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Fabrication and Characterization of Triterpenoid Oleanolic Acid NIOSOMES

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KEYWORDS

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ABSTRACT:

Introduction: Phytoconstituents, derived from plants, exhibit diverse pharmacological activities; however, their medical application is frequently impeded by challenges such as poor solubility, limited bioavailability, rapid metabolism, and inadequate targeting to specific sites of action. Oleanolic acid (OA), a natural triterpenoid compound abundant in various plants such as olive oil, garlic, and apples, has emerged as a focal point in cancer research, particularly in the realm of liver cancer therapy. However, its application faces several challenges, including low solubility, limited bioavailability, and the emergence of drug resistance mechanisms. To surmount these hurdles, the formulation of niosomes encapsulating oleanolic acid presents a promising strategy, offering a means to enhance solubility, augment bioavailability, and potentially circumvent issues related to drug resistance.

Objectives: Oleanolic acid has a very low water solubility of about 1 ug/mL and poor permeability which usually results in low oral bioavailability and limited use in clinical treatments. Incorporating OA in niosomal vesicular drug delivery system solubility might be increase which result in increase in bioavailability of OA.

Methods: Niosomes of oleanolic acid was prepared by modified ethanol injection followed by solvent evaporation method. A different combination of span 60 and cholesterol were made to obtained optimized niosomes, span 60, Cholesterol and drug were dissolved separately in ethanol. Cholesterol solution was firstly mixed with span 60. The drug solution was then added to surfactant mixture. The resulting mixture was then added in aqueous phase with help of syringe (flow rate 1.5 mL/min) under constant stirring at 800 rpm on magnetic stirrer. The stirring was continued till 4-6 hours for complete removal of organic phase.

Results: Formulated OA loaded niosmoes were characterized for PDI, average particle size, zeta potential and % entrapment efficiency (%EE). On the basis of these parameter a optimized batch is selected. The optimized batch were further used all other characterization such as, SEM, TEM, invitro drug release etc.

Conclusions: OA loaded niosomes were formulated by modified ethanol injection method. The prepared niosomes were characterized using PDI, average particle size, zeta potential and % entrapment efficiency (%EE), SEM, TEM, invitro drug release techniques. The formulated niosomes of OA shows sustained release of drug from niosomal vesicle.

1. Introduction

Developing innovative drug delivery systems for phytoconstituents necessitates a multidisciplinary approach that amalgamates expertise from pharmaceutical sciences, chemistry, pharmacology, and material science (1). Phytoconstituents, derived from

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plants, exhibit diverse pharmacological activities; however, their medical application is frequently impeded by challenges such as poor solubility, limited bioavailability, rapid metabolism, and inadequate targeting to specific sites of action (2). Consequently, the primary objective in devising effective drug delivery systems for phytoconstituents is to address these hurdles and enhance their therapeutic efficacy (1). Phytoniosomes present a groundbreaking strategy in drug delivery, merging the advantages of niosomes, characterized by non-ionic surfactant-based vesicles, with the therapeutic promise of phytoconstituents sourced from plants (3). Engineered as specialized vesicular systems, phytoniosomes are tailored to encapsulate and transport phytoconstituents, including flavonoids, alkaloids, terpenoids, and polyphenols, to specific target sites within the body for diverse therapeutic intents. Phytoconstituents have long been recognized for their pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties (4). Nevertheless, their clinical translation is frequently impeded by obstacles such as poor aqueous solubility, limited bioavailability, rapid metabolism, and systemic toxicity (5). Through the encapsulation of phytoconstituents within niosomal vesicles, phytoniosomes present a viable solution by offering numerous advantages that mitigate these limitations and augment their therapeutic efficacy.

