REVIEW ARTICLE

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Lipospheres and Pro-Nanolipospheres: Advances in Drug Delivery Systems

Abeer Osama Motawee [a], El-Sayed Khafagy [b, c], , Ahmed R. Gardouh [b,d*], Mamdouh Mostafa Ghorab [b]

[a] Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Heliopolis University, Cairo, Egypt; abeermotawea@gmail.com; [b] Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt; [c] Department of Pharmaceutics, College of Pharmacy, Prince Sattam bin Abdulaziz University, Al-kharj 11942, Saudi Arabia; [d] Department of Pharmaceutical sciences, Faculty of pharmacy, Jadara university, Irbid 21110, Jordan.

Received on: 15-01-2023

Revised on: 29-01-2023

Accepted on: 02-02-2023

*Correspondence Author:

E-mail:

ahmed_mahmoud@pharm.suez.edu.eg

Tel. : +2 01013790828

Abstract

The usefulness of nanotechnology nowadays in improving the performance of different active pharmaceutical ingredients (API) has generated a great deal of excitement worldwide. These nano-systems offer the advantage of higher bioavailability, lower toxicity, and the ability to deliver drugs with low half-life or low permeability. Lipid nano-based carriers have been extensively used for their ability to protect the API from degradation and their safety as these used lipids are generally regarded as safe. Lipospheres and Pro-nanolipospheres are among the promising systems for the delivery of water-insoluble drugs. They are composed of aqueous dispersible particles with a rigid hydrophobic lipid centre, coated by an outer protective phospholipid exterior. These lipospheres and Pro-nanolipospheres have the advantage of being easy to scale up, high dispersibility in aqueous media, and improved physical stability, making them the perfect candidate for the delivery of different APIs using different routes including oral, parenteral, intranasal and topical routes.

Keywords: Lipospheres, lipids, Pre-concentrate, Delivery system, bioavailability

1. Introduction

Lipids are usually used to enhance drug absorption and permeability, thus there rising usage in formulating lipid nanoparticles, nanostructured lipid carriers and lipospheres (Dixit et al., 2017). Moreover, the presence of these lipids protects the loaded drugs from chemical and enzymatical degradation, making them quite advantageous for oral drug delivery systems. Oral drug delivery is one of the most commonly used routes for drug administration due to its ease of use, patient acceptance and non-invasiveness. To formulate a drug for oral administration, bioavailability should be considered since it reflects the proportion of the dose that can reach systemic circulation and provide the expected therapeutic effect (Rabbie et al., 2016). Many drugs that are currently on the market or newly developed have poor bioavailability, meaning they do not reach the minimum effective concentration in the bloodstream due to factors such as limited

solubility, poor absorption, and rapid metabolism. Additionally, some drugs may have unpredictable fluctuations in the bloodstream, making it difficult to establish a relationship between the dose and the response. (Elgart et al., 2012)

2. Lipospheres

Lipospheres were first introduced by Abraham J. Domb, and they were formulated to contain an antigen which were used as vaccines for animals (Abraham J. Domb, 1994). They are made of a solid hydrophobic lipid core (triglycerides) that is solid at room temperature and may melt or remain solid at body temperature. The outer surface of the lipospheres is stabilized by a layer of embedded phospholipid molecules which may either entrap the drug or enrich its coat with it (Elgart et al., 2012; Yalavarthi et al., 2014). Their internal core contains the drug dissolved or dispersed, and their particle sizes range from 0.01 to 100 µm. (Bekerman et al., 2004; Elgart et al., 2012; Khopade & Jain, 1997)

Lipospheres are one of the lipid-based drug delivery systems complying well with the needs of a drug delivery system, as they have high dispersibility in aqueous media, easy in their preparation and scaling up, and their high ability to entrap hydrophobic drugs; enhancing the solubility and permeability characteristics of especially class II and IV drug candidate (Ganesan & Allimalarkodi, 2015; Kumar et al., 2021).

2.1. Composition of lipospheres:

Lipospheres are a combination of solid inner core with single layer phospholipid exterior. Many solid lipids were previously used as the main lipid component in the formulation to create its solid lipid core, such as trilaurin, tripalmitin, tristearin, tricaprin, stearic acid, ethyl stearate, and hydrogenated vegetable oil. As for the phospholipids, they can be either purified and obtained from natural origin such as soyabean phosphatidyl choline and hydrogenated soyabean phosphatidyl choline and their derivatives, or synthesized such as distearoyl phosphatidylcholine, dimyristoyl phosphatidylcholine and dipalmitoyl phosphatidylcholine which proved great efficacy as a coating layer (Elgart et al., 2012; Yalavarthi et al., 2014).

