REVIEW ARTICLE

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Nanostructured Lipid Carriers as Novel Drug Delivery System: A Review

Mohamed F. Zaky[1], Mohamed A. Megahed[1], Taha M. Hammady[2], Shadeed Gad[2], Mamdouh Mostafa Ghorab[2]*

[1]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Egyptian Russian University, Cairo 11829, Egypt; [2]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt.

Received on: 21. 05. 2023

Revised on: 02. 06. 2023

Accepted on: 06. 06. 2023

*Correspondence Author:

Tel: +2 01003934422

Fax : + 20643230741

E-mail address:

shaded_abdelrahman@pharm.suez.edu.eg

Abstract

Notwithstanding the physiological hurdles in the gastrointestinal tract, enteral delivery is the most practical route of drug administration. Lipid-based formulations known as nanostructured lipid carriers (NLCs) have received much research as drug-delivery nanovesicles. Due to their superior physical stability, biocompatibility, and enhanced drug-loading capacity, NLCs are preferred over other conventional lipidic nano-formulations, such as liposomes, solid lipid nanoparticles (SLNs), and nanoemulsions. NLCs are considered a promising strategy for oral bioavailability (BAV) enhancement of drugs; this could be due to the benefits of nanomaterials as well as the properties of the lipidic composition of the vesicles, which prevent enzyme degradation, mask unpleasant taste, and being favorably taken up to the lymphatic system through chylomicrons. This review particularly emphasizes their applications as oral drug delivery systems; this review discusses the most recent developments in using NLCs as nanocarriers and their composition, preparation, and characterization techniques.

Keywords: Nanostructured lipid carriers; preparation; characterization; oral drug delivery; applications.

1. Introduction

The oral route is the most preferred medication absorption choice because it is convenient for administration, making the patients more compliant, non-sophisticated to use, and the safest method of drug delivery. Additionally, it gives systemic effects via intestinal absorption, making it the preferred route of administration for common therapeutics, including antidiabetics, antihypertensives, and anticancer drugs (Yasir & Asif, 2010). However, the oral route suffers from drawbacks such as slow onset of action and low bioavailability. Also, the physiochemical properties of medications with low solubility or low permeability in the gastrointestinal tract (GIT) hinder absorption, resulting in inappropriate therapeutic action at the targeted site (Stillhart et al., 2020). Therefore, it is essential to consider the factors above to provide adequate therapeutic effects and increase the bioavailability of orally administered drug carriers. Lipid-based nanoparticles, which are classified as

liposomes, nanoemulsions, solid lipid nanoparticles (SLNs), as well as nanostructured lipid carriers (NLCs), are thought to have more advantages over other nanoparticles because they can be prepared without organic solvents and can withstand preparation conditions (Doktorovova et al., 2014; Samimi et al., 2019). The first generation of lipid nanoparticles, SLNs, was introduced in the 1900s. They are composed of water-dispersed solid lipids and stabilized using surfactants or co-surfactants, giving nanosized vesicles incorporating the drugs (Pardeike et al., 2009). Being biocompatible and easy to scale up while preserving low production cost, SLNs gained more attention than other lipid-based nanocarriers (Khosa et al., 2018; Samimi et al., 2019). Unfortunately, this carrier is limited by the crystalline nature of its solid lipids at room temperature. Also, the rearrangement of the crystalline structure upon storage leads to leakage of an incorporated drug and particle aggregation (Mehnert & Mäder, 2012; Weber et al., 2014).

Further research to address these SLNs shortcomings eventually resulted in the development of the NLCs, the next generation of lipid-based nanoparticles. NLCs share a crucial character with SLNs, a colloidal arrangement of water-dispersed lipids with the assistance of emulsifiers. NLCs differ from SLNs in that liquid lipids replace a portion of the solid lipids; thus, the resulting mixture would be in an amorphous liquid

This review discusses the novel lipid-based crystal form in the room and at physiological temperatures (Li et al., 2017). Additionally, these liquid lipids disrupt the lattice's structure and form imperfect architecture. This eventually increases the percentage of drugs loaded in NLCs compared to SLNs (H. Muller et al., 2011). Also, the formed lipid matrix will hinder solid lipids' recrystallization, making the system more stable during storage and decreasing the amount of drug extruded (S. Khan et al., 2015).nanoparticles, and nanostructured lipid carriers, focusing on their use as an oral drug delivery system. The classification, composition, and fabrication methods of NLCs are highlighted and discussed. The characterization of NLCs with focused attention to the formulation parameters effects on the developed nanocarriers is also emphasized.

Composition and Classification of NLCs

Diverse solid lipids (SL), liquid lipids (LL), and surfactants are blended in particular proportions and dispersed in aqueous solutions to form NLCs. When drug molecules are incorporated into NLCs, the selected components must be biocompatible, non-toxic, and appropriate for systemic administration (Khosa et al., 2018). Examples of LL, SL, and surfactants utilized in developing NLCs are listed in Table 1.

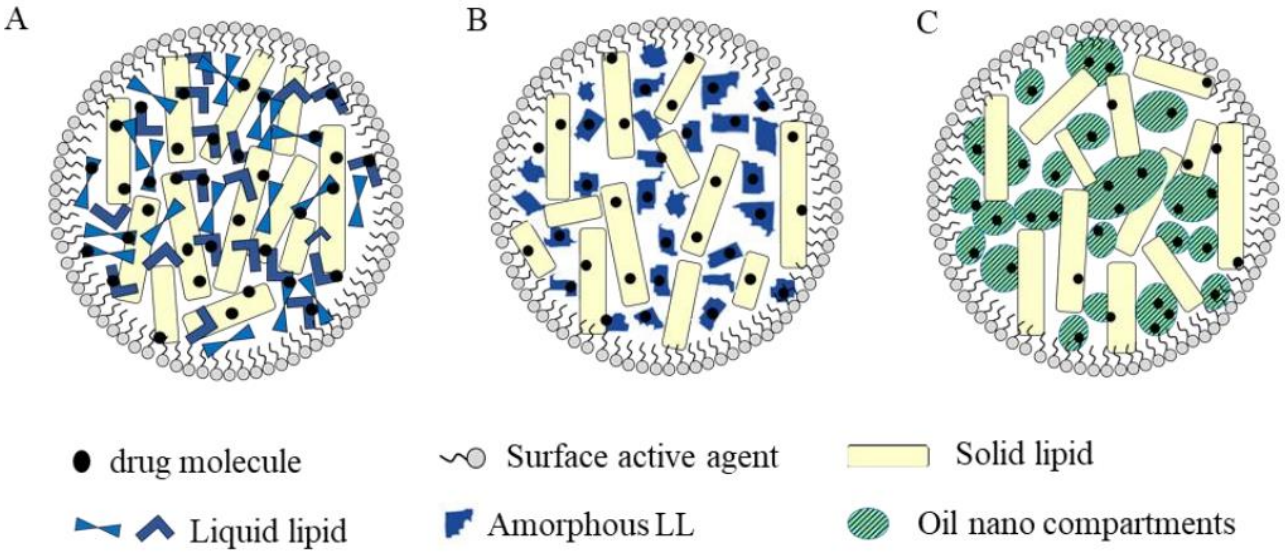

Figure 1. Different types of NLCs. (A) Imperfect; (B) amorphous; and (C) oil-in-fat-in-water (Haider et al., 2020)

