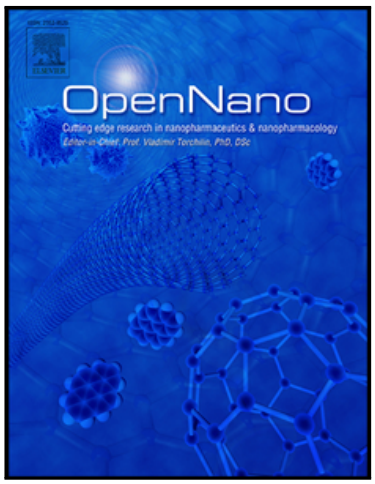


Nanostructured lipid carriers and their potential applications for versatile drug delivery via oral administration

Nguyen Hong Van , Nguyen Thuy Vy , Vo Van Toi , Anh Hoang Dao , Beom-Jin Lee

PII: S2352-9520(22)00026-3
DOI: https://doi.org/10.1016/j.onano.2022.100064
Reference: ONANO 100064

To appear in: *OpenNano*

Received date: 5 June 2022
Revised date: 30 July 2022
Accepted date: 4 August 2022

Please cite this article as: Nguyen Hong Van , Nguyen Thuy Vy , Vo Van Toi , Anh Hoang Dao , Beom-Jin Lee , Nanostructured lipid carriers and their potential applications for versatile drug delivery via oral administration, *OpenNano* (2022), doi: https://doi.org/10.1016/j.onano.2022.100064

Highlights:

- Advantages of NLCs for oral administration: NLCs absorption mechanism, sensory masking, enzyme degradation prevention, P-glycoprotein (P-gp) efflux circumvention
- Methods to improve oral NLCs drug loading, targeting effect and stability: hydrophobic ion pairing, surface modification for target position, transformation into powder form
- Site specific delivery and organ/cancer targeting of NLCs through oral administration

Nanostructured lipid carriers and their potential applications for versatile drug delivery via oral administration

Nguyen Hong Van[1,*], Nguyen Thuy Vy[1], Vo Van Toi[1], Anh Hoang Dao[2], Beom-Jin Lee[3]

[1]*Pharmaceutical Engineering Laboratory, School of Biomedical Engineering, International University - Vietnam National University Ho Chi Minh City (VNU-HCM) Ho Chi Minh City, Viet Nam*

[2]*Department of Formulation and Processing, National Institute of Medicinal Materials, Hanoi 100000,Vietnam;*

[3]*Bioavailability Control Laboratory, College of Pharmacy, Ajou University, Suwon 443-749, Republic of Korea*

[*]Corresponding address: chodomthanyeu@gmail.com

Abstract

Enteral administration is the most convenient route despite the gastrointestinal physiological barriers. Nanostructured lipid carriers (NLCs) have emerged as a promising strategy for improving therapeutic compound oral bioavailability, not only due to nanomaterial advantages, but also due to lipid ingredients themselves, such as preventing enzyme degradation, taste masking, and especially favorable uptake by chylomicron pathways to the lymphatic drainage system. Therefore, NLCs have been employed for systemic absorption improvement, site-specific treatment of the digestive system, and especially targeting delivery to the liver, brain, cancer ulcer, and so on through oral uptake. Lipids, surfactants, and other materials like lipophilic counter ions or coating polymers are considered for oral NLCs formulation design. A variety of NLCs fabrication methods and stability enhancement techniques by transforming NLCs into powder form or hydrophobic -ion pairing are discussed. Hence, this review aims to provide an overview of the current state of the art of NLCs and their modern techniques and applications in oral drug administration, which will advocate their extended use in the future.

Keywords: nano lipid carrier, oral administration, hydrophylic ion pairing, site specific, targeting

1. Introduction

Oral route is the most accepted drug uptake option due to its ease of administration, providing patient compliance, the simplest use, and the safest means of administration. It offers systemic effects through absorption in the gastrointestinal tract, thereby being chosen as the uptake route for common treatments, even for long-term treatment of some chronical diseases involving diabetes, hypertension, cardiovascular ailments, and cancer. However, oral administration may result in low bioavailability and a slow onset of action. Besides, the hindrance of the physiochemical nature of drugs that are either or both low solubility and low permeability [1] in the gastrointestinal tract (GIT) leads to poor absorption and, subsequently, insufficient therapeutic action in the targeting site. There are several hurdles from the physiology of GIT challenging drugs to overcome before reaching the systemic circulation. Firstly, the physiological lumen fluid is prone to multi-pH and food enzyme intervention can cause drug degradation chemically, which alters unpredictably the pharmacological effects. Secondly, the physical barriers to absorption, including mucus, unstirred water layer, and gut wall cell line membrane, are semipermeable, which only allows selective molecules to penetrate during the short dosage transit throughout GIT. Thirdly, despite successful delivery through these barriers, drugs encounter pre-systemic metabolism by the liver or by enzymes, present in the intestinal and colon mucosa. The CYP3A4 family of enzymes, for example, dominates drug metabolism. Lastly, drugs across the membrane are prevented from intercellular transport by P-glycoprotein (P-gp), highly expressed on the apical surface of columnar cells in the jejunum and tumor cells, pumping drugs back into the intestinal lumen or out of cancer cells. Furthermore, the absorption also varies, depending on other non-disease factors such as age and sex as well as the disease status of patients [2, 3]. Therefore, these above factors should be taken into account to provide enough therapeutic effects in order to improve the bioavailability of oral drug carriers.

Scientists concentrate on ideal strategies for drug transport. From the early 1900s, with the proliferation of nanotechnology, several colloidal delivery vehicles on a nanoscale incorporating drugs have been broadly researched, including polymeric nanoparticles, liposomes, niosomes, and lipid-based nanoparticles (LBNs), including solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) [4]. With the combination of natural lipids and surfactants as main ingredients, LBNs shed light on encapsulated drug delivery systems, especially for overcoming GIT hurdles. They are considered to possess more advantages than other nanoparticles (NPs) due to their organic solvent-free preparation and also their ability to withstand factorial degradation [5, 6]. SLNs were first introduced in the 1900s [7], and the first generation of lipid nanocarriers were made of solid lipids, dispersed in water and stabilized by the addition of surfactants or co-surfactants, resulting in nanosized vesicles with drugs encapsulated inside [8-10]. SLNs are capable of conveying hydrophilic molecules, even peptides, genes, and vaccines. When they were first introduced, SLNs got more attention than other lipid-based colloidal carriers because they are biocompatible and easy to scale up in mass production while keeping a low cost of ingredients [5, 11]. However, the crystalline nature of solid lipids at ambient temperature presents limitations to this carrier. Their bulk structure of the carrier system is like a "symmetric brick wall", in which just a few available spaces for free molecules are embbed in, and consequently unsatisfying drug loading efficacy [12]. Furthermore, they will rearrange the crystalline lattice to get more stable and form gelation in the dispersed phase. This leads to drug leakage out of carriers and particle aggregation during storage time [13, 14].

Because of these drawbacks of SLNs, further studies to modify them finally led to the introduction of the next LBNs generation – nanostructured lipid carriers (NLCs). NLCs share a fundamental identity with SLNs, a colloidal system of lipids dispersed in a watery phase with the help of emulsifiers. The distinguishing point is that a part of solid lipids is replaced by liquid lipids, resulting in the lipid matrix still being liquid or amorphous at room temperature and physiological temperature [5, 15]. The addition of liquid lipids alters the core structure of NLCs. Besides the presence of crystal-like solid lipid composition, liquid lipids cause disruption partly in the lattice structure and form crystal imperfection structures in NLCs. The integrated drugs are distributed more in oil, and the imperfect structure of NLCs is capable of encapsulating more drugs. As a result, the amount of drug loading in

NLCs would be significantly higher than in SLNs [12]. This lipid matrix also prevents polymorphic changes of solid lipids by lowering the level of recrystallization to get more stable after a long storage time, which then limits drug extrusion. The amorphous clusters of the NLCs could carry active pharmaceutical ingredients (APIs) molecules better inside [16]. Like SLNs, NLCs show substantial favorability for lipophilic drugs due to the hydrophobic nature of the carrier [17]. The process of adding more methods, like conjugation, would make it easier to put hydrophilic drugs into LBNs [18]. In this review, novel LBNs, a combination of solid and liquid lipid into a promising vehicle named nanostructured lipid carriers (NLCs) are described in detail about their formulations and superior characteristics, especially the high drug payload features of NLCs due to the presence of liquid lipid solubilizing drugs, when compared to parent lipid-based carriers, that is, solid lipid nanoparticles (SLNs). The utilization of NLCs has been an all-around approach for optimizing carriers due to supreme drug entrapment and for both local treatments, targeting action sites to organs like the brain [19], liver [20], kidney [21] and even cancer, or simply intensifying the effectiveness of bioactive agents via oral means. This paper briefly discusses a succinct introduction of NLCs involved in components and their integration with various techniques and highlights outstanding applications that illustrate the tremendous potential of incorporating drugs into NLCs for oral drug delivery systems.

2. Advantages of nanostructured lipid carriers regarding oral delivery

2.1. Higher entrapment efficiency (EE) and drug loading capacity (LC)

As aforementioned, NLCs resolve the shortcomings of SLNs by offering extensive drug loading, better release profile, and stability owing to the employment of a blend of solid lipid and liquid lipid in their formulations. Many studies have done a comparison on SLNs and NLCs in which the results indicated that NLCs is the better carrier. For example, in the study of ketoconazole inclusion in different formulations of SLNs and NLCs, it was shown that drug EE in NLCs (95.3%) was higher than in SLNs (87.3%) despite equivalent lipid occupation. The reason is explained by the disordered structure because of the presence of liquid lipid in NLCs providing more space for drug loading [22]. A similar conclusion was drawn in some other studies about nisoldipine [23, 24]. Another study of erlotinib encapsulated in NLCs and liposomes also supported the superior loading capacity of NLCs (4.35%) than liposomes (3.40%), with the former being smaller and more homogeneous [25]. The

presence of liquid lipid lowers the melting point of the systems, and this favors lipophilic molecules capable of dissolving well since they are still in the liquid state during the cooling process. Therefore, NLCs also prevent the immediate drug extrusion, thereby remaining high loading capacity of drugs.

2.2. Control of drug release pattern

In the release profile, NLCs perform a biphasic release pattern, bursting initially and sustaining steady afterwards. The liquid lipid situated in the exterior layers of the NLCs generates drug-enriched packing, resulting in burst drug release at the beginning by diffusion mechanism or matrix erosion by lipolysis [17]. This is followed by solid core lipid discharge drugs consistently. This feature can be utilized to fabricate NLCs with the desired release patterns by varying the amount between solid and liquid lipid composition. In addition, the smaller size of NLCs also has the advantage of a faster release than SLNs due to the larger surface area [26], allowing for a complete release in GIT before gastric emptying taking place.

2.3. Long shelf-life storage

The inhibition of recrystallization in the NLCs delivery system is an attractive pattern because the recrystallization process of solid lipid molecules to develop crystallinity after extended periods of storage causes significant drug leakage. However, the NLCs model is a matrix of solid and liquid lipids that avoids supersaturation of the solid lipid composition and retains the nano-sized drop of mixture consistent, or with minor variation in polymorphism, allowing the system to hold the drug inside for a longer period. After 8 weeks of storage at high temperature 40°C, EFV-NLCs persisted in EE and LC at 93% and 9.2%, respectively, compared to EFV-SLNs at 88% and 8.6%, respectively, and the degree of crystallinity of NLCs was slightly lower than SLNs, indicating preferable inclusion of EFV in NLCs [27].

2.4. pH and enzyme degradation prevention

The highly acidic fluid in the stomach and the presence of enzymes in the small intestine are generally some of the limitations when it comes to oral delivery of bioactives, such as peptides and vitamins, which are susceptible to degradation by proteases and gastric acid. The goal of NLCs is to safely deliver safety these bioactives through gastric before reaching duodenum and jejunum. LBNs were stable in a wide range of gastrointestinal pH range, from 1.2-8, maintained up to 8 hours in mice

gastrointestinal tract, and penetrate into the base of intestinal villi and colon [28]. In simulated gastrointestinal fluid test, with NLCs incorporating vitamin D3 thanks to Tween 80 and stearic acid in composition, only smaller than 4% of vitamin D3 was released even after 8 hours of digestion [29]. In another study of insulin encapsulated in NLCs (INS-NLC), in both simulated gastrointestinal fluid test and simulated intestinal fluid test, INS-NLCs showed much better degradation by pancreatin anticipation than insulin suspension (INS-S). In the former test, INS-S was completely degraded while INS-NLCs still remained at over 80% intact insulin after 4 hours; and in the latter test, INS_NLCs persisted at 50% of intact insulin after 4 hours whereas no INS-S could be detected after 0.5 hour incubation [30].

2.5. P-glycoprotein (P-gp) efflux circumvention

P-gp is the one efflux pump protein on the apical membrane area of enterocytes of the gut wall. As part of their function, they return drugs to the bowel, resulting in multi-drug resistance. For this reason, P-gp efflux may be one of the main causes of poor systemic reaching of drugs from intestinal absorption. Be that as it may, the excipients used in the NLCs formulation somehow can modulate or inhibit this efflux transport and may significantly alter transcellular pathways of these NPs. A great number of studies has mentioned this striking characteristic in certain surfactants including non-ionic surfactants Polysorbate [31], Poloxamer 407 [32], Cremophor EL, and Solutol HS 15 [33]. Furthermore, incorporating poorly soluble P-gp substrates into NLCs themselves could prevent this pump transportation. For example, saquinavir (SQV) is a P-gp substrate. SQV-NLCs with a diameter of 247nm and a surfactant content of 1.5% (w/v) were incubated with the P-gp inhibitor verapamil, but there was no significant difference in permeability rate regardless of incubation with or without the inhibitor. Therefore, it was suggested that the formulation of NLCs could avoid this efflux and exploit transcellular pathways by caveolae- and clathrin-mediated [34].

2.6. Sensory masking

One considerable advantage of NLCs is sensory masking for foods or drugs. The undesirable sensory features (taste, odor, color, and texture), despite the health promotion effects, of these bio-active agents limit their oral uses to some extent, especially, for the pediatric patient. For instance, phenolic family compounds are famous for their antioxidant, antiviral, anti-inflammatory, or even anticancer,

but their direct consumption is limited due to low solubility in water and unpalatability. Therefore, bioactive agents encapsulated in lipid-based nano-delivery systems like NLCs can be an efficient strategy to enhance their solubility within the lipid matrix and, favorably, conceal their unwanted sensory qualities while preserving their nutritional benefits during processing. Recently, hydrochlorothiazide was developed in form of NLCs oral liquid formulation for effective and stable dosage for pediatric therapy [35]. In another study, NLCs was applied for color masking of betel nut extract for oral and dermal administration routes [36]. The incorporating in NLCs could mask off-flavor and yellowness of polyphenol hesperetin when fortified in milk [37] or cover the unpleasant taste of thymol in nutrition snack bar [38]. In addition, the utilization of lipids in the NLCs formulations is good for taste masking of bitter drugs, since the criterion in oral drugs is palatable [39].

2.7. NLCs disposition mechanism through gastrointestinal absorption

After oral administration, NLCs is required to remain stable under the influence of oral cavity enzyme, gastric juice, stomach mobility, bowel peristalsis, then get contact with mucus layer and finally the villi in the small intestine wall. Many findings have been studied to understand the mechanisms of drug absorption and transport to reach the systemic circulation through GIT. For NLCs, the absorption is initiated through multiple pathways such as transcellular, paracellular and lymphatic uptake [40] (Fig.1.)

The villi are composed of specialized endothelial cells called enterocytes, each of which has a capillary and lymphatic duct called the lacteal. As reported, drugs are absorbed through transcytosis and endocytosis by enterocytes. LBNs endocytosis is mainly regulated by micropinocytosis, clathrin-mediated and caveolae-mediated pathways [40]. As for the transcytosis pathway, nonspecific passive diffusion is the main mechanism by which these NPs are transported. Because of the hydrophobic nature of the lipid carriers, these particles can easily pass through the apical cell membrane to enter the cell [41]. *In-vitro* observation, LBNs were destined to the endoplasmic reticulum [42]. Another finding is that the organelles endoplasmic reticulum, Golgi apparatus, and microtubules transport NLCs from the apical to the basolateral compartments of the cell and expel them [43]. Regarding paracellular pathways, the opening and closing of tight junction between endothelial cells allow

molecules get through. However, this pathway is preferable for hydrophilic drug molecules, with a diameter of less than 1nm and a weight of less than 200 Da [44, 45].

