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Study on Formulation Approach for Nlcs

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Abstract

NLCs have provoked the incessant impulsion for the development of safe and valuable drug delivery systems owing to their exceptional physicochemical and then biocompatible characteristics. Throughout the earlier period, a lot of studies recounting NLCs based formulations have been noticeably increased. They are binary system which contains both solid and liquid lipids aiming to produce less ordered lipidic core. Their constituents particularly influence the physicochemical properties and effectiveness of the final product. NLCs can be fabricated by different techniques which are classified according to consumed energy. More utilization NLCs is essential due to overcome barriers surrounded by the technological procedure of lipid-based nanocarriers' formulation and increased information of the core mechanisms of their transport via various routes of administration. They can be used in different applications and by different routes such as oral, cutaneous, ocular and pulmonary. This review article seeks to present an overview on the existing situation of the art of NLCs for future clinics through exposition of their applications which shall foster their lucid use. The reported records evidently demonstrate the promise of NLCs for innovate therapeutic applications in the future.

Keyword: NLCs, Drug delivery system, Lipid nanocarriers, Application of NLCs

INTRODUCTION

With the help of nanotechnology tools, oncologists can target breast cancer cells while offering various advantages, such as the co-delivery of two or more drugs, dose reduction, and reduce systemic side effects (Wan *et al*., 2019). Different types of nanomaterials are being investigated for therapeutic applications in breast cancer. While nanotechnology-based drug delivery against cancer is in the market or trials, success against cancer depends on early detection. Nanotechnology thus provides novel molecular agents and nanodevices that could enable oncologists to diagnosis breast cancer at its initial stages and allow continuous monitoring during treatment. In the context of cancer detection, nanoparticles with contrasting characteristics are employed as molecular imaging agents to identify tumor cells based on cancer-associated genetic mutations or functional characteristics (Rajitha *et al*., 2021). The initial properties of the nanomaterials examined were based on their physical, mechanical, electrical, magnetic, chemical, and biological applications. However, today attention has been focused on their pharmaceutical applications, especially in the field of drug delivery. Nanotechnology plays an important role in the field of drug delivery and targeting. Several delivery systems have been used to deliver therapeutic agents to the body to achieve the desired effect. This includes studies on the size control of materials at the atomic and molecular levels (Han *et al*., 2019).

Several challenges of using large-scale carriers in drug delivery, include reduced bioavailability, *in vivo* stability, solubility and intestinal absorption, therapeutic effectiveness, systemic side effects, sustained and targeted site delivery, and plasma fluctuations of drugs. Recently, severalresearchers have developed nanoscale drug delivery systems to address these challenges by designing and fabricating nanostructures with superior properties (Prasanna *et al*., 2018). It hasbeen reported that the nanostructures can protect the drug from biodegradation in the gastrointestinal tract and blood, and this nanoscale formulation can deliver the drug to targets in different parts of the body (Shrivastava *et al*., 2020). It enhances the therapeutic effects of the drug and helps reduce unwanted side effects. With the help of nanomaterials, it is also possible to deliver drugs that are sparingly soluble in water and to deliver drugs that bypass the liver and prevent first-pass metabolism (Das *et al*., 2020). Owing to their unique absorption processes, such as absorptive endocytosis, nanomaterials help to boost the oral bioavailability of drugs, which means that they reside in the blood for a long time and can regulate the ingested drug and release it into the bloodstream, leading to decreased plasma fluctuations and

decreased side effects. Nanoscale nanostructures enter the tissue and are quickly absorbed by the cells, allowingrapid drug delivery to the target sites (Hossen *et al*., 2018).