Incorporating phytoconstituents into niosomes is commonly accomplished through techniques such as thin-film hydration, sonication, or extrusion, facilitating the entrapment of both hydrophilic and hydrophobic phytoconstituents within the vesicular bilayers or aqueous compartments of the niosomes (6). As a result, the resultant phytoniosomes demonstrate enhanced stability, solubility, and bioavailability, along with targeted delivery of phytoconstituents to specific tissues or cells, thereby amplifying their pharmacological effects while mitigating systemic toxicity. Phytoniosomes offer a platform for optimization through adjustments in composition, size, surface charge, and the integration of targeting ligands to fine-tune their physicochemical properties and biodistribution for specific therapeutic applications. These adaptable drug delivery systems hold immense promise across a spectrum of biomedical fields, cancer, cardiovascular diseases, inflammatory disorders, neurodegenerative diseases, and microbial infections (7).

Among the phytoconstituents, oleanolic acid, a natural triterpenoid compound abundant in various plants such as olive oil, garlic, and apples, has emerged as a focal point in cancer research, particularly in the realm of liver cancer therapy (8). However, its application faces several challenges, including low solubility, limited bioavailability, and the emergence of drug resistance mechanisms (7). To surmount these hurdles, the formulation of niosomes encapsulating oleanolic acid presents a promising strategy, offering a means to enhance solubility, augment bioavailability, and potentially circumvent issues related to drug resistance (8).

2. Objectives

Oleanolic acid has a very low water solubility of about 1 ug/mL and poor permeability which usually results in low oral bioavailability and limited use in clinical treatments (12). Incorporating OA in niosomal vesicular drug delivery system solubility might be increase which result in increase in bioavailability of OA.

3. Methods

All research chemicals were purchased from Merck and Cosmo Chem Pvt. Ltd. and used as such for the formulation. Oleanolic acid was purchased from yucca enterprises, Mumbai.

Preparation of nano-niosomes of oleanolic acid:

Niosomes of oleanolic acid was prepared by modified ethanol injection followed by solvent evaporation method. A different combination of span 60 and cholesterol were made to obtained optimized niosomes (table 1). Span 60, Cholesterol and drug were dissolved separately in ethanol. Cholesterol solution was firstly mixed with Span 60. The drug solution was then added to surfactant mixture. The resulting mixture was then added in aqueous phase with help of syringe (flow rate 1.5 mL/min) under constant stirring at 800 rpm on magnetic stirrer. The stirring was continued till 4-6 hours for complete removal of organic phase. The formed niosomes were used for further characterization.

4. Characterization of OA niosomes

Melting Point:

A small amount of oleanolic acid (2-4 mg) was put into a small closed-end capillary tube and gently tapped on the bench top until the sample is at the

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bottom of the tube. The capillary was placed in melting point apparatus and melting point was recorded.

Solubility:
A solvent under consideration (water & ethanol) was saturated with the drug powder and the vials were allowed to stand at temperature (37°C) and kept for 48 hrs for equilibrium with occasionally shaking. Equilibrium solubility was determined by taking supernatant liquid and analysing it on Shimadzu UV double beam spectrophotometer.

Calibration curve:
Calibration curve was constructed in phosphate buffer at pH 7.4 at concentration rang of 5 to 50 µg/mL.

FTIR:
Kbr were previously activated. The sample (1mg) of drug and other excipients and physical mixture of drug-excipient were triturated with Kbr and pellet was formed. This pellet was then filled into die and analysed by FTIR spectrometry in the range of 400-4000 nm.

Particle size, polydispersity index (PDI), and ʒ-potential:
The mean particle size, polydispersity index (PDI), and ʒ-potential of the formulations were characterized by Nano-ZS Zetasizer. The instrument was operated by measuring the autocorrelation function at 90° and temperature of 25 °C. The average size and standard error were measured by the instrument fitting data. Each experiment was conducted in triplicate

Entrapment efficiency:
Niosomal sample (2ml) was taken into a centrifuge tube. Centrifuge the sample at a 18000 rpm for 25 min. after centrifugation, carefully remove the supernatant without disturbing the pellet at the bottom of the tube. The free drug concentration in the supernatant was determined by UV spectrophotometry at 279 nm. The entrapment efficiency (EE) percentage was calculated as follows:

EE% = (Total drug- Free drug)/ Total drug×100

SEM
Surface morphology of the formulation was carried out using scanning electron micro-scope (SEM). The formulation was poured into circular aluminum plate and dried in vacuum oven to form a dry film which was then observed under the scanning electron microscope (FEI, Quantum 200E Instrument).