NLCs enhance the oral bioavailability of either hydrophobic drugs or poorly soluble drugs by facilitating intraduodenal absorption through the lymphatic system in the intestinal. Because of their large surface area, lipid moieties, and bio-adhesion properties [46], NLCs are likely to prolong their residence time at the intestinal villus, allowing them to reach the brush border site. The triglycerides (TG) composition of lipids in NLCs, undergone lipase enzyme and high shear motion of stomach contractions, breakdown into diglycerides (DG) and monoglycerides [2]. At the duodenum section, the presence of DG and MG induces bile salts and lipase/co-lipase enzyme secretion by gall bladder and pancreas, which is like the physiological mechanism of high-fat meal digestion, then consecutively forming micelles vesicles. These micelles convey the lipid fragment through the unstirred water layer and enter the enterocytes by passive or facilitated diffusion and active transport. In the enterocytes, bile salts are left behind and DG and MG are converted into fatty acid with different carbon chain lengths. Medium chain lipids tend to enter directly systemic circulation while long chain lipids are re-esterified and associated with cholesterols and phospholipid, provoke the formation of chylomicrons, which follow into lacteal then thoracic lymphatic system [40, 47, 48]. Besides that, M cell of Peyer's patches is also a promising target for NLCs to lymphatic transport. M cells play a role in conveying insoluble molecules and xenobiotics into circulation. With high transcytosis and low intracellular lysosomal activity, M cells capture LBNs into Peyer's patches. These LBNs are then phagocytosed by dendritic cells, and transported to afferent lymph then thoracic lymph [49]. This lymphatic transport will join in the systemic circulation at the junction of the left internal jugular and left subclavian vein. It is necessary to note that although the absorption pathways of foreign compounds including carbohydrates, proteins, and fats in the intestinal always destinate to systemic uptake, lipids are an exception which is favorable uptake by chylomicron pathways to lymphatic drainage system. As a result of the involvement of lipid components, facilitating dietary fat uptake mechanism, and avoiding first pass metabolism, the use of NLCs to incorporate pre-systemic metabolism susceptible drugs is advantageous.

3. NLCs materials for oral administration

The main components of NLCs are solid and liquid lipids, surfactants, and water. Due to their different characteristics in molecules, charge, steric structure, melting point, hydrophilic-hydrophobic nature, to name a few, the selection of types, occupied ratio of these components and the preparation method determine characteristics of NLCs. This is performed in characterization parameters such as particle size (PS), polydispersity index (PDI), zeta potential (ZP), entrapment efficiency (EE), loading capacity (LC), release profile (RP), morphology, stability, cytotoxicity, and pharmacokinetic-pharmacodynamic profiles. With bioactives properties, lipid based and surfactants decide NLCs types, which could be categozied into the imperfection type [50, 51], the amorphous type [52, 53], and the multiple type oil-in-solid fat-in-water (O/F/W) type [54].

3.1. Lipids

The proportional range between solid lipids and oils in NLCs formulations is from 70:30 to 99.9:0.1.. Solid lipids commonly used are triglycerides (e.g. tripalmitin, tristearin, trilaurin), partial glycerides (e.g.Witepsol 85E, Imwitor, Compritol® 888 ATO), fatty acids (e.g. palmitic acid, stearic acid), hard waxes (e.g.,glyceryl monostearate, glyceryl behenate), sterols (e.g.cholesterol) [55, 56]. Oils or liquid lipids include the mixture of unsaturation mono-, di- and triglycerides of fatty acids with different chain lengths [57, 58]. Overall, the inclusion of lipid ingredients, especially for oral use, is regulated under GRAS (Generally Recognised As Safe) criteria. Several considerations in physiological tolerance, physiochemical structure, drug solubility, and lipid miscibility are necessary for desire formulation [59] *(Table 1)*.

The lipid composition and melting point alter NLCs size and drug loading [60, 61]. Short chain length lipids may endure higher solubility than the longer ones. For example, lipophilic statins (rosuvastatin) showed higher solubility in liquid lipid than solid lipid, in which it was more soluble in capryol-90 and oleic acid than Witepsol and glyceryl monolaurate. Besides, lipids with emulsifying ability enhance the solubility of drugs, as the solubility of rosuvastatin in capryol-90 is higher than in oleic acid, which manifested greater EE in the formulation contained capryol-90 than oleic acid. The combination of cetyl alcohol and stearic acid inhibited size growth during storage as well as performed better permeation of progesterol through duodenum in *ex-vitro* release test compared to NLCs made with only stearic acid [62]. Lipids ingredient can alter release profile in GIT, in which

long carbon chains (C-chain) of fatty acids in triglycerides are degraded slower than ones with shorter C-chains [63]. Additionally, the lipid functional groups also alter NLCs surface charge as well as NLCs structure, for example: oleic acid, carrying hydroxyl groups lowered NLCs zeta potential -20 mV in comparison to Mygliol 812, and located at the interface of NLCs while the latter one was in the blend lipid mixture [54].

Plant-extracted oils have been commonly used in traditional medicine from ancient. More than 8000 phytochemicals in plants have been reported for protection against pathogen in human [64]. For this reason, NLCs could be an ideal approach, where these edible oils with biological effects play a role as liquid compartment, for incorporating and delivering these essential oils in oral administration for antibacterial, antifungal, and antioxidant purposes. Ribeiro et al exerted a screening of 28 essential oils for anti-bacteria activity of *Campylobacter jejuni (CJ)*, causing gastroenteritis and autoimmune diseases in humans, and employed nanotechnology to optimize NLCs orally formulation. As a result, the NLCs with the combination of ucuuba butter as solid lipid and essential oil olibanum as liquid lipid as well as bioactive agent showed a significant effect against CJ *in-vitro* disc diffusion test compared to free oil and can improve the permeation through biofilm matrix, formed by CJ as a survival strategy in a hostile environment [65]. Essentiol oil with biological effects is highly appropriate to be encapsulated in lipid based nano carrier. The incorporation of cinnamon essential oil in NLCs was beneficial for the stability enhancement of essential oils and oxidative prevention during the storage period, which is promising for orally administrated supplements [66].

3.2. Surfactants and co-surfactant

NLCs appear into lipid-dispersed-in-water form. Therefore, the presence of surfactants is that rapidly adsorb on the interfaces and lower interfacial tension between two distinguished phases. Hydrophilic – hydrophobic balance (HLB) indicator of surfactant significantly affect on various NPs properties including PS, ZP, PDI and EE [67]. Apart from that, the colloidal structure is determined by the molecular structure of surfactant, which is referred to critical packing parameter (Cpp), [68]. Regrading to the influence on size and polydispersity of NPs, smaller diameter could be favored by larger head group and longer tail chain of surfactant molecules [69]. This is due to the large polar

head allowing tighter binding with water while long hydrocarbon chain firm more rigid packing within surfactant film.

When it comes to utilizing an amount of surfactant in NLCs, concentration must be considered as its potential toxicity to biological membranes. Low surfactant concentration causes less irritant, however, might result in the poor repulsion between particles during the recrystallization process causing aggregation later. In contrast, a high concentration of surfactants can completely cover the surfaces of particles, however, the excessive surfactants would form micelles, which may destabilize the colloidal system by flocculation due to the depletion force of free micelles [70]. In general, the amount of surfactants in LBNs colloidal system varies from 0.5% to 5% of total volume [11].

In NLCs preparation, surfactants could be divided into 3 classes: neutral surfactant, ionic surfactant and non-ionic surfactant. These surfactants accumulate on the Stern's layer of the particle and prevent particles aggregation during storage time due to the electrostatic repulsion between identical electric charges. Non-ionic surfactants, on the other hand, can offer steric stabilization by forming a densely packed surfactant film around NLCs. Overall, mixed surfactants are usually developed for the desired physical properties owing to their synergistic effects. Lecithin, a neutral surfactant and Pluronic F68, a non inonic surfactant, in the atorvastatin-NLCs result in the smallest size, considered PDI whereas encapsulated drugs up to over 90% [71]. Ionic surfactants carry positive or negative charge on their molecules. There are common anion surfactant names such as sodium dodecyl sulfate, sodium oleate, and sodium taurodeoxycholate. Furthermore, in the study on the effect of charge on praziquantel loaded NLCs, the author found that charged NLCs were superior in entrapment efficiency of drugs; inhere, *in vivo* histopathological examination, positive charge NLCs particles confirmed the better improvement in liver lesions than the negative counterpart [72]. Non-ionic surfactants, like Poloxamers, Polysorbates, sorbitan esters, Brij78, Tego care 450, and Solutol HS15, are widely used in the formulation of NLCs [73]. The advantages of non-ionic surfactants over the ionic one is, firstly, not susceptible to the pH and electrolyte concentration; and secondly, they are less toxic to the body, which is more acceptable for higher concentration use.

Co-surfactants are agents used to augment the solubility of drugs in LBNs, or rather NLCs. In addition, co-surfactant increases the interfacial fluidity and flexibility by entering the rigid surfactant

film, thereby allowing more movement of surfactant during the lipid recrystallization, consequently minimizing the possibility of particle size increase. Both surfactant and co-surfactant would be adsorbed on the surface cover of the NPs, they lower the high interfacial energy, which is likely resulting in particle agglomeration, and generate a mechanical barrier enhancing the thermodynamic stability of the whole system. Familiar co-surfactants used in NLCs include butanol [74], glycerol, propylene glycol [75], low molecular weight polyethylene glycol (PEG 400) [76] Transcutol [35, 77] and soy lecithin [78].

Surfactants could alter the drug transportation through gastrointestinal tract, by regulating activities of transpoter and carriers [79]. About over hundred surfactants could reduce the efflux activity of P-glycoprotein, which suggests the addition of surfactants in formulations of P-glycoprotein substrates. Among Tween 20, Tween 80, Myrj 52, and Brij 30, Tween 80 exhibited strongest effect not only on increasing P-gp substrate absorption, but also disminishing drug efflux in the secretory direction on Caco -2 cells [80]. Moreover, the number of surfactants and co-surfactant, such as Cremophor EL, Labrasol, Poloxamer 407, Polysorbate 80, PEG 400… have ability to inhibit multidrug resistance-associated protein 2 - MRP2. Lower HLB surfactants, like Spans, showed weaker influences on multidrug resistance modulating activities [81]. The roles of surfactant is pivotal important for the absorption of hydrophobic drugs, which known as suitable agents for loading in LBNs. With the presence of bile salts, surfactants release from the interface of NLCs in to instinal lumen might enhance the micellization of drugs, resulted in higher drug cargo through intestinal membranes.

4. NLCs fabrication methods

4.1. NLCs usual preparation methods

NLCs were formed with various methods, from methods with no organic solvent requirements including high shear homogenization, ultrasonication, pressure homogenization, microemulsion, phase inversion temperature, as well as organic solvent involvement methods such as emulsification solvent and solvent injection (Fig.2).

4.1.1. High energy input method

Although this method requires a high energy supply, it is the most preferred because of its feasibility to be scaled up to an industrial scale and no requirement of the involvement of organic solvents, thus

generating highly stable particles and minimal toxicity. This includes high speed homogenization, ultrasonication, and hot/cold high-pressure homogenization, where the last ones are considered as most effective in preparation with desire particle size and narrow size-range distribution

High shear/ high speed homogenization: this is the simple and cost-effective method. In brief, a molten lipidic phase after being brought to temperature 5-10°C higher than melting point is mixed with drug. A watery phase containing surfactant(s) is also heated to similar temperature subsequently adding to molten lipid mixture, then it is homogenized using high shear force. The turbulent swirl would fabracite hot oil-in-water emulsion. NLCs then form by cooling these dispersions [82]. To obtain the narrower distribution of particle size, they usually combine this with further ultrasonication [83], which breaks down droplets into smaller ones as well as collapses existing bubbles.

Ultrasonication: the preparation of this method is somewhat like hot homogenization. Briefly, drug dissolved in molten lipid is dispersed in solution including surfactant, both is preheated at over melting point of lipid, to form pre-emulsion. This pre-emulsion is sonicated for a specific period at a specified volume of power [84]. After that, it cools down immediately to room temperature to obtain NLCs [85]. However, a long time of sonication can cause metal contamination to the final product by the sonicated probe.

Hot high-pressure homogenization: Lipidic phase is melted above melting point 5-10°C then mixing with hydrophobic drugs [86]. Simultaneously, an aqueous phase containing surfactants is preheated at equivalent temperatures. The lipidic phase is then distributed in a watery phase utilizing high-speed stirring to form pre-emulsion. Following that, the pre-emulsion is homogenized extensively using a high-pressure (100-2000 bar) homogenizer, which results in nano-emulsion afterwards. Once the emulsion is cooled down to low temperature [87], it develops NLCs structure. However, the heat during the process would be susceptible to thermo-sensitive bioactive agents and lessen the activity of some specific emulsifiers.

Cold high-pressure homogenization: This method is an alternative solution for the drawbacks of hot homogenization method. To be specific, a mixture of molten lipids and drugs is solidified by liquid nitrogen or dry ice [88]. The resulting solid mixture is milled finely and dispersed in a cold surfactant solution. This is completed by high-pressure homogenization process [89]. This method would avoid

the undesired heat; however, the final particle size may be bigger and more heterogeneous than hot homogenization does.

4.1.2. Lower energy input method

Microemulsion: is the most simple and chosen method for NLCs preparation [35, 90]. Melted lipid is mixed into the warmed oil, and the drug is dissolved in it. Dissolving surfactant in distilled water produces an aqueous phase. Both stages are heated and kept at a high temperature. At the same temperature, the lipid phase is introduced to the aqueous phase while being mechanically stirred, allowing the creation of a microemulsion [56]. This heated microemulsion is stirred into the chilly water. In this case, dilution with a significant amount of cold water (20-50 times the volume of microemulsion [89] is necessary to precipitate microemulsion globules into NLCs. Dilution with a high volume of water reduces the concentration of actives. As a result, additional formulation concentration or lyophilization is required. However, a large amount of surfactant may be considered for safety using.

Double emulsion: watery phase containing hydrophilic bio-active ingredients dispersed in lipidic phase (molten lipid + lipophilic drugs + hydrophobic surfactants) leads to the formation of water-in-oil emulsion [54]. After that, the primary emulsion is dispersed once more in an aqueous phase containing hydrophilic surfactants to form W/O/W emulsion. Two dispersion processes utilize sonication for homogenization [91]. The advantage of this method is to enhance the drug loading of hydrophilic drugs as well as to be potential strategies for co-loading various bio-active agents.

Phase inversion temperature: with the temperature-dependent polyoxyethylated surfactant, it decreases their HLB value at high temperature and vice versus. The phase inversion method employs this phenomenon to firstly synthesize W/O emulsions by incorporating lipid, water, and surfactants at high temperatures. This emulsion is then rapidly cooled while stirring constantly to convert to an O/W emulsion when the temperature drops down afterwards [92, 93]. At this low temperature, tiny lipid droplets recrystallize and generate NLCs. This method requires little energy without organic solvents. Nevertheless, the stability of the colloidal system virtually dependents on the control of the temperature cycle [94]. NLCs resulted from this method has significantly small size, below 50 nm and

stable, but the high ratio of mixed surfactant (more than 65% of total lipid base) must be taken into consideration [95].

Membrane contractor: molten lipid phase is pressed through a specialized porous membrane [96]. Lipid droplets pressed through the pores are swiped by the turbulence rotation of the watery phase, contained surfactants, underneath the membrane. The emulsion then brings to cool down for the NLCs formation [97]. The limitations of this method are sophisticated system and pores of membrane prone to clogging

4.1.3. Preparation with organic solvent involvement

Emulsification solvent diffusion: The solvent diffusion method employs water miscible organic solvents such as methanol, ethanol, and acetone [98], among others. The drug and lipids are introduced in a single or mixed organic phase in this technique. To achieve a distinct lipid phase, this is sonicated and kept at a high temperature. The aqueous phase is made by adding a suitable stabilizer/surfactant and is maintained at the same temperature as the lipid phase. At a high temperature, the organic-lipid phase is introduced to the aqueous phase while being constantly stirred. To obtain NLCs, this dispersion is agitated at room temperature for cooling and evaporation of the organic solvent [99].

Emulsification solvent evaporation: Instead of utilizing water miscible organic solvents like emulsification solvent diffusion method, water immiscible organic solvents such as chloroform, cyclohexane, dichloromethane [100], DMSO [101], and others are used to dissolve drugs and lipids in this technique [102]. Pre-emulsion resulted from mixing bioactive agents and lipid, surfactant with the heat often going through probe sonication [103] or high pressure homogenization [104] to obtain reasonable size and polydispersion index. The primary restriction of the solvent diffusion and evaporation approach is the use of organic solvents, as residues of them may remain in the formulations.