Nanotechnology improves the performance and acceptability of dosage forms by increasing the efficacy, safety, and liability of the patient and reducing medical costs. It may also boost the efficiency of medicines that are unable to reach the clinical trial stage (Safari *et al*., 2014). For more demanding traditional medicines used in the treatment of chronic diseases such as cancer, asthma, hypertension, HIV and diabetes, nanotechnology promises to become the drug delivery of choice. Traditional chemotherapeutic agents are distributed non-specifically to the body, affecting both cancer cells and normal cells, reducing the viable dose in tumors and resulting in sub-optimal therapy due to unnecessary toxicity (Afzal *et al*., 2021). Targeted molecular therapyshould resolve the lack of precision of traditional chemotherapeutic agents. Resistance can bypass the cytotoxicity of traditional chemotherapies, but it can also circumvent the cytotoxicity of these latest molecularly targeted therapies (Florence, 2018). In the biomedical field, nano- formulations include nanoparticles, nanocapsules, nanospheres, nanosuspensions, solid lipid nanoparticles, nanostructured lipid carriers, nanoemulsions, nanomedicine and more have been used for the delivery of therapeutics drug to treat breast cancer (Dinparvar *et al*., 2020).

Formulation Approach for NLCs

Recently, high potential for drug delivery has been attributed to drug carriers with lipid particulates, especially nanometer-ranged colloidal carriers. Lipid particulates include Solid Lipid Nanoparticles (SLNs), Nanostructured Lipid Carriers (NLCs), Lipid Drug Conjugates (LDCs), Lipid Nanocapsules, Nanoemulsions and Liposomes (Alhalmi et al., 2021). Lipid- based nanoformulations have significant potential for oral delivery of hydrophilic and lipophilic drugs, as well as biologicals, and are widely regarded as a superior drug delivery system to colloidal delivery systems. Their benefits in controlling drug release, increase stability, high loading, low toxicity, making them a promising candidate for use as targeted carriers for the treatment of various diseases (Emad et al., 2021). Lipid particulates consists of various types of lipids which are regarded as GRAS (Generally regarded as safe) category and are biodegradable as well as biocompatible in nature (Gaba et al., 2015). NLCs have attracted much interest over the last few years due to their unique properties and behaviors resulting from their small size (Khan et al., 2020). NLCs are made from biocompatible and biodegradable ingredients and are able to incorporate both lipophilic and hydrophilic bioactive substances (Khosa et al., 2018). They made of physiological lipids triglycerides (e.g., tristearin, compritol 888 ATO, and dynasan112), cholesterol, cholesterol butyrate, cetyl palmitate, and stearic acid that are dispersed in a surfactant solution (Tapeinos et al., 2017). In addition to solid lipid components of NLCs, liquid lipids such as (cetiol, almond oil, peanut oil, corn oil, soybean oil, olive oil, oleic acid, sesame oil, L Phosphatidyl choline, soy lecithin, speziol® EOL NF, mygliol® 812 N, suppocire® NC, tegosoft®, and capmul® MCM C8, which are used in combination with solid lipids to ensure a higher drug encapsulation capacity and higher stability during storage (Li et al., 2017).

SLNs produced from solid lipid enlisted various disadvantages like expulsion of drug during storage and poor drug loading capacity. As a result, second-generation lipid nanoparticles known as NLCs have been designed to overcome the disadvantages of SLNs. NLCs were synthesized by combining solid and liquid lipids, which leads to the formation imperfections in the lipid matrix (Thapa et al., 2018). These imperfections in the lipid matrix prevent drug spillage during long-term storage and result in a higher drug payload, which is theprimary reason for choosing NLCs over SLNs (Alhalmi et al., 2022).

Herein, we hypothesized that encapsulation of RLX and NRG into the NLCs could enhance theoral bioavailability of both drugs by enhancing its solubilization in the intestine and byincreasing the intestinal permeability. NLCs loaded with RLX-NRG were prepared via hot homogenization followed by ultrasonication, optimized via Central Composite

Design (CCD),and then characterized in terms of a variety of parameters. Additionally, for proof of the concept, various studies were conducted; *in vitro*, *ex vivo*, and *in vivo* pharmacokinetics. The study's findings are depicted graphically in **Figure 1.1**.

