty acids are in high demand nowadays due to their documented role in promoting human health. Nevertheless, formulation stability is one of the most encountered issues when assessing the long-term stability, toxicity, and efficacy of n-3 PUFA throughout processing and storage. This can be further complicated by the low bioavailability of n-3 PUFA due to their hydrophobic nature and digestion-dependent absorption in the GI. These limitations can be overcome by utilizing advanced encapsulation systems, that convert n-3 PUFAs into various colloidal structures such as nanoemulsions, liposomes, and lipid-based nanocarriers. Recently, significant research has been conducted to explore solid lipid nanoparticles (SLNs) and nanostructure carriers (NLCs) as lipid based nanocarrier systems for improving the dispersibility, stability, and bioavailability of n-3 PUFA. Alternatively, the bioactivity of n-3 PUFA can be improved through achieving selective uptake in specific tissue by modifying the n-3 PUFA composition and positional distribution to produce structured n-3 PUFA formulations. Interestingly, the bioactivity and stability of these formulations are supported by pre-clinical evidence. However, their pharmacokinetics evaluation in humans is poorly investigated. The following sections highlight the current advancement in improving the delivery of n-3 PUFA via solid lipid nanoparticles (SLNs), nanostructure carriers (NLCs), and structured lipid formulations.

4.1. Encapsulation of n-3 PUFA in solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs)

SLNs and NLCs are comprised of a lipid matrix that consists of both solid and/or liquid lipids, an emulsifier, a co-emulsifier, an aqueous phase, and active ingredients. These systems can improve the bioavailability of hydrophobic bioactive ingredients by increasing their solubility and permeability across GI tract. NLCs, which are the next generation of SLNs, encompass both liquid and solid lipids within their structure, resulting in enhanced drug loading and stability. In general, their lipid matrix can be constituted by either a single solid lipid or a combination of diverse lipid classes having distinct melting temperatures. These lipid classes predominantly include triacylglycerols (TAGs), partial glycerides, fatty acids, steroids, and waxes. (Mehnert & Mäder, 2012) (da Silva Santos, Ribeiro, & Santana, 2019) In fact, oils rich in n-3 PUFA can be incorporated directly as the liquid lipid in the production of NLCs, which can generate a less-ordered crystalline structure and increase n-3 PUFA encapsulation efficiency. This NLC approach has been shown to achieve high encapsulation efficiency (>95 %) and prevent photooxidation of krill oil (Zhu, Zhuang, Luan, Sun, & Cao, 2015).

SLNs or NLCs are often stabilized by a layer of surfactant, which can be comprised of either a single surfactant, or a combination of surfactants. This layer serves to stabilize the interfacial phase between the molten lipid and aqueous phase, thus stabilizes formed emulsion. The encapsulation of n-3 PUFA within SLNs or NLCs imparts high stability for these unsaturated fatty acids due to the crystallization of the lipid phase, which slows down the interaction of prooxidants with n-3 PUFA (Tang, Chen, & Dong, 2023). For instance, using high-melting-temperature lecithins as emulsifiers were found to inhibit the oxidation of encapsulated n-3 PUFA within NLCs carriers due to the creation of interfacial solid surfactant layer (Salminen, Helgason, Kristinsson, Kristbergsson, & Weiss, 2013). Similarly, the encapsulation of DHA and ALA in SLNs consisting of triglycerides improved the oxidative stability of n-3 PUFA compared to unformulated oils as determined using infrared spectra analysis of SLNs (Holser, 2012).

The generation of SLNs and NLCs involves employing methods analog to those employed in the creation of emulsions or nanoemulsions. However, in this case, homogenization is conducted at a temperature exceeding the lipid phase's melting point. Among the various techniques utilized for preparation, high pressure homogenization (HPH) and ultrasonic homogenization are widely acknowledged as the most effective methods for producing SLNs and NLCs. This is primarily due to their frequent utilization in large-scale production in food industry (Ashfaq et al., 2023).

There exist two approaches of high-pressure homogenization (i.e., hot, and cold methods). Method selection should be guided by the thermal stability of the active ingredient, both methods can be employed to fabricate SLNs and NLCs depending on the stability of the ingredients. The hot HPH process is suitable for incorporating heat-stable ingredients and is typically conducted at temperatures above the lipid phase's melting point. On the other hand, cold HPH is more suitable for encapsulation of thermal-sensitive ingredients (da Silva Santos et al., 2019) This can be achieved at low temperatures to prevent the oxidation of sensitive n-3 PUFA that predominate at higher thermal processing conditions. In this case, rapid cooling of molten mixtures is attained in liquid nitrogen or dry ice, then the solid matrix is ground into microparticles, followed by dispersion and homogenization in emulsifier solution at room temperature. Aside From HPH, ultrasound homogenization involves treating a pre-emulsion consisting of a molten lipid matrix with high-intensity ultrasonic waves in order to generate O/W nanoemulsions. This is followed by homogenization using ultrasound to produce solidified SLNs and NLCs. Today, Successful encapsulation of