Solvent injection: the basic pre-steps of this method are similar to the emulsification solvent diffusion method [105]. The difference is that the lipid phase is introduced by going through a needle then into an aqueous phase being stirred at a specified velocity [106]. Following injection, the oil droplet is formed when it is out of the needle. In this method, solvent type and surfactant concentration are

critical for particle size and PDI [107]. The effect of solvent injection parameters on NLCs properties, such as pH, temperature, viscosity and dispersion energy in fabrication steps, or total lipid ratio, liquid lipid content and drug involvement in formulation design, were discussed in the previous review [108].

4.2. Additional techniques in NLCs fabrication

4.2.1. Hydrophobic ion pairing

Hydrophilic drugs hardly solubilize in the lipidic phase, restricting its encapsulated efficiency into LBNs. However, it can be incorporated by augmentation the lipophilic of drugs by techniques such as hydrophobic ion paring, then encapsulated in LBNs for better solubility [109]. It has been found that paring these ionized drugs with other hydrophobic substances containing opposite charged groups can improve their incorporation in LBNs (Fig. 3). As the ionic interaction between charged groups of drugs and counterion substance would form "new" complex drug to which expresses better partition coefficient in non-watery phase. Consequently, with enhanced lipophilicity, the complex drug could be loaded in the lipidic carrier with better retention time. Moreover, the molecular structure of complex after complexation is so-called amphiphile with hydrophilic drug end and hydrophobic alkyl chain of counterion substance end. As a result, permeability improvement across the membrane can be seen, enabling better bioavailability in general. The list of counterion pairing with drugs was reported elsewhere. They can be either anionic or cationic and typically contain one or more charged groups. Ionic surfactants, fatty acids (oleic acid, stearic acid, deoxycholic acid, and their salts), sulfates, phospholipids (dimyristoyl phosphatidyl glycerol), anionic polymer (dextran sulfate) have been extensively used as cationic counterion while quaternary amines and alkylamines are commonly used as cationic counterion [110].

For example, all-trans retinoic acid (ATRA) was ion paring with a lipophilic amine (benethamine) then loaded in NLCs [111]. Compared to blank NLCs (131 ± 4 nm), NLCs loaded ATRA complexing with and without benethamine increased in mean size (154 ± 4nm and 171 ± 18nm, respectively), this was observed that ATRA crystals were still present in the external phase of the latter and subsequently leading to be greater in size, no crystal could be seen in case of the former. The results on encapsulated efficiency showed similar observation where that of ATRA-benethamine NLCs

reached at 79% while only-drug NLCs stood at 15%. In another study, oleic acid (OA) was utilized as anion counterion of Doxorubicin (DOX) before loading into NLCs [112]. The lipophilicity of OA-DOX complex was confirmed by the saturability test, OA-DOX showed 6-fold and 3-fold increases in superior solubility than pure DOX in n-octanol and in medium chain glycerides media. Noticeably, insulin – ion paired complex with sodium dodecyl sulfate (SDS) was formed and improved log P of insulin from -1.8 to 2.1 for ensuring drug loading ability in NLCs [30], significantly improved intestinal cell membrane permeability [113].

4.2.2. Surface modification

Surface charge modification

It is important to note that the surface charge of a NPs is one of the major factors influencing to the physical stability of the colloidal dispersion and its cellular uptake capacity. There is inevitably no exception for nano-dispersion like NLCs. During the storage period, aggregation can be caused by destabilized elements: Ostwald ripening, coalescence, flocculation, coagulation, creaming and sedimentation. Charge agents could be incorporated with NLCs to maintain a high surface charge ensuring the electrostatic repulsion between particles. Moreover, positively charged NPs are prone to adhere to the negatively charged cell membrane, which facilitated a longer retention time on cells (**Fig.3**). Some charge modifiers are widely used including s stearyl amine, cetylpyridinium chloride, dicetyl phosphate, N, N-di- (beta stearoyl). In the study NLCs with olazapine, stearyl amine was incorporated in the organic phase with other lipid ingredients dispersed with the presence of pluronic F-68 as a nonionic surfactant [114]. The surface charge of batches with stearyl amine resulted more positively than that of non-stearyl amine (28.39mV vs. 10.90mV), indicating physical stability. Another study on the effect of charge on praziquantel NLCs having the preparation with Precitol ATO, oleic acid and nonionic surfactants, however, further integrated stearyl amine and dicetyl phosphate as positive and negative charge agents, respectively [72]. Although both NLCs formulations with charge agents demonstrated well drug-encapsulated, positively charged praziquantel-NLCs, was superior in destroying infection *S. mansoni* worm compared to negative charged and non-charged NLCs, even *in vivo*, similarly superior results were confirming by histopathology examination on rats. Chitosan, a well-known polymer for the formation of NPs surface

positive charge, was used to improve oral NLCs effectiveness [115, 116]. Chitosan coated NLCs loading amphotericin-B had EE at 86.0% while LC at 11.0%, and marginally reduced to 10.2% LC after 15 months. Compared to uncoated NLCs, the coated one showed greater bio-adhesion to intestinal rat lining (84.2% vs. 55.8%). This explained by the positive charge of chitosan retaining the electrostatic repulsion during storage as well as being better affinity to the negative-charge instinct of cells [115]. Surface modification with chitosan at optimum concentration at 0.1% reversed the surface charge of NLCs from negative value to positive value, with zeta potential over 30mV, and promoted the cellular uptake of ovalbumin antigen on RAW264.7 [116].

Surface modification for targeting delivery

Further coating polymers of NLCs takes advantage of the polymer by endurance during gastrointestinal processing and by the ability to control sudden burst release caused by lipid-based nanostructures as reported. Moreover, surface modification of NLCs by polymers can be utilized for enhancing mucoadhesion, targeting specific sites and prolonging blood circulation (Fig. 3). As it enables to be altered the molecular weight and structure of polymers, this combination allows to tailor the designed drug delivery system with desired therapeutic outcome. An array of both natural and synthesized polymers is employed for this fabrication. Specifically, commonly used polymers include polysaccharides, such as chitosan [66], alginate [117], dextran [118], polyethylene glycol (PEG) [119], polyesters [120] and co-polymers [121] their derivatives [122] are utilized for stabilizing and coating materials in integration with NLCs. Coating with PEG enabled LBNs passing through the mucus of gastrointestinal tract, reaching the epithelial cells and further to circulation systems [28]. Hyaluronic acid decorating on lipid nanocarriers NLCs have been extensively used for specific targeting to CD44 biomarker, which is overexpressed in colon cancer [123, 124]. Gelatin, a porotein polymer was combined with lipid to form LBNs loading zidovudine for HIV/AIDS therapy. This modified LBNs expressed significantly better cellular uptake on MCF-7 and neuro 2a brain cells [125].

Besides marketed polymer, novel synthetic polymers, mostly from polyethylene glycol (PEG) were investigated for targerting and stealth effect. In a study, doxorubicin loaded NLCs, folic acid conjugated PEG 2000 was coating for the active targeting purpose [126]. All the formulations with

PEG functionalization had particle size ranging 220nm-280nm and to be considered as homogeneous (PDI<0.18) and stable, these indicators were recorded as negligible change after 42 days of storage in room temperature. Functionalized particles caused greater cellular internalization on MDA-MB231 cells about 1.5 times compared with non-functional one. Besides, the use of hydrophilic polymers as "stealth molecules" prevents NPs uptake by the reticuloendothelial system (RES), in other words, the decoration by these polymers could help in lengthen time half-life plasma. There have been commonly investigated on PEG [127] and its derivatives listed dipalmitoyl-phosphatidyl-ethanolamine conjugated with polyethylene glycol 2000 (DPPE-PEG2000), distearoyl-phosphatidyl-ethanolamine N-poly(ethyleneglycol) 2000 (DSPE-PEG2000), stearic acid-PEG-2000 (SA-PEG2000), α-methoxy-PEG-2000-carboxylic acid-α-lipoamino acids (mPEG2000-C-LAA18), and α-methoxy-PEG-5000-carboxylic acid-α-lipoamino acids(mPEG5000-C-LAA18) [123, 128].

4.3. Transformation NLC into powder form

In the aqueous dispersion of NLCs, it either has the presence of other colloidal structures such as micelles, mixed micelles, liposomes and nano-emulsion which affect the stability of whole systems. During the storage, some transformations may occur within the system to gain higher thermodynamic stability, however, this can result in several relevant stability issues such as increasing particle size, then leading to widening the size distribution range, caused by Oswald ripening or coalescence, gelation of dispersion caused by the lipid bridge between the particles and drug expulsion out of the lipid matrix mainly cause by the recrystallization of lipids. The parameters of PS, PDI, ZP or DSC are usually references for determining the stability of dispersion systems like NLCs.

The long-term stability of NLCs is heading to aggregation like SLNs as reported. It can be attributed to several variables of the material properties or the preparation. Regarding the lipid concentration, highly concentrated occupied lipid dispersion was observed more stable than that of low concentration, with no change in the size of the particle after dilution. This was explained that free movement of particles in dispersion with a low concentration of lipid accelerates aggregation by collisions and peri-kinetic flocculation, whereas lipid dispersion in more dense occupation forms pearl chain-like so that minimizes the collisions [26]. Another factor possibly relevant to stability is pre-solidifying temperature. After the dispersion is done, it requires the lowering of temperature to

solidify solid lipid composition forming NLCs microstructure. The study of rambutan kernel NLCs found that when dispersion was pre-solidified at 5°C, it provided superior stability compared to one pre-solidified at 25°C [129]. This could be at lower temperature 5°C; the higher degree of supercooling accelerates the rate of nucleation faster than the growth of crystal. For this reason, even if the temperature gets back to room temperature, only the surrounding crystals will be decayed whilst the nuclei inside preserving consistently after 28 days. Storage temperature also influences the status of NLCs stability. The comparison of PS and PDI of NLCs formulated from trimyristin as solid and 6 different chemical molecules liquid lipids showed that NLCs were well preserved at 20°C rather than 4°C. The PS just slightly increased and PDI was insignificantly changed after 6 months of storage at ambient temperature [130]. This could probably be the lower temperature leading to polymorphism transition of lipid matrix then recrystallization of the drug. And lastly, as mentioned above, the electric charge on the particle surfaces would be maintained at enough repulsive interaction to keep the whole system stable, this is utilized using emulsifiers.

4.3.1. Spray-drying

Spray drying techniques are utilized for prolonging the stability of NLCs (Fig. 2). Spray drying converts liquid material into powder one, which takes advantage of less mobility within molecules, less transport and storage cost. However, regarding NLCs dispersion, the melting point of used lipid should be high because the high temperature of hot air during the spray drying process may expose the NLCs in being prone to polymorphism transition. For example, the NLCs of fish oil, with the incorporation of tristearin as a carrier and the help of maltodextrin as a protective material, was optimized at the temperature of inlet and out hot air are 140°C and 65°C, respectively. The results of unchanged physical stability were verified after 71 days of storage [131]. Similarly, NLCs also preserved well their diameter size at relatively small (<220nm) and performed good flowability after spray drying with the addition of sodium chloride as the excipient [132]. Therefore, the application of spray drying techniques is promising for stable bioactive encapsulated in NLCs when the related parameter such as material, temperature, and excipient are well investigated to optimize the physical state of the product afterwards.

4.3.2. Lyophilization

Like spray drying, lyophilization or freeze-drying has been gaining more attraction as strategies to prolong the stability of liquid-like material (Fig. 2). Under a vacuum, frozen products will be dehydrated and turn into powder. The process requires addition of cryoprotectants (such as carbohydrate trehalose, mannitol, sucrose, and glucose [133, 134]) to prevent the mechanical stress of ice on NPs, for the remarkable protective activity being observed in case of trehalose [134], and the growth of particle. In stability study of lopinavir loaded NLCs, freeze drying aided in minimizing the growth of particles, while there was no significant change in PDI, ZP and drug content inside the carriers under storage temperature of approximately 5°C [135].

5. Applications of NLCs via oral administration

NLCs have been successfully applied to improve effectiveness of bioactives not only at local gastrointestinal tract (GIT), but also at targeting sites such as brain, liver, cancer ulcer and so on (Fig.4.)

5.1. Site-specific NLCs for gastrointestinal tract

Many studies have been done for investigation and characterization of NLCs encapsulating drugs for GIT related inflammation with or without modified surface of NLCs (Table 2).

pH media variation throughout the GIT is one of the unpredicted physiochemical change factors once drug is orally administrated. On the other hand, this feature could be utilized in pH-dependent control release system, which means the design of drug carriers would release drugs in foreseeable conditions owing to specific pH. 5-Fluorouracil loaded NLCs was successfully developed with Eudragit S-100 decorated surface as a potential strategy for colon cancer targeting treatment. Eudragit S-100 is known as a delayed release polymer for colon targeting, where pH medium is higher than 7. The results of *in vitro* release in 24 hours at colon region showed a selective release profile. Furthermore, pharmacokinetic parameters on mice revealed higher maximum drug concentration in plasma (C_{max}) (2.54 folds), area under curve (AUC) (11 folds), and longer time for maximum concentration (T_{max}) (16 folds) and mean residence time (MRT) (4.32 folds) than drug solution. Thus the authors confirmed the better spatial and temporal bioavailability of this novel NLCs in colonic region [136]. Another study utilized Eudragit as coating for spray-dried BUD-NLCs pellets, the *in vitro* release observed that there was prevention release on the upper part (low pH) from the pellet. Therefore,

developed system could be promising for colonic with higher pH targeting release [137]. There was another synthesized polymer being used as coating, for example polyethylene glycol (PEG), for NLCs incorporating the chemotherapeutic agent, docetaxel (DOX). Inhere, cysteine was functionalized with PEG for the bio-adhesion coating [138]. The results showed not only the permeation through intestine increased significantly when compared with unmodified NLCs and drug solution, coated DOX-NLCs but also exhibited more intense fluorescence markers distributed throughout the intestinal tract after 4 hours of oral administration. This indicates that thiolated NLCs improve intestinal absorption by establishing disulfide bonding with the intestinal tract and promising for targeting local delivery due to the high affinity of modified particles to the mucus. Several studies on the ability to protect the gastrointestinal tract from factors that cause lesion were thoroughly conducted. For instance, pumpkin seed oil (PSO)-based, which is an alleviation of gastric ulcer as well as has multiple therapeutic effects such as anti-inflammation, antibacterial and anti-oxidant [139], was successfully played a role as both liquid lipid ingredient and therapeutic agent formulated in NLCs for peptic ulcer diseases induced by NSAID (indomethacin). The mice given PSO-NLCs orally had significantly fewer mucosal lesions and less gastric oxidative stress than the induced-group given only raw seed oil ($p < 0.01$) and the control group (no induction and no treatment) ($p < 0.001$). The histopathology photomicrographs of the stomach section depicted that PSO-NLCs offer well protection to the mucosal barrier and induce the re-epithelialization of colonic tissue. Furthermore, no inflammation or infiltrates in lamina propria could be detected [140]. The prior relevant model had been done that coconut oil based NLCs was formulated to incorporated (TMQ) for ethanol-induced ulcer management. The authors also acquire the similar result that optimal formulated (TMQ)-NLCs and (TMQ) suspension treatment demonstrated more effective protection against mucosal ulcer on ethanol-induced ulcer model than non-treatment, with respect to the former of 78.95% ($p < 0.01$) and the latter of 30.87% inhibition ($p < 0.05$). The histopathology evaluation of stomach also manifested noticeably prevention effects of NLCs [141]. Several relevant strategies have been developed elsewhere [142].

Regarding ulcerative colitis disease, OLE-loaded NLCs for reducing inflammation in acute colitis was prepared successfully with almost absolute entrapment efficiency and narrow size distribution (PDI

~0.2). *In vitro* test of anti-inflammatory and antioxidant potential surprisingly revealed that both blank NLCs and oleuropein-loaded NLCs had significant inhibition ($p<0.05$) on TNF-α secretion, a type of cytokine responding to inflammation, whereas pure OLE did not. From the *in vivo* study, the authors concluded the ability of orally administrated OLE-loaded NLCs can reduce inflammation and oxidative stress in DSS-induced acute colitis on mice, proven by results of myeloperoxidase activity reduction, cytokine-associated tissues attenuation and reactive oxidative species generation inhibition [143]. Budesonide was also loaded into NLCs with coumarin-6 dye to determine the percentage of dose delivered to organs. The inflammatory-induced rats with NLCs treated showed Elisa assays and histological examination significantly suppression on pre-inflammatory cytokines (e.g., TNF-α, IL-1β) at colon compared to the untreated group. In addition, the specific retention at colon area was confirmed by the amount of detected coumarin-6 NLCs at cecum and colon remained up to 12 hours after oral administration. The NLCs tended to penetrate the mucosa of the induced group while just accumulated on villi in case of the control group [144]. Another study NLCs loading curcumin also indicated the sustaining of NLCs at inflamed areas in the mice colon over 12 hours, and was the only form of the carrier (when being compared with nano-capsule and self-nanoemulsifying system in this study) exhibiting considerably inhibition on the expression of inflammation in term of the histological score, TNF-α level after 8 days of treatment [145].