Table 1. Ingredients used in the development of NLCs

Type	Name	References
Solid lipids	Glyceryl monostearate	(Ding et al., 2017)
	Glyceryl Tri-decanoate	(Sun et al., 2014)
	Glyceryl tripalmitate	(Chen et al., 2015)
	Glyceryl behenate (Compritol® ATO)	(Y. Wang et al., 2015)
	Stearic acid	(Abdolahpour et al., 2017)
	Glyceryl distearate (Precirol® ATO 5)	(Carvajal-Vidal et al., 2019)
Liquid lipids	Oleic acid	(Zaky et al., 2022)
	Alpha-tocopheryl acetate	(Sun et al., 2014)
	Squalene	(Y. P. Fang et al., 2011)
	Medium chain triglycerides (MCT)	(Sabzichi et al., 2016)
	PEG-8 caprylic (Labrasol®)	(Carvajal-Vidal et al., 2019)
	Propylene glycol dicaprylocaprate	(Cirri et al., 2012)
	Soy lecithin (Epikuron®)	(Beloqui et al., 2016)
Surfactants	Soybean phosphatidylcholine	(Sun et al., 2014)
	Hydrogenated Soybean phosphatidylcholine	(Y. P. Fang et al., 2011)
	Lecithin	(Abdolahpour et al., 2017)
	Solutol® HS 15	(Zhang et al., 2008)
	Pluronic® F-68 (Poloxamer 188)	(***Fang et al., 2012***)
	Pluronic® F127 (Poloxamer 407)	(Cirri et al., 2018)
	Tween® 80	(Cirri et al., 2018)
	Cremophor® RH40 (PEG-40 Hydrogenated Castor Oil)	(Sabzichi et al., 2016)
	Kolliphor® EL (Polyoxyl castor oil)	(Abdelbary & Haider, 2013)

According to the formulation characteristics and the nature of lipid composition, NLCs can be divided, as displayed in Figure 1, into three groups: imperfect, amorphous, and multiple structures. The imperfect NLCs are created by combining lipids made up of different fatty acids or carbon chains with different lengths and saturations, thus, resulting in imperfections in the crystal lattice and, subsequently, accommodation of lipid-soluble drugs (Müller *et al.*, 2002). Regarding the amorphous type, mixing LL with SL, which forms amorphous composition, results in a non-crystalline structureless matrix and minimizes drug expulsion. Multiple NLCs are composed of multiple LL nano-compartments enclosed in a matrix of SL in the form of oil in fat in water forms.

These LL nano-compartments increase the percent of lipophilic drug encapsulated due to the higher solubility of lipid-soluble drugs in LL than SL. Furthermore, the solid matrix surrounding the nanovesicles functions as a barrier against drug expulsion and controls drug release (Haider et al., 2020).

Methods of Preparation of NLCs

Techniques utilized in NLCs preparation could be classified into three major categories: high energy, low energy, and organic solvents approaches. Figure 2 displays the most widely used techniques for preparing NLCs, and critical aspects of selected techniques are discussed below.