TEM
Morphology of the prepared niosomes was observed by Transmission Electron Microscopy (TEM). Drug loaded niosomes were diluted with distilled water and sonicated. Few drops of the diluted niosomes were placed on a corbon coated copper grid, which was then placed in the sample holder to capture the images of formulated particles.

In- vitro drug release:
A closed dialysis bag containing 2mg drug and equivalent amount of niosomal formulation was placed in a beaker holding 100 ml PBS 7.4 buffers s. The buffer was magnetically stirred at 150 rpm at 37 °C. At specific time intervals, 1 ml of the solution was removed and immediately replaced by fresh buffer. The drug concentration was subsequently determined spectrophotometrically at 279 nm. The percentages of the released amount of the drug in the buffer are presented as the cumulative release %. The mechanism of release of OA-niosomes was determined by applying mathematical models like zero order kinetics, first order kinetics, higuchi kinetics and korsmeyer peppas models.

5. Results & Discussion:

Melting Point:
The melting point of drug was found tobe 312-315^{O}C. Which confirm the oleanolic acid.

Solubility:
The oleanolic acid was found tobe very less soluble in water whereas in ethanol the solubility was found tobe 8 mg/mL.

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Calibration curve:

Calibration curve was constructed in phosphate buffer at pH 7.4 at concentration rang of 5 to 50 µg/mL and it shows linear relationship with R^2 = 0.9945.

FTIR:

The FTIR spectra of OA, excipients and Physical mixture of OA and excipients were recorded. OA shows characteristics carboxylic acid -OH stretch at 3442.31 $^{cm-1}$ and C=O stretch at 1694.16 $^{cm-1.}$ Cholesterol shows characteristics -OH peak at 3423.99 $^{cm-1.}$ Span40 shows acetic anhydride peak at 1742.37 $^{cm-1}$ and -OH peak at 3322.75 $^{cm-1.}$ All the peaks of OA, span 40 and cholesterol were reappeared in FTIR of physical mixture. Hence, it was confirmed that all excipients and drug was compatible with each other.

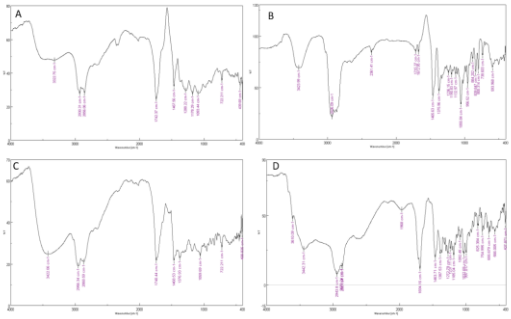

Figure 1: A) Span 60; B) Cholesterol; C) Physical mixture; D) Pure OA.

Particle size, polydispersity index (PDI), and ƺ-potential:

The particle size and PDI of OA loaded niosomal formulation was measured by using Nano-ZS Zetasizer. The average particle size was found to range from 97.3± 8.16 to 162.1± 15.82 (Table 1). The PDI value were found between 0.005 ± 0.104 and 0.378 ± 0.091 (Table 1). This demonstrated monodisperse sample with homogenous population of particle and is considered tobe an acceptable nanocarrier for drug delivery system (9). Niosomes composed of surfactants (span 60) with a lower HLB (6.7) value are expected to have smaller particle size than those with higher HLB values (10). One the basis of particle size, zeta potential and entrapment efficiency the F6 batch was considered as optimized batch. The particle size and PDI of optimized batch F6 was recorded in figure 2.

All the OA loaded niosomes exhibited negative zeta potential value from -18.4 ± 2.82 to -29.1 ± 3.66 (Table 1). The zeta potential of optimized batch F6 was recorded in figure 3. Negative zeta potential value might be due to free hydroxyl group present in cholesterol, non-ionic surfactant molecules and addition of negatively charge inducing molecule i.e. DCP. The higher negative charge might be reducing the tendency of niosome aggregation which result in increase in stability of niosomes during storage condition.