The formulation may also contain an emulsifier or a surfactant to create a homogeneous coating around the core substance, ensure stability and to enhance the ability of the drug to be partitioned between the lipid and aqueous phases (Manogna & Sagar, 2019; Yalavarthi et al., 2014). Moreover, they affect significantly the particle size, surface charge and the prolonged stability of the prepared lipospheres (Rout et al., 2022). Non-ionic surfactants are the most preferred type for their safety and efficacy, including sorbitan derivatives, PEGilated fats, Tween® and Span®, Cremophor® and LipoPeg (Elgart et al., 2012). Moreover, pH-adjusting agents, osmotic pressure controllers and preservatives may be added to liposphere preparation (M. R. Singh et al., 2012).

2.2. Methods of preparation of Lipospheres

2.2.1. High-pressure homogenization method

The first step used in this technique is the incorporation of the drug into bulk lipid by dissolving or dispersing the drug in lipid melt. The produced liquid is pushed through a narrow gap (in the range of a few microns) of a high pressure (100–2000 bar). Accordingly, the fluid accelerates on a very short distance to a very high velocity (over 1000 km/h), creating extremely high levels of shear stress and cavitation forces that break apart particles and decrease their size to a size smaller than a micron. (W Mehnert & Mäder, 2001; Wolfgang Mehnert & Mäder, 2012). This method could be applied on hot or cold.

2.2.1.1. Hot homogenization

This method depends on the preparation of a pre-emulsion by dissolving the drug in the molten solid carrier (lipid or polymer) which is kept at a temperature 5–10°C above its melting point. A hot buffer solution is quickly added to the lipid mixture along with phospholipids and the mixture is blended for 2-5 minutes with a homogenizer. Afterwards, this hot pre-emulsion is passed through high-pressure homogenizer and kept at the same temperature. The homogenization process may be repeated several times using high temperatures till the required size range is obtained. The resulting emulsion is then rapidly cooled down, during which the lipospheres form solid matrix (Domb, 2006; Kasongo et al., 2012; Niranjan Patra & Routray, 2018).

2.2.1.2. Cold homogenization

This method was established to overcome problems of the hot homogenization method, such as temperature-induced drug degradation, drug distribution to the aqueous phase and the complex crystallization step that results in drug modifications. Primarily, the drug is solubilized or dispersed into the molten lipid bulk, and then it's rapidly cooled employing dry ice or liquid nitrogen, which results in the drug being evenly distributed within the lipid matrix. The resulting solid lipid matrix is then ground into micron-sized

particles using a mortar mill or ball mill. Afterwards, these particles are dispersed in a chilled emulsifier solution. The suspension is subjected to high-pressure homogenization at a low temperature of 5 – 10°C below the lipid melting point to obtain a nano-sized dispersion system (Jaspart et al., 2005; Kasongo et al., 2012; Wolfgang Mehnert & Mäder, 2012).

2.2.2. Melt Dispersion Method

In this method, the lipid or lipid mixture is melted and maintained at a temperature slightly above the melting point of the lipid, in which the drug is dispersed. This mixture is emulsified with an external aqueous phase containing a suitable surfactant and phospholipids and it's maintained at a temperature nearly or slightly higher than the lipid phase. The formed emulsion is continuously agitated with a mechanical stirrer, then the formulation is immediately cooled by submerging it in an ice bath with continuous agitation to produce a uniform dispersion of lipospheres (Manogna & Sagar, 2019; Natarajan et al., 2013; Yalavarthi et al., 2014).

2.2.3 Solvent evaporation method

The solvent evaporation method is an alternative to the melt dispersion method for heat-sensitive drugs as it reduces exposure to high temperatures. This method is based on the evaporation of the organic solvent in which lipids are dissolved, allowing the formation of solid Lipospheres (Cortesi, 2002; Manogna & Sagar, 2019).

In the solvent evaporation method, the lipophilic drug, altogether with the solid carrier and phospholipids, is dissolved in an organic solvent. The most commonly used organic solvents include ethanol, ethyl acetate, acetone or dichloromethane. Then this mixture is emulsified in an external aqueous phase by the agitation of two immiscible phases in the presence of an emulsifier. Further emulsification can be done by high-pressure homogenizer or another homogenization technique, then the organic solvent is evaporated and the formed solid is mixed with a warm buffer solution until a homogeneous liposphere dispersion is attained (Domb, 2006; M. R. Singh et al., 2012).

2.2.4. Supercritical fluid method

The supercritical fluid method was invented to prevent contamination by using an organic solvent, by extracting any traces of the organic solvent using a supercritical fluid. The drug and lipid are dissolved in a suitable organic solvent then it's emulsified with an aqueous phase to produce an emulsion with a discontinuous phase of micelles comprised of organic solvent, drug and lipid. Lastly, this formed emulsion is treated with a supercritical fluid (SCF) under certain conditions of pressure and temperature. SCF like CO_2 facilitates the extraction of the organic solvent and precipitation of the solid lipid nanoparticles containing the drug in the aqueous dispersion (Shukla et al., 2011; Yalavarthi et al., 2014).

