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Formulation, Development And Characterization Of Nanostructured Lipid Carriers For Exemestane

Mr. Avinash B. Swami[1], Dr. Shivappa N. Nagoba[2]*

[1,2*]Department of Pharmaceutics, Channabasweshwar Pharmacy College (Degree), Latur Latur-413512, Maharashtra, India

***Corresponding Author:** Prof. Dr. Nagoba Shivappa N.
*M. Pharm, Ph.D. Professor and Head, Department of Pharmaceutics, Channabasweshwar Pharmacy College (Degree), Kava Road, Latur-413512, Dist. Latur. (MS) Tel: (+91)-2382-641008 (O), (+91)-2382-240008(O), Fax: +91-2382-243855, Mobile: +919637908901E-mail: nagobashivraj@gmail.com,nshivraj11@rediffmail.com

ABSTRACT:

Aim & Objective: This study aimed to formulate, develop and characterize nanostructured lipid carriers (NLCs) for delivering exemestane (EXE), a BCS Class-IV anticancer drug with poor bioavailability, to enhance its therapeutic efficacy.
Methods: NLCs formulated using melt emulsification with cetylpalmitate (solid lipid), almond oil (liquid lipid), cetyl alcohol (surfactant). Developed formulations were tested for appearance, pH, entrapment efficiency, particle size, polydispersity index (PI), zeta potential, *in vitro* diffusion, and stability.
Results: All formulated NLCs appeared as milky white dispersions with a pH of 4-5. Entrapment efficiency for EXE ranged from 59.64% to 80.82%, with NLCEXE2 showing the highest at 80.82%. Particle sizes ranged from 79.90 nm to 694.00 nm; NLCEXE2 had a size of 103.30 nm and a PI of 0.409. Its zeta potential was -32.54 mV, indicating stability. NLCEXE2 had the highest diffusion efficiency (88.42%), while NLCEXE7 had the lowest (60.14%) over 12 hours. Stability studies confirmed that NLCEXE2 maintained its quality over 6 months.
Conclusion: This research highlights NLCs potential as a delivery system for poorly soluble drugs like exemestane, improving therapeutic efficacy. Further research and clinical trials are needed to confirm these findings and facilitate practical applications.

KEYWORDS: Exemestane; solvent evaporation; cetylpalmitate; *in vitro* diffusion.

INTRODUCTION:

Delivering anticancer drugs effectively remains a significant challenge due to issues like poor solubility and limited bioavailability in traditional drug delivery systems. Exemestane (EXE), an oral steroidal aromatase inhibitor used in the adjuvant treatment of hormone-receptor-positive breast cancer in postmenopausal women, exemplifies these challenges. EXE irreversibly binds to the active site of the aromatase enzyme, leading to permanent inhibition, but is classified as a BCS Class-IV drug, characterized by poor solubility and bioavailability.[1,2]

Nanostructured lipid carriers (NLCs) offer a promising solution by enhancing the stability, loading capacity, and controlled release of such drugs. These carriers combine solid and liquid lipids, providing a robust delivery system that addresses the solubility and bioavailability issues associated with EXE. The formulation and optimization of NLCs are crucial for maximizing their effectiveness in drug delivery, involving the selection of appropriate lipids and surfactants, fine-tuning formulation parameters, and performing rigorous characterizations.[3-6]

This study aims to formulate and characterize NLCs containing EXE to improve its delivery and therapeutic efficacy. Through a nanostructured NLCs, preparation using a solvent evaporation method, and detailed characterization, this research seek to identify the optimal formulation for enhancing EXE's bioavailability and effectiveness. This research could advance the field of nanomedicine and offer a significant improvement in cancer treatment protocols.

MATERIALS AND METHODS:

Exemestane (EXE) was generously provided by Cipla Ltd., Mumbai. The other chemicals used include cetylpalmitate, almond oil, cetyl alcohol, and chloroform, all were of AR grade.