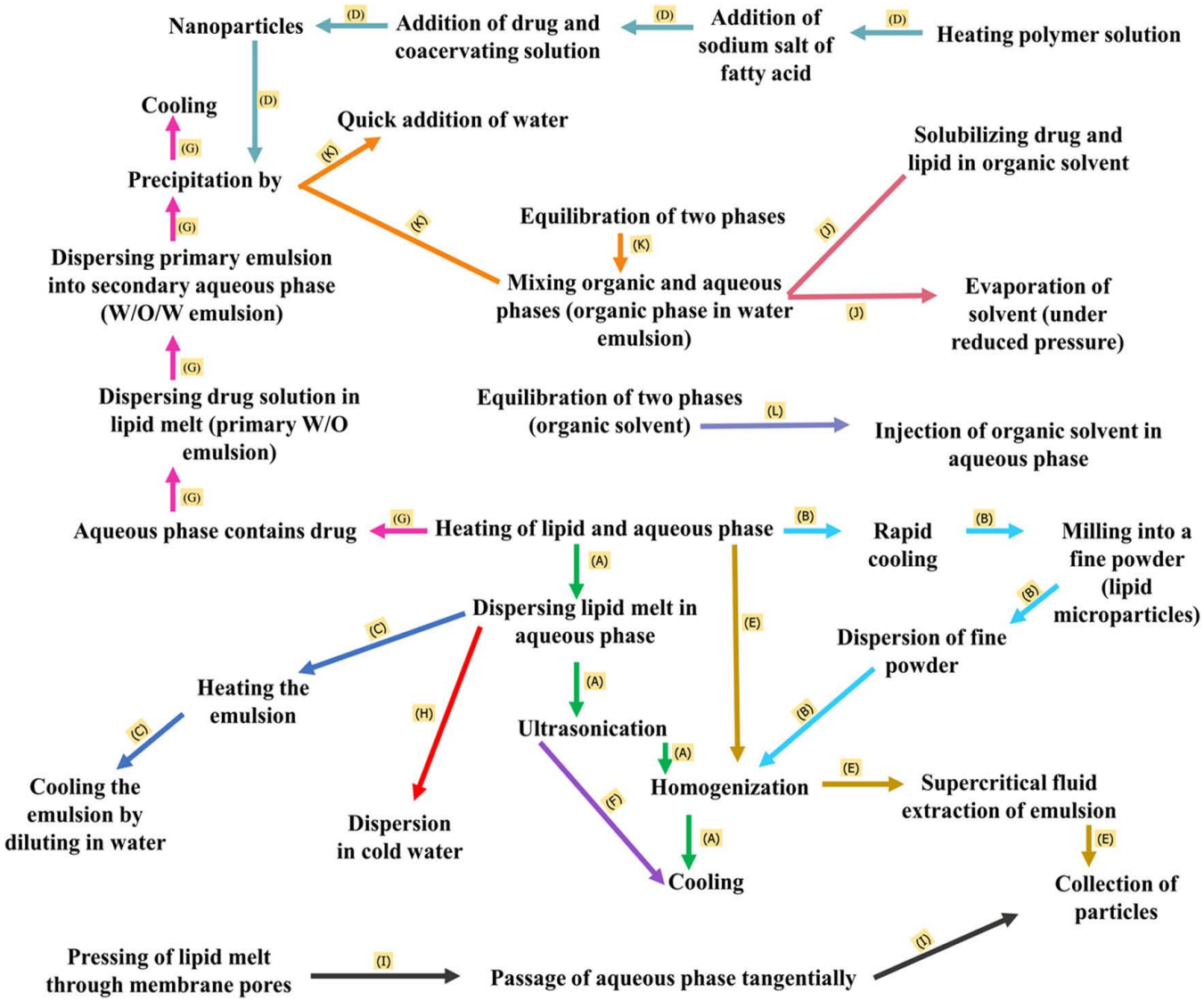

Figure 2. Methods of preparation of NLCs. (a) Hot homogenization technique, (b) cold homogenization technique, (c) phase inversion temperature (PIT) technique, (d) coacervation technique, (e) supercritical fluid (SCF) technique, (f) high shear homogenization/ultrasonication technique, (g) double emulsion technique, (h) microemulsion technique, (i) membrane contactor technique, (j) emulsification–solvent evaporation technique, (k) emulsification solvent diffusion technique, and (l) solvent injection technique (Javed et al., 2022).

High-energy approaches

High-pressure homogenization (HPH) technique

HPH method is still one of the most popular NLCs preparation methods because it requires less time to produce than other methods, scales up more quickly, and does not use solvents (Cirri et al., 2018). HPH technique is classified into both hot and cold protocols. In the heated HPH procedure, solid lipids are first melted, then combined with liquid added to the mixture to form a pre-emulsion homogenized at high temperatures, yielding NLCs. On the other hand, in cold HPH, the lipid mixture is mixed, after melting, to form a dispersion of microparticles; a cold surfactant solution is mixed with the formed dispersion to give pre-suspension. Finally, the pre-suspension is homogenized at room temperature to give NLCs (Carvajal-Vidal et al.,

lipids and drugs. Hot aqueous surfactant solution is

Melt emulsification homogenization technique.

This technique uses a probe sonication method to disperse SL, LL, and the drug into an aqueous surfactant solution. Solid NLCs are obtained when this mixture is cooled to a low temperature. This method's primary advantage is avoiding high temperatures (Javed et al., 2022).

Low-energy approaches

Microemulsion technique

A thermodynamically stable transparent oil in water microemulsion is produced by melting the lipid carrier slightly beyond its melting point, then adding the drug, surfactant, and deionized water. The produced microemulsion is immediately dispersed in ice-cold water, and a gentle stirring is applied to yield NLCs dispersion (Mendes et al., 2019). The main advantage of this method is the simplicity in production, while the drawbacks are the high amount of surfactants and co-surfactants utilized (Joshi & Patravale, 2008).

Membrane contractor technique

Little droplets are formed when a lipid is carried through a membrane's pores at a pressure stabilizing the system's temperature above the lipid's melting point. These droplets are swept along the aqueous phase when cycled through the membrane. NLC are formed when the resultant preparation cooled down to ambient temperature. Factors influencing the particle size of the produced NLCs include the aqueous phase's flow rate, the temperature of different phases, lipid phase pressure, and membrane pore size (Charcosset *et al.*, 2005).

Approaches with organic solvents

Solvent emulsification evaporation technique

Chloroform or cyclohexane, as water-immiscible organic solvents, are used to dissolve the lipid, emulsified in an aqueous surfactant phase using continuous stirring until the production of O/W emulsion. At decreasing pressure, lipid precipitation occurs as a result of solvent evaporation. The use of organic solvent is a significant drawback; however, this technique eliminates the heat stress (Vitorino et al., 2014).

Supercritical fluid technique

With this method, the medication and lipid material are dissolved in an organic solvent, like chloroform, with the help of a suitable surfactant, creating an organic solution. The aqueous phase is mixed with this organic solution, and the resultant mixture is homogenized under high pressure to create an O/W emulsion. With a constant flow rate, this emulsion is then introduced to the extraction column at one end while the supercritical fluid is injected from the other. The process is continued until the complete extraction of solvent occurs, and NLCs are produced (Li et al., 2017).

Characterization of Drug-Loaded NLCs

Particle Size and Morphology

The range of size distribution and particle size significantly impacts NLC stability. Narrow-distributed small particles are more stable, have less tendency to aggregate, and are more physically stable during storage. Additionally, particle solubility, release rate, and biocompatibility are affected by particle size. Generally, the particle size of NLCs ranges from 10 nm to 1000 nm. In order to enhance cellular uptake of chemotherapeutics-loaded NLCs, a range of 50-300 nm is usually targeted, while sizes above 300 nm are utilized for intestinal drug delivery to give sustained release (Üner, 2015).

Their shape affects the drug loading, entrapment efficiency, cellular uptake, and targeting potential of NLCs (Truong et al., 2014). Studies of the shapes of NLCs, utilizing transmission electron microscope (TEM) or scanning electron microscope (SEM), revealed that spherical NLCs have a lower surface area in comparison with anisometric particles that need higher surfactants concentration for stability (Tamjidi et al., 2013).

Surface Charge

The surface charge provides crucial information about the dispersion of NLCs, particle aggregation, and long-term stability. Zeta potential (ZP) is the parameter used to measure the surface charge of the particles. ZP depends on the ionic strength of ions that surround the NLCs, their type, and the medium's pH (Xu, 2008). Decreased particle aggregation and increased electrostatic repulsion results from greater surface charge. Generally, a ZP of ±20 mV or more is required to give stable NLCs dispersion (Gonzalez-Mira et al., 2010).