n-3 PUFA in SLNs or NLCs has been achieved using various methods, including Hot HPH[106], hot homogenization combined with microfluidization (Holser, 2012), or Hot shear homogenization and microfluidization (Salminen et al., 2013). Tweens and lecithin are frequently used emulsifiers to stabilize n-3 PUFA in various triglyceride matrices, such as Tripalmitin, tristearin, and triolein. Typically, the successful encapsulation of n-3 PUFA in SLNs and NLCs is impacted by different formulation factors, including lipid phase properties, type of emulsifiers, and oil-to-lipid ratio. Therefore, excipient selection should be based on comprehensive understanding of their impact on the performance of candidate formulation.

4.2. Structured lipids

Structured lipids are chemically or enzymatically modified lipids that display altered composition and/or the positional distribution of specific fatty acid(s) (Bandarra et al., 2016). The utilization of structured lipids has emerged as a promising strategy to boost the therapeutic effects of n-3 PUFA by facilitating selective uptake in specific tissues, particularly in the brain. This group of lipids offers a broad range of modified chemical forms such as triglycerides, phospholipids, or related derivatives which are reported to retain higher bioavailability and superior anti-inflammatory, and antioxidant effects compared to traditional n-3 PUFA (Balakrishnan, Kannan, & Govindasamy, 2021; Mora, Arola, Caimari, Escoté, & Puiggròs, 2022).

The positional distribution of n-3 PUFA is a key factor for optimizing their activity according to the positioning of the fatty acid within the glycerol backbone of the triglyceride molecule on three distinct locations: sn-1, sn-2, and sn-3. During digestion, fatty acids are released from the sn-1 and sn-3 positions, whereas fatty acids of the sn-2 position are mainly absorbed. The impact of n-3 PUFA positional distribution on absorption was previously demonstrated in several experimental studies. For example, a significant absorption of DHA at the sn-2 position was observed compared to DHA-diacylglycerol (DAG) and DHA-ethyl ester (Banno, Doisaki, Shimizu, & Fujimoto, 2002; Valenzuela, Valenzuela, Sanhueza, & Nieto, 2005).

Structured phospholipid derivatives have also been extensively investigated for their potential to effectively accumulate DHA n-3PUFA in the brain tissue by overcoming the blood-brain barrier (BBB). Early studies have shown a 10-fold increased uptake of DHA when introduced at the sn-2 of 1-lyso,2-acyl-glycerophosphocholine in rat brains compared to corresponding non-esterified fatty acids (Thies, Pillon, Moliere, Lagarde, & Lecerf, 1994). Similarly, oral administration of ^{13}C-DHA bound to phosphatidylcholine (PC) to piglets for 16 days, resulted in a 1.9-fold increase in DHA accumulation gray matter area of the brain. On the other hand, this was not achieved with TAG-13C-DHA. AceDoPC® is a stabilized version of the physiologically active 2-DHA-LysoPC derivative (refer to Fig. 4). It's designed to prevent the relocation of DHA from the sn2 position by introducing an acetyl group at the sn1 position. The activity of AceDoPC® was investigated In-vivo using various experimental models (Chauveau et al., 2011; Hachem et al., 2016).According to Chauveau et al. (2011), AceDoPC ®exhibited higher neuroprotection activity in a post-ischemic stroke compared to non-esterified DHA, in rats (Chauveau et al., 2011).

Synthesis of n-3 PUFA structured lipids can be achieved via various approaches, commonly through chemical or enzymatic methods. Chemical methods are traditionally used due to low cost, ease of processing, and capability for large-scale manufacturing. Nevertheless, the chemical approach has several limitations such as lack of specificity and extreme processing temperature which can lead to a degradation of sensitive products (Zhou, Lee, Mao, Wang, & Zhang, 2022). Therefore, significant research is currently being conducted to explore application of enzymatic approaches to prepare a wide range of structured lipids. This method offers several advantages over chemical synthesis, including its minimal byproduct formation and high specificity of lipases to bind n-3 PUFA on lipid molecules (Castejón & Señoráns, 2020).