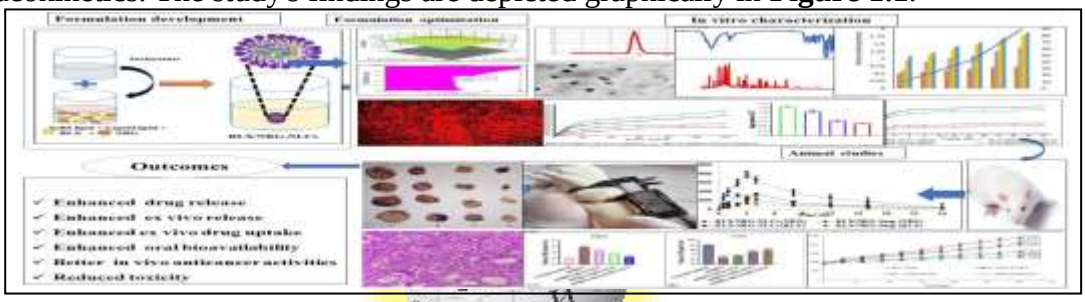

Figure 1.1. Graphical representation of the study.

Materials and Methods

Raloxifene hydrochloride (RLX) was gifted by Aarti Drugs Ltd., (New Delhi, India), while naringin hydrate (NRG), DMBA, and KBr were obtained from Tokyo Chemical Industry CO., Ltd (Tokyo, Japan). Labrasol®, Labrafac®,Precirol ATO 5®, Transutol®, Labrafil®, Caproyl 90®, Capmul MCM®, Gelucire 50/13®, Gelucire 48/16®, and Compritol 888 ATO® were generously provided by (Gattefosse, France). Soyabean oil, peanut oil sesame oil, corn oil, Glyceryl monostearate, and sunflower oil were purchased from Loba Chemie (Chennai, India). Methanol, acetonitrile, ethyl oleate, PEG 400, Tween 80®, D-mannitol, methylene chloride, and hydrochloric acid were received from central drug house Pvt. Ltd. (Chennai, India). Almond oil, olive oil, castor oil, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from SRL. Pvt. Ltd. (Chennai, India). Oleic acid, rhodamine B, and stearic acid were received from Sigma® Aldrich (New Delhi, India). The other materials utilized in this work were of standard analytical quality and used as received from their commercial source.

Excipients screening

An essential pre-formulation step in the fabrication of successful NLCs is selecting appropriateexcipients. We consider the solubility of RLX and NRG in different liquid oils while choosinga liquid lipid for the RLX/NRG-NLCs formulation. In this context, an extra quantity of RLX and NRG were added to 2 mL of chosen oil (viz. oleic acid, sesame oil, ethyl oleate, sunflower oil, rose oil, Capryol 90, corn oil, olive oil, almond oil, castor oil, Labrafac, Capmul MCM) in small glass vials and then mixed briefly on a vortex mixer (Sphinix Pvt. Ltd, Mumbai, India), followed by 72 h of stirring at 25 °C in a mechanical shaker (Grower enterprises, Chennai, India). Following that, the tested samples were spun at 12000 rpm for half-hour using a high- speed centrifuge (Remi Pvt Ltd, Mumbai, India) before being analyzed. Thereafter, 200 µl from the supernatant layer was collected and diluted in 5 mL methanol and estimated for RLX and NRG quantity using a spectrophotometric apparatus (1700-UV, Shimadzu Corporation, Japan) at 289 nm and 284 nm, respectively.

Likewise, RLX and NRG solubility tests were conducted in various solid lipids (Compritol ATO 888, GMS, Stearic acid, Gelucire 50/13, Gelucire 48/16, and Precirol ATO 5) to find the best solid lipid suitable to load maximum quantity of RLX and NRG in the NLC formulation. Briefly, we take a known quantity of both drugs (5 mg) and place it separately in glass vials, towhich 50 mg of each solid lipid was added individually. The blender of lipid and RLX or NRGwas heated in reciprocally agitated bath water to a temperature that was 10 °C above the melting temperature of the solid lipid. Following that, the solid lipid was added by 50 mg at each time until the 5 mg of drugs had been entirely dissolved and the molten lipid had formeda transparent dispersion (Rojekar et al., 2021).