5.3. NLCs for bioavailability improvement via oral administration

Oral drug delivery is usually limited by the poor drug physiochemical properties and the nature of GIT barriers. NLCs has been one of the promising carriers for broad applications for oral bioavailability enhancement. It has led to the development of various systems to facilitate the absorption of gypenosides (GPS) [146], nimodipine (NMP) [147], biochanin A (BCA) [148], perphenazine (PPZ) [104], candesartan cilexetil (CC) [149], ergosterol [150] [151] ***(Table 3)***. Accordingly, Anwar et al. synthesized a CC-NLCs was statistically proved to be able to be absorbed through GIT effectively, where the intestinal was a preference in absorption than that of the stomach. The monolayer Caco2 uptake test supported the idea of internalization of NLCs through enterocytes [149]. Similarly, the study of drug NMP incorporated NLCs also revealed the primary absorption route through enterocytes, which facilitates relative BA 160% further than plain drug [147]. In

another research, the combination of bile salts in the formulation of NLCs augmented the bioavailability of active agent GPS more than 8.5 times more than the pure powder dosage form. This may be the result of the help of bile salts inducing the lipid absorption mechanism in the duodenum [146]. Many more research studies in design carriers formulation have been reported about successful incorporating drugs in NLCs can improve bioavailability, as well as prolong the presence of drugs in blood circulation, which was demonstrated in study of BCA-NLCs [148].

5.4. Liver – targeting NLCs via oral administration

Hepatic diseases are non-cure diseases until now, primarily caused by viruses, drugs or poisons, cancer, and inherited factors. On the contrary, the metabolism in the liver sometimes hinders the effectiveness of drug treatment, even triggers a worse scenario for the liver itself. Combining drugs in NLCs should be a novel strategy to improve the therapeutic efficacy in this case. Numerous research studies have been executed on various trigger-induced hepatic lesion models to investigate related diseases ***(Table 3)***. For example, hepatic infection is also prevented by adefovir dipivoxil (AD) NLCs [152], which is observed to have superior uptake by the liver after administration. Inhere, the amount of radio-iodinated rose Bengal, which its uptake implicates normal hepatic function, was found to significantly increase the liver uptake of AD-NLCs treated unhealthy-mice (22% injected dose/gram liver) compared to that of untreated unhealthy-group (9% injected dose/gram liver) and healthy-group (26% injected dose/gram liver), indicating the efficacy of hepatic protection by utilizing nano-carrier to deliver targeting liver via intestinal absorption. Similarly, a successfully synthesized praziquantel (PZQ) NLCs with the positive charge on the surface of the particle was formulated resulting in a conclusion that positively charged particles reduced 62.1% of the granuloma in the liver caused by *S. mansoni* infection on mice [72]. In another study, naringenin (NGN) loaded in NLCs was used to treat excessive lipid in liver [153], NGN can be delivered sufficiently to liver and decrease 12.5 folds lipid deposition as much as that of plain drug on liver of mice. Moreover, with the incorporation with NLCs, NGN was found to be able could mitigate the nonalcoholic fatty disease at the low dose of 12.5mg/kg body weight, which was more effective than pure drug at doses of 50mg/kg and 100mg/kg.

5.5. Brain – targeting NLCs via oral administration

For brain delivery via oral administration, besides overcoming the GIT barriers, drugs are usually hampered by blood brain barrier (BBB) before reaching to targeting site inside. This brain membrane is even more highly selective which is the cause of poor delivery of drugs to the brain. Regardless, relevant investigations about NLCs proposed the results surpassing these hurdles ***(Table 3)***. In the study of brain diseases, some incorporation were established with promising results for the new treatments, such as atazanavir (ATZ) NLCs for neuro-AIDS [154], temazepam (TZP) NLCs for insomnia [155] and lopinavir (LPV) NLCs for HIV-associated neurocognitive disorder [156], all showed the preference in brain distribution at 10 times, 3.4 times and 2.8 times, respectively, than those of their own plain drug suspension. Herein, the first study evident the brain uptake of ATZ-NLCs was superior observed by confocal microscopy and concluded that NLCs could cross BBB and then accumulate drug in tissue inside; the drug concentration-time profile in brain also witnessed the evident that after 24 hours of administration, ATZ in NLCs formulation could deposit more in the brain (62.57 ± 2.43 µg/mL) than in suspension (only 7.54 ± 1.68 µg/mL). The two latter studies are seen as the relevant results, where gamma scintigraphy image of TZP-NLCs showed the radiolabeled agent accumulating more in brain, indicating the higher concentration of TZP in the brain area after 4 hours of oral administration. Regarding LPV-NLCs, it was found that with the help of Tween 80 coating for brain targeting [157], it was delivered to the brain via oral mean with the level of about 45mg/mL, better than non-incorporated LPV delivery via oral and even via intravenous means. In another study, the restoration of brain chemicals such as significantly depleted glutathione and dopamine and considerably decreased of the lipid peroxidation indicated the effectiveness of lipid formulation of RP treated Parkinson's Disease on mice [158]. In terms of biochemical, histological, and immune-histochemical alterations, NLCs were the safest formulation when compared to carbamazepine (CBZ) suspension and the market product (Tegretol^{CR}). Because CBZ toxicity was directly related to the reactive metabolite carbamazepine-10,11-epoxide [159], the authors hypothesized that bypassing the liver via NLCs would potentially minimize toxicity, though they did not measure serum carbamazepine-10,11-epoxide concentrations.[87]. In case of berberin loaded NLCs for Alzheimer's treatment, the best formulation had homogenous size around 186 nm and high

EE, 88%, improved *in vivo* behaviour in Albino Wistar rats, evaluated by passive avoidance test and Morris water maze test [19].

5.6. NLCs for cancer treatment via oral administration

Other oral dosage forms of NLCs have been applied to chemotherapeutic agents ***(Table 3)***. Rahman and coworkers designed zerumbone (ZER) loaded NLCs and test its antileukemia effects on WEHI-3B cell-induced murine leukemia model [160]. As a result, the effectiveness of ZER-NLCs in inhibiting the growth of leukemia cells was experimentally proved *in vitro* and *in vivo* which then explained that they induce a mitochondrial-independent apoptosis pathway. In another study, auraptene (ART) NLCs was proposed to ameliorate testosterone-induced benign prostatic hyperplasia, a common disease in middle-aged men [161]. Treatment with ART-NLCs (dose 5–10 mg/kg) significantly reduced testosterone-induced granuloma growth. Furthermore, they modulated the chain of inflammation less severely. In breast cancer, citral (CT) NLCs was investigated in the 4T1 breast cancer- induced murine mouse model [162]. The results could be promising for new breast chemotherapy where CT-NLCs substantially inhibited inflammation at the targeting site, evenly prevented metastasis effectively. Raloxifene–loaded NLCs were more toxic to MCF-7 cells and improved *in vivo* AUC nearly 5 times more than the suspension form, indicating that these NLCs could be used in breast cancer via oral uptake [163].

5.7. NLCs for other diseases via oral administration

Currently, NLCs are being tested as new therapeutic strategies in a variety of diseases ***(Table 3)***. For example, antimalaria combined drugs artemether/lumefantrine (ARM/LFN) were encapsulated in NLCs [164]. In the clinical simulation protocol, ARM/LFN-NLCs in soft capsules and tablets were seen to be effective in parasite clearance at doses lower 10 times more than the marketed dose (Actizo DT) owing to the sustained release property of lipid carrier. For another disease, hyperlipidemia, the incorporation of the hypolipidemic agents such as ezetimibe [165] and β-sitosterol [166] in NLCs revealed a significant reduction, after 14 days and 60 days of oral administration, respectively, of levels of total cholesterol and low-density lipoprotein cholesterol, which are the main cause of disease, observed by *in vivo* studies on induced-fatty diet mice. This suggested that NLCs could favor

the uptake of those active ingredients overcoming GIT hurdles, thereby raising the drug concentration in plasma, which have an effect in reducing cholesterol level. In addition, due to the bioavailability enhancement by combining LBNs, olmesartan medoxomil + concavalin-A (OL+ CoA) NLCs was found to reduce 37% the blood pressure level in comparison with orally administrating pure drug suspension; inhere, the presence of CoA as lectin was supposed to attribute to facilitating drug absorption by intestinal lectin receptor-mediated pathway [167].

5.8. NLCs for nutraceutical delivery

Besides APIs with therapeutic effects, current research has also focused on enhancing the absorption of compounds with nutritional and medical benefits for the body, called nutraceuticals for short. These compounds are found in a wide range of components such as peptides, carotenoids, polyphenols, fatty acids, vitamins, and minerals, to name a few. They do, however, have limits, such as low bio-accessibility and poor solubility in low-fat environments. In addition, the oxidation susceptibility of these compounds is a hurdle to their storage without special concerns [168]. For these reasons, a lipophilic delivery carrier like NLCs promotes these nutraceuticals in a variety of absorption ways ***(Table 3)***. Some of nutritional bio-actives encapsulated in NLCs and their improvements are described somewhere and will be discussed below.

Phenolic family is the most ubiquitous source, which is found in plants such as fruits, vegetables, cereals, and teas. Phenolic compounds have many wonderful benefits for health in terms of antioxidant, anticarcinogenic, anticancer, antibacterial, and anti-inflammatory activities [169]. Quercetin (QUC), a flavonoid found in grains, fruits, and red onions, is a prime example of a potential bio-active with various good effects on health. In the study of QUC-NLCs, the authors suggested that thanks to the lower ordered crystallinity structure, the excellent solubilization and antioxidant properties of QUC-NLCs were observed. Moreover, this lowered the lipid oxidation in comparison with a conventional emulsion [170]. Other compounds, that although they are micronutrients, bring to health enormously good effects. Tocopherol is a lipid-soluble chemical as complementary medicine for chronic diseases and omega-3 fatty acids, a natural unsaturated lipid found in sea fish, is famous for cardiovascular diseases. Although they are all found in food, the daily dietary intake is not enough for their significant effects; thus, they should be contained in supplementary pills. Recently, there was

a co-incorporation of omega-3 fish oil/ α-tocopherol in NLCs (O-T-NLCs) for the investigation of their characteristics [171]. The results demonstrated better long-term stability of 75 days and oxidative stability than other formulations without encapsulation. Furthermore, they had seen the O-T-NLCs inhibit the primary oxidative products, which suggests the fortified strategies for foodstuffs that are prone to easily being oxidized.

5.9. NLCs for peptides and proteins delivery via oral administration

Biotechnological development has suggested a new therapeutic strategy in addition to pharmaceutical small molecules, peptides and proteins. This novel finding takes advantage in targeting delivery and treatment since peptides and proteins is likely to be highly selective and highly specific to some well-defined mechanism of body reaction rather than conventional drugs [172]. However, peptides and proteins are prone to degradable by body's enzymes and poorly absorbable across cell membrane. Their duration in systemic circulation is rapidly shortened under renal clearance [173]. These are critical hindrances when it comes to applying macro-molecules into non-invasive therapy.

Incorporating peptides and proteins into/with NLCs has been becoming one of the favored approaches among current nanocarrier system. Although its number of publications is limited, NLCs are still promising for oral peptide and protein delivery thanks to its advantages such as burst release control pattern, degradation prevention and absorption acceleration across intestinal membrane, which is stated above of this article. NLCs have been employed for the delivery of peptide molecules such as self-replicating RNA , siRNA [174], glutathione [21], insulin [30, 175] thanks to enzyme protection property of lipid base via oral administration. On the other aspect, NLCs is also applied for vaccine formulation. The current study on ovalbumin (OVA) antigen model showed effective uptake by macrophage and promoted mice immunization by the formulation of antigen encapsulated inside chitosan modified NLCs [116]. Another approach is to complex NLCs and mRNA (mRNA-NLCs) [176]. The purpose of this complexion is not only to prevent degradation after administration but also to facilitate for long-term storage issue. The study resulted in excellent thermostability of mRNA-NLCs in which lyophilized form was stable at room temperature up to 8 months and at refrigerated temperature up to 21 months while remaining treatment efficacy in vivo. Notably, two authorized Covid-19 vaccines, mRNA-1273 [177] and BNT162 [178], use LBNs to deliver antigen mRNA.

Besides, other great deals of studies have shown the benefits of applying NLCs in vaccines such as similar delivering of replicating viral RNA [179] and HIV p24 peptide [180] vaccines, through intramuscular and intraperitoneal injections, respectively. Oral LBNs loading self-replicating RNA (saRNA) were successfully fabricated for SARS-CoV-2 vaccination. These saRNA LBNs boosted high levels of SARS-CoV-2 IgG and IgA antibodies and effectively treated both SARS-CoV-2 variants alpha and delta in mice [181]. However, there has been not enough data to prove the efficacy of oral NLCs coated for vaccines yet. Nevertheless, differentiated M-cells and Peyer's patches on the intestinal wall are known to be an effective gut immune system, closely related to systemic autoimmunity. Based on the absorption pathways of NLCs at GIT, including assisted M-cells, as discussed above, NLCs could be an expecting strategy in the development and application of oral vaccines as well as other perspectives of oral drug delivery.

6. Future perspectives

NLCs has been investigating improve therapeutic effectiveness of numerous bioactive chemicals following oral route, including site-specific cure as well as biodistribution rearrangement, resulting in organ targeting such as brain, liver, kidney and so on. Specifically, NLCs system could be designed further for either active or passive targeting and imaging [182]. Besides functionalizing the NLCs using ligands for suppressing drug resistance [174], the strategy to engineer imaging markers such as superparamagnetic iron oxide detected by magnetic resonance imaging [183], which can provide useful information for evaluating the distributing ability of carriers to action sites. Magnetic NLCs could also be used as a triggering factor for controlled release [182]. Thus, this appears to be a promising tool for *in vivo* distribution observation, diagnosis, and treatment of diseases. Lipid ingredient is biodegradable, biocompatible, and simple to attain the necessary nanometric size, making it a unique drug carrier for targeted treatment. However, the appearance of surfactant for forming and stabilizing NLCs suspension may cause potential toxicity to the body. Novel lipid based materials has been synthesized for NLCs modification with superior structure, improve EE and especially targeting properties. For example PEG-SA-UA, (ursodeoxycholic acid) for loading asiatic acid for liver fibrosis [20], (gastric cancer peptide GX1) GX1-SA-PEG2000 for enhancing antitumor activity of paclitaxel [184]. Since NLCs increase the amount of drug transported via the intestinal

lymphatic system, it is the optimum drug delivery system for lymph targeting via oral route like atazanavir (ATV) NLCs expressed higher bioavailability than ATV suspension in the chylomicron flow block model [185].

NLCs fabrication technique has been improved for better LC, stability and industrial application. Recently, nanospray dryer has drawn a lot of attention since gaining the benefits of the nanosized nozzle and structural modification, it is possible to directly convert pure chemical mixtures into dry submicron-sized powders with electrostatic particle collector [150]. This supports the application of the possibility of large-scale production of NPs in foreseeable future, especially NLCs. [186]. Besides, spray chilling has been applied in probiotics and nutraceuticals and plant natural compounds for large scale [187]. Noticably, hydrophobic ion paring is newly useful tool to enable entrapment of hydrophilic drugs in to NLCs [188].

Besides, the development and popularity of evaluation techniques including physicochemical characterization: atomic force microscopy (AFM) [189], molecules interaction, drug loading and pharmacokinetics investigation give better clarification of the NLCs formation and absorption. NLCs for oral administration have been evaluated by numerous specific methods including *in vitro* mucoadhesive study [138], *in situ* intestinal perfusion test [146], *in vivo* gastrointestinal irritancy and liver damage [190], *in vitro* release by dialysis bag in medium mimicking gastrointestinal pH [35], various *in vitro* cell culture models [191], *ex vivo* and *in situ* experiments on each intestinal segment [146, 147]. Regardless of how sophisticated an *in vitro* model is, *in vivo* studies will inevitably be crucial to analyze the realistic therapeutic effect of an oral delivery system. Since oral NPs administration is most discovered in the GIT, several imaging methods focus entirely on the stomach, small intestine, and colon [146]. Moreover, pharmacological models corresponding to therapeutic effects such as memorization increase [19], liver fibrosis treatment [20], or induced disease models [140]…should be carry out for better understanding of NLCs carrier biodistribution and therapeutic enhancement. Besides, it is necessary to have more pre-clinical and clinical results of LBNs, since LBNs are the latter fabricated nanosystems as compared to other nanocarriers. Moreover, absorption and targeting pathways for LBNs should be clarified, which may provide better knowledge bioactive pharmacokinetics after oral administration.