3. Pro-nanolipospheres (PNLs)

Pro-nanolipospheres are formed from lipids (natural or synthetic), with surfactants, phospholipids and co-solvents. They are especially useful in encapsulating lipophilic drugs. Upon contact with any aqueous media, they can form spontaneous o/w emulsions encapsulating the drug within, and thus they are referred to as "preconcentrate" (Bruni et al., 2018; Cherniakov, Izgelov, Domb, et al., 2017; Elgart et al., 2012; Hoffman et al., 2014). The main *in situ* method that was developed for the preparation of PNL depends on producing a dispersible pre-concentrate system (Bekerman et al., 2004; Hoffman et al., 2014). PNL preparation depends on the use of a surfactant mixture, with high and low hydrophilic-lipophilic balance (HLB) as Tween and Span (Hoffman et al., 2014). Also, the solvent used should be an organic amphiphilic one miscible with all the formulation components (Hoffman et al., 2014).

4. Advantages of lipospheres and PNLs

Lipospheres have several advantages over other particulate delivery systems including improved physical stability as they prevent particle coalescence, reducing drug mobility after its entrapment and thus reducing drug leakage (Bhosale et al., 2016) and high drug loading capacity (Yalavarthi et al., 2014).

Lipospheres play an important role in enhancing the aqueous solubility of poorly water-soluble drugs which leads to the improvement of their bioavailability especially oral bioavailability (Maheen et al., 2020; Yalavarthi et al., 2014). Moreover lipospheres help in enhancing patient compliance as they lead to long-term control over drug plasma levels which was clear in different drug classes such as anti-inflammatory drugs, antibiotics, local anaesthetics, anticancer agents and many others (Yalavarthi et al., 2014), thus

helping in decreasing dose frequency and associated side effects (Maheen et al., 2020). Lipospheres are also known for their proved safety and biocompatibility as they are composed of physiological components and excipients which has GRAS status (Generally accepted and safe) (Domb, 2006; Hanif et al., 2021). They have also shown improved stability in addition to their ease and lower cost of production (Hanif et al., 2021).

5. Applications of lipospheres and PNLs

Lipospheres have been used to incorporate and deliver many drugs through different routes of administration including oral, ocular, topical and parenteral routes.

5.1. Oral drug delivery:

The oral route is the most common route for drug administration for its safety, ease of use and improved patient compliance in comparison with other routes (Lennernäs et al., 2007). Despite this, it has been reported that many compounds do not progress to advanced stages of research and development due to their limited bioavailability and significant variation in absorption among patients after oral administration. Therefore, using lipospheres loaded with drugs has been effective in addressing this crucial oral bioavailability problem.

One of the first drugs formulated as lipospheres after extensive research was done by Hoffman *et al.* was Carbamazepine (CBZ) (Barakat & Yassin, 2006), an effective antiepileptic drug that suffered high dosing frequency, leading to poor patient compliance and fluctuations in plasma concentrations that might cause intermittent side effects. CBZ was successfully incorporated into lipospheres by the melt-dispersion method with an extended-release profile, which was considered to be a promising result for formulation use in other *in vivo* studies.

Also, Resveratrol was incorporated into acoustically active lipospheres, and ultrasound waves were used to promote its cardiovascular targeting and thrombose treatment (Fang et al., 2007).

The anti-hypertensive drug Lercanidipine was formulated as Buoyant lipospheres which helped in a more controlled release pattern of the drug when prepared using suitable amounts of cetostearyl alcohol and poloxamer® 407 (Pandit & Patil, 2009).

Moreover, lipospheres were used to encapsulate antibiotics such as Ceftriaxone, a third-generation cephalosporin (Attama et al., 2009) and Gentamicin which is active against a variable range of gram-positive and gram-negative bacteria (Momoh & Esimone, 2012)

Some anti-hyperlipidemic drugs such as Fenofibrate were formulated also as lipospheres which showed a superior lowering of serum cholesterol levels when compared with the marketed product (Saroja & Lakshmi, 2013).

Nebivolol (NEB) is another antihypertensive drug of poor bioavailability that was encapsulated into liposphere by Hanif et al. (Hanif et al., 2019) and then further research was done by combining NEB with Ivabradine (IVB) and co-loading into lipospheres which produced a prolonged release formulation with improved bioavailability when compared to the marketed oral tablets(Hanif et al., 2021).

More research was done by Maheen and Rasul using the antihypertensive drug enalapril maleate – an ACE inhibitor- that was formulated as liposphere and showed a sustained-release pattern that led to a maximum benefit of controlling blood pressure with the least side effects and cost for hypertensive patients (Maheen & Rasul, 2020).

Antidiabetic drugs were target medications for the encapsulation into lipospheres, as it's one the most widespread chronic diseases with the lowest bioavailability. Glipizide is a BSC class II drug with a short half-life that leads to its frequent administration, was encapsulated in lipospheres that showed sustained anti-diabetic effects when administered orally in rats (Shivakumar et al., 2007). Saxagliptin (SG) is an antidiabetic drug that acts as a DPP-4 inhibitor that has low oral bioavailability and a short half-life. When it was formulated as a liposphere, it showed significant improvement in bioavailability and achieved a slower and controlled release of (SG) from the optimized lipospheres, thus decreased administration frequency (Rasul et al., 2021).

