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RESEARCH ARTICLE

Oleanolic Acid Loaded Niosomes as an Effective Phytochemical Nanocarrier: Physicochemical Characterization and Cytotoxicity Assay on HepG2 Cell Line

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Abstract

Background: Oleanolic acid is a triterpenoid with significant anticancer properties, but its clinical application is limited due to its poor solubility and bioavailability. **Aim:** This study explores the use of Oleanolic acid-loaded niosomes as a nanocarrier to boost its effectiveness against liver cancer. **Methods:** Niosomes were created *via* ethanol injecting work flow with cholesterol and Span 60. The formulations analysed for average particle diameter, polydispersity index, particle charge, entrapment efficiency and release profile. Cytotoxic influence of the Oleanolic acid-loaded niosomes was assessed using the HepG2 liver cancer cell line with the MTT assay. **Results:** The optimized niosomes possessed an average vesicular size of 113.3±9.38 nm, a zeta potential of -29.1±3.66 mV and entrapment efficiency of 91.51±2.61%. They showed a sustained release of up to 78.84±0.26% of Oleanolic acid over 24 hours. The niosome formulation significantly decreased HepG2 cell viability as compare to free oleanolic acid and cisplatin, exhibiting a IC_{50} of 36.44 µg/mL, 43.78 µg/L and 74.18 µg/mL respectively, indicating improved shows anticancer efficacy compared to free Oleanolic acid. **Conclusion:** These outcomes imply that Oleanolic acid-loaded niosomes enhance solubility, stability and anticancer activity, making them a promising approach for liver cancer treatment. Further *in vivo* investigations are crucial to definitively assess their clinical effectiveness.

Major Finding: This study demonstrates that oleanolic acid-loaded niosomes enhance the drug's stability, solubility and gives controlled release. The formulation exhibited significant cytotoxicity against the HepG2 cell line, highlighting its potential as an effective nanocarrier for anticancer therapy.

Keywords: Cytotoxicity, Drug Content, Drug Delivery, HepG2, Liver Cancer, MTT, Niosomes, Oleanolic Acid

1. Introduction

Developing innovative drug delivery systems for phytoconstituents requires a multidisciplinary approach that combines knowledge from pharmaceutical sciences, chemistry, pharmacology, and materials science. Oleanolic Acid (OA) is diversely present in numerous medicinally productive herbs. Its broad spectrum of pharmacological effects, including anti-inflammatory, antineoplastic, and immune-modulating actions, has significantly heightened researchers interest. However, the inherent structural properties of oleanolic acid pose challenges for its direct use in new drug formulations, thus limiting its application[1].

Among of actions the distinguishing effects of OA is its hepatoprotector characteristic. However, OA is categorized as a biopharmaceutical class IV drug, characterized by very less water solubility and permeability. This limitation impairs its absorption and bioavailability, constraining its biological effectiveness.

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Researchers are exploring various techniques to boost the water solvation and hence bioavailability of the triterpenoid OA by preparing nanosponge[2], nanosuspension[3], solid dispersion, chemically modified derivatives, phospholipid complexes, nanoscale formulations *etc*[4]. To overcome these obstacles, modern preparation techniques such as non-ionic vesicular system can be used (niosomes).

The utilization of niosomal delivery system for oleanolic acid offers a promising solution to the challenges of poor water solubility and low permeability associated with this compound. Niosomes are non-ionic surface-active agent based vesicular carriers that can wrap hydrophobic drugs, such as oleanolic acid, thereby enhancing their solubility and improving their permeability across biological membranes. By incorporating oleanolic acid into niosomes, it is possible to increase its bioavailability and optimize its therapeutic potential, particularly for targeting liver-related disorders where effective delivery is crucial[5]. OA effectively inhibits Hepatocellular Carcinoma (HCC) by triggering apoptosis and halting the cellular cycle in both engrafted tumours and HepG2 cells. OA triggers cellular self-destruction through the mitochondrion-associated route, as demonstrated by the suppression of the Akt/mTOR signalling, mitochondrial impairment, temporary ATP level spikes, an increased Bax/Bcl-2 ratio, enhanced cytochrome c discharge, and enabling of cysteine proteases and poly (ADP-ribose) polymerase (PARP)[6].

In this research, OA niosomes were developed via a modified ethanol injection method and thoroughly characterized using techniques such as particle size average, polydispersity index, zeta potential assessment, infrared spectroscopy (FTIR), Differential Scanning Calorimetric analysis (DSC). Furthermore, *in vitro* release of oleanolic acid from formulation were carried out to provide an in-depth evaluation of the niosome delivery system. The cytotoxicity of the niosomes was screened on HepG2 cells using the MTT assay[7].

2. Materials and Methods

2.1 Materials

Sorbitan monostearate (Span 60), Cholesterol, ethanol was obtained from New Neeta Chemical Pune. Oleanolic acid was purchased from Yucca Enterprises Mumbai. MTT reagent and dimethyl sulfoxide were obtained from Himedia. The HepG2 cultured cells was obtained from the NCCS, Pune.