Degree of Crystallinity

The effectiveness of therapeutic drug encapsulation and the rate of release from NLCs are substantially impacted by the crystal lattice's structure and the condition of its lipid composition. Generally, the entrapment of drugs is enhanced by the defects in the NLC crystal lattice. Differential scanning calorimetry (DSC) can be applied to investigate the condition of the lipid components because different lipids have varying melting points and melting enthalpies (Sanjula et al., 2009). Storage time, amount of drug encapsulated, and the formulation viscosity significantly affect the NLCs crystallinity.

Due to improved drug entrapment and housing in the lipid matrix, employing SL with many crystal lattice defects can increase drug encapsulation and chemical stability in NLCs (Teeranachaideekul *et al.*, 2007).

Entrapment Efficiency Percentage (EE %)

It is crucial to measure the amount of drug encapsulated within the NLCs. EE% can be measured either directly by measuring the entrapped drug in the NLC dispersion or indirectly by measuring the difference between total added and un-entrapped free drugs. The rate of drug release and concentration gradient is affected by the EE%. Generally, if the entrapped drug is more than 60% of the total added drug, a successful preparation technique was utilized in developing the NLCs (Jiang et al., 2015).

Stability

Encapsulating therapeutic moieties in NLCs enhances their physical as well as chemical stability. Drug-loaded NLCs' stability is affected by storage pH and temperature. A study of quercetin-loaded NLCs revealed that a low temperature of 4 °C results in a stable system for 28 days, while a temperature of 22 °C reduces this time to only ten days. This could be because increasing temperature resulted in hydrogen bond breakage of the surfactant molecules at the lipid-water interface (Lee & Koo, 2005). Also, the ZP of particles was decreased upon storage at 22 °C for a long duration; thus, aggregation and agglomeration of the particles occurred (Chen et al., 2015).

Drug Release from NLCs

The analysis of drug molecule release from NLCs in vitro can be used to forecast how well they will perform in vivo. The dialysis method is typically used to determine the total amount of therapeutic agent released from NLCs. Briefly, NLCs loaded with the drug are placed in appropriate dialysis bags, then immersed in a phosphate buffer while shaking at 37 °C. At pre-determined time intervals, samples from the release media are withdrawn and replaced by the new buffer solution. Sink conditions are achieved by keeping a suitable temperature and constant stirring rate (Y. P. Fang et al., 2011).

Several investigations have demonstrated that a biphasic pattern of drug release from NLCs, which involves an initial burst release behavior of the loaded medication followed by a sustained release phase, is usually observed. The accumulation of the drug molecules in the outer layer of NLCs is responsible for this initial burst release. This accumulation is due to lipid crystallization which results in phase separation and subsequently causes fast drug release. On the other hand, the drug entrapped in the NLCs' core is the reason for the sustained slow-release phase, either by diffusion of the drug molecules or erosion of the lipid matrix (Fang et al., 2012).

Applications of NLCs through oral administration

During oral delivery, NLCs must maintain stability while being affected by the saliva, gastric juice, stomach movement, bowel peristalsis, mucus layer, and the small intestine's villi. Numerous studies were carried out to explore the drug absorption mechanism and drug transport to reach blood circulation through the gastrointestinal tract (GIT). As displayed in Figure 3, multiple pathways are supposed to initiate drug absorption from NLCs, including paracellular, transcellular, and lymphatic uptake (Nguyen et al., 2022). NLCs have been used successfully to enhance the efficacy of drug molecules locally in the GIT and systemically at targeting sites, including the liver, brain, cancer cells, etc.

Site-specific NLCs for gastrointestinal tract

Numerous types of research were done to investigate and characterize NLCs, whether surface-modified or not, encapsulating medicines for GIT-related inflammation. One of the physiological factors that influence the physicochemical characteristics of the active drug is the pH variation along the GIT. This factor can be utilized in developing pH-dependent controlled drug delivery systems, which means a drug release at specific pH. Eudragit S-100 is a polymer applied for delayed release polymer and colon targeting approaches, with a pH of more than 7. A potential targeted system for colon cancer treatment with 5-Fluorouracil was developed utilizing Eudragit S-100. The in-vivo pharmacokinetic (PK), study of the drug, following oral administration to mice revealed that PK parameters such as maximum plasma drug concentration (Cmax) and area under the curve (AUC) were increased by 2.54- and 11-folds, respectively, compared to drug solution. Additionally, mean residence time (MRT) and time to reach maximum concentration (Tmax) were prolonged by 4.32- and 16-fold, respectively, compared with the drug solution. These results led to the confirmation of better bioavailability as well as the delayed release of the drug in the colon (Sinhmar et al., 2018).