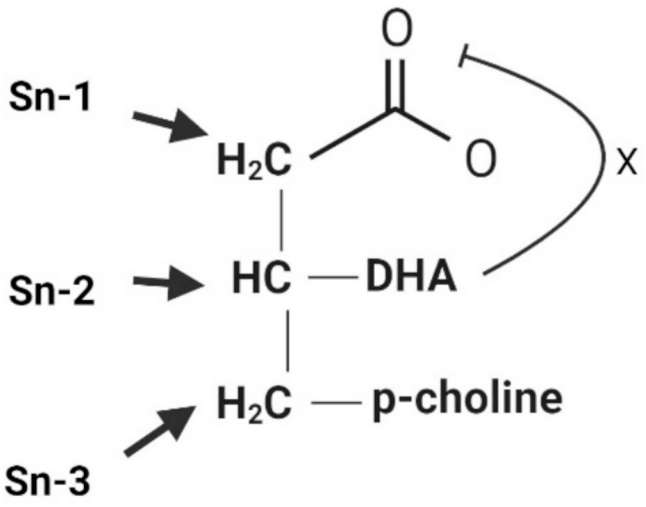

Fig. 4. Stabilization of DHA at sn-2 position in 1-acetyl,2-docoshexaenoyl-glycerophosphocholine or AceDoPC®. Created with BioRender.com

Among biocatalysts, lipases have demonstrated remarkable versatility due to their regiospecificity in the modification of oils, resulting in the production of high-value products rich in EPA + DHA. Furthermore, the utilization of milder conditions in enzymatic synthesis enhances the stability of synthesized n-3 PUFA compared to production by chemical methods. For instance, following a two-step esterification process with Rhizopus delemar, the DHA content reached 91 %, with a yield of 88 %, exceeding those achieved through chemical hydrolysis for DHA concentrate (Shimada, Maruyama, Sugihara, Moriyama, & Tominaga, 1997).

5. Challenges in developing optimal formulations of n-3 PUFA

5.1. In- vitro models and poor IVIVC

The challenges to the successful development of bioavailable n-3 PUFA formulations are significant, particularly for establishing valid in vitro-in vivo correlations (IVIVC). IVIVC is a mathematical model that bridges in-vitro properties of specific formulas with an in-vivo response. This can be a promising approach for predicting the pharmacokinetic performance of various n-3 PUFA formulations, thus improving the selection of candidate formulation based on different critical design factors using suitable in-vitro models. However, the development of validated in-vitro models for optimizing formulation development remains a major challenge due to the inability of most in-vitro models to fully replicate the process of lipid digestion and absorption (Huang et al., 2021; Kollipara & Gandhi, 2014). This can be further complicated by the inability to mimic various physiological conditions affecting overall absorption occurring in GI tract, including variation in pH, availability of enzymes (i.e., lipases and proteases), simulating fasted/fed state conditions, and transit time (Guerra et al., 2012).

pH-stat lipolysis model is commonly used to assess the performance and establish potential IVIVC for lipid-based formulations (LBFs). During this testing, LBFs are dispersed into a medium mimicking intestinal fluid, then digestion is initiated by the addition of lipase leading to free fatty acid release, leading to a drop in the pH, the digestion rate then can be indirectly calculated using the addition rate of sodium hydroxide that reacted with released fatty acids.

Established IVIVC for lipid-based formulations involves predicting the rank ordering of drug absorption following formulation digestion, rather than constructing a strong point-to-point correlation. For instance, the absolute bioavailability of the poorly soluble drug danazol was directly correlated with the percentage of lipids in the formulation

and the amount of danazol that is soluble in the micellar phase after conducting a suitable in-vitro lipolysis model (Larsen, Holm, Pedersen, & Müllertz, 2008). These challenges highlight the complexity of developing strong IVIVC for n-3 PUFA formulations and the need for further investigations to establish a suitable in-vitro model that replicates the complex process of lipid digestion, solubilization, and absorption in humans.

5.2. *In vivo pharmacokinetic assessment of n-3 PUFAs*

The accurate estimation of pharmacokinetic parameters following oral administration of n-3 PUFA can be challenging and requires a careful consideration of several physiological and dietary factors. As a result, when designing a pharmacokinetic study for n-3 PUFA, factors such as administered dose, treatment length, food intake state, and participant selection should be accounted for formulation effects, aiming to reduce inter and intra-subject variabilities that influence the pharmacokinetic profile of targeted n-3 PUFA. These variabilities include age, sex, metabolic variations, and individual baseline levels of n-3 PUFA (de Groot, Emmett, & Meyer, 2019; Schuchardt & Hahn, 2013).