Herbal extract often constitutes of various chemicals, different about molecular weight and hydrophobicity. NLCs has been powerfully alternative approach for carry multi plant bioactives agents, for example: Diosgenin and *Glycyrrhiza glabra* extract [192], with or without other drugs, like antibiotics [193] as sensory masking, enzyme degradation protection and flexible physical structure. Incorporation hydrophilic and hydrophobic phytoagents from Diosgenin and yam into NLCs resulted in better sustained release and antioxidant activity than single one loaded NLCs.

Using peptides and proteins as novel therapy for high specificity and low side-effects has been a promising trend for pre-clinical and clinical in recent decades. However, peptides and proteins are extremely susceptible to environment, which has required exceptional storage condition and has limited their use for widely routes of administration. The lipid carriers are able to prevent peptides and proteins encapsulated inside from being reactive and being chemically transformed under harsh condition throughout GIT. The studies on glutathione [21] and insulin [30, 175] can be promising evidence, where these proteins were protected from low pH, lipolytic and proteolytic enzymes when orally administrating. Moreover, hydrophobic ion technique can alter the hydrophilicity of peptides and proteins, resulting in significantly higher loading in LBNs [194]. Additionally, transformation peptide loaded NLCs in to powder form could prolong the storage time without losing their therapeutic effects after reconstitution, as has been studied on adjuvant ovalbumin [116] and mRNA [176].

Additionally, solid dosage forms loading NLCs is critical factor for therapeutic performance of NLCs through oral administration. In the study about NLCs loaded glargine insulin, neither NLCs suspension nor tablet containing NLCs powder showed considerable gastrointestinal absorption and antihyperglycemic effectiveness on diabetic rats. Exceptionally, only capsule loaded glargine insulin NLCs significantly reduce glycemic index in diabetics rat, thanks to the large size of this dosage form, leading to longer retention time and higher uptake at the stomach-duodenum tract [175].

7. Conclusion

In summary, NLCs may be a promising solution among enteral drug delivery systems. NLCs poses unique characteristics to overcome the limitations of oral administration: protecting from pH, enzyme activity, enhancing the absorption from the gastrointestinal tract via intestinal lymphatic system

transportation, improve biocompatibility and bioavailability of drugs thanks to lipid nature. Absolutely, NLCs also carry nanaomaterials positive points, a flexible system suitable for hydrophobic chemical encapsulation, and maintain reasonable hydrophylic compounds holding by hydrophobic ion pariring technique or blending with polymer. A diversity of bioactives agents has been loaded in NLCs, individual or multiple compounds for various treatment in oral route. Site - specific NLCs for major treatment of gastric and colon diaseases, while systematic delivery not only improve drug bioavailbility for cure of malaria, anaphylactic shock, hypertension, etc but also could bring therapeutic agents reach the targeting organs such as brain, liver and kidney and cancer tumors. Studies have shown that oral NLCs would shine brighter, even further in a variety of applications, especially for chronic diaseases, in the future with the supporting of more modern technology in production, evaluation for industrial concern.

Acknowledgments

This research is funded by Vietnam National University Hochiminh City (VNU-HCM) under grant number NCM2020-28-01.

Disclosure statement

References

1. Yasir, M., et al., *Biopharmaceutical classification system: An account.* International Journal of PharmTech Research, 2010. **2**(3): p. 1681-1690.

2. Taylor, K.M. and M.E. Aulton, *Aulton's Pharmaceutics E-Book: The Design and Manufacture of Medicines*. 5 ed. 2017: Elsevier Health Sciences. 19.

3. Stillhart, C., et al., *Impact of gastrointestinal physiology on drug absorption in special populations—An UNGAP review.* European Journal of Pharmaceutical Sciences, 2020. **147**: p. 105280.

4. Shirodkar, R.K., et al., *Solid lipid nanoparticles and nanostructured lipid carriers: emerging lipid based drug delivery systems.* Pharmaceutical Chemistry Journal, 2019. **53**(5): p. 440-453.

5. Samimi, S., et al., *Lipid-based nanoparticles for drug delivery systems.* Characterization and biology of nanomaterials for drug delivery, 2019: p. 47-76.

6. Doktorovova, S., E.B. Souto, and A.M. Silva, *Nanotoxicology applied to solid lipid nanoparticles and nanostructured lipid carriers–a systematic review of in vitro data.* European Journal of Pharmaceutics and Biopharmaceutics, 2014. **87**(1): p. 1-18.

7. Gasco, M.R., *Method for producing solid lipid microspheres having a narrow size distribution*. 1993, Google Patents.

8. Müller, R.H., K. Mäder, and S. Gohla, *Solid lipid nanoparticles (SLN) for controlled drug delivery–a review of the state of the art.* European journal of pharmaceutics and biopharmaceutics, 2000. **50**(1): p. 161-177.

9. Pardeike, J., A. Hommoss, and R.H. Müller, *Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products.* International journal of pharmaceutics, 2009. **366**(1-2): p. 170-184.

10. Lucks, S. and R. Muller, *Medication vehicles made of solid lipid particles (solid lipid nanospheres-SLN)*. 1996, Google Patents.

11. Khosa, A., S. Reddi, and R.N. Saha, *Nanostructured lipid carriers for site-specific drug delivery.* Biomedicine & Pharmacotherapy, 2018. **103**: p. 598-613.

12. H Muller, R., R. Shegokar, and C. M Keck, *20 years of lipid nanoparticles (SLN & NLC): present state of development & industrial applications.* Current drug discovery technologies, 2011. **8**(3): p. 207-227.

13. Weber, S., A. Zimmer, and J. Pardeike, *Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) for pulmonary application: a review of the state of the art.* European Journal of Pharmaceutics and Biopharmaceutics, 2014. **86**(1): p. 7-22.

14. Mehnert, W. and K. Mäder, *Solid lipid nanoparticles: production, characterization and applications.* Advanced drug delivery reviews, 2012. **64**: p. 83-101.

15. Müller, R., et al., *Solid-liquid (semi-solid) liquid particles and method of producing highly concentrated lipid particle dispersions.* German patent application, 2000. **199**(45,203.2).

16. Li, Q., et al., *A review of the structure, preparation, and application of NLCs, PNPs, and PLNs.* Nanomaterials, 2017. **7**(6): p. 122.

17. Khan, S., et al., *Nanostructured lipid carriers: An emerging platform for improving oral bioavailability of lipophilic drugs.* International journal of pharmaceutical investigation, 2015. **5**(4): p. 182-191.

18. Irby, D., C. Du, and F. Li, *Lipid–drug conjugate for enhancing drug delivery.* Molecular pharmaceutics, 2017. **14**(5): p. 1325-1338.

19. Raju, M., et al., *Berberine loaded nanostructured lipid carrier for Alzheimer's disease: Design, statistical optimization and enhanced in vivo performance.* Life Sciences, 2021. **285**: p. 119990.

20. Zhang, Y.-W., et al., *Liver-targeted delivery of asiatic acid nanostructured lipid carrier for the treatment of liver fibrosis.* Drug Delivery, 2021. **28**(1): p. 2534-2547.

21. Ahmad, A.M., et al., *Nano-Structured Lipid Carrier-Based Oral Glutathione Formulation Mediates Renoprotection against Cyclophosphamide-Induced Nephrotoxicity, and Improves Oral Bioavailability of Glutathione Confirmed through RP-HPLC Micellar Liquid Chromatography.* Molecules, 2021. **26**(24): p. 7491.

22. Dudhipala, N. and A.A. Ay, *Amelioration of ketoconazole in lipid nanoparticles for enhanced antifungal activity and bioavailability through oral administration for management of fungal infections.* Chemistry and Physics of Lipids, 2020. **232**: p. 104953.

23. Dudhipala, N., K.Y. Janga, and T. Gorre, *Comparative study of nisoldipine-loaded nanostructured lipid carriers and solid lipid nanoparticles for oral delivery: preparation, characterization, permeation and pharmacokinetic evaluation.* Artificial cells, nanomedicine, and biotechnology, 2018. **46**(sup2): p. 616-625.

24. Poonia, N., et al., *Nanostructured lipid carriers: versatile oral delivery vehicle.* Future science OA, 2016. **2**(3): p. FSO135.

25. Abedi Gaballu, F., et al., *Comparative of in-vitro Evaluation between Erlotinib Loaded Nanostructured Lipid Carriers and Liposomes against A549 Lung Cancer Cell Line.* Iranian journal of pharmaceutical research : IJPR, 2019. **18**(3): p. 1168-1179.

26. Müller, R., M. Radtke, and S. Wissing, *Nanostructured lipid matrices for improved microencapsulation of drugs.* International journal of pharmaceutics, 2002. **242**(1-2): p. 121-128.

27. Makoni, P.A., K. Wa Kasongo, and R.B. Walker, *Short Term Stability Testing of Efavirenz-Loaded Solid Lipid Nanoparticle (SLN) and Nanostructured Lipid Carrier (NLC) Dispersions.* Pharmaceutics, 2019. **11**(8): p. 397.

28. Ball, R.L., P. Bajaj, and K.A. Whitehead, *Oral delivery of siRNA lipid nanoparticles: fate in the GI tract.* Scientific reports, 2018. **8**(1): p. 1-12.

29. Park, S.J., et al., *Development of nanostructured lipid carriers for the encapsulation and controlled release of vitamin D3.* Food Chemistry, 2017. **225**: p. 213-219.

30. Shahzadi, I., et al., *Nanostructured Lipid Carriers (NLCs) for Oral Peptide Drug Delivery: About the Impact of Surface Decoration.* Pharmaceutics, 2021. **13**(8): p. 1312.

31. Al-Ali, A.A.A., et al., *Nonionic surfactants increase digoxin absorption in Caco-2 and MDCKII MDR1 cells: Impact on P-glycoprotein inhibition, barrier function, and repeated cellular exposure.* International Journal of Pharmaceutics, 2018. **551**(1): p. 270-280.

32. Johnson, B.M., W.N. Charman, and C.J. Porter, *The impact of P-glycoprotein efflux on enterocyte residence time and enterocyte-based metabolism of verapamil.* J Pharm Pharmacol, 2001. **53**(12): p. 1611-9.

33. Zordan-Nudo, T., et al., *Effects of nonionic detergents on P-glycoprotein drug binding and reversal of multidrug resistance.* Cancer Res, 1993. **53**(24): p. 5994-6000.

34. Beloqui, A., et al., *Mechanism of transport of saquinavir-loaded nanostructured lipid carriers across the intestinal barrier.* Journal of Controlled Release, 2013. **166**(2): p. 115-123.

35. Mura, P., et al., *Evaluation and comparison of solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) as vectors to develop hydrochlorothiazide effective and safe pediatric oral liquid formulations.* Pharmaceutics, 2021. **13**(4): p. 437.

36. Thepwatee, S., et al. *Color masking of betel nut extract using encapsulation technology for nutricosmeceutical applications.* in *Key Engineering Materials*. 2019. Trans Tech Publ.

37. Fathi, M. and J. Varshosaz, *Novel hesperetin loaded nanocarriers for food fortification: Production and characterization.* Journal of Functional Foods, 2013. **5**(3): p. 1382-1391.

38. Baqeri, F., et al., *The effect of gelatin and thymol‐loaded nanostructured lipid carrier on physicochemical, rheological, and sensory properties of sesame paste/date syrup blends as a snack bar.* Journal of texture studies, 2020. **51**(3): p. 501-510.

39. Banerjee, S., et al., *Lipids for Taste masking and Taste assessment in pharmaceutical formulations.* Chemistry and Physics of Lipids, 2021. **235**: p. 105031.

40. Salah, E., et al., *Solid lipid nanoparticles for enhanced oral absorption: A review.* Colloids and Surfaces B: Biointerfaces, 2020: p. 111305.

41. Camenisch, G., et al., *Estimation of permeability by passive diffusion through Caco-2 cell monolayers using the drugs' lipophilicity and molecular weight.* European journal of pharmaceutical sciences, 1998. **6**(4): p. 313-319.

42. Zhang, Z., et al., *Solid lipid nanoparticles loading candesartan cilexetil enhance oral bioavailability: in vitro characteristics and absorption mechanism in rats.* Nanomedicine: Nanotechnology, Biology and Medicine, 2012. **8**(5): p. 740-747.

43. Chai, G.-H., et al., *Transport mechanisms of solid lipid nanoparticles across Caco-2 cell monolayers and their related cytotoxicology.* ACS applied materials & interfaces, 2016. **8**(9): p. 5929-5940.

44. Nellans, H.N., *(B) Mechanisms of peptide and protein absorption:(1) Paracellular intestinal transport: modulation of absorption.* Advanced drug delivery reviews, 1991. **7**(3): p. 339-364.

45. Ma, T. and J.M. Anderson, *Tight junctions and the intestinal barrier*, in *Physiology of the gastrointestinal tract*. 2006, Elsevier Inc. p. 1559-1594.

46. Ali, H. and S.K. Singh, *Biological voyage of solid lipid nanoparticles: a proficient carrier in nanomedicine.* Therapeutic delivery, 2016. **7**(10): p. 691-709.

47. C Silva, A., et al., *Lipid-based nanocarriers as an alternative for oral delivery of poorly water-soluble drugs: peroral and mucosal routes.* Current medicinal chemistry, 2012. **19**(26): p. 4495-4510.

48. Charman, W.N., *Lipid vehicle and formulation effects on intestinal lymphatic drug transport*, in *Lymphatic transport of drugs*. 2019, Routledge. p. 113-179.

49. Lin, C.-H., et al., *Recent advances in oral delivery of drugs and bioactive natural products using solid lipid nanoparticles as the carriers.* Journal of food and drug analysis, 2017. **25**(2): p. 219-234.

50. Ortiz, A.C., et al., *Development of a Nanostructured Lipid Carrier (NLC) by a Low-Energy Method, Comparison of Release Kinetics and Molecular Dynamics Simulation.* Pharmaceutics, 2021. **13**(4): p. 531.

51. Varrica, C., et al., *Topical Allopurinol-Loaded Nanostructured Lipid Carriers: A Novel Approach for Wound Healing Management.* Bioengineering, 2021. **8**(12): p. 192.

52. Chu, C.C., C.P. Tan, and K.L. Nyam, *Development of nanostructured lipid carriers (NLCs) using pumpkin and kenaf seed oils with potential photoprotective and antioxidative properties.* European Journal of Lipid Science and Technology, 2019. **121**(10): p. 1900082.

53. Araujo, V.H.S., et al., *The influence of NLC composition on curcumin loading under a physicochemical perspective and in vitro evaluation.* Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2020. **602**: p. 125070.

54. Cavalcanti, S., et al., *Multiple Lipid Nanoparticles (MLN), a New Generation of Lipid Nanoparticles for Drug Delivery Systems: Lamivudine-MLN Experimental Design.* Pharmaceutical research, 2017. **34**(6).

55. Gaba, B., et al., *Nanostructured lipid (NLCs) carriers as a bioavailability enhancement tool for oral administration.* (1521-0464 (Electronic)).

56. Das, S. and A. Chaudhury, *Recent advances in lipid nanoparticle formulations with solid matrix for oral drug delivery.* Aaps Pharmscitech, 2011. **12**(1): p. 62-76.

57. Souto, E.B. and R.H. Müller, *Lipid nanoparticles: effect on bioavailability and pharmacokinetic changes.* Handb Exp Pharmacol, 2010(197): p. 115-41.

58. Hauss, D.J., *Oral lipid-based formulations.* Adv Drug Deliv Rev, 2007. **59**(7): p. 667-76.

59. Elmowafy, M. and M.M. Al-Sanea, *Nanostructured lipid carriers (NLCs) as drug delivery platform: Advances in formulation and delivery strategies.* Saudi Pharmaceutical Journal, 2021.

60. Makwana, V., et al., *Solid lipid nanoparticles (SLN) of Efavirenz as lymph targeting drug delivery system: Elucidation of mechanism of uptake using chylomicron flow blocking approach.* International journal of pharmaceutics, 2015. **495**(1): p. 439-446.

61. Vitorino, C., et al., *Co-encapsulating nanostructured lipid carriers for transdermal application: from experimental design to the molecular detail.* Journal of controlled release, 2013. **167**(3): p. 301-314.

62. Elmowafy, M., et al., *Fatty alcohol containing nanostructured lipid carrier (NLC) for progesterone oral delivery: in vitro and ex vivo studies.* Journal of Drug Delivery Science and Technology, 2018. **45**: p. 230-239.

63. Pokharkar, V., A. Patil-Gadhe, and G. Kaur, *Physicochemical and pharmacokinetic evaluation of rosuvastatin loaded nanostructured lipid carriers: Influence of long-and medium-chain fatty acid mixture.* Journal of Pharmaceutical Investigation, 2018. **48**(4): p. 465-476.

64. Klančnik, A., et al., *Anti-adhesion activity of phytochemicals to prevent Campylobacter jejuni biofilm formation on abiotic surfaces.* Phytochemistry Reviews, 2021. **20**(1): p. 55-84.