Through the use of various solid-to-liquid lipid ratios, we were able to determine if the optimized binary lipid combination was physically compatible. The binary mixtures were melted with mixing for 1h and then cooled down to room temperature before being visually examined in front of a white sheet to observe the physical integrity of the lipid mixture. The

binary mixture of selected lipids that did not disclose any phase separation was selected for the fabrication of NLCs loading RLX and NRG.

The surfactants have been chosen based on their ability to emulsify the optimal binary mixture of solid lipids and liquid lipids. Surfactants (Tween 60, Tween 80, Transcutol, Labrasol, and Span 20) were used for the screening. A weighted quantity of 300 mg of the optimized binary lipid mixture was dispersed by 20 mL of methylene chloride and 10 mL of surfactant solution (5% w/v). Following that, the solution was heated to 50 °C for 20 min, or till all the methylene chloride had been evaporated. Thereafter, 2 mL of this solution was blended with 10 mL of double-distilled water, and the % transmittance (% T) at 517 nm was estimated utilizing a UV spectrophotometer (Garcês et al., 2018). Furthermore, it was essential to consider the solubilityprofiles of the drugs in surfactants and the HLB values of the surfactants while choosing surfactants.

Experimentation design

In this research, design and optimization of RLX/NRG-NLCs was carried out by central composite design (CCD), and the data was interpreted by (Design Expert® software, 13-version, Stat-Ease, USA). CCD is a combination of mathematical and statistical approaches that are useful for modeling and analyzing situations that are composed of combinations of variables. It is a very adaptable design that helps for the examination of many process factors in a variety of experimental circumstances (Beg, Swain, et al., 2019). The interest response can be investigated using a CCD evaluation that measures how much influence it has on process variables. As an outcome, the number of research experiments that are necessary to identify a mathematical pattern in the experimental design is greatly decreased, allowing for evaluating the variable elements and their optimal level required for a specific response (Javed et al., 2019)(Beg, Hasnain, et al., 2019). Here, several preliminary tests in the process formulation were conducted to identify the method elements that displayed a statistically significant influence on the outcomes. The amount of lipids (mg), the amount of surfactants (mg), and sonication time (min) were the process parameters (independent variables) that were determined from the preliminary experiments.While the RLXand NRG quantities, and ratioof solid lipid to liquid lipid, as well as the ratio of Tween 80 to Labrasol, were all deemed fixedvalues.

In this study, as shown in **Table 1.1**. CCD with 3–factor 3–level was used for screening and optimization of the process variables for preparation of RLX/NRG-NLCs and to test the influence of the amount of lipids (solid lipid and liquid lipid) (A), amount of surfactants (Tween 80 and Labrasol) (B), and the sonication time (C) on the dependent variables (responses), which include the particle size (Y1), PDI (Y2), % entrapment efficiency of RLX (% EE) (Y3), and % entrapment efficiency of NRG (% EE) (Y4). Twenty different designs of the runs were performed to choose the appropriate model and to limit the experimental error. Using a correlation and optimization technique, three-dimensional (3D) surface response plots were evaluated for correlation and optimization of various factors. Moreover, the Design Expert and variance analysis (ANOVA) was used in this study to evaluate the statistical relevance of each model coefficient.

Table 1.1: Independent variables and their actual levels in the CCD.

Factors		**Levels**	
Independent Variables	Low Level (-1)	Medium Level (0)	High Level (+1)
A = Weight of lipid (mg)	200	300	400
B = Weight of surfactant (mg)	100	150	200
C = Sonication time (min)	1	2	3
Dependent Variables		**Desired outcomes**	
Y1 = Particle size (nm)		Minimize	
Y2 = Polydisperisbility index (PDI)		Minimize	
Y3 = % Entrapment efficiency of RLX		Maximize	
Y4 = % Entrapment efficiency of NRG		Maximize	