Another critical point that affects the quality of hypertensive patients' life, is the co-existence of diabetes mellitus with hypertension which increases the risk of stroke and other cardiovascular complications. Thus, combination therapy is considered a good choice for the management of both conditions. Accordingly, Maheen et al. formulated both Saxagliptin and enalapril in an optimized liposphere formulation and showed a significant enhancement in the oral bioavailability of both drugs compared to the marketed oral tablets (Maheen et al., 2020).

5.2. Topical drug delivery

Topical delivery systems are favoured for localizing the action of the drug to the skin surface

to avoid the side effects of oral drug administration. Additionally, topical drug delivery systems are essential for the treatment of skin conditions like psoriasis and candidiasis.
The liposphere gel formulation is beneficial in the ease of topical application, deeper skin penetration and gradual release of drugs (Mestry et al., 2020).

Aceclofenac lipospheres were prepared to be used topically for their anti-inflammatory effect, to create a sustained release formulation to overcome the side effects of the oral administration of aceclofenac. The formulation proved to have a promising effect on the delivery of Aceclofenac due to its high entrapment efficiency and stability (Nasr et al., 2008).
Thymoquinone (TMQ) was formulated as lipospheres for topical use since it had poor water solubility and was characterized by its light and pH sensitivity. TMQ lipospheres promoted deep skin penetration and slow release which increased their anti-inflammatory and anti-psoriatic activity(Jain et al., 2017).
Another liposphere gel formulation was prepared for the treatment of psoriasis using Commiphora mukul and Quercetin. This formulation showed an enhanced anti- psoriatic efficacy in the *in vivo* study done on Imiquimod- induced psoriasis-like skin of mice model (Mestry et al., 2020).
Clotrimazole anti-candidal study confirmed that its encapsulation in lipospheres improved its activity against *Candida albicans*. Since it was formulated in a gel form, it could be suitable for vaginal application (Esposito et al., 2018).
Kenechukwu *et al.* formulated miconazole nitrate lipospheres to be used in the treatment of oral candidiasis by its application as a mucoadhesive gel. By comparing the activity of the formulated lipospheres with the marketed oral gel Daktarin®, it showed a more controlled release pattern of miconazole (Kenechukwu et al., 2022).

5.3. Pulmonary Drug Delivery

Pulmonary delivery is a common drug administration technique to manage different lung diseases such as tuberculosis (TB). Rifampicin is one of the important drugs used for the management of pulmonary TB infections. Rifampicin was encapsulated into lipospheres by the use of cyclodextrin and vitamin C, and the study showed the potential of this delivery system for the better management of TB as it showed superbly in vitro antimycobacterial effectiveness (C. Singh et al., 2015).

5.4. Intranasal delivery

Quetiapine Fumarate is an antipsychotic drug that suffers poor brain uptake when administered orally, thus the drug's efficacy may be reduced as a significant portion of the drug does not reach the brain when taken orally. Additionally, oral administration can also cause unwanted side effects, such as sedation and weight gain.
Zaki *et al.* found that the liposphere-based formulation was able to effectively target the brain through the intranasal route, thus lipospheres loaded Quetiapine Fumarate showed high drug loading capacity and better stability and were able to improve the brain uptake and the efficacy of the drug (Zaki et al., 2022). The study also showed that this strategy was a promising alternative to traditional oral administration.

1.1. Parenteral Drug Delivery

The parental administration of lipophilic compounds is one of the challenges currently facing pharmaceutical research and industry. This happens due to their limited water solubility which requires the inclusion of solubilizing agents, some of which can be toxic(Elgart et al., 2012).
Therefore, a safe and effective delivery system is essential for the parenteral delivery of lipophilic compounds. A major advantage of using lipid-based systems, like lipospheres, is that they are composed of biodegradable and well-tolerated lipid substances and excipients that are compliant with regulatory standards. (GRAS) (Elgart et al., 2012).
Lipospheres were used for the parenteral delivery of some anaesthetic's drugs for pain management such as lidocaine and bupivacaine to sustain their effect for several days following a single injection to decrease the frequency of administration (Hersh et al., 1992; Toongsuwan et al., 2004).
Numerous antibiotics as ofloxacin and oxytetracycline and antifungal agents, such as nystatin were incorporated into lipospheres and used mainly for developing long-acting formulations for farm animal studies (Domb, 2006; Saxena, 2021). Oxytetracycline was studied for its long acting effect on camel and turkeys and both showed the needed sustained effect (Domb, 1995; Oukessou et al., 1992).
Donepezil is used in the treatment of Alzheimer's patients, thus formulating it in a depot form is one of the great targets for these patients to decrease the frequency of administration and improve patient compliance. Accordingly, Intramuscular and subcutaneous liposphere injection helped in overcoming the burst release effect of the in-situ