2.2 Preparation of Nano-niosomes of Oleanolic Acid by Modified Ethanol Injection Method[8,9]

Oleanolic acid niosomal formulations were formulated using a modified ethanol injecting approach. Precise quantities of non-ionic surfactant (Span 60), cholesterol, and oleanolic acid were dissolved in ethanol in varying weight ratios, ranging from 1:1:1 to 6:6:1, as detailed in Table 1. The cholesterol solution was first mixed with Span 60. The drug solution was then combined to a surfactant composition. The composition was then infused in the aqueous medium at a rate of 1.5 mL/minute using a syringe pump while maintaining a temperature of 60°C and stirring continuously at 800 revolutions per minutes using a magnetic stirrer. The niosomes formed spontaneously upon contact of the organic solution with the aqueous phase[10]. Once the niosomes were formed the organic phase *i.e.* ethanol was removed with the help of rotary evaporator under reduced pressure. The formed niosomes were used for further characterization.

3. Characterization of Formulation

3.1 Melting Point

A small quantity of oleanolic acid was loaded into a capillary tube (closed-end) and gently tapped on the bench to position it at the bottom. The tube was placed in a device that measures melting points. The temperature was recorded as the substance started to melt and continued until it was completely liquid.

Table 1. Formulation table of Oleanolic acid niosomes

Sr. No.	Formulation Code	Span 60	Cholesterol	Oleanolic Acid
1	F1	1	1	1
2	F2	1	2	1
3	F3	2	2	1
4	F4	3	3	1
5	F5	4	4	1
6	F6	5	5	1
7	F7	6	6	1

3.2 Linearity Curve

A linearity curve was developed in phosphate buffer having pH 7.4, covering a concentration range from 5 to 50 µg/ml.

3.3 Particle Size Average, Polydispersity Index and Zeta-potential

Niosomal formulations were analysed for mean particle diameter, PDI, and ζ-potential using a Nano-ZS Zetasizer. Measurements were conducted by evaluating the autocorrelation function at a 90° angle and a 25°C temperature. The instrument provided the average particle size along with standard error based on the fitted data. Each experiment was performed in triplicate to ensure accuracy[10].

3.4 Fourier Transformation Infrared Spectroscopy (FTIR)

FTIR was utilized to procure a spectrum of individual niosomal constituents of *i.e* oleanolic acid, span 60 and cholesterol, a physical mixture of niosomes component and optimized OA loaded niosomes. The spectra were obtained using the KBr disc method under a hydraulic pressin the range of 400-4000 nm. FTIR analysis was performed to investigate drug-excipient compatibilityand examine the structural features of the formed niosomes[11].

3.5 Differential Scanning Calorimetry (DSC)

The thermal characterization of Oleic Acid (OA), Span 60, cholesterol, their composition, and the improved OA-entrapped noisome compositionwere assessed using Differential Scanning Calorimetry (DSC). A sample weighing near about 5 mg was analysed in a packed aluminium pan with a pinhole, in a dry nitrogen flow at 50 mL/min. The temperature was raised from ambienttemperature to 5°C above the samples melting point and held for 5 minutes. Subsequently, the sample was chilled to 223 K (-50°C) and maintained at this temperature for 15 minutes before being reheated to 5°C above its melting point. Thermal management *i.e.*, inducing heat and chilling were conducted at a rate of 10°C/min.

3.6 Entrapment Efficiency

Entrapment efficiency was assessed using a centrifugation technique. A specified amount of niosomal suspension was transferred for centrifugation. Centrifuge the sample at high rpm for 25 min. After centrifugation, carefully remove the supernatant. Examined the transparent supernatant solution using UV spectrophotometry at 210 nm to find out the free drug concentration. Percent Entrapment Efficiency (%EE) was then found out using following formula[12].

$$\% \text{ Entrapment Efficiency} = \frac{Total\ drug - free\ drug}{Total\ drug} x10$$

3.7 *In Vitro* Drug Release

A pre-treated dialysis bag comprising 2 mg of drug and an equivalent quantity of niosomal preparation was immersed in a flask comprising 100 mL of PBS. The buffer was stirred continuously at 150 rpm and sustained at 37°C. At specified intervals, 1 mL of the solution was taken out and refilled with freshly prepared PBS. The amount of the active component in the samples was assessed using UV method at 210 nm. The percent active component release from the niosome formulation was computed. The release kinetics of OA-niosomes were analysed by leveraging different simulation, such as zero-order, first-order, Higuchi, and Korsmeyer-Peppas models[13,14].

3.8 *In Vitro* Cytotoxicity Study

3.8.1 Preparation of Cells

HepG2 liver cancer cells were maintained in Modified Eagle Medium (MEM) fortified with dispensable amino acids and 10% (v/v) bovine serum. The cells were left for incubation at 37°C 5% carbon dioxide environment, with the medium being refreshed every 2 to 3 days.