The accurate estimation of n-3 PUFA levels can also be influenced by the ability to detect measurable changes in their blood concentrations following oral administration. This can be further complicated by the variability in the incorporation and elimination rate of investigated fatty acid into different plasma fractions such as plasma triglycerides (TAG), phospholipids (PL), or free non-esterified fatty acids fraction (NEF). According to a meta-analysis, it was suggested that the suitable biological markers for DHA uptake are plasma triacylglycerol DHA and plasma phospholipid DHA, or free non-esterified fatty acids fraction (NEF), and for EPA, plasma phospholipid EPA appears to be an effective marker. (Fekete, Marosvölgyi, Jakobik, & Decsi, 2009) On the other hand, long-term uptake is usually performed to assess the overall biodistribution state of measured n-3 PUFA, which usually lasts several weeks and can better predict the biodistribution state of fatty acids by reflecting the overall metabolic fate and deposition in target tissues. This can be also achieved by monitoring n-3 PUFA levels in red blood cells which are less affected and more stable to acute and short-term fatty acid dosing. (Harris & Thomas, 2010) A comparative study by Harris et al., investigated the effect of consuming a large dose of n-3 PUFA on measured red blood cells and total plasma concentration of EPA + DHA. In this study, 20 healthy volunteers received 3.6 g of EPA/DHA with a standardized breakfast. The plasma concentration of EPA and DHA 6 h post-dosing increased by 47 % (95 % confidence interval [CI], 24 % to 73 %), and the plasma EPA and DHA percentage of total fatty acids increased by 19 % (95 % CI, 4.7 % to 36 %). However, at 24 h, the percentage of EPA + DHA in red blood cells was unaffected (−0.6 % confidence interval [CI] −2.6 % to 1.5 %). This suggests that the short-term increase in plasma levels of n-3 PUFA may not accurately reflect the long-term uptake and incorporation into cellular membranes, particularly EPA + DHA percent in total red blood cells (RBC) fatty acids (known as omega-3 index). The long-term uptake study design allows for the accurate estimation of n-3 PUFA status in various tissues and holds significant implications for various physiological processes, such as reflecting the risk factor for death from coronary heart disease (CHD). [126] This approach serves as a valuable tool to assess the formulation's impact on the estimated pharmacokinetic parameters of n-3 PUFA following long-term uptake as well.

6. Conclusions & Future directions

n-3 PUFA are essential bioactive ingredients that play various biological functions, however achieving optimal blood and tissue concentrations remains a major challenge. This review investigated the impact of each specific design of several commercially applied formulation strategies (microemulsions, nanoemulsions, self-emulsifying systems, gelled emulsion, and microencapsulation) on improving the pharmacokinetics properties of n-3 PUFA. Among these systems, self-emulsifying drug delivery systems (SEDDS) show the most promising results for enhancing n-3 PUFAs pharmacokinetics. Nevertheless, to achieve a better understanding of how SEDDS formulation design influences pharmacokinetics, a multifaceted and systematic approach is required. Specifically, a thorough screening of various formulation components- including the oil phase, surfactants, and co-surfactants- must be carefully planned and guided by testing under suitable in-vitro models. This facilitates understanding the impact of utilizing various oil sources of n-3 PUFA with various physicochemical on the solubilization, stability and release profile of n-3 PUFA. Moreover, biosafety and potential toxicity of formulations should be carefully considered, particularly when selectin excipients. For example, excipients that are GRAS (Generally Recognized As Safe) can help minimize toxicity risks, ensuring the formulations are both safe and effective for use. Additionally, the selection of surfactant(s), along with their surface activity, surfactant(s) to oil ratio, and interfacial coverage, which play a critical role in emulsification, must be carefully considered under simulated gastrointestinal models. Furthermore, the role of co-surfactants in fine tuning the interfacial properties and enhancing the solubilization capacity SEDDS should not be underestimated. Investigating combinations and ratios of surfactants to co-surfactants could lead to optimized formulations with superior bioavailability and pharmacokinetic profiles.

Advanced in-vitro models, such as simulated gastrointestinal fluids and dynamic digestion systems, should be employed to assess these variables. These models can provide insights into how n-3 PUFA formulations behave under physiological conditions, thus facilitating the construction of validated in vitro-in vivo correlations (IVIVC). This approach holds promise for predicting the pharmacokinetics performance of various n-3 PUFA formulations through enhancing the selection of candidate formulations based on different critical design factors using suitable in vitro models.

Lipid carriers like solid lipid nanoparticles (SLNs) and nanostructure lipid carries (NLCs), alongside with structured lipids, are considered highly promising formulation strategies for n-3 PUFA. These strategies offer significant advantages in terms of enhancing stability and improving the biodistribution of orally administered n-3 PUFA. However, further investigations are required to understand the impact of these formulation strategies on digestibility, uptake, and overall pharmacokinetics of n-3 PUFA.

CRediT authorship contribution statement

Amer Abdelhafez: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Zahra Khabir:** Writing – review & editing, Formal analysis. **Clive A. Prestidge:** Writing – review & editing, Supervision. **Alfonso Garcia-Bennett:** Writing – review & editing, Supervision, Funding acquisition. **Paul Joyce:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Paul Joyce reports financial support was provided by Australian Research Council. Paul Joyce reports financial support was provided by The Hospital Research Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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