Donepezil formulation, and achieving depot effect and controlled release when compared to that of subcutaneous injection (Yehia et al., 2012a, 2012b).
Based on the research work that was initiated by Hoffman *et al*. in 2014, PNLs managed to improve the oral bioavailability of the drug by various mechanisms such as increasing GI drug solubilization, reducing the intra-enterocyte metabolism and reducing P-gp efflux activity. On top of that, PNL reduced the high variability for Class II compounds which made this delivery system suitable for drugs which are P-gp and CYP3A4 substrates as they are characterized by low oral bioavailability and erratic absorption (Hoffman et al., 2014).
In the last twenty years, extensive clinical research was done on PNL and advanced PNL systems that used penetration enhancers and one of the most studied drugs was the immunosuppressant drug Cyclosporin A (CsA), which was initially formulated by Bekerman *et al.* as oral liposphere nano-dispersion that showed comparable bioavailability with Neoral® (Bekerman et al., 2004). Furthermore, Avramoff *et al.* studied the impact of the various formulation parameters and stability of CsA oral liposphere pro-dispersion (Avramoff et al., 2012). Finally Cyclosporin A(CsA) PNL succeeded to conquer challenges and reached clinical application to be commercially available as Deximune® at different dosages of 25mg, 50mg and 100 mg (Dexcel Pharma Ltd.) (Atsmon et al., 2018; Rout et al., 2022).

Tacrolimus, an efficient immunosuppressant drug having poor bioavailability and poor dose-plasma drug concentration correlation (Elgart et al., 2012) was also formulated as PNL to be used orally and showed higher bioavailability when compared to Prograf® control capsules (Hoffman et al., 2014). Another study was done by Jain *et al.* to evaluate the effect of Tacrolimus and curcumin co-loaded PNL formulation in the management of Psoriasis by its application topically as a gel, and it exhibited a better anti-psoriatic effect of the established formulation (Jain et al., 2016).
Also, cannabinoids like Cannabidiol (CBD) an extremely lipophilic drug (log P ~6) with very poor bioavailability(Izgelov et al., 2020) formulated as PNL and studied for the improvement in its pharmacokinetic profile (Hoffman et al., 2014), then cannabidiol altogether with Δ9-Tetrahydrocannabinol were incorporated into a more advanced oral PNL system by combining it with different absorption enhancers (Curcumin, piperine and Resveratrol) and it showed a great increase in its bioavailability using a rat model (Cherniakov, Izgelov, Domb, et al., 2017). And finally, the bioavailability of the optimized PNL formulation known as PTL401 (THC-CBD-Piperine-PNL) was studied and compared to the Sativex® spray using a human model(Atsmon et al., 2018; Cherniakov, Izgelov, Barasch, et al., 2017).
Other drugs were formulated as PNL such as amiodarone HCl and talinolol. Upon conducting pharmacokinetic studies for both drugs, amiodarone-PNL showed significant improvement in its bioavailability and fewer fluctuations in the plasma concentrations which might improve the drug safety profile as Amiodarone is known for its narrow therapeutic index. It's worth mentioning that this improved bioavailability was obtained also by even the simultaneous administration of Amiodarone with blank nano-lipospheres. Also, talinolol-PNL showed improved bioavailability as it showed higher AUC and C_{max} when compared to Talinolol (Hoffman et al., 2014).
Amiodarone PNL was also administered intravenously and showed higher plasma concentrations at the central compartment during the first disposition phase when compared to IV Amiodacore® (Hoffman et al., 2014).
PNLs can act as a platform for the delivery of different drugs along with absorption enhancers, thus resolving some absorption problems like phase II metabolism. Raloxifene co-administration with piperine-PNL showed a 2-fold increase in its oral bioavailability (Izgelov et al., 2018).

6. Conclusion

Developing new drug delivery systems became a necessity in the pharmaceutical industry, nearly of equal importance as the development of new active drug molecules, to improve the bioavailability, and have the ability to maintain therapeutic drug levels at the intended site of action for an adequate duration and thus comes the role of lipid-based lipospheres and pro-nano lipospheres. In addition, these systems have the advantage of being proven safe and biocompatible as they are composed of physiological components and GRAS excipients, making them valuable drug delivery systems for various therapeutic applications.

7. Declaration of interest

The authors declare that they have no conflicts of interest concerning the preparation of this manuscript.

8. References

Abraham J. Domb. (1994). LIPOSPHERE CARRIERS OF VACCINES (Patent No. 5340588).

Atsmon, J., Cherniakov, I., Izgelov, D., Hoffman, A., Domb, A. J., Deutsch, L., Deutsch, F., Heffetz, D., & Sacks, H. (2018). PTL401, a New Formulation Based on Pro-Nano Dispersion Technology, Improves Oral Cannabinoids Bioavailability in Healthy Volunteers. Journal of Pharmaceutical Sciences, 107(5), 1423–1429.