65. Ribeiro, L.N.d.M., et al., *Nanocarriers from natural lipids with in vitro activity against Campylobacter jejuni*. Frontiers in Cellular and Infection Microbiology, 2021. **10**: p. 827.

66. Bashiri, S., et al., *Preparation and characterization of chitosan-coated nanostructured lipid carriers (CH-NLC) containing cinnamon essential oil for enriching milk and anti-oxidant activity.* LWT, 2020. **119**: p. 108836.

67. Shahraeini, S.S., et al., *Atorvastatin Solid Lipid Nanoparticles as a Promising Approach for Dermal Delivery and an Anti-inflammatory Agent.* AAPS PharmSciTech, 2020. **21**(7): p. 1-10.

68. Scano, A., et al., *Microemulsions: the Renaissance of ferrite nanoparticle synthesis.* Journal of nanoscience and nanotechnology, 2019. **19**(8): p. 4824-4838.

69. Lopez-Quintela, M.A., *Synthesis of nanomaterials in microemulsions: formation mechanisms and growth control.* Current opinion in colloid & interface science, 2003. **8**(2): p. 137-144.

70. Mohammadi, M., E. Assadpour, and S.M. Jafari, *Encapsulation of food ingredients by nanostructured lipid carriers (NLCs)*, in *Lipid-based nanostructures for food encapsulation purposes*. 2019, Elsevier. p. 217-270.

71. Elmowafy, M., et al., *Atorvastatin-loaded nanostructured lipid carriers (NLCs): strategy to overcome oral delivery drawbacks.* Drug delivery, 2017. **24**(1): p. 932-941.

72. Said, A.R., et al., *Nanostructured lipid carriers for enhanced in vitro and in vivo schistosomicidal activity of praziquantel: effect of charge.* Drug Development and Industrial Pharmacy, 2021. **47**(4): p. 663-672.

73. Fang, C.-L., S. A Al-Suwayeh, and J.-Y. Fang, *Nanostructured lipid carriers (NLCs) for drug delivery and targeting.* Recent patents on nanotechnology, 2013. **7**(1): p. 41-55.

74. Suksaeree, J., et al. *Stability Test of Nanostructured Lipid Carriers-Loaded Mefenamic Acid prepared by Microemulsion Technique*. in *IOP Conference Series: Materials Science and Engineering*. 2020. IOP Publishing.

75. Ramli, S., et al., *The influence of surfactant/co-surfactant hydrophilic-lipophilic balance on the formation of limonene-based microemulsion as Vitamin C carrier.* Sains Malaysiana, 2019. **48**(5): p. 1035-1042.

76. Al-Sarraf, M.A., A.A. Hussein, and K.K. Al-Kinani, *Formulation, Characterization, and Optimization of Zaltoprofen Nanostructured Lipid Carriers (NLCs).*

77. Mangla, B., et al., *Lipid-nanopotentiated combinatorial delivery of tamoxifen and sulforaphane: ex vivo, in vivo and toxicity studies.* Nanomedicine, 2020. **15**(26): p. 2563-2583.

78. Sahib, A.S., et al., *Preparation and In-vitro Hematological Evaluation of Chlorambucil Loaded Lipid Nanoparticles.*

79. Al-Ali, A.A.A., et al., *Nonionic surfactants modulate the transport activity of ATP-binding cassette (ABC) transporters and solute carriers (SLC): Relevance to oral drug absorption.* International journal of pharmaceutics, 2019. **566**: p. 410-433.

80. Yaghoobian, M., et al., *The impact of surfactant composition and surface charge of niosomes on the oral absorption of repaglinide as a BCS II model drug.* International Journal of Nanomedicine, 2020. **15**: p. 8767.

81. Lo, Y.-l., *Relationships between the hydrophilic–lipophilic balance values of pharmaceutical excipients and their multidrug resistance modulating effect in Caco-2 cells and rat intestines.* Journal of Controlled Release, 2003. **90**(1): p. 37-48.

82. Souto, E.B., et al., *Key production parameters for the development of solid lipid nanoparticles by high shear homogenization.* Pharmaceutical development and technology, 2019. **24**(9): p. 1181-1185.

83. Zardini, A.A., et al., *Production and characterization of nanostructured lipid carriers and solid lipid nanoparticles containing lycopene for food fortification.* Journal of food science and technology, 2018. **55**(1): p. 287-298.

84. Ajiboye, A.L., et al., *Olanzapine Loaded Nanostructured Lipid Carriers via High Shear Homogenization and Ultrasonication.* Scientia Pharmaceutica, 2021. **89**(2): p. 25.

85. Jannin, V., et al., *Evaluation of the digestibility of solid lipid nanoparticles of glyceryl dibehenate produced by two techniques: Ultrasonication and spray-flash evaporation.* European Journal of Pharmaceutical Sciences, 2018. **111**: p. 91-95.

86. Pereira, R.R., et al., *Ucuùba Fat Characterization and Use to Obtain Lipid Nanoparticles by High‐Pressure Homogenization with Full Factorial Design.* Chemical Engineering & Technology, 2021. **44**(6): p. 1009-1016.

87. Ana, R., et al., *Rethinking carbamazepine oral delivery using polymer-lipid hybrid nanoparticles.* International journal of pharmaceutics, 2019. **554**: p. 352-365.

88. Hanifiyah, I.A., N. Rosita, and T. Purwanti, *Production Method of Nanostructured Lipid Carrier (NLC): Hot and Cold Homogenization Against NLC-Coenzyme Q10 Characteristics.* Journal of Computational and Theoretical Nanoscience, 2021. **18**(1-2): p. 26-31.

89. Mehnert, W. and K. Mäder, *Solid Lipid Nanoparticles: Production, Characterization and Applications. Ad-vanced Drug Delivery Reviews, 47, 165-196*. 2001.

90. Joshi, M.D., R.H. Prabhu, and V.B. Patravale, *Fabrication of nanostructured lipid carriers (NLC)-based gels from microemulsion template for delivery through skin*, in *Pharmaceutical Nanotechnology*. 2019, Springer. p. 279-292.

91. Mazur, K.L., et al., *Diethyldithiocarbamate loaded in beeswax-copaiba oil nanoparticles obtained by solventless double emulsion technique promote promastigote death in vitro.* Colloids and Surfaces B: Biointerfaces, 2019. **176**: p. 507-512.

92. Heurtault, B., et al., *A novel phase inversion-based process for the preparation of lipid nanocarriers.* Pharmaceutical research, 2002. **19**(6): p. 875-880.

93. Roger, K., B. Cabane, and U. Olsson, *Formation of 10− 100 nm size-controlled emulsions through a sub-PIT cycle.* Langmuir, 2010. **26**(6): p. 3860-3867.

94. Izquierdo, P., et al., *Formation and stability of nano-emulsions prepared using the phase inversion temperature method.* Langmuir, 2002. **18**(1): p. 26-30.

95. Gomes, G., et al., *β-carotene and α-tocopherol coencapsulated in nanostructured lipid carriers of murumuru (Astrocaryum murumuru) butter produced by phase inversion temperature method: characterisation, dynamic in vitro digestion and cell viability study.* Journal of microencapsulation, 2019. **36**(1): p. 43-52.

96. D'oria, C., et al., *Preparation of solid lipid particles by membrane emulsification—Influence of process parameters.* Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2009. **338**(1-3): p. 114-118.

97. Charcosset, C., A. El-Harati, and H. Fessi, *Preparation of solid lipid nanoparticles using a membrane contactor.* Journal of controlled release, 2005. **108**(1): p. 112-120.

98. Khan, S., et al., *Tacrolimus-loaded nanostructured lipid carriers for oral delivery–optimization of production and characterization.* European Journal of Pharmaceutics and Biopharmaceutics, 2016. **108**: p. 277-288.

99. Trotta, M., F. Debernardi, and O. Caputo, *Preparation of solid lipid nanoparticles by a solvent emulsification–diffusion technique.* International journal of pharmaceutics, 2003. **257**(1-2): p. 153-160.

100. Emami, J., H. Yousefian, and H. Sadeghi, *Targeted nanostructured lipid carrier for brain delivery of artemisinin: design, preparation, characterization, optimization and cell toxicity.* Journal of Pharmacy & Pharmaceutical Sciences, 2018. **21**(1s): p. 225s-241s.

101. Fathi, H.A., et al., *Nanostructured lipid carriers for improved oral delivery and prolonged antihyperlipidemic effect of simvastatin.* Colloids and Surfaces B: Biointerfaces, 2018. **162**: p. 236-245.

102. Sjöström, B., et al., *Structures of nanoparticles prepared from oil-in-water emulsions.* Pharm Res, 1995. **12**(1): p. 39-48.

103. Shirazi, A.S., et al., *SN38 loaded nanostructured lipid carriers (NLCs); preparation and in vitro evaluations against glioblastoma.* Journal of Materials Science: Materials in Medicine, 2021. **32**(7): p. 1-12.

104. Saghafi, Z., et al., *Preparation, characterization, and in vivo evaluation of perphenazine-loaded nanostructured lipid carriers for oral bioavailability improvement.* Drug Development and Industrial Pharmacy, 2021. **47**(3): p. 509-520.

105. Poonia, N., et al., *Resveratrol loaded functionalized nanostructured lipid carriers for breast cancer targeting: Systematic development, characterization and pharmacokinetic evaluation.* Colloids and Surfaces B: Biointerfaces, 2019. **181**: p. 756-766.

106. Schubert, M. and C. Müller-Goymann, *Solvent injection as a new approach for manufacturing lipid nanoparticles–evaluation of the method and process parameters.* European journal of pharmaceutics and biopharmaceutics, 2003. **55**(1): p. 125-131.

107. Schubert, M.A. and C.C. Müller-Goymann, *Solvent injection as a new approach for manufacturing lipid nanoparticles – evaluation of the method and process parameters.* European Journal of Pharmaceutics and Biopharmaceutics, 2003. **55**(1): p. 125-131.

108. Duong, V.-A., T.-T.-L. Nguyen, and H.-J. Maeng, *Preparation of solid lipid nanoparticles and nanostructured lipid carriers for drug delivery and the effects of preparation parameters of solvent injection method.* Molecules, 2020. **25**(20): p. 4781.

109. Phan, T.N.Q., I. Shahzadi, and A. Bernkop-Schnürch, *Hydrophobic ion-pairs and lipid-based nanocarrier systems: The perfect match for delivery of BCS class 3 drugs.* Journal of Controlled Release, 2019. **304**: p. 146-155.

110. Ristroph, K.D. and R.K. Prud'homme, *Hydrophobic ion pairing: encapsulating small molecules, peptides, and proteins into nanocarriers.* Nanoscale Advances, 2019. **1**(11): p. 4207-4237.

111. Silva, E.L., et al., *Nanostructured lipid carriers loaded with tributyrin as an alternative to improve anticancer activity of all-trans retinoic acid.* Expert review of anticancer therapy, 2015. **15**(2): p. 247-256.

112. Zhao, S., et al., *Doxorubicin hydrochloride-oleic acid conjugate loaded nanostructured lipid carriers for tumor specific drug release.* Colloids and Surfaces B: Biointerfaces, 2016. **145**: p. 95-103.

113. Muntoni, E., et al., *Lipid nanoparticles as vehicles for oral delivery of insulin and insulin analogs: preliminary ex vivo and in vivo studies.* Acta diabetologica, 2019. **56**(12): p. 1283-1292.

114. Jawahar, N., et al., *Enhanced oral bioavailability of an antipsychotic drug through nanostructured lipid carriers.* International journal of biological macromolecules, 2018. **110**: p. 269-275.

115. Ling, J.T.S., C.J. Roberts, and N. Billa, *Antifungal and mucoadhesive properties of an orally administered chitosan-coated amphotericin B nanostructured lipid carrier (NLC).* AAPS PharmSciTech, 2019. **20**(3): p. 1-11.

116. Gao, X., et al., *Chitosan modified squalene nanostructured lipid carriers as a promising adjuvant for freeze-dried ovalbumin vaccine.* International Journal of Biological Macromolecules, 2021. **188**: p. 855-862.

117. Senna, J.P., et al., *Dual alginate-lipid nanocarriers as oral delivery systems for amphotericin B.* Colloids and Surfaces B: Biointerfaces, 2018. **166**: p. 187-194.

118. Khan, A.A., et al., *Lyophilized hybrid nanostructured lipid carriers to enhance the cellular uptake of verapamil: Statistical optimization and in vitro evaluation.* Nanoscale research letters, 2018. **13**(1): p. 1-16.

119. Nahak, P., et al., *Orcinol glucoside loaded polymer-lipid hybrid nanostructured lipid carriers: Potential cytotoxic agents against gastric, colon and hepatoma carcinoma cell lines.* Pharmaceutical research, 2018. **35**(10): p. 1-10.

120. Zhang, L., et al., *Folate-modified lipid–polymer hybrid nanoparticles for targeted paclitaxel delivery.* International journal of nanomedicine, 2015. **10**: p. 2101.

121. Yin, J., et al., *Cholate-modified polymer-lipid hybrid nanoparticles for oral delivery of quercetin to potentiate the antileukemic effect.* International journal of nanomedicine, 2019. **14**: p. 4045.

122. Joyce, P., et al., *Polymer lipid hybrid (PLH) formulations: a synergistic approach to oral delivery of challenging therapeutics*, in *Delivery of Drugs*. 2020, Elsevier. p. 1-27.

123. Negi, L.M., et al., *Hyaluronic acid decorated lipid nanocarrier for MDR modulation and CD-44 targeting in colon adenocarcinoma.* International journal of biological macromolecules, 2015. **72**: p. 569-574.

124. Qu, C.-Y., et al., *Engineering of lipid prodrug-based, hyaluronic acid-decorated nanostructured lipid carriers platform for 5-fluorouracil and cisplatin combination gastric cancer therapy.* International journal of Nanomedicine, 2015. **10**: p. 3911.

125. Joshy, K., et al., *Gelatin modified lipid nanoparticles for anti-viral drug delivery.* Chemistry and physics of lipids, 2017. **207**: p. 24-37.

126. Moraes, S., et al., *Targeted nanostructured lipid carriers for doxorubicin oral delivery.* International Journal of Pharmaceutics, 2021. **592**: p. 120029.

127. Kim, C.H., et al., *pH-sensitive PEGylation of RIPL peptide-conjugated nanostructured lipid carriers: Design and in vitro evaluation.* International journal of nanomedicine, 2018. **13**: p. 6661.

128. Shah, R., et al., *Lipid nanoparticles: Production, characterization and stability*. 2015: Springer.

129. Witayaudom, P. and U. Klinkesorn, *Effect of surfactant concentration and solidification temperature on the characteristics and stability of nanostructured lipid carrier (NLC) prepared from rambutan (Nephelium lappaceum L.) kernel fat.* J Colloid Interface Sci, 2017. **505**: p. 1082-1092.

130. Houacine, C., D. Adams, and K.K. Singh, *Impact of liquid lipid on development and stability of trimyristin nanostructured lipid carriers for oral delivery of resveratrol.* Journal of Molecular Liquids, 2020. **316**: p. 113734.

131. Salminen, H., et al., *Influence of spray drying on the stability of food-grade solid lipid nanoparticles.* Food Research International, 2019. **119**: p. 741-750.

132. Mozaffar, S., et al., *A new approach for drying of nanostructured lipid carriers (NLC) by spray-drying and using sodium chloride as the excipient.* Journal of Drug Delivery Science and Technology, 2021. **61**: p. 102212.

133. Santiago, R.R., et al., *Nanostructured lipid carriers containing Amphotericin B: Development, in vitro release assay, and storage stability.* Journal of Drug Delivery Science and Technology, 2018. **48**: p. 372-382.

134. Rouco, H., et al., *A Traffic Light System to Maximize Carbohydrate Cryoprotectants' Effectivity in Nanostructured Lipid Carriers' Lyophilization.* Pharmaceutics, 2021. **13**(9): p. 1330.

135. Khan, A.A., et al., *Freeze-dried lopinavir-loaded nanostructured lipid carriers for enhanced cellular uptake and bioavailability: statistical optimization, in vitro and in vivo evaluations.* Pharmaceutics, 2019. **11**(2): p. 97.

136. Borderwala, K., et al., *Eudragit S-100 Surface Engineered Nanostructured Lipid Carriers for Colon Targeting of 5-Fluorouracil: Optimization and In Vitro and In Vivo Characterization.* AAPS PharmSciTech, 2021. **22**(6): p. 1-15.

137. Sinhmar, G.K., et al., *Process, optimization, and characterization of budesonide-loaded nanostructured lipid carriers for the treatment of inflammatory bowel disease.* Drug development and industrial pharmacy, 2018. **44**(7): p. 1078-1089.