3.8.2 MTT Assay Procedure

Cells were arranged in well plates at a concentration of 100,000 cells per well in 100 µL of medium. The plates were incubated at 37°C at 5% carbon dioxide environment for 24 hrs. After the incubation period cells were observed for half confluence monolayer. After draining the culture medium, the cells received treatment with 7 different concentration of standard, pure oleanolic acid and niosomal formulation of oleanolic acid. Cisplatin is used as standard. Cells in cell culture medium without any test item incubated and under the same conditions served as control. Plates were incubated at 37°C and 5% CO_2 for 24 hrs. After 24 hrs, cells were observed under

an inverted microscope for changes in morphology or death if any. After observation, culture medium was removed and 100ul fresh medium added with 10ul mg/ml MTT reagent in each well. Plated were incubated for 4 hrs at 37°C in 5% CO_2 incubator. 100ul solubilization (DMSO) was added into each well. Plates were allowed to stand for 1 hr at 37°C in 5% CO_2. Once the formazan purple crystals were fully dissolved, absorbance was determined at 570 nm using a microplate reader. IC_{50} levels were assessed by logarithmic graph of the test compound concentrations against the percentage of cell survival. The cell survival percentage was determined using the following formula.

$$\text{Percent Cell Survival}(\%) = \frac{Absorbance\,of\,test}{Absorbance\,of\,control} x100$$

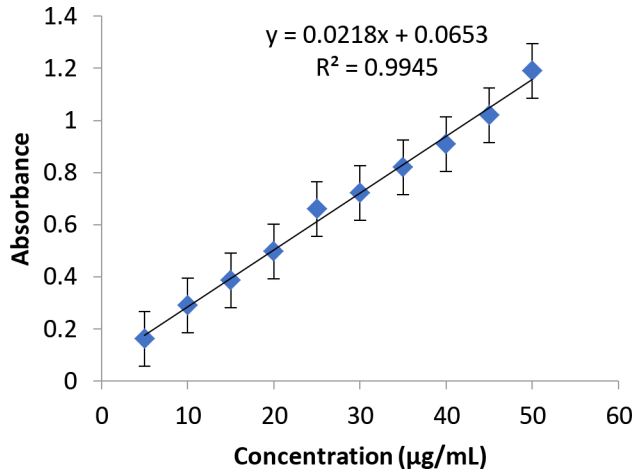
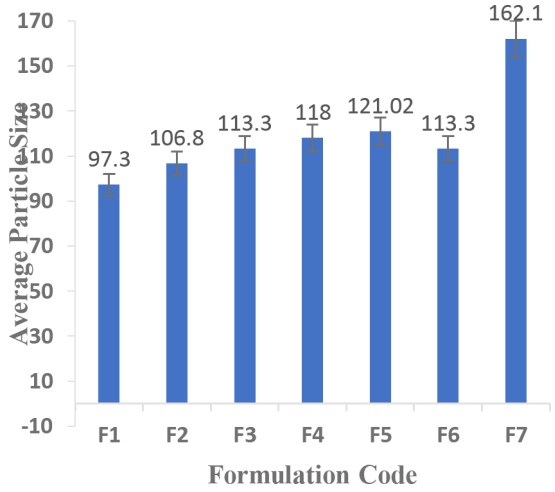


Figure 1. Linearity curve of Oleanolic acid.



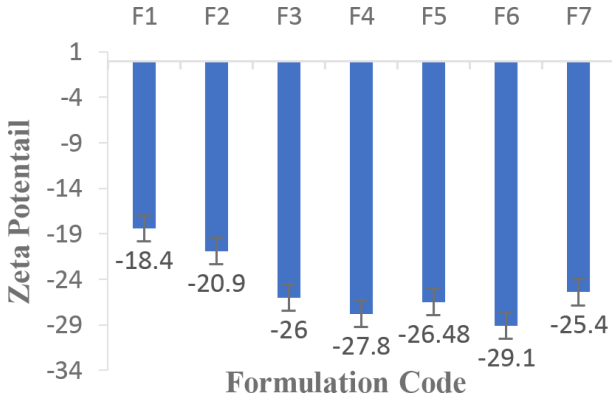


Figure 2. Mean particle size and zeta potential of different formulation batches.

4. Results and Discussion

4.1 Melting Point

The melting point of OA was observed to be 312-315 °C, which confirms its identity as oleanolic acid.

4.2 Linearity Curve

Linearity curve was developed in PBS solution at pH 7.4 at concentration rang of 5 to 50 µg/mL (Figure 1) and it shows linear relationship with $R^2 = 0.9945$.

4.3 Average Particle Size, Zeta-potential and Polydispersity Index (PDI)

The particle diameter average and PDI of OA loaded niosomal formulation was measured by using Nano-ZS Zetasizer. The particle size mean was found in the range of 97.3± 8.16 to 162.1± 15.82 (Figure 2). Smaller particle size is desirable for niosomal formulation as they may enhance the bioavailability of entrapped drug by improving the penetration through different biological barriers and enhancing the cellular uptake of drug particle. Nanosized particles comprises a more area of surface to volume ratio which may enhance interaction between niosomes and targeted cells and tissue[15]. Niosomal composition comprises lower HLB (4.7) value surface active agent (span 60) with anticipated to possess lower particle size than those with greater HLB values[16].