Attama, A. A., Okafor, C. E., Builders, P. F., & Okorie, O. (2009). Formulation and in vitro evaluation of a PEGylated microscopic lipospheres delivery system for ceftriaxone sodium. Drug Delivery, 16(8), 448–457.

Avramoff, A., Khan, W., Ezra, A., Elgart, A., Hoffman, A., & Domb, A. J. (2012). Cyclosporin pro-dispersion liposphere formulation. Journal of Controlled Release, 160(2), 401–406.

Barakat, N. S., & Yassin, A. E. B. (2006). In vitro characterization of carbamazepine-loaded Precifac lipospheres. Drug Delivery: Journal of Delivery and Targeting of Therapeutic Agents, 13(2), 95–104.

Bekerman, T., Golenser, J., & Domb, A. (2004). Cyclosporin Nanoparticulate Lipospheres for Oral Administration. Journal of Pharmaceutical Sciences, 93(5), 1264–1270.

Bhosale, U. M., Galgatte, U. C., & Chaudhari, P. D. (2016). Development of pioglitazone hydrochloride lipospheres by melt dispersion technique: Optimization and evaluation. Journal of Applied Pharmaceutical Science, 6(1), 107–117.

Bruni, N., Della Pepa, C., Oliaro-Bosso, S., Pessione, E., Gastaldi, D., & Dosio, F. (2018). Cannabinoid Delivery Systems for Pain and Inflammation Treatment. Molecules, 23(10), 2478.

Cherniakov, I., Izgelov, D., Barasch, D., Davidson, E., Domb, A. J., & Hoffman, A. (2017). Piperine-pro-nanolipospheres as a novel oral delivery system of cannabinoids: Pharmacokinetic evaluation in healthy volunteers in comparison to buccal spray administration. Journal of Controlled Release, 266(September), 1–7.

Cherniakov, I., Izgelov, D., Domb, A. J., & Hoffman, A. (2017). The effect of Pro Nano Lipospheres (PNL) formulation containing natural absorption enhancers on the oral bioavailability of delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) in a rat model. *European Journal of Pharmaceutical Sciences*, *109*(May 2018), 21–30.

Cortesi, R. (2002). Production of lipospheres as carriers for bioactive compounds. *Biomaterials*, *23*(11), 2283–2294.

Dixit, N., Gupta, A., Asija, R., & Sharma, M. (2017). Liposphere : a Noval Approach of Drug Delivery. *World Journal of Pharmaceutical and Medical Research*, *3*(6), 124–130.

Domb, A. J. (1995). Long acting injectable oxytetracycline-liposphere formulations. *International Journal of Pharmaceutics*, *124*(2), 271–278.

Domb, A. J. (2006). Lipospheres for Controlled Delivery of Substances. In S. Benita (Ed.), *Microencapsulation; Methods and Industrial Applications* (2nd editio, pp. 297–316). Taylor & Francis Group.

Elgart, A., Cherniakov, I., Aldouby, Y., Domb, A. J., & Hoffman, A. (2012). Lipospheres and pro-nano lipospheres for delivery of poorly water soluble compounds. *Chemistry and Physics of Lipids*, *165*(4), 438–453.

Esposito, E., Sguizzato, M., Bories, C., Nastruzzi, C., & Cortesi, R. (2018). Production and characterization of a clotrimazole liposphere gel for Candidiasis treatment. *Polymers*, *10*(2), 1–16.

Fang, J. Y., Hung, C. F., Liao, M. H., & Chien, C. C. (2007). A study of the formulation design of acoustically active lipospheres as carriers for drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, *67*(1), 67–75.

Ganesan, V., & Allimalarkodi, S. (2015). Liposphere: A versatile drug delivery system - an over view. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, *6*(4), 1753–1762.

Hanif, M., Khan, H. U., Afzal, S., Majeed, A., Iqbal, N., Afzal, K., Andleeb, M., Rauf, A., & Farooq, A. (2019). Formulation, characterization and optimization of nebivolol-loaded sustained

release lipospheres. *Tropical Journal of Pharmaceutical Research*, *18*(2), 223–231.

Hanif, M., Khan, H. U., Maheen, S., Shafqat, S. S., Shah, S., Masood, S. A., Abbas, G., Rizwan, M., Rasheed, T., & Bilal, M. (2021). Formulation, characterization, and pharmacokinetic evaluation of Ivabradine-Nebivolol co-encapsulated lipospheres. *Journal of Molecular Liquids*, *344*, 117704.

Hersh, E. V, Maniar, M., Green, M., & Cooper, S. A. (1992). Anesthetic Actvity of te Lipospheres Bupivacane Delivery System in te Rat. In *Anesth Prog* (Vol. 39).

Hoffman, A., Domb, A. J., Elgart, A., & Cherniakov, I. (2014). *Formulation and method for increasing oral bioavailability of drugs* (Patent No. US 20140348926A1).