Pre-formulation characterization:

Drug authentication by FTIR:

To verify the identity of EXE, Fourier-transform infrared (FTIR) spectroscopy was performed using a Perkin Elmer FTIR instrument. The EXE sample was scanned across a range of 4000 to 400 cm^{-1}. The resulting spectra were compared to the standard profiles outlined in the Indian Pharmacopoeia (IP) and the United States Pharmacopeia (USP) to confirm its authenticity.[7,8]

Drug – Excipients compatibility study:

To detect any potential interactions between EXE and other excipients, a compatibility study was conducted. EXE and the excipients were mixed in a 1:1 ratio and analyzed using FTIR in the mid-infrared range of 4000 to 400 cm^{-1}. Changes in the FTIR spectra, such as the appearance or disappearance of peaks, were monitored to identify any interactions.[7,8]

Ultraviolet spectroscopy:

EXE was calibrated using ultraviolet (UV) spectroscopy with a SHIMADZU instrument. The drug was diluted in PBS at pH 7.4 to prepare concentrations ranging from 0.2 to 1 µg/ml. The UV absorbance was measured in the wavelength range of 400 to 200 nm. A plot of absorbance versus concentration was created to establish the linearity and determine the λmax.[8,9]

Method of preparation:

Nanostructured lipid carriers (NLCs) have formulated by melt emulsification as detailed in Table 1. Cetylpalmitate (solid lipid), almond oil (liquid lipid), mixed and melted at 80°C. Aq. Solution of cetyl alcohol (surfactant) is also heated at same temperature. Both this melts were mixed along with EXE. The mixture was stirred with a magnetic stirrer to produce a milky white dispersion of NLCs, which was subsequently subjected to probe sonication to achieve a uniform particle size.[9-12,22]

Table 1: Formulation table of NLCs

Batch	Cetyl Palmitate (mg)	Almond oil (ml)	Cetyl alcohol (mg)	Chloroform (ml)	Water (ml)
NLCEXE1	30	15.5	3.25	10	Upto100
NLCEXE2	35	15.5	3.25	10	Upto100
NLCEXE3	40	15.5	3.25	10	Upto100
NLCEXE4	45	30	1.5	10	Upto100
NLCEXE5	50	30	1.5	10	Upto100
NLCEXE6	55	30	1.5	10	Upto100
NLCEXE7	60	30	1.5	10	Upto100

Characterizations:

Visual appearance and pH:

Each formulated batch was visually observed to assess its appearance.[13-15] The pH of all formulations was measured using a digital pH meter. 5% aqueous solutions of formulated batch were prepared prior to pH detection.[13-15]

Drug entrapment efficiency:

To assess how well the drug was trapped in the Nano Lipid Carriers (NLCs), centrifugation method was used. The NLC dispersion was centrifuged at 4°C for 90 minutes with a Remi centrifuge. After centrifugation, the clear liquid (supernatant) and the solid part (sediment) were separated. Finally, absorbances have measured of each part at 216 nm with a UV spectrophotometer to calculate the drug content.[16-20,22]

Particle size and Polydispersity index (PI):

The sample was diluted to 0.1% with water and filtered. It was then placed in a cuvette and analyzed with a HORIBA SZ-100 to determine the particle size distribution and polydispersity index by measuring scattered light intensity. Lesser particle size was desired to characterize nanostructure of NLCs.[16-20,22]

Zeta potential:
The zeta potential was measured based on the electrophoretic mobility of the particles. The sample was diluted to a suitable concentration and introduced to the HORIBA SZ-100. Values displayed on monitor were noted. Values other beyond and below 0 are characteristics of stable dispersion.[16-20,22]

% *In vitro* diffusion:
% *In vitro* diffusion study characterizes how NLCs (nanostructured lipid carriers) diffuse using a setup with a cellophane membrane separating two compartments, kept at body temperature. NLC sample is placed in one compartment (Donar) and allowed it to diffuse through the membrane into the other (Receiver). At regular intervals, samples have taken from the receiving compartment, replaced them with fresh media, and diluted with 10 mL of buffer. Samples taken have analyzed on UV spectroscopy to measure the amount of EXE that had diffused over various time intervals.[16-20,22]

Stability study:
Accelerated stability have carried out on optimized NLC formulation using accelerated conditions, following ICH guideline Q1A(R2). The study characterized the formulation's appearance, pH, drug entrapment, and diffusion over time to ensure its stability.[17-22]

RESULTS AND DISCUSSION:

Drug authentication by FTIR:
FTIR spectroscopy of EXE was conducted and peaks were correlated to identify chemical structure of EXE depending on peak intensity obtained. The provided frequencies correspond to various vibrational modes in EXE, particularly focusing on ketones, aldehydes, and aromatic systems. At 1731.15 cm^{-1}, specify the carbonyl stretch (C=O) typical of ketones or aldehydes, indicating a strong, distinct vibrational signature for this functional group. The frequency at 1655.47