The PDI is a dimensionless value that reflects consistency of particle size distribution in a niosomal formulation. The PDI value were found between 0.005 ± 0.104 and 0.378 ± 0.09. This indicates monodisperse sample with homogenous population of particle and is

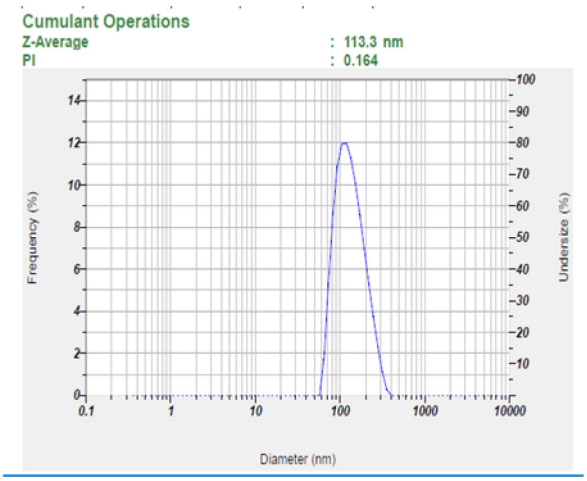


Figure 3. Particle size average and PDI of F6 batch.

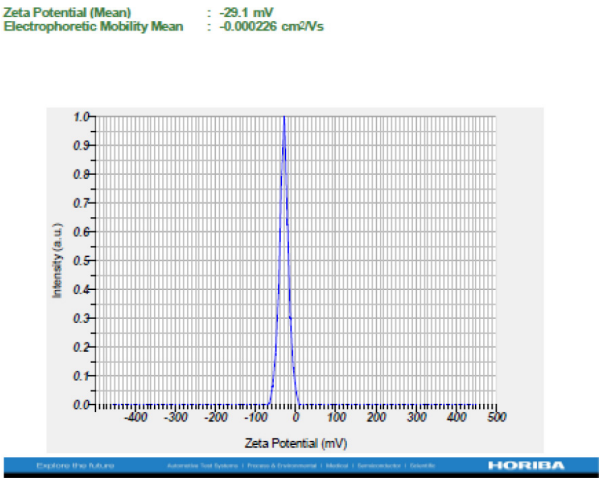


Figure 4. Zeta potential of F6 batch.

an acceptable nanocarrier for drug delivery system[17]. A lower PDI is also indicative of a homogenous niosomal population, which is important for achieving reliable and reproducible therapeutic outcomes. In contrast, a high PDI value denotes a broad size scattering, which can lead to inconsistent drug delivery and reduced formulation stability. The F6 batch was deemed the optimized formulation based on its size of particles, zeta potential, and efficiency of entrapment. The particle size and PDI of refined batch F6 displayed in Figure 3. All the OA loaded niosomes exhibited negative zeta potential value from -18.4 ± 2.82 to -29.1 ± 3.66 (Figure 2). The zeta potential of optimized batch F6 was recorded in Figure 4. 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 niosomes aggregation which result in increase in stability of niosomes during storage condition.

4.4 Entrapment Efficiency

Factors influencing the effectiveness of entrapment include vesicle size, type of surfactant, and concentration of cholesterol[18]. The % EE was found to be in the range of 36.71 ± 1.86 to 91.51±2.61 (Figure 5). Formulation F6 shows maximum entrapment efficiency. This is attributed to the blend of non-ionic surface-active agent and cholesterol, which leads to the formation of extra stable and more impermeable niosome vesicles. Cholesterol stabilizes the bilayer, prevent the leakage of vesicle.

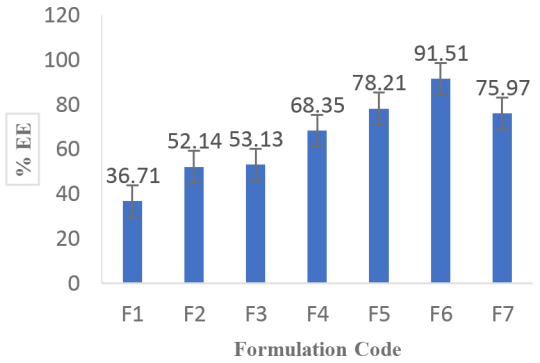


Figure 5. % Entrapment efficiency (%EE) of different formulation batches.