Izgelov, D., Cherniakov, I., Aldouby Bier, G., Domb, A. J., & Hoffman, A. (2018). The Effect of Piperine Pro-Nano Lipospheres on Direct Intestinal Phase II Metabolism: The Raloxifene Paradigm of Enhanced Oral Bioavailability. *Molecular Pharmaceutics*, *15*(4), 1548–1555.

Izgelov, D., Shmoeli, E., Domb, A. J., & Hoffman, A. (2020). The effect of medium chain and long chain triglycerides incorporated in self-nano emulsifying drug delivery systems on oral absorption of cannabinoids in rats. *International Journal of Pharmaceutics*, *580*(December 2019), 119201.

Jain, A., Doppalapudi, S., Domb, A. J., & Khan, W. (2016). Tacrolimus and curcumin co-loaded liposphere gel: Synergistic combination towards management of psoriasis. *Journal of Controlled Release*, *243*, 132–145.

Jain, A., Pooladanda, V., Bulbake, U., Doppalapudi, S., Rafeeqi, T. A., Godugu, C., & Khan, W. (2017). Liposphere mediated topical delivery of thymoquinone in the treatment of psoriasis. *Nanomedicine: Nanotechnology, Biology and Medicine*, *13*(7), 2251–2262.

Jaspart, S., Piel, G., Delattre, L., & Evrard, B. (2005). Solid lipid microparticles: formulation, preparation, characterisation, drug release and applications. *Expert Opinion on Drug Delivery*, *2*(1), 75–87.

Kasongo, K. W., Müller, R. H., & Walker, R. B. (2012). The use of hot and cold high pressure homogenization to enhance the loading capacity and encapsulation efficiency of nanostructured lipid carriers for the hydrophilic antiretroviral drug, didanosine for potential administration to paediatric patients. *Pharmaceutical Development and Technology*, *17*(3), 353–362.

Kenechukwu, F. C., Flora Kalu, C., Momoh, M. A., Onah, I. A., Attama, A. A., Chima Okore, V., & Ng, A. A. A. (2022). Novel Bos indicus Fat-Based Nanoparticulate Lipospheres of Miconazole Nitrate as Enhanced Mucoadhesive Therapy for Oral Candidiasis. *Biointerface Research in Applied Chemistry*, *13*(1), 24.

Khopade, A. J., & Jain, N. K. (1997). Long-circulating lipospheres targeted to the inflammed tissue. *Die Pharmazie*, *52*(2), 165–166.

Kumar, A., Jain, U. K., & Patel, A. (2021). Formulation Development and Evaluation of Liposphere of Poor Water Soluble Drug for Hyperlipidemia. *Journal of Drug Delivery and Therapeutics*, *11*(2), 23–30.

Lennernäs, H., Abrahamsson, B., Persson, E. M., & Knutson, L. (2007). Oral drug absorption and the Biopharmaceutics Classification System. *Journal of Drug Delivery Science and Technology*, *17*(4), 237–244.

Maheen, S., & Rasul, A. (2020). Formulation, characterization and statistical optimization of enalapril-loaded lipospheres. *Bioinspired, Biomimetic and Nanobiomaterials*, *9*(4), 202–212.

Maheen, S., Rasul, A., Hanif, M., & Khan, H. U. (2020). Lipospheres for Simultaneous Controlled Release and Improved Pharmacokinetic Profiles of Saxagliptin-Enalapril: Formulation, Optimization, and Comparative In Vitro-In Vivo Evaluation. *AAPS PharmSciTech*, *21*(5), 188.

Manogna, K., & Sagar, S. V. (2019). A DETAILED REVIEW ON LIPID BASED CARRIER DRUG DELIVERY SYSTEM: LIPOSPHERES. *International Journal of Scientific Research*, *8*(7), 39–42.

Mehnert, W, & Mäder, K. (2001). Solid lipid nanoparticles: production, characterization and

applications. *Advanced Drug Delivery Reviews*, *47*(2–3), 165–196.

Mehnert, Wolfgang, & Mäder, K. (2012). Solid lipid nanoparticles: Production, characterization and applications. *Advanced Drug Delivery Reviews*, *64*, 83–101.

Mestry, M., Rane, M., & Bajaj, A. (2020). Commiphora mukul and Quercetin Loaded Liposphere Gel: Potential Treatment for Psoriasis. *Indian Journal of Pharmaceutical Education and Research*, *54*(3), 654–667.

Momoh, M. A., & Esimone, C. O. (2012). Phospholipon 90H (P90H)–based PEGylated microscopic lipospheres delivery system for gentamicin: an antibiotic evaluation. *Asian Pacific Journal of Tropical Biomedicine*, *2*(11), 889–894.

Nasr, M., Mansour, S., Mortada, N. D., & El Shamy, A. A. (2008). Lipospheres as Carriers for Topical Delivery of Aceclofenac: Preparation, Characterization and In Vivo Evaluation. *AAPS PharmSciTech*, *9*(1), 154–162.