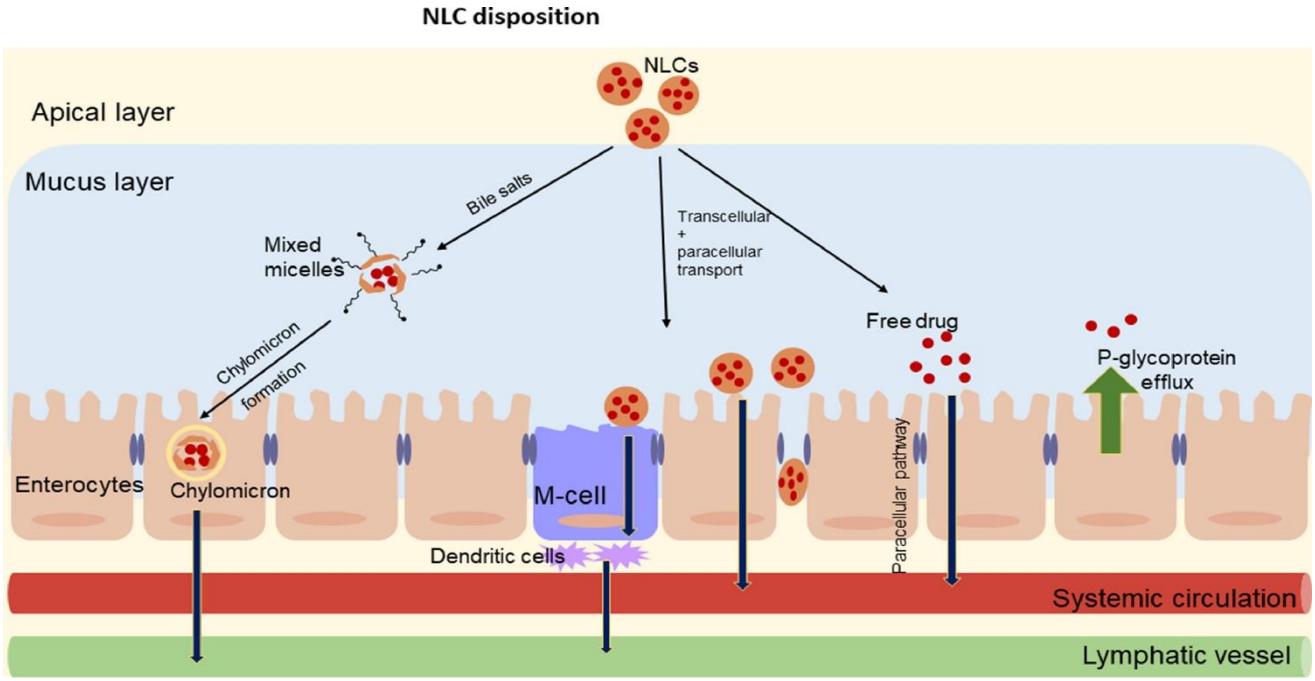

Figure 3. Absorption pathways of NLCs through the intestinal wall (Nguyen et al., 2022)

Site-specific NLCs for gastrointestinal tract

Numerous types of research were done to investigate and characterize NLCs, whether surface-modified or not, encapsulating medicines for GIT-related inflammation. One of the physiological factors that influence the physicochemical characteristics of the active drug is the pH variation along the GIT. This factor can be utilized in developing pH-dependent controlled drug delivery systems, which means a drug release at specific pH. Eudragit S-100 is a polymer applied for delayed release polymer and colon targeting approaches, with a pH of more than 7. A potential targeted system for colon cancer treatment with 5-Fluorouracil was developed utilizing Eudragit S-100. The in-vivo pharmacokinetic (PK), study of the drug, following oral administration to mice revealed that PK parameters such as maximum plasma drug concentration (Cmax) and area under the curve (AUC) were increased by 2.54- and 11-folds, respectively, compared to drug solution. Additionally, mean residence time (MRT) and time to reach maximum concentration (Tmax) were prolonged by 4.32- and 16-fold, respectively, compared with the drug solution. These results led to the confirmation of better bioavailability as well as the delayed release of the drug in the colon (Sinhmar et al., 2018).

NLCs for bioavailability enhancement through oral administration

Oral delivery of drugs is hindered by the nature of various GIT barriers and the poor physicochemical properties of the drug. NLCs are confirmed to be one of the leading drug carriers that are applied for enhanced oral bioavailability. Various poorly bioavailable drugs were successfully incorporated into NLC carriers, which enhanced their oral BAV. Accordingly, incorporating nimodipine in an NLC carrier enhanced oral absorption and BAV by 160% compared to plain drugs (Q. Wang et al., 2013). Similarly, the oral BAV of the poorly water-soluble anticoagulant drug, apixaban, was enhanced utilizing NLC technology. The AUC and Cmax of the drug-loaded NLCs were found to be increased by 8- and 2.67-folds, respectively, relative to oral apixaban suspension (Zaky et al., 2022). Furthermore, candesartan-loaded NLCs were developed by Anwar et al. and proved to be better absorbed from the intestine in which the enterocytes successfully internalized the drug-loaded NLCs, which was evidenced by Caco-2 cells uptake study (Arpagaus et al., 2018). Other researchers reported that incorporating drugs in NLCs enhanced their oral BAV and prolonged the drug release; this was apparent in a study of biochanin A- loaded NLCs (Anwar et al., 2020).

Liver-targeting NLCs through oral administration

Viruses, medications or poisons, cancer, and genetic factors are the leading causes of hepatic disorders, which are currently incurable diseases. Contrarily, the liver's metabolism occasionally interferes with drug treatment effectiveness and even sets off a worst-case scenario for the liver. In this case, therapeutic efficacy should be increased

through drug-loaded NLCs. For instance, adefovir dipivoxil NLCs, reported to have more excellent absorption by the liver, could prevent hepatic infection. It was proved that high hepatic uptake of the drug-loaded NLCs occurred compared to a control group. This indicates successful liver targeting through intestinal absorption of the nanocarrier (Hu et al., 2021).

Brain-targeting NLCs through oral administration

Drugs are typically prevented from reaching their targeted sites inside the brain due to the presence of the blood-brain barrier, which results in poor delivery to the brain of orally administered drugs. Nonetheless, proper research on NLCs suggested outcomes that overcame these obstacles. Several incorporations were developed to research brain disorders with positive outcomes for novel therapies. Examples are temazepam-loaded NLCs for the management of insomnia (Eleraky et al., 2020), atazanavir NLCs for AIDS (S. A. Khan et al., 2020), and lopinavir-loaded NLCs for the treatment of HIV-related neurocognitive disease (Garg et al., 2019). All the studies showed enhanced brain uptake of the drugs by 3.4-, 10-, and 2.8-folds for temazepam, atazanavir, and lopinavir NLC nanovesicles, respectively, compared to drug suspensions.

NLCs for cancer treatment through oral administration

Some chemotherapeutic agents were incorporated into NLC carriers with proven enhanced anticancer activity in a study by Rahman et. Al., zerumbone, an antileukemic agent, was successfully incorporated into NLCs with enhanced efficacy in the murine leukemia model (Nguyen et al., 2022). In another study, in middle-aged men, NLC vesicles loaded with auraptene was effective against benign prostatic hyperplasia, induced by testosterone (Almukadi et al., 2021).

Future perspectives

NLCs have been found to increase the therapeutic potency of various oral bioactive substances, including site-specific treatment and biodistribution rearrangement, which results in organ targeting for the liver, brain, etc. The NLCs system might be improved to include active or passive targeting and imaging (Zhu et al., 2018). Functionalizing NLCs could be applied to suppress drug resistance by targeting the site of action and by engineering imaging markers used to diagnose critical diseases (Muntoni et al., 2021). Furthermore, magnetic NLCs can be utilized for controlled drug delivery as a triggering factor. Thus, functionalizing NLC carriers could be a promising method for disease diagnosis, treatment, and *in vivo* drug distribution observation.