4.5 Fourier Transformation Infrared Spectroscopy (FTIR)

Figure 6 shows FTIR spectra of main components of niosomes (pure oleanolic acid, span60 and cholesterol) and lyophilized powder of formed niosomes were recorded. OA shows characteristics carboxylic acid stretch of OH at 3442cm^{-1}, stretch of CH and aromatics at 2943cm^{-1}, stretch of C=O at 1694cm^{-1}, bend of CH_2 at 1463cm^{-1}, stretch of C-O at 1092cm^{-1} and bend of C-H at 754cm^{-1}. Cholesterol shows characteristics OH peak at 3423cm^{-1}, stretch of CH and aromatics at 2936cm^{-1}, stretch of C=C at 1672cm^{-1}, bend of CH_2 at 1465cm^{-1}, bend of CH_3 at 1375cm^{-1}, stretch of C-O at 1055cm^{-1} and bend of C-H at 738cm^{-1}. Span 60 shows OH peak at 3322cm^{-1}, CH stretch and aromatics at 2930cm^{-1}, stretch of C=O ester at 1742cm^{-1}, CH_3 bend at 1288cm^{-1} and stretch of C-O at 1178cm^{-1}. The FTIR spectrum of the physical mixture clearly exhibited all prominent peaks of OA, Span 60 and cholesterol. The absence

of new peaks confirms that there were no chemical interactions between OA and the other additives. In the FTIR spectrum of OA niosomes there was shift in wave number related to OH stretch from 3422cm^{-1} to 3370cm^{-1}, CH stretch from 2956cm^{-1} to 2939cm^{-1}, C=O ester from 1740cm^{-1} to 1684cm^{-1}, CH_2 bend from 1468cm^{-1} to 1457cm^{-1}, CH_3 bend from 1376cm^{-1} to 1387cm^{-1} and C-O stretch from 1059cm^{-1} to 1082cm^{-1}. All of which confirmed the presence of drug and niosomes vesicles in the structure of final niosomes. Since from above interpretation it can be concluded that OA is encapsulated inside niosome vesicles.

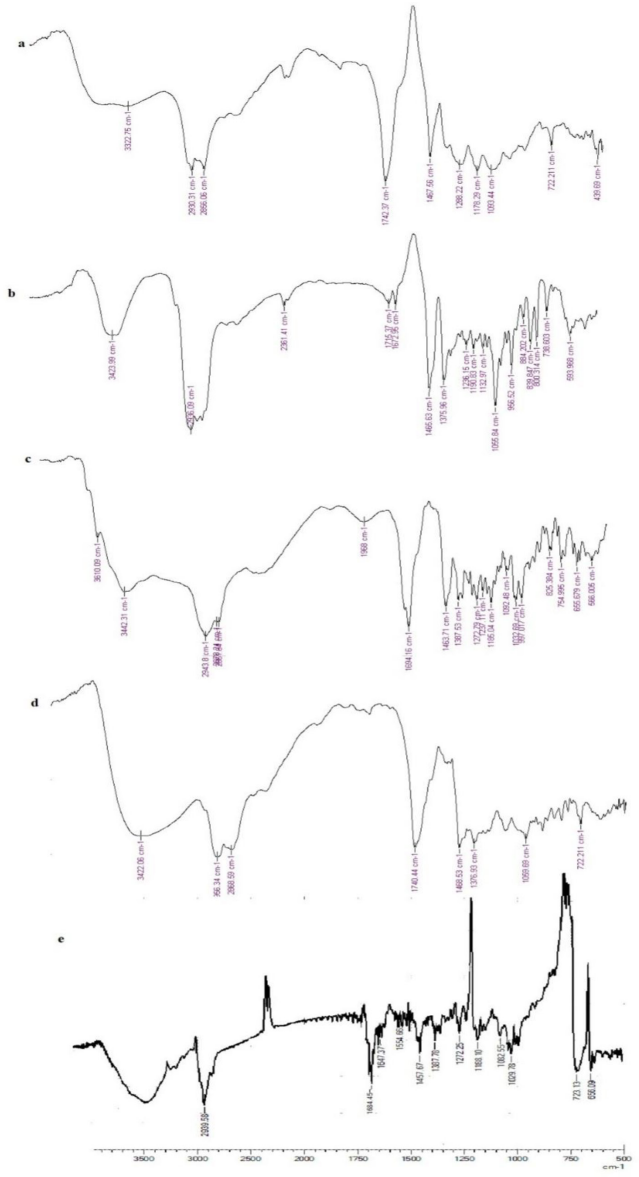


Figure 6. FTIR spectra of **(a)** Span60 **(b)** Cholesterol **(c)** Pure OA **(d)** Physical mixture **(e)** Niosome formulation.

4.6 Differential Scanning Calorimeter (DS)

Figure 7 presents the DSC thermograms for Span 60, cholesterol, pure OA, the physical mixture, and the optimized OA-loaded niosomes. Sharp endothermic peaks were observed for OA at 312.12°C, cholesterol at 148.88°C, and Span 60 at 57.87°C. These distinct peaks were also present in the physical mixture. However, the DSC thermogram for the OA-loaded niosomes showed a complete disappearance of OA's endothermic peak and a shift in Span 60's major endothermic peak from 57.87°C to 50.51°C. This absence of OA's peak indicates that the drug may present in amorphous form and is effectively encapsulated within the niosomal vesicles.