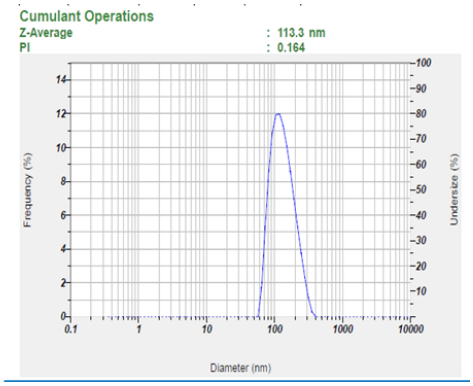

Figure 2: Particle size and PDI of F6 batch

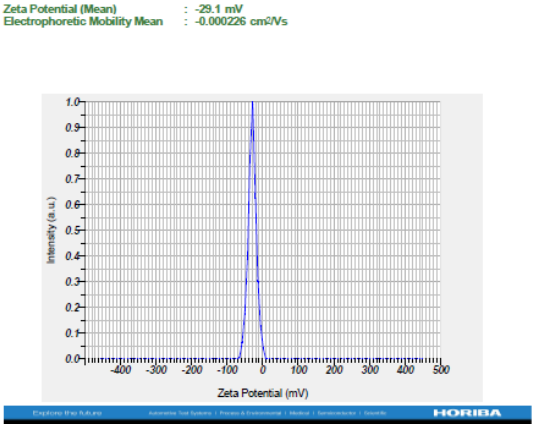

Figure 3: Zeta potential of F6 batch

Entrapment efficiency:

Vesicle size, surfactant type and cholesterol concentration are the factors affecting the effectiveness of entrapment (11). Based on table no 1 the % EE was found to be in the range of 36.71 ± 1.86 to 91.51±2.61. Formulation F6 shows maximum entrapment efficiency. It is due to a

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combination of non-ionic surfactant and cholesterol in niosomes result in more stable and less leaky niosomal vesicles. Cholesterol stabilizes the bilayer, prevent the leakage of vesicle.

Table 1: Particle size, PDI, zeta potential and %EE

Formulation Code	Span 60: Cholesterol:Oleonolic acid (% w/w/w)	Average particle size (nm)	Polydispersity index	Zeta potential (mV)	Entrapment efficiency (%
F1	1:1:1	97.3± 8.16	0.378 ± 0.091	-18.4± 2.82	36.71 ± 1.86
F2	1:2:1	106.8 ± 8.72	0.005 ± 0.104	-20.9±2.14	52.14 ± 3.47
F3	2:2:1	113.3± 12.78	0.208 ± 0.11	-26.0 ± 2.62	53.13 ± 2.41
F4	3:3:1	118± 9.91	0.0.43 ±0.124	-27.8 ± 3.51	68.35 ± 3.28
F5	4:3:1	121.02± 11.47	0.117 ± 0.116	-26.48 ± 3.42	78.21 ± 2.73
F6	**5::5:1**	**113.3± 9.38**	**0.164 ± 0.107**	**-29.1 ± 3.66**	**91.51 ± 2.61**
F7	6:6:1	162.1± 15.82	0.114 ± 1.22	-25.4 ± 2.27	75.97 ± 2.15

SEM & TEM: Microscopic images captured with a light microscope (Figure 4) provided insights into the shape of the formulated niosomal vesicles. Additionally, the niosomal formulation underwent characterization for morphology utilizing both SEM and TEM techniques. SEM revealed a crystal structure of the niosomes, showcasing their smooth surface. Meanwhile, TEM micrographs offered sub-nanometre resolution, revealing spherical, multilamellar vesicles (Figure 4). These images indicated there is no structural deformations, hence confirming the stability of the niosomal formulation.