Conclusion

To sum up, NLCs are a promising alternative to other enteral drug delivery technologies, to sum up. NLCs have unique qualities that help them overcome the drawbacks of oral administration, including lipid nature, protection from pH and enzyme activity, enhancement of absorption from the GI tract through intestinal lymphatic transportation, and improved bioavailability and biocompatibility of drugs. Moreover, NLCs have the advantages of nanomaterials, including a flexible structure appropriate for encapsulating hydrophobic chemicals and maintaining reasonable hydrophilic compound retention via polymer blending or hydrophobic ion pairing. Various bioactive substances have been loaded into NLCs as single or multiple molecules for oral treatments. Additionally, site-specific NLCs for the primary treatment of colon and gastric diseases, and systematic delivery that increases drug bioavailability for the treatment of thrombosis, hypertension, etc., as well as the potential to deliver therapeutic agents to the targeted organs like the brain, liver, and cancer tumors, have been developed. Studies have demonstrated that, in the future, with more advanced technology in production and evaluation for industrial applications, oral NLCs will shine even brighter in various applications, notably for chronic diseases.

References

Abdelbary, G., & Haider, M. (2013). In vitro characterization and growth inhibition effect of nanostructured lipid carriers for controlled delivery of methotrexate. *Pharmaceutical Development and Technology*, *18*(5), 1159–1168. https://doi.org/10.3109/10837450.2011.614251

Abdolahpour, S., Toliyat, T., Omidfar, K., Modjtahedi, H., Wong, A. J., Rasaee, M. J., Kashanian, S., & Paknejad, M. (2017). Targeted delivery of doxorubicin into tumor cells by nanostructured lipid carriers conjugated to anti-EGFRvIII monoclonal antibody. *Artificial Cells, Nanomedicine, and Biotechnology*, *46*(1), 89–94. https://doi.org/10.1080/21691401.2017.1296847

Almukadi, H., Eid, B. G., Shaik, R. A., Abdel-

Naim, A. B., & Esmat, A. (2021). Auraptene nanoparticles ameliorate testosterone-induced benign prostatic hyperplasia in rats: Emphasis on antioxidant, anti-inflammatory, proapoptotic and PPARs activation effects. *Biomedicine & Pharmacotherapy*, *143*, 112199. https://doi.org/10.1016/J.BIOPHA.2021.112199

Anwar, W., Dawaba, H. M., Afouna, M. I., Samy, A. M., Rashed, M. H., & Abdelaziz, A. E. (2020). Enhancing the Oral Bioavailability of Candesartan Cilexetil Loaded Nanostructured Lipid Carriers: In Vitro Characterization and Absorption in Rats after Oral Administration. *Pharmaceutics*, *12*(11), 1047. https://doi.org/10.3390/PHARMACEUTICS12111047

Arpagaus, C., Collenberg, A., Rütti, D., Assadpour, E., & Jafari, S. M. (2018). Nano spray drying for encapsulation of pharmaceuticals. *International Journal of Pharmaceutics*, *546*(1–2), 194–214. https://doi.org/10.1016/J.IJPHARM.2018.05.037

Beloqui, A., Solinís, M. Á., Rodríguez-Gascón, A., Almeida, A. J., & Préat, V. (2016). Nanostructured lipid carriers: Promising drug delivery systems for future clinics. *Nanomedicine: Nanotechnology, Biology and Medicine*, *12*(1), 143–161. https://doi.org/10.1016/J.NANO.2015.09.004

Carvajal-Vidal, P., Fábrega, M. J., Espina, M., Calpena, A. C., & García, M. L. (2019). Development of Halobetasol-loaded nanostructured lipid carrier for dermal administration: Optimization, physicochemical and biopharmaceutical behavior, and therapeutic efficacy. *Nanomedicine: Nanotechnology, Biology and Medicine*, *20*, 102026. https://doi.org/10.1016/J.NANO.2019.102026

Charcosset, C., El-Harati, A., & Fessi, H. (2005). Preparation of solid lipid nanoparticles using a membrane contactor. *Journal of Controlled Release*, *108*(1), 112–120. https://doi.org/10.1016/J.JCONREL.2005.07.023

Chen, Y., Pan, L., Jiang, M., Li, D., & Jin, L. (2015a). Nanostructured lipid carriers enhance the bioavailability and brain cancer inhibitory efficacy of curcumin both in vitro and in vivo. *Drug Delivery*, *23*(4), 1383–1392. https://doi.org/10.3109/10717544.2015.1049719

Cirri, M., Bragagni, M., Mennini, N., & Mura, P. (2012). Development of a new delivery system consisting in "drug – in cyclodextrin – in nanostructured lipid carriers" for ketoprofen topical delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, *80*(1), 46–53. https://doi.org/10.1016/J.EJPB.2011.07.015

Cirri, M., Maestrini, L., Maestrelli, F., Mennini, N., Mura, P., Ghelardini, C., & Mannelli, L. D. C. (2018). Design, characterization and in vivo evaluation of nanostructured lipid carriers (NLC) as a new drug delivery system for hydrochlorothiazide oral administration in pediatric therapy. *Drug Delivery*, *25*(1), 1910–1921. https://doi.org/10.1080/10717544.2018.1529209/SUPPL_FILE/IDRD_A_1529209_SM5294.DOCX

Ding, X., Xu, X., Zhao, Y., Zhang, L., Yu, Y., Huang, F., Yin, D., & Huang, H. (2017). Tumor targeted nanostructured lipid carrier co-delivering paclitaxel and indocyanine green for laser triggered synergetic therapy of cancer. *RSC Advances*, *7*(56), 35086–35095. https://doi.org/10.1039/C7RA06119F

Doktorovova, S., Souto, E. B., & Silva, A. M. (2014). Nanotoxicology applied to solid lipid nanoparticles and nanostructured lipid carriers – A systematic review of in vitro data. *European Journal of Pharmaceutics and Biopharmaceutics*, *87*(1), 1–18. https://doi.org/10.1016/J.EJPB.2014.02.005

Eleraky, N. E., Omar, M. M., Mahmoud, H. A., & Abou-Taleb, H. A. (2020). Nanostructured Lipid Carriers to Mediate Brain Delivery of Temazepam: Design and In Vivo Study. *Pharmaceutics*, *12*(5), 451. https://doi.org/10.3390/PHARMACEUTICS12050451

Fang, C.-L., A. Al-Suwayeh, S., & Fang, J.-Y. (2012). Nanostructured Lipid Carriers (NLCs) for Drug Delivery and Targeting. *Recent Patents on Nanotechnology*, *7*(1), 41–55. https://doi.org/10.2174/187221013804484827

Fang, Y. P., Lin, Y. K., Su, Y. H., & Fang, J. Y. (2011). Tryptanthrin-Loaded Nanoparticles for Delivery into Cultured Human Breast Cancer Cells, MCF7: the Effects of Solid Lipid/Liquid Lipid Ratios in the Inner Core. *Chemical and Pharmaceutical Bulletin*, *59*(2), 266–271.