4.7 *In Vitro* Release of Drug

Release of oleanolic acid of optimized F6 batch of niosome and from drug aqueous suspension was executed in phosphate buffer at pH 7.4 (Figure 8). The niosomes shows a biphasic active ingredient discharge profile. There was an initial rapid release of drug 18.84 ± 0.36% in first hour, followed by a gradual release that persisted up to 24 hours, with a total release of 78.84 ± 0.26%. The initial burst is due to the rapid release



Figure 7. DSC spectra of **(a)** Span60 **(b)** Cholesterol **(c)** Pure OA **(d)** Physical mixture **(e)** Niosome formulation.

of OA molecules loosely attached to the niosome surface. In contrast, the sustained release is due to the gradual diffusion of OA from the niosomal core, which is influenced by the vesicular composition and encapsulation efficiency[18,19]. This prolonged release profile makes niosomes a promising carrier system for drug molecules, especially in targeting chronic diseases, where prolonged drug exposure is required for efficacy.

4.8 Release Kinetic Behaviour

Mathematical models are essential for elucidating the manner of drug getting released from preparation. They serve as critical tools for understanding these mechanisms, with each model providing a release equation that is used to precisely fit the data and reveal the release dynamics. From the value of correlation coefficient (r^2) an optimized equation was chosen.

Several kinetic simulations were used to analyse the *in vitro* discharge data. The Zero-order, First-order, Higuchi, and Korsmeyer-Peppas kinetic model were applied to drug release from niosomes, to determine the specific mechanism of drug release from the niosomal formulation[20]. As can be seen from result in Table 2, the *in vitro* release of OA from niosomes was best explained by Higuchi model for selected formulation owing to highest value of r^2.

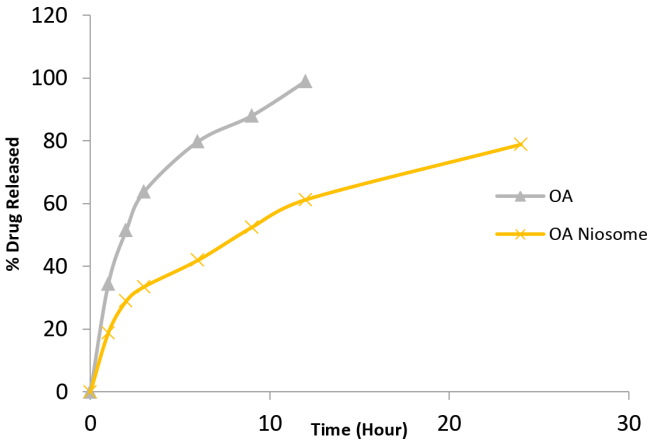


Figure 8. Release of drug from F6 batch and aqueous drug suspension.

4.9 *In Vitro* Cytotoxicity Study

Entrapment of hydrophobic drug into niosomes vesicles can enhance the solubility and improved interaction with niosomes and malignant cells. This may result into the increased cell toxicity of resulting formulation[21,22]. To validate this rational, we have studied cell cytotoxicity of different concentration of free OA, OA loaded niosomes and standard cisplatin in the range of concentration (10-1000 µg/ml) by performing MTT test on HepG2 cells for 24 hrs (Figure 9). For comparison we have chosen the concentration 100 µg/

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

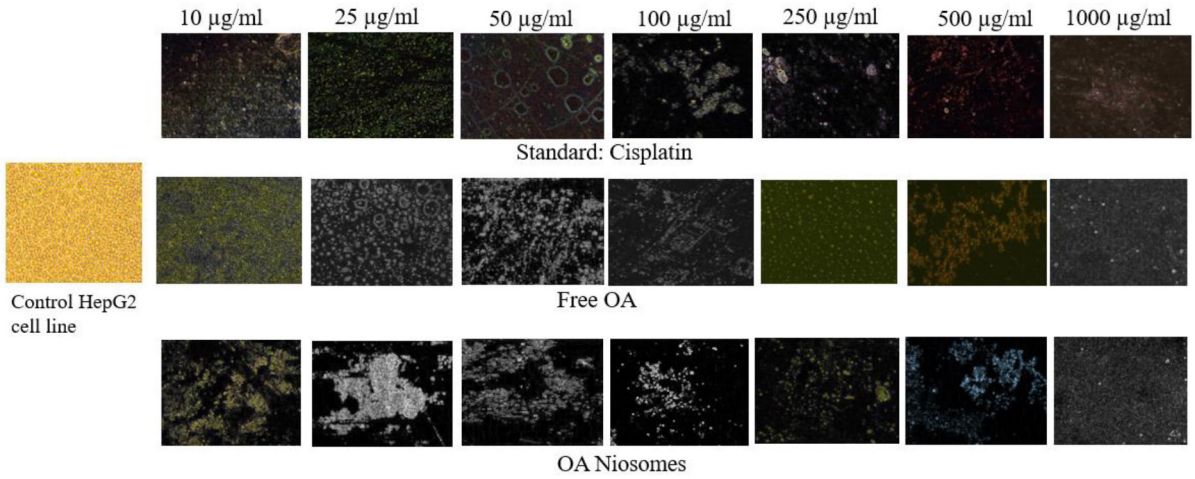


Figure 9. Morphological changes observed at different concentration of standard (Cisplatin), free OA and OA niosomes.