Formulation of RLX/NRG-NLCs

NLCs loading both RLX and NRG (RLX/NRG-NLCs) and Blank-NLC (without RLX and NRG) were made by hot homogenization with probe sonication as reported previously by (Mangla et al., 2020) with some alterations. Briefly, solid lipid Compritol and liquid lipid oleic acid (total of 200-400 mg) at a 3:1 ratio were mixed and heated with stirring till melting at 75- 80 °C, and NRG and/ or RLX (20 mg and/ or 10 mg respectively), were incorporated into the lipid phase. To create the aqueous phase, Tween 80 and Labrasol (100-200 mg, in a fixed weight ratio of 1:1) were mixed in double-distilled water (20 mL), which was pre-heated to 75- 80 °C. Subsequently, the surfactant aqueous phase was gently dropped dropwise onto the lipid phase and homogenized at 1200 rpm with continuous heating for 20 min. Using a probe sonicator, the proemulsion was further sonicated (work time 20 sec, rest time 5 sec) for 2-4 min, allowing it to settle down to room temperature while being gently agitated. A similar procedure was used to produce RLX-NLCs, NRG-NLCs, and blank NLCs.

Lyophilization of RLX/NRG-NLCsformulations

To lyophilize the RLX/NRG-NLCs, a cryoprotectant comprising 2% (w/v) pure D-mannitol was incorporated. Briefly, RLX/NRG-NLCs were frozen in a petri dish for 24 h, at -20 ^{o} C and the frozen samples were dried under vacuum in a freeze-dryer (Lab Conco., LPYH, Lock 6, USA) for dryness up to 24 h.

***In vitro* characterization of RLX/NRG-NLCs**

X-Ray Diffraction

The X-ray diffractometer (Siemens, D5000, Munich, Germany) was used to characterize the crystalline structure of RLX, NRG, Compritol® 888 ATO, the physical mixture, lyophilized blank NLCs, and lyophilized RLX/NRG-NLCs. Cu-Kα radiation (40 kV; 40 mA) was applied to the powder samples at a scan rate of 0.02°/second over a 2/min, range of 5°–50° (Dudhipala et al., 2020).

Fourier transform infrared spectroscopy

FTIR spectrum of pure materials (RLX, NRG, Compritol, and D-mannitol) and lyophilized (blank NLC & RLX/NRG-NLCs) was generated using the KBr press method and conducted onan IR spectrophotometer (Shimadzu Corp, Kyoto, Japan). The tested powder samples were triturated with 50 times their weight in potassium bromide and pressed into small pellets usinga mini-press under extremely high pressure (3000 psi). The spectra were obtained in the 400– 4000 cm^{-1} wave range (Rajput et al., 2019).

Differential scanning calorimetry

Materials in bulk form (RLX, NAR, compritol 888, and mannitol), as well as lyophilized RLX/NAR-NLCs, were put in aluminum pans for DSC testing, and the results have been reported (DSC 60, Shimadzu, Japan). The heating rate was 10 ℃ per minute, with a nitrogen flow rate of 50 ml per minute. The temperature scan range was performed between 40 and 300 °C (Sun et al., 2021).icroscopic evaluation

RLX/NRG-NLCs morphological surface of the optimized formulations evaluated by transmission electron microscopy (TEM) (Tecnai G20 HR-TEM, Thermo Scientific, USA) and scanning electron microscopy (SEM) (Zeiss EVO 18, Gemini 5, and Germany). The RLX/NRG-NLCs were previously diluted 1:100 by distilled water before use. For TEM analysis, three drops of the diluted sample were dispersed on the surface of the copper grids carbon-coated (mesh 300); the sample was stained with a few drops of 2% (w/v) phosphotungstic acid, and then kept to dry overnight at room temperature. The dried residue of the sample was displayed under the TEM. For SEM analysis, three drops of RLX/NRG-NLCs were placed on a double-sided carbon tape mounted on an aluminum stud, then vacuum coated with gold for 5 min. The dried sample of the optimized RLX/NRG-NLCs was displayed under SEM (Pakdaman et al., 2021).

Particle size, polydispersity index, and zeta potential determination

The intensity average of particle size, PDI, and zeta potential of the optimized

RLX/NRG- NLCs was analyzed by the dynamic light scattering principle using a zeta-sizer device (Malvern Zeta-Sizer, England). Before the measurements, all of the tested samples have been dispersed with 50- folds of Milli-Q water to create 2% uniform dispersion. All obtained values are the mean of three measurements.

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