https://doi.org/10.1248/CPB.59.266

Garg, B., Beg, S., Kumar, R., Katare, O. P., & Singh, B. (2019). Nanostructured lipidic carriers of lopinavir for effective management of HIV-associated neurocognitive disorder. *Journal of Drug Delivery Science and Technology*, *53*, 101220. https://doi.org/10.1016/J.JDDST.2019.101220

Gonzalez-Mira, E., Egea, M. A., Souto, E. B., Calpena, A. C., & García, M. L. (2010). Optimizing flurbiprofen-loaded NLC by central composite factorial design for oculardelivery. *Nanotechnology*, *22*(4), 045101. https://doi.org/10.1088/0957-4484/22/4/045101

H. Muller, R., Shegokar, R., & M. Keck, C. (2011). 20 Years of Lipid Nanoparticles (SLN & NLC): Present State of Development & Industrial Applications. *Current Drug Discovery Technologies*, *8*(3), 207–227. https://doi.org/10.2174/157016311796799062

Haider, M., Abdin, S. M., Kamal, L., & Orive, G. (2020). Nanostructured Lipid Carriers for Delivery of Chemotherapeutics: A Review. *Pharmaceutics*, *12*(3), 288. https://doi.org/10.3390/PHARMACEUTICS12030288

Hu, R., Liu, S., Anwaier, G., Wang, Q., Shen, W., Shen, Q., & Qi, R. (2021). Formulation and intestinal absorption of naringenin loaded nanostructured lipid carrier and its inhibitory effects on nonalcoholic fatty liver disease. *Nanomedicine: Nanotechnology, Biology and Medicine*, *32*, 102310. https://doi.org/10.1016/J.NANO.2020.102310

Javed, S., Mangla, B., Almoshari, Y., Sultan, M. H., & Ahsan, W. (2022). Nanostructured lipid carrier system: A compendium of their formulation development approaches, optimization strategies by quality by design, and recent applications in drug delivery. *Nanotechnology Reviews*, *11*(1), 1744–1777. https://doi.org/10.1515/NTREV-2022-0109

Jiang, H., Pei, L., Liu, N., Li, J., Li, Z., & Zhang, S. (2015). Etoposide-loaded nanostructured lipid carriers for gastric cancer therapy. *Drug Delivery*, *23*(4), 1379–1382. https://doi.org/10.3109/10717544.2015.1048491

Joshi, M., & Patravale, V. (2008). Nanostructured lipid carrier (NLC) based gel of celecoxib. *International Journal of Pharmaceutics*, *346*(1–2), 124–132. https://doi.org/10.1016/J.IJPHARM.2007.05.060

Khan, S. A., Rehman, S., Nabi, B., Iqubal, A., Nehal, N., Fahmy, U. A., Kotta, S., Baboota, S., Md, S., & Ali, J. (2020). Boosting the Brain Delivery of Atazanavir through Nanostructured Lipid Carrier-Based Approach for Mitigating NeuroAIDS. *Pharmaceutics*, *12*(11), 1059. https://doi.org/10.3390/PHARMACEUTICS12111059

Khan, S., Baboota, S., Ali, J., Khan, S., Narang, R. S., & Narang, J. K. (2015). Nanostructured lipid carriers: An emerging platform for improving oral bioavailability of lipophilic drugs. *International Journal of Pharmaceutical Investigation*, *5*(4), 182–191. https://doi.org/10.4103/2230-973X.167661

Khosa, A., Reddi, S., & Saha, R. N. (2018). Nanostructured lipid carriers for site-specific drug delivery. *Biomedicine & Pharmacotherapy*, *103*, 598–613. https://doi.org/10.1016/J.BIOPHA.2018.04.055

Lee, C. S., & Koo, J. (2005). A review of acitretin, a systemic retinoid for the treatment of psoriasis. *Expert Opinion on Pharmacotherapy*, *6*(10), 1725–1734. https://doi.org/10.1517/14656566.6.10.1725

Li, Q., Cai, T., Huang, Y., Xia, X., Cole, S. P. C., & Cai, Y. (2017a). A Review of the Structure, Preparation, and Application of NLCs, PNPs, and PLNs. *Nanomaterials*, *7*(6), 122. https://doi.org/10.3390/NANO7060122

Mehnert, W., & Mäder, K. (2012). Solid lipid nanoparticles: Production, characterization and applications. *Advanced Drug Delivery Reviews*, *64*(SUPPL.), 83–101. https://doi.org/10.1016/J.ADDR.2012.09.021

Mendes, I. T., Ruela, A. L. M., Carvalho, F. C., Freitas, J. T. J., Bonfilio, R., & Pereira, G. R. (2019). Development and characterization of nanostructured lipid carrier-based gels for the transdermal delivery of donepezil. *Colloids and Surfaces B: Biointerfaces*, *177*, 274–281. https://doi.org/10.1016/J.COLSURFB.2019.02.007

Müller, R. H., Radtke, M., & Wissing, S. A.

(2002). Nanostructured lipid matrices for improved microencapsulation of drugs. *International Journal of Pharmaceutics*, *242*(1–2), 121–128. https://doi.org/10.1016/S0378-5173(02)00180-1

Muntoni, E., Anfossi, L., Milla, P., Marini, E., Ferraris, C., Capucchio, M. T., Colombino, E., Segale, L., Porta, M., & Battaglia, L. (2021). Glargine insulin loaded lipid nanoparticles: Oral delivery of liquid and solid oral dosage forms. *Nutrition, Metabolism and Cardiovascular Diseases*, *31*(2), 691–698. https://doi.org/10.1016/J.NUMECD.2020.09.020

Nguyen, V. H., Thuy, V. N., Van, T. V., Dao, A. H., & Lee, B. J. (2022). Nanostructured lipid carriers and their potential applications for versatile drug delivery via oral administration. *OpenNano*, *8*(August), 100064. https://doi.org/10.1016/j.onano.2022.100064

Pardeike, J., Hommoss, A., & Müller, R. H. (2009). Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. *International Journal of Pharmaceutics*, *366*(1–2), 170–184. https://doi.org/10.1016/J.IJPHARM.2008.10.003