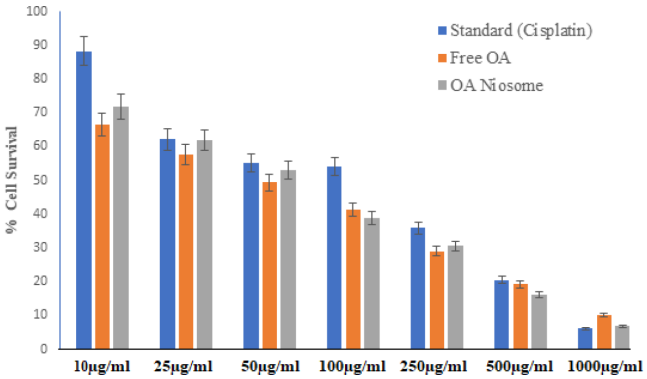


Figure 10. Percentage of Cell survival of standard (Cisplatin), free OA and OA niosomes.

ml for standard, free OA, and noisome formulation. The cytotoxicity of OA increases as it is loaded in niosomes. The percentage cell toxicity at 100 µg/ml was 54.18%, 41.34 %, 39.03% for standard, free OA and OA niosomes respectively. It is shown that cell cytotoxicity of OA loaded niosomes is significantly greater than that of free OA. The cell cytotoxicity increases as a function of drug concertation as shown in Figure 9 and 10. The IC_{50} value of OA niosomes free drug and cisplatin is 36.44µg/ml, 43.78 µg/ml and 74.18µg/ml respectively. Formulated OA niosomes have a lower IC_{50} value which indicate the formulation is more effective for inhibiting the cancer cell growth. It might be due to improved interaction of OA loaded niosomes to cancers cells. From this it can be concluded that loading of hydrophobic drug into niosomes increases its efficacy.

5. 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. *Invitro* drug release of optimized batch *i.e.* F6 demonstrated biphasic discharge 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%. Cell cytotoxicity of OA loaded niosomes is significantly surpassing than that of OA alone. The IC_{50} value of OA niosomes was 36.44**µg/ml and for free drug was** 43.78**µg/ml. Formulated OA** niosomes have a lower IC_{50} value which indicate the formulation is more effective for inhibiting the cancer cell growth. It might be due to improved interaction of OA loaded niosomes to cancers cells. This demonstrates that the niosomal formulation enhances the cytotoxic effects of chemotherapeutic agents on cancer cells, potentially lowering the required dose and reducing the likelihood of side effects.

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7. Author Contribution

Sonali Devne: Whole research work was conducted; Vrushali Kashikar provided essential guidance throughout the research work, offering expertise in study design, experimental methodology and data interpretation; Shashikant Dhole: Reviewed the results and approved the final version of the manuscript; Jyoti Jawale contributed significantly to the discussion and interpretation of the results. All authors have read and agreed to the published version of the manuscript.

8. References

1. Feng A, Yang S, Sun Y, Zhang L, Bo F, Li L. Development and evaluation of oleanolic acid dosage forms and its derivatives. Biomed Res Int. 2020: p.1308749. https://doi.org/10.1155/2020/1308749
2. Li W, Ng KY, Heng PWS. Development and evaluation of optimized sucrose ester stabilized oleanolic acid nanosuspensions prepared by wet ball milling with design of experiments. Biol Pharm Bull. 2014; 37(6):926-937. https://doi.org/10.1248/bpb.b13-00864
3. Li W, Das S, Ng KY, Heng PWS. Formulation, biological and pharmacokinetic studies of sucrose ester-stabilized nanosuspensions of oleanolic acid. Pharm Res. 2011; 28:2020-2033. https://doi.org/10.1007/s11095-011-0428-3
4. Liu Y, Luo X, Xu X, Gao N, Liu X. Preparation, characterization and *in vivo* pharmacokinetic study of