138. Fang, G., et al., *Cysteine-functionalized nanostructured lipid carriers for oral delivery of docetaxel: a permeability and pharmacokinetic study.* Molecular pharmaceutics, 2015. **12**(7): p. 2384-2395.

139. Bardaa, S., et al., *Oil from pumpkin (Cucurbita pepo L.) seeds: evaluation of its functional properties on wound healing in rats.* Lipids in health and disease, 2016. **15**(1): p. 1-12.

140. Ahmed, O.A.A., et al., *Pumpkin Oil-Based Nanostructured Lipid Carrier System for Antiulcer Effect in NSAID-Induced Gastric Ulcer Model in Rats.* International journal of nanomedicine, 2020. **15**: p. 2529-2539.

141. Fahmy, U.A., et al., *Optimization of thymoquinone-loaded coconut oil nanostructured lipid carriers for the management of ethanol-induced ulcer.* AAPS PharmSciTech, 2020. **21**: p. 1-10.

142. Mishra, R.K., et al., *Lipid-based nanocarrier-mediated targeted delivery of celecoxib attenuate severity of ulcerative colitis.* Materials Science and Engineering: C, 2020. **116**: p. 111103.

143. Huguet-Casquero, A., et al., *Oral delivery of oleuropein-loaded lipid nanocarriers alleviates inflammation and oxidative stress in acute colitis.* International Journal of Pharmaceutics, 2020. **586**: p. 119515.

144. Beloqui, A., et al., *Budesonide-loaded nanostructured lipid carriers reduce inflammation in murine DSS-induced colitis.* International journal of pharmaceutics, 2013. **454**(2): p. 775-783.

145. Beloqui, A., et al., *A comparative study of curcumin-loaded lipid-based nanocarriers in the treatment of inflammatory bowel disease.* Colloids and Surfaces B: Biointerfaces, 2016. **143**: p. 327-335.

146. Yang, G., et al., *Formulation design, characterization, and in vitro and in vivo evaluation of nanostructured lipid carriers containing a bile salt for oral delivery of gypenosides.* International journal of nanomedicine, 2019. **14**: p. 2267.

147. Teng, Z., et al., *Preparation and characterization of nimodipine-loaded nanostructured lipid systems for enhanced solubility and bioavailability.* International journal of nanomedicine, 2019. **14**: p. 119.

148. Wang, Q., et al., *Nanostructured lipid carriers as a delivery system of biochanin A.* Drug delivery, 2013. **20**(8): p. 331-337.

149. Anwar, W., et al., *Enhancing the oral bioavailability of candesartan cilexetil loaded nanostructured lipid carriers: In vitro characterization and absorption in rats after oral administration.* Pharmaceutics, 2020. **12**(11): p. 1047.

150. Arpagaus, C., et al., *Nano spray drying for encapsulation of pharmaceuticals.* International journal of pharmaceutics, 2018. **546**(1-2): p. 194-214.

151. Dong, Z., S. Iqbal, and Z. Zhao, *Preparation of ergosterol-loaded nanostructured lipid carriers for enhancing oral bioavailability and antidiabetic nephropathy effects.* AAPS PharmSciTech, 2020. **21**(2): p. 1-11.

152. Abd El-Halim, S.M., et al., *Stabilized oral nanostructured lipid carriers of Adefovir Dipivoxil as a potential liver targeting: Estimation of liver function panel and uptake following intravenous injection of radioiodinated indicator.* DARU Journal of Pharmaceutical Sciences, 2020. **28**(2): p. 517-532.

153. Hu, R., et al., *Formulation and intestinal absorption of naringenin loaded nanostructured lipid carrier and its inhibitory effects on nonalcoholic fatty liver disease.* Nanomedicine: Nanotechnology, Biology and Medicine, 2021. **32**: p. 102310.

154. Khan, S.A., et al., *Boosting the brain delivery of Atazanavir through nanostructured lipid carrier-based approach for mitigating neuroaids.* Pharmaceutics, 2020. **12**(11): p. 1059.

155. E Eleraky, N., et al., *Nanostructured lipid carriers to mediate brain delivery of temazepam: Design and in vivo study.* Pharmaceutics, 2020. **12**(5): p. 451.

156. Garg, B., et al., *Nanostructured lipidic carriers of lopinavir for effective management of HIV-associated neurocognitive disorder.* Journal of Drug Delivery Science and Technology, 2019. **53**: p. 101220.

157. Sun, W., et al., *Specific role of polysorbate 80 coating on the targeting of nanoparticles to the brain.* Biomaterials, 2004. **25**(15): p. 3065-71.

158. Dudhipala, N. and T. Gorre, *Neuroprotective effect of ropinirole lipid nanoparticles enriched hydrogel for parkinson's disease: In vitro, ex vivo, pharmacokinetic and pharmacodynamic evaluation.* Pharmaceutics, 2020. **12**(5): p. 448.

159. Martins, I., M. Marques, and A. Antunes, *Protein adducts from carbamazepine-10, 11-epoxide, the major metabolite of the antiepileptic drug carbamazepine: possible biomarkers of toxicity.* Toxicology Letters, 2015. **2**(238): p. S359.

160. Rahman, H.S., et al., *Antileukemic effect of zerumbone-loaded nanostructured lipid carrier in WEHI-3B cell-induced murine leukemia model.* International journal of nanomedicine, 2015. **10**: p. 1649.

161. Almukadi, H., et al., *Auraptene nanoparticles ameliorate testosterone-induced benign prostatic hyperplasia in rats: Emphasis on antioxidant, anti-inflammatory, proapoptotic and PPARs activation effects.* Biomedicine & Pharmacotherapy, 2021. **143**: p. 112199.

162. Nordin, N., et al., *Antitumor and anti-metastatic effects of citral-loaded nanostructured lipid carrier in 4T1-induced breast cancer mouse model.* Molecules, 2020. **25**(11): p. 2670.

163. Soni, N.K., et al., *Nanostructured lipid carrier potentiated oral delivery of raloxifene for breast cancer treatment.* Nanotechnology, 2020. **31**(47): p. 475101.

164. Prabhu, P., et al., *Artemether□ lumefantrine nanostructured lipid carriers for oral malaria therapy: Enhanced efficacy at reduced dose and dosing frequency.* International journal of pharmaceutics, 2016. **511**(1): p. 473-487.

165. Shevalkar, G. and P. Vavia, *Solidified nanostructured lipid carrier (S-NLC) for enhancing the oral bioavailability of ezetimibe.* Journal of Drug Delivery Science and Technology, 2019. **53**: p. 101211.

166. Soleimanian, Y., et al., *β-Sitosterol Loaded Nanostructured Lipid Carrier: Physical and Oxidative Stability, In Vitro Simulated Digestion and Hypocholesterolemic Activity.* Pharmaceutics, 2020. **12**(4): p. 386.

167. Beg, S., et al., *Nanocolloidal lipidic carriers of olmesartan medoxomil surface-tailored with Concavalin-A for lectin receptor targeting.* Nanomedicine, 2018. **13**(24): p. 3107-3128.

168. Jafari, S.M., *An overview of nanoencapsulation techniques and their classification.* Nanoencapsulation technologies for the food and nutraceutical industries, 2017: p. 1-34.

169. Laura, A., et al., *Phenolic compounds*, in *Postharvest physiology and biochemistry of fruits and vegetables*. 2019, Elsevier. p. 253-271.

170. Huang, J., et al., *Nanostructured lipid carrier (NLC) as a strategy for encapsulation of quercetin and linseed oil: Preparation and in vitro characterization studies.* Journal of Food Engineering, 2017. **215**: p. 1-12.

171. Shahparast, Y., et al., *Preparation, physicochemical characterization and oxidative stability of omega-3 fish oil/α-tocopherol-co-loaded nanostructured lipidic carriers.* Advanced pharmaceutical bulletin, 2019. **9**(3): p. 393.

172. Craik, D.J., et al., *The future of peptide- based drugs.* Chemical biology & drug design, 2013. **81**(1): p. 136-147.

173. Moroz, E., S. Matoori, and J.-C. Leroux, *Oral delivery of macromolecular drugs: Where we are after almost 100 years of attempts.* Advanced drug delivery reviews, 2016. **101**: p. 108-121.

174. Majumder, J. and T. Minko, *Multifunctional Lipid-Based Nanoparticles for Codelivery of Anticancer Drugs and siRNA for Treatment of Non-Small Cell Lung Cancer with Different Level of Resistance and EGFR Mutations.* Pharmaceutics, 2021. **13**(7): p. 1063.

175. Muntoni, E., et al., *Glargine insulin loaded lipid nanoparticles: Oral delivery of liquid and solid oral dosage forms.* Nutrition, Metabolism and Cardiovascular Diseases, 2021. **31**(2): p. 691-698.

176. Gerhardt, A., et al., *A flexible, thermostable nanostructured lipid carrier platform for RNA vaccine delivery.* Molecular Therapy-Methods & Clinical Development, 2022. **25**: p. 205-214.

177. Baden, L.R., et al., *Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine.* New England Journal of Medicine, 2021. **384**(5): p. 403-416.

178. Polack, F.P., et al., *Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine.* New England Journal of Medicine, 2020.

179. Erasmus, J.H., et al., *A nanostructured lipid carrier for delivery of a replicating viral RNA provides single, low-dose protection against Zika.* Molecular Therapy, 2018. **26**(10): p. 2507-2522.

180. Bayon, E., et al., *Overcoming immunogenicity issues of HIV p24 antigen by the use of innovative nanostructured lipid carriers as delivery systems: evidences in mice and non-human primates.* NPJ vaccines, 2018. **3**(1): p. 1-14.

181. Mohammadi, G., Z.S. Koranni, and A. Jebali, *The oral vaccine based on self-replicating RNA lipid nanoparticles can simultaneously neutralize both SARS-CoV-2 variants alpha and delta.* International Immunopharmacology, 2021. **101**: p. 108231.

182. Yoozbashi, M., et al., *Magnetic nanostructured lipid carrier for dual triggered curcumin delivery: Preparation, characterization and toxicity evaluation on isolated rat liver mitochondria.* Journal of Biomaterials Applications, 2021: p. 08853282211034625.

183. Zhu, X., et al., *SPIO-loaded nanostructured lipid carriers as liver-targeted molecular T2-weighted MRI contrast agent.* Quantitative imaging in medicine and surgery, 2018. **8**(8): p. 770.

184. Jian, Y., et al., *A Gastric Cancer Peptide GX1-Modified Nano-Lipid Carriers Encapsulating Paclitaxel: Design and Evaluation of Anti-Tumor Activity.* Drug Design, Development and Therapy, 2020. **14**: p. 2355.

185. Gurumukhi, V.C. and S.B. Bari, *Quality by design (QbD)–based fabrication of atazanavir-loaded nanostructured lipid carriers for lymph targeting: bioavailability enhancement using chylomicron flow block model and toxicity studies.* Drug Delivery and Translational Research, 2021: p. 1-23.

186. Assadpour, E. and S.M. Jafari, *Advances in spray-drying encapsulation of food bioactive ingredients: From microcapsules to nanocapsules.* Annual review of food science and technology, 2019. **10**: p. 103-131.

187. Reque, P.M. and A. Brandelli, *Encapsulation of probiotics and nutraceuticals: Applications in functional food industry.* Trends in Food Science & Technology, 2021.

188. Lages, E.B., et al., *pH-sensitive doxorubicin-tocopherol succinate prodrug encapsulated in docosahexaenoic acid-based nanostructured lipid carriers: An effective strategy to improve pharmacokinetics and reduce toxic effects.* Biomedicine & Pharmacotherapy, 2021. **144**: p. 112373.

189. Falsafi, S.R., et al., *Morphology and microstructural analysis of bioactive-loaded micro/nanocarriers via microscopy techniques; CLSM/SEM/TEM/AFM.* Advances in colloid and interface science, 2020. **280**: p. 102166.

190. Zhu, Y., et al., *Nanostructured lipid carriers as oral delivery systems for improving oral bioavailability of nintedanib by promoting intestinal absorption.* International Journal of Pharmaceutics, 2020. **586**: p. 119569.

191. Zhang, Q., et al., *Nanostructured lipid carriers with exceptional gastrointestinal stability and inhibition of P-gp efflux for improved oral delivery of tilmicosin.* Colloids and Surfaces B: Biointerfaces, 2020. **187**: p. 110649.

192. Lacatusu, I., et al., *Multifaced Role of Dual Herbal Principles Loaded-Lipid Nanocarriers in Providing High Therapeutic Efficacity.* Pharmaceutics, 2021. **13**(9): p. 1511.

193. Sharaf, M., et al., *Co-delivery of hesperidin and clarithromycin in a nanostructured lipid carrier for the eradication of Helicobacter pylori in vitro.* Bioorganic Chemistry, 2021. **112**: p. 104896.

194. Haddadzadegan, S., F. Dorkoosh, and A. Bernkop-Schnurch, *Oral delivery of therapeutic peptides and proteins: Technology landscape of lipid-based nanocarriers.* Advanced Drug Delivery Reviews, 2022: p. 114097.

195. Makeen, H.A., et al., *Preparation, Characterization, and Anti-Cancer Activity of Nanostructured Lipid Carriers Containing Imatinib.* Pharmaceutics, 2021. **13**(7): p. 1086.

196. Elmowafy, M., et al., *Multifunctional carbamazepine loaded nanostructured lipid carrier (NLC) formulation.* International journal of pharmaceutics, 2018. **550**(1-2): p. 359-371.

197. Chakraborty, S., et al., *Inhibitory effect of a new orally active cedrol-loaded nanostructured lipid carrier on compound 48/80-induced mast cell degranulation and anaphylactic shock in mice.* International journal of nanomedicine, 2017. **12**: p. 4849.

List of tables

List of figures

Table 1. List of excipients of NLCs

Ingredients	Examples	References
Solid lipid	Triglycerides and partial glycerides (e.g tributyrin, Compritol) Fatty acids (e.g. stearic acid, Lauric acid) Fatty alcohols (e.g.cetyl alcohol, cerostearyl alcohol) Waxes (e.g. beewax, carnauba wax,) Butters (e.g. ucuuba butter, cacao butter, murumuru butter) Sterols (e.g Cholesterol)	[65], [50, 195],[63],[62],[196], [66], [95]
Liquid lipid	Triglycerides (e.g. Miglyol®812) Fatty acids (e.g. oleic acid) Essential oils (e.g. Oblibanum) Vegetable oils (e.g. sweet almond oil, pumpkin seed oil, coconut oil, sesame oil)	
Surfactant	Neutral (e.g. lecithin) Ionic (e.g. sodium dodecyl sulfate, sodium oleate, and sodium taurodeoxycholate) Non-ionic (e.g. Poloxamers, Polysorbates Brij78, Tego care 450, and Solutol HS15, PEG-25 stearate)	
Co-surfactant	Butanol, glycerol, propylene glycol, low molecular weight polyethylene glycol, Transcutol, and soy lecithin	[75],[74],[76], [78]

Table 2. Oral NLCs for local treatment at gastrointestinal tract

Cargos	Surface Modifier	Specific site	Testing model	Purpose of study	References
5-Fluorouracil (5-FL)	Eudragit S-100	Colon	In vitro release In vivo (Albino Wistar rats)	Control release only at colonic medium	[136]
Pumpkin Oil (PSO)	-	Stomach	In vivo (male Wistar rats)	Protecting gastric mucosa from ulcer lesion related to NSAID-induced factor	[140]
Thymoquinone (TMQ)	-	Stomach	In vivo (Male Sprague–Dawley rats)	Protecting gastric mucosa from ulcer lesion related to ethanol-induced factor	[141]
Celecoxib (CLC)	Eudragit S-100	Colon		Anti-ulcer activity related to DSS-induced colonic ulcer	[142]
Oleuropein (OLE)	-	Colon		Alleviation in inflammation and oxidative stress in acute colitis	[143]
Budesonide [177]	-	Colon	In vitro (J774 murine macrophages) in vivo (C57BL/6 female mice)	Prolonging time retention at mucus, enhancing permeation and inflammation cytokines suppression	[144]
Curcumin (CUR)	-	Colon	In vitro (J774 murine macrophages and Caco-2 cells) In vivo (C57BL/6 female mice)	Inhibition on expression of inflammation reaction	[145]
Budesonide	Eudragit S-100	Colon	In vitro (Eudragit E100 coated pellet release in simulated GIT pH condition)	Prevent premature release in upper part (in vitro simulation)	[137]
Docetaxel	Cy-PEG-MSA (amphiphilic thiolated polymer)	Intestine	In vitro (mucus glycoprotein assay) Ex vivo permeation and bidistribution (Male Sprague-Dawley (SD) rats)	Muco-adhesion of was significantly increased and the absorption of was greatly improved in total intestinal segments	[138]