Sabzichi, M., Samadi, N., Mohammadian, J., Hamishehkar, H., Akbarzadeh, M., & Molavi, O. (2016). Sustained release of melatonin: A novel approach in elevating efficacy of tamoxifen in breast cancer treatment. *Colloids and Surfaces B: Biointerfaces*, *145*, 64–71. https://doi.org/10.1016/J.COLSURFB.2016.04.042

Samimi, S., Maghsoudnia, N., Eftekhari, R. B., & Dorkoosh, F. (2019). Lipid-Based Nanoparticles for Drug Delivery Systems. *Characterization and Biology of Nanomaterials for Drug Delivery: Nanoscience and Nanotechnology in Drug Delivery*, 47–76. https://doi.org/10.1016/B978-0-12-814031-4.00003-9

Sanjula, B., Shah, F. M., Javed, A., & Alka, A. (2009). Effect of poloxamer 188 on lymphatic uptake of carvedilol-loaded solid lipid nanoparticles for bioavailability enhancement. *Journal of Drug Targeting*, *17*(3), 249–256. https://doi.org/10.1080/10611860902718672

Sinhmar, G. K., Shah, N. N., Chokshi, N. V., Khatri, H. N., & Patel, M. M. (2018). Process, optimization, and characterization of budesonide-loaded nanostructured lipid carriers for the treatment of inflammatory bowel disease. *Drug Development and Industrial Pharmacy*, *44*(7), 1078–1089. https://doi.org/10.1080/03639045.2018.1434194

Stillhart, C., Vučićević, K., Augustijns, P., Basit, A. W., Batchelor, H., Flanagan, T. R., Gesquiere, I., Greupink, R., Keszthelyi, D., Koskinen, M., Madla, C. M., Matthys, C., Miljuš, G., Mooij, M. G., Parrott, N., Ungell, A. L., de Wildt, S. N., Orlu, M., Klein, S., & Müllertz, A. (2020). Impact of gastrointestinal physiology on drug absorption in special populations—An UNGAP review. *European Journal of Pharmaceutical Sciences*, *147*, 105280. https://doi.org/10.1016/J.EJPS.2020.105280

Sun, M., Nie, S., Pan, X., Zhang, R., Fan, Z., & Wang, S. (2014). Quercetin-nanostructured lipid carriers: Characteristics and anti-breast cancer activities in vitro. *Colloids and Surfaces B: Biointerfaces*, *113*, 15–24. https://doi.org/10.1016/J.COLSURFB.2013.08.032

Tamjidi, F., Shahedi, M., Varshosaz, J., & Nasirpour, A. (2013). Nanostructured lipid carriers (NLC): A potential delivery system for bioactive food molecules. *Innovative Food Science & Emerging Technologies*, *19*, 29–43. https://doi.org/10.1016/J.IFSET.2013.03.002

Teeranachaideekul, V., Müller, R. H., & Junyaprasert, V. B. (2007). Encapsulation of ascorbyl palmitate in nanostructured lipid carriers (NLC)—Effects of formulation parameters on physicochemical stability. *International Journal of Pharmaceutics*, *340*(1–2), 198–206. https://doi.org/10.1016/J.IJPHARM.2007.03.022

Truong, N. P., Whittaker, M. R., Mak, C. W., & Davis, T. P. (2014). The importance of nanoparticle shape in cancer drug delivery. *Expert Opinion on Drug Delivery*, *12*(1), 129–142. https://doi.org/10.1517/17425247.2014.950564

Üner, M. (2015). Characterization and imaging of solid lipid nanoparticles and nanostructured lipid carriers. *Handbook of Nanoparticles*, 117–141. https://doi.org/10.1007/978-3-319-15338-4_3/FIGURES/12

Vitorino, C., Almeida, A., Sousa, J., Lamarche, I., Gobin, P., Marchand, S., Couet, W., Olivier, J. C.,

& Pais, A. (2014). Passive and active strategies for transdermal delivery using co-encapsulating nanostructured lipid carriers: In vitro vs. in vivo studies. *European Journal of Pharmaceutics and Biopharmaceutics*, *86*(2), 133–144. https://doi.org/10.1016/J.EJPB.2013.12.004

Wang, Q., Cheng, H., Zhou, K., Wang, L., Dong, S., Wang, D., & Chen, W. (2013). Nanostructured lipid carriers as a delivery system of biochanin A. *Drug Delivery***,** *20*(8), 331–337. https://doi.org/10.3109/10717544.2013.838716

Wang, Y., Zhang, H., Hao, J., Li, B., Li, M., & Xiuwen, W. (2015). Lung cancer combination therapy: co-delivery of paclitaxel and doxorubicin by nanostructured lipid carriers for synergistic effect. *Drug Delivery***,** *23*(4), 1398–1403. https://doi.org/10.3109/10717544.2015.1055619

Weber, S., Zimmer, A., & Pardeike, J. (2014). 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*, *86*(1), 7–22. https://doi.org/10.1016/J.EJPB.2013.08.013

Xu, R. (2008). Progress in nanoparticles characterization: Sizing and zeta potential measurement. *Particuology*, *6*(2), 112–115. https://doi.org/10.1016/J.PARTIC.2007.12.002

Zaky, M. F., Megahed, M. A., Hammady, T. M., Yasir, M., & Asif, M. (2010). Biopharmaceutical Classification System :An Account Article in. *International Journal of PharmTech Research*, *73*, 945. https://www.researchgate.net/publication/266213316

Gad, S., Ghorab, M. M., & El-Say, K. M. (2022). Tailoring Apixaban in Nanostructured Lipid Carrier Enhancing Its Oral Bioavailability and Anticoagulant Activity. *Pharmaceutics*, *15*(1), 80. https://doi.org/10.3390/PHARMACEUTICS15010080

Zhang, X. G., Miao, J., Dai, Y. Q., Du, Y. Z., Yuan, H., & Hu, F. Q. (2008). Reversal activity of nanostructured lipid carriers loading cytotoxic drug in multi-drug resistant cancer cells. *International Journal of Pharmaceutics*, *361*(1–2), 239–244. https://doi.org/10.1016/J.IJPHARM.2008.06.002

Zhu, X., Deng, X., Lu, C., Chen, Y., Jie, L., Zhang, Q., Li, W., Wang, Z., Du, Y., & Yu, R. (2018). SPIO-loaded nanostructured lipid carriers as liver-targeted molecular T2-weighted MRI contrast agent. *Quantitative Imaging in Medicine and Surgery*, *8*(8), 770. https://doi.org/10.21037/QIMS.2018.09.03