PVP-modified oleanolic acid liposomes. Int J Pharm. 2017; 517(1-2):1-7. https://doi.org/10.1016/j.ijpharm.2016.11.056
5. Uchegbu IF, Vyas SP. Non-ionic surfactant based vesicles (niosomes) in drug delivery. Int J Pharm. 1998; 172(1-2):33-70. https://doi.org/10.1016/s0378-5173(98)00169-0
6. Wang X, Bai H, Zhang X, Liu J, Cao P, Liao N, Zhang W, Wang Z, Hai C. Inhibitory effect of oleanolic acid on hepatocellular carcinoma *via* ERK-p53-mediated cell cycle arrest and mitochondrial-dependent apoptosis. Carcinog. 2013; 34(6):1323-1330. https://doi.org/10.1093/carcin/bgt058
7. Chen Y, Liu J, Yang X, Zhao X, Xu H. Oleanolic acid nanosuspensions: Preparation, *in-vitro* characterization and enhanced hepatoprotective effect. J Pharm Pharmacol. 2005; 57(2):259-264. https://doi.org/10.1211/0022357055407
8. Pando D, Matos M, Gutierrez G, Pazos C. Formulation of resveratrol entrapped niosomes for topical use. Colloids Surf B Biointerfaces. 2015; 128:398-404. https://doi.org/10.1016/j.colsurfb.2015.02.037
9. Estupinan OR, Garcia-Manrique P, Blanco-Lopez MDC, Matos M, Gutierrez G. Vitamin D3 loaded niosomes and transfersomes produced by ethanol injection method: Identification of the critical preparation step for size control. Foods. 2020; 9(10):1367. https://doi.org/10.3390/foods9101367
10. Pham TT, Jaafar-Maalej C, Charcosset C, Fessi H. Liposome and niosome preparation using a membrane contactor for scale-up. Colloids Surf B Biointerfaces. 2012; 94:15-21. https://doi.org/10.1016/j.colsurfb.2011.12.036
11. Afreen U, Fahelelbom KM, Shah SNH, Ashames A, Almas U, Khan SA, Yameen MA, Nisar N, Asad MHHB, Murtaza G. Formulation and evaluation of niosomes-based chlorpheniramine gel for the treatment of mild to moderate skin allergy. J Exp Nanosci. 2022; 17(1):467-495. https://doi.org/10.1080/17458080.2022.2094915
12. Manconi M, Sinico C, Valenti D, Loy G, Fadda AM. Niosomes as carriers for tretinoin. I. Preparation and properties. Int J Pharm. 2002; 234(1-2):237-248. https://doi.org/10.1016/s0378-5173(01)00971-1
13. Danaei M, Dehghankhold M, Ataei S, Davarani FH, Javanmard R, Dokhani A, Khorasani S, Mozafari MR. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharm. 2018; 10(2):57. https://doi.org/10.3390/pharmaceutics10020057
14. Nowroozi F, Almasi A, Javidi J, Haeri A, Dadashzadeh S. Effect of surfactant type, cholesterol content and various downsizing methods on the particle size of niosomes. Iran J Pharm Res. 2018;17(Suppl2):1-11. PMCID: PMC6447874. PMID: 31011337.
15. Xu YQ, Chen WR, Tsosie JK, Xie X, Li P, Wan JB, He CW, Chen MW. Niosome encapsulation of curcumin: characterization and cytotoxic effect on ovarian cancer cells. J Nanomater. 2016; 2016(1):6365295.
16. Xi J, Chang Q, Chan CK, Meng ZY, Wang GN, Sun JB, Wang YT, Tong HH, Zheng Y. Formulation development and bioavailability evaluation of a self-nanoemulsified drug delivery system of oleanolic acid. AAPS PharmSciTech. 2009; 10:172-182. https://doi.org/10.1208/s12249-009-9190-9
17. Feng A, Yang S, Sun Y, Zhang L, Bo F, Li L. Development and evaluation of oleanolic acid dosage forms and its derivatives. Biomed Res Int. 2020: p.1308749. https://doi.org/10.1155/2020/1308749
18. Alkilani AZ, Abu-Zour H, Alshishani A, Abu-Huwaij R, Basheer HA, Abo-Zour H. Formulation and evaluation of niosomal alendronate sodium encapsulated in polymeric microneedles: *In vitro* studies, stability study and cytotoxicity study. Nanomater. 2022; 12(20):3570. https://doi.org/10.3390/nano12203570
19. Reddy LH, Murthy RSR. Etoposide-loaded nanoparticles made from glyceride lipids: Formulation, characterization, *in vitro* drug release, and stability evaluation. AAPS PharmSciTech. 2005; 6(24). https://doi.org/10.1208/pt060224
20. Aldawsari MF, Khafagy ES, Moglad EH, Lila AS. Formulation optimization, *in vitro* and *in vivo* evaluation of niosomal nanocarriers for enhanced topical delivery of cetirizine. Saudi Pharm J. 2023; 31(9):101734. https://doi.org/10.1016/j.jsps.2023.101734
21. Shukla SK, Kulkarni NS, Chan A, Parvathaneni V, Farrales P, Muth A, Gupta V. Metformin-encapsulated liposome delivery system: An effective treatment approach against breast cancer. Pharm. 2019; 11(11):559. https://doi.org/10.3390/pharmaceutics11110559
22. Hajizadeh MR, Parvaz N, Barani M, Khoshdel A, Fahmidehkar MA, Mahmoodi M, Torkzadeh-Mahani M. Diosgenin-loaded niosome as an effective phytochemical nanocarrier: Physicochemical characterization, loading efficiency, and cytotoxicity assay. Daru. 2019; 27:329-339. https://doi.org/10.1007/s40199-019-00277-0