Natarajan, S., Lakshmanan, P., Nerkar, N., Bajaj, A., Shrikhande, S., & Jain, D. (2013). Effect of Processing Variables on Characterization of Ofloxacin Loaded Lipospheres Prepared by Melt Dispersion Technique. *Current Drug Delivery*, *10*(5), 517–526.

Niranjan Patra, C., & Routray, S. B. (2018). Solid lipid-based delivery system for oral delivery of drugs: A review. *Asian Journal of Pharmaceutics*, *12*(4), S1135–S1145.

Oukessou, M., Uccelli-Thomas, V., & Toutain, P. L. (1992). Pharmacokinetics and local tolerance of a long-acting oxytetracycline formulation in camels. *American Journal of Veterinary Research*, *53*(9), 1658–1662.

Pandit, S. S., & Patil, A. T. (2009). Formulation and in vitro evaluation of buoyant controlled release lercanidipine lipospheres. *Journal of Microencapsulation*, *26*(7), 635–641.

Rabbie, S. C., Martin, P. D., Flanagan, T., Basit, A. W., & Standing, J. F. (2016). Estimating the variability in fraction absorbed as a paradigm for informing formulation development in early clinical drug development. *European Journal of Pharmaceutical Sciences*, *89*, 50–60.

Rasul, A., Maheen, S., Khan, H. U., Rasool, M., Shah, S., Abbas, G., Afzal, K., Tariq, F., Shahzadi, I., & Asad, M. H. H. Bin. (2021). Formulation, Optimization, In Vitro and In Vivo Evaluation of Saxagliptin-Loaded Lipospheres for an Improved Pharmacokinetic Behavior. *BioMed Research International*, *2021*, 1–17.

Rout, A., Mohapatra, M., Kumar, L. A., & Patro, S. K. (2022). Recent Trends In Nanoliposphere. *Journal of Pharmaceutical Negative Results*, *13*, 3792–3797.

Saroja, C., & Lakshmi, P. krishnamani. (2013). Formulation and optimization of fenofibrate lipospheres using Taguchi's experimental design. *Acta Pharmaceutica*, *63*(1), 71–83.

Saxena, M. (2021). LIPOSPHERE - LIPID BASED DRUG DELIVERY SYSTEM FOR DELIVERY OF POORLY WATER SOLUBLE COMPOUNDS : A Review. *World Journal of Pharmaceutical Research*, *10*(10), 258–279.

Shivakumar, H., Patel, P., Desai, B., Ashok, P., & Arulmozhi, S. (2007). Design and statistical optimization of glipizide loaded lipospheres using response surface methodology. *Acta Pharmaceutica*, *57*(3), 269–285.

Shukla, D., Chakraborty, S., Singh, S., & Mishra, B. (2011). Lipid-based oral multiparticulate formulations-advantages, technological advances and industrial applications. In *Expert Opinion on Drug Delivery* (Vol. 8, Issue 2, pp. 207–224).

Singh, C., Koduri, L. V. S. K., Singh, A., & Suresh, S. (2015). Novel potential for optimization of antitubercular therapy: Pulmonary delivery of rifampicin lipospheres. *Asian Journal of Pharmaceutical Sciences*, *10*(6), 549–562.

Singh, M. R., Pradhan, K., & Singh, D. (2012). Lipid Matrix Systems with Emphasis on Lipid Microspheres: Potent Carriers for Transcutaneous Delivery of Bioactive. *Curr Drug Deliv*, *9*(3), 243–254.

Toongsuwan, S., Li, L. C., Erickson, B. K., & Chang, H. C. (2004). Formulation and characterization of bupivacaine lipospheres. *International Journal of Pharmaceutics*, *280*(1–2),

57–65.

Yalavarthi, P., Dudala, T., Mudumala, N., Pasupati, V., Thanniru, J., Vadlamudi, H., & Yaga, G. (2014). A perspective overview on lipospheres as lipid carrier systems. *International Journal of Pharmaceutical Investigation*, *4*(4), 149.

Yehia, S. A., Elshafeey, A. H., & Elsayed, I. (2012a). A novel injectable in situ forming poly-DL-lactide and DL-lactide/glycolide implant containing lipospheres for controlled drug delivery. *Journal of Liposome Research*, *22*(2), 128–138.

Yehia, S. A., Elshafeey, A. H., & Elsayed, I. (2012b). Biodegradable donepezil lipospheres for depot injection: optimization and in-vivo evaluation. *Journal of Pharmacy and Pharmacology*, *64*(10), 1425–1437.

Zaki, R. M., Aldawsari, M. F., Alossaimi, M. A., Alzaid, S. F., Devanathadesikan Seshadri, V., Almurshedi, A. S., Aldosari, B. N., Yusif, R. M., & Sayed, O. M. (2022). Brain Targeting of Quetiapine Fumarate via Intranasal Delivery of Loaded Lipospheres: Fabrication, In-Vitro Evaluation, Optimization, and In-Vivo Assessment. *Pharmaceuticals*, *15*(9), 1083.