Table 3. Oral NLCs for versatile drug delivery

Abbreviations (PK: pharmacokinetics, PD: pharmacodynamics, BC: Biochemical, BA: Bioavailability)

Purposes	**Disease**	**Drug and Bioactive**	**Detection methods**	**Research outcomes**	**Sources**
Bioavailability improvement		Gypenosides (GPS) + bile salt	PK model-independent method fluorescence images *in situ* intestinal perfusion	In rats, PK study reveal GPS-NLC increase 8.5 folds BA as much as GPS powder *In vivo* imaging and *in situ* perfusion indicate GPS-NLCs provide better GPS absorption through intestinal wall	[146]
		Nimodipine (NMP)	*In situ* intestinal perfusion *In vivo* PK study	*In situ* perfusion reveal primarily absorption of NMP-NLCs in intestinal Relative BA of NMP-NLCs was 160% to that of NMP suspension	[147]
		Biochanin a (BCA)	*In vivo* PK study	BCA-NLCs demonstrated greater AUC value and prolonged blood circulation better than BCA suspension	[148]
		Perphenazine (PPZ)	*In vivo* PK study	The best formulation of PPZ-NLCs improved over 3 in oral BA than plain drug suspension	[104]
		Candesartan cilexetil (CC)	*In vivo* PK study and *in situ* absorption study Caco-2 cell monolayer uptake study	The CC-NLCs show twice times BA better than CC suspension Cellular uptake of CC-NLCs indicated the enterocytes internalization	[149]
		Ergosterol [150]	*In vivo* PK study *In vitro* PD study	Relative BA of ERG-NLCs was 277.56% to that of raw ERG *In vitro* PD study specify effective inhibition ability of ERG-NLCs in suppressing high-glucose-stimulated mesangial cells over proliferation and extra-cellular matrix accumulation	[151]
Liver delivery	Infection (Hepatitis B)	Adefovir dipivoxil (AD)	*In vivo* assessment for hepatoprotective effectiveness through selective uptake of radio-iodinated rose Bengal dye of liver cell	Significant increase of dye uptake at liver confirmed the effectiveness of orally administrated AD-NLCs delivery targeting to liver. (biodistribution)	[152]
	Hepatic Infection	Praziquantel (PZQ)	*In vivo* parasitological examination and histopathological study	The proportion of granuloma in liver site was seen to be reduce to 62.1% (positive PQZ-NLCs) compared to 35.2% of PZQ suspension A considerable improvement in histopathological regarding positive PZQ-NLCs treated on *S. mansoni* infected mice	[72]
	Nonalcoholic fatty liver	Naringenin (NGN)	*In vitro* and *in vivo* transepithelial absorption model BC analysis on liver	NGN-NLCs increased solubility by 115.7 folds, transepithelial 1.2-fold, intestinal absorption by 1.3-fold and oral bioavailability by 3.7-fold compared to the pure NGN.	[153]

				Distribute well in liver than pure NGN	
Brain delivery	NeuroAIDS	Atazanavir (ATZ)	*In vitro* histopathological examination and confocal microscopic blood brain barrier assessment *In vivo* dug contained in brain estimation	More intense fluorescence dye in brain was observe in case of ATZ-NLCs rather than ATZ suspension Drug concentration with respect to ATZ-NLCs was found remained almost 10 times as much as that of ATZ suspension Biodistributed (brain/blood)	[154]
	Insomnia	Temazepam (TZP)	*In vivo* Gamma Scintigraphy Imaging and brain biodistribution study	The gamma scintigraph images provide evident that TZP-NLCs accumulated more in brain than TZP suspension Relative drug bioavailability enhanced (292.7%) and relative rate of brain uptake improved 3.4 folds in comparison with TZP suspension	[155]
	Parkinson's Disease	Ropinirole (RP)	*In vivo* PK study and biochemical studies in brain Blood serum	The oral bioavailability of RP-NLCs enhanced 2.7 times as opposed to RP suspension and there was chemical restoration in brain of mice group treated with RP-NLCs	[158]
	HIV-associated neurocognitive disorder	Lopinavir (LPV)	*In vivo* PK and brain biodistribution studies	The brain biodistribution of orally administrated LPV-NLCs manifested about 45 folds as much as that of plain LPV via oral and 2.35 folds that via parental administration.	[156]
	Epilepsy	Carbamazepine (CBZ)	*In vivo* biodistribution study	CBZ-loaded NLCs prompted an equal brain disposition as CBZ suspension at lower dose administration Pk study on retention time, distribute and C max	[87]
Common diseases treatment	Malaria	artemether/ lumefantrine (ARM/LFN)	Antimalarial efficacy studies and clinical simulation protocol	ARM/LFN-NLCs showed therapeutic efficacy at low dose (1/10) and 10 folds less frequent daily dose compared to marketed dose	[164]
	Anaphylactic shock	Cedrol (CR)	*In vivo* simulated-anaphylactic shock mice reaction after orally administrating various CR dose	The survival time increased from 28 minutes of mice pretreated CR suspension to 75 minutes of those with CR-NLCs	[197]
	Cholesterol	Ezetimibe (EZE)	*In vivo* PK and PD studies	A significant decline in total level of cholesterol when rats administrated EZE-NLCs	[165]
	Hypertension	Olmesartan medoxomil + Concavalin-A (OL +	*In vivo* PK, PD, BD and BC evaluations	Drug loaded NLCs was witnessed blood pressure reducing 37% compared to pure drug	[167]

		CoA)			
	Hypocholesterolemic	β-sitosterol	*In vivo* diet study	On rats with high cholesterol diet, a considerable decrease in total level of cholesterol and low-density lipoprotein cholesterol observed in formulated NLCs administration compared to vehicle and plain drug administration after period of 30 and 60 days	[166]
Cancer targeting	Breast	Citral (CT)	*In vivo* assessment on murine model	Citral-NLCs significantly inhibited inflammation and hampered metastasis of cell in murine model	[162]
	Leukemia (Blood)	Zerumbone (ZER)	Histopathology, transmission electron microscopy, and Tdt-mediated dUTP nick-end labeling analyses Western blotting and reverse-transcription quantitative polymerase chain reaction assays	The substantial reduction in the number of leukemia cell in spleen	[160]
	Benign prostatic hyperplasia (Prostate)	Auraptene (ART)	Histopathological examination BC and weight assessment	Auraptene-NLC protects rats against BPH produced by testosterone through anti-inflammatory, antioxidant, and proapoptotic actions, as well as PPAR family activation.	[161]
Nutraceutical absorption enhancement		Quercetin (QUC) + linseed oil	*In vitro* antioxidant activity	QUC-NLCs enhanced solubility and antioxidant activity as well as hampered the lipid oxidation than emulsion	[170]
		Omega-3 fish oil/ α-Tocopherol (O-T-NLCs)	Storage stability test Lipid oxidation products tests	O-T-NLCs revealed long term stability after 75 days and effectively prevented the primary oxidative products	[171]
Peptides and proteins delivery	Nephrotoxicity (by anticancer drug)	Glutathion (GSH)	*In vivo* study on mice with renal damage caused by cyclophosphamide	Oral GSH NLCs expressed strong antioxidant and anti-inflammation activities on kidney cells and kidney functions, compared to no effect of GSH only.	[21]
	Diabetics	Insulin	*In vitro* lipolysis, proteolysis study and cytotoxicity study on Caco-2 cells	Insulin NLCs had no chemical linkage for enzyme degradation and could be considered as no toxicity for cells.	[30]

	Diabetics	Glargine insulin	*In vivo* study in streptozotocin-induced diabetic rats	Glargine insulin was loaded in NLCs, turned into solid dosage forms (tablet, capsules). NLCs suspensions expressed slight hypoglycaemic effects by gavage feeding, while only capsules showed considerably effects of glargine insulin on diabetic rats	[175]

Figure 1

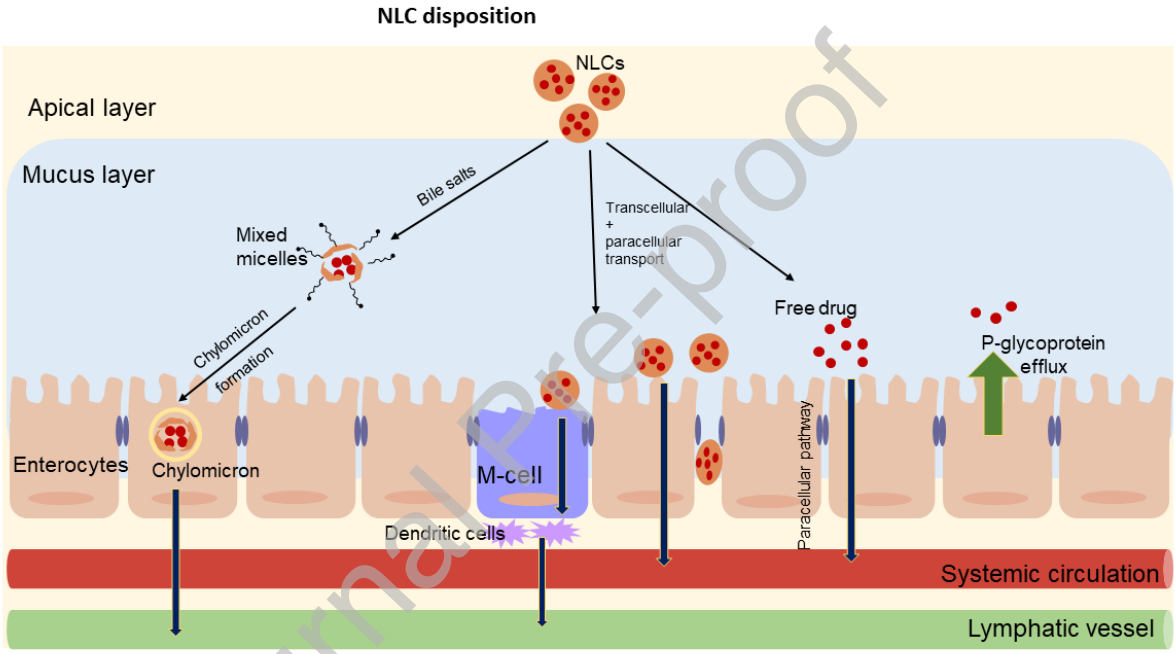


Figure 2

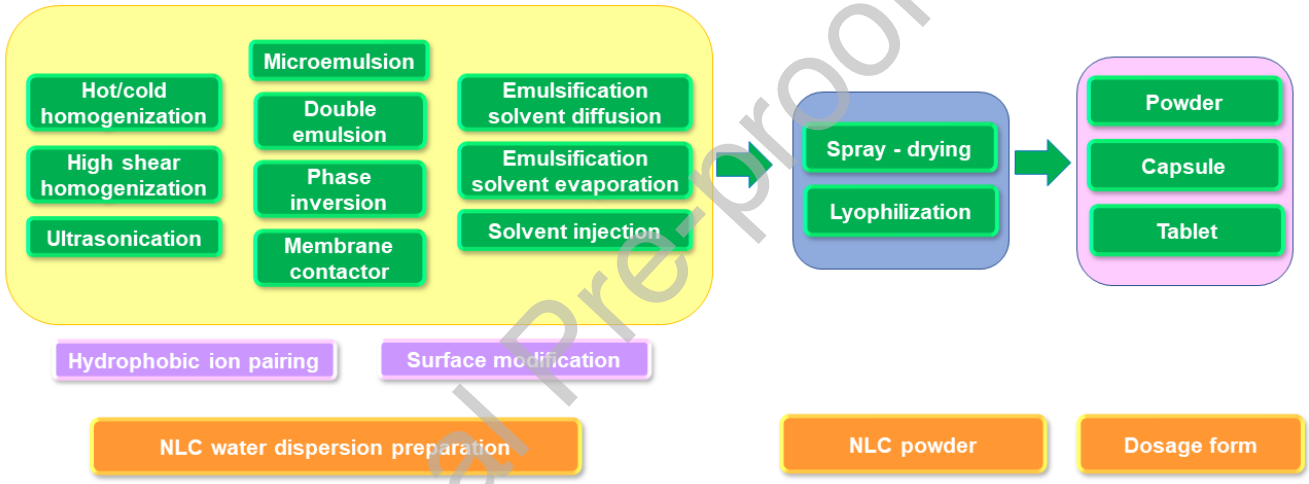


Figure 3

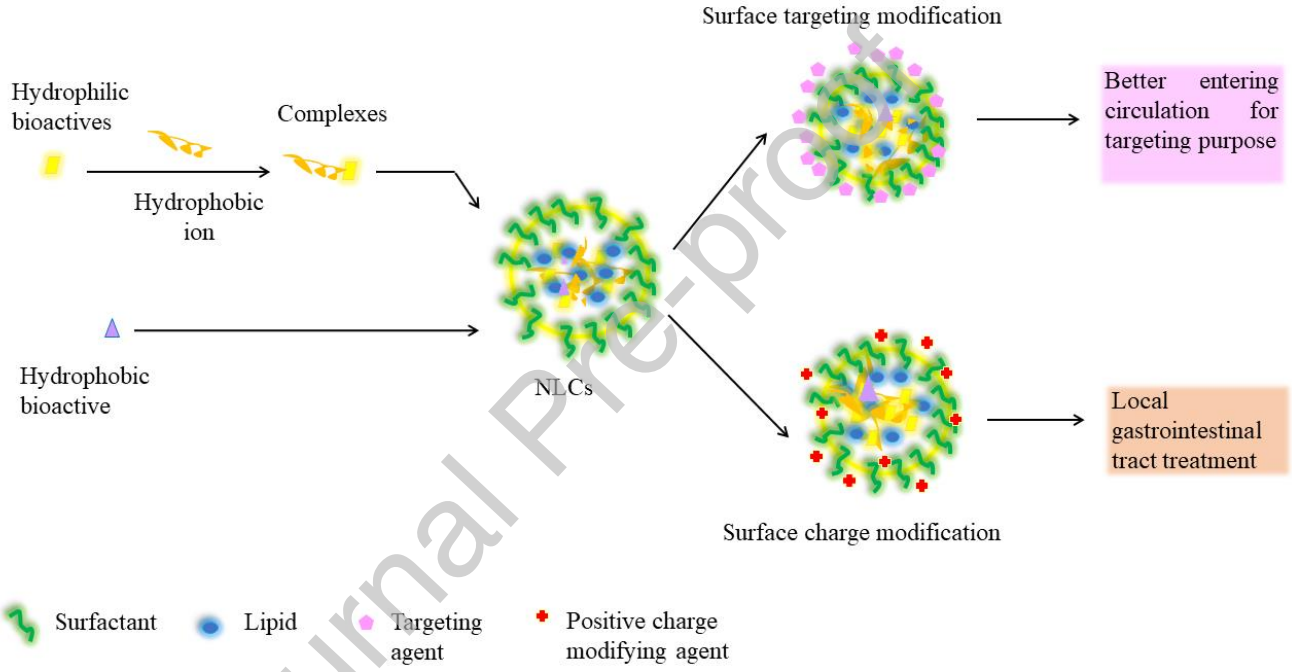
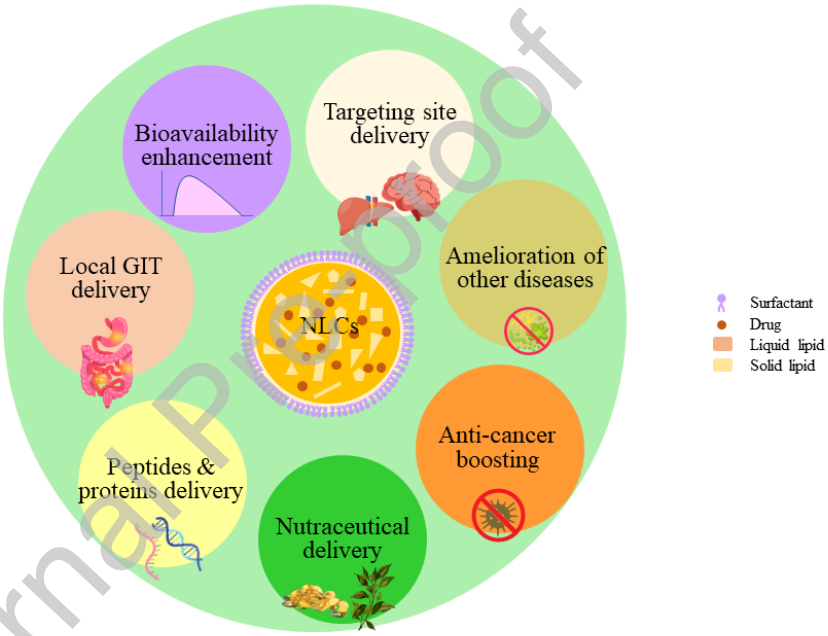


Figure 4



Graphical Abstract