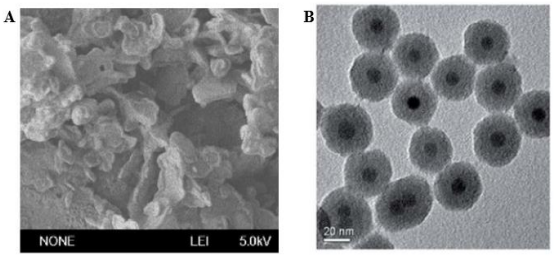

Figure 4: A) SEM photograph of OA Niosome B) TEM photograph of OA Niosome

In- vitro drug release:

The release data of OA niosomes of F6 batch and drug suspension in phosphate buffer at pH 7.4 are presented in figure 4. The in-vitro drug release of formulated niosomes showed biphasic release pattern. The initial burst drug release from formulation is 18.84 ± 0.36 % in 1 hour followed by sustained release of drug was observed till 24 hours. The drug release in 24 hours was 78.84 ± 0.26%.

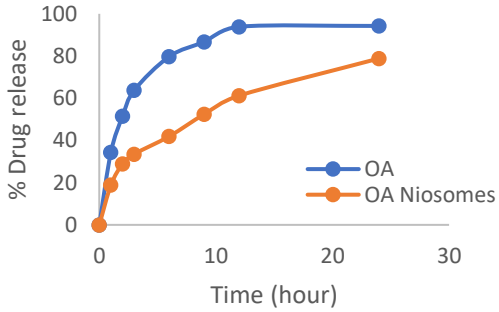

Figure 5: In vitro drug release of F6 batch and drug suspension

Release Kinetic Behaviour:

Mathematical models play a vital role in the interpretation of mechanism of drug release from a dosage form. It is an important tool to understand the drug release kinetics of a dosage form. The in vitro release data was applied to different kinetic models i.e. zero order, first order, higuchi and korsmeyer peppas to determine the mechanism of release of drug from niosomal formulation. Mechanism was interpreted in the form of graphical presentation and evaluated by correlation coefficient (r^2) represented in Table 2. It was found that in vitro release of drug from niosomes was best explained by Higuchi model for selected formulation. The correlation coefficient was found to be 0.9903.

Table 2: Release kinetics

Kinetic model	R^2
Zero order kinetic	0.8485
First order kinetics	0.9838
Higuchi	0.9903
Korsmeyer Peppas	0.5579

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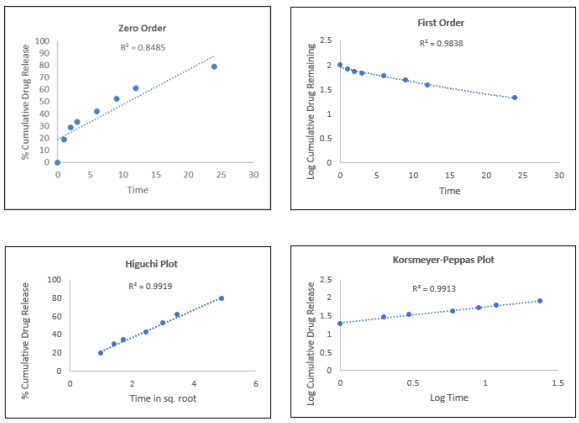

Figure 6: Release kinetic of optimized OA loaded niosomes

Conclusion:

In an effect to enhance the solubility and bioavailability of oleanolic acid, OA loaded niosomes were formulated by modified ethanol injection method. The prepared niosomes were shows an average particle size from 97.3± 8.16 to 162.1± 15.82. Zeta potential value from -18.4 ± 2.82 to -29.1 ± 3.66. % drug entrapment efficiency was found between 36.71 ± 1.86 to 91.51±2.61. SEM and TEM shows a spherical structure of OA loaded niosomes. Invitro drug release of optimized batch i.e. F6 showed biphasic release pattern. The initial burst drug release from formulation is 18.84 ± 0.36 % in 1 hour followed by sustained release of drug was observed till 24 hours. The drug release in 24 hours was 78.84 ± 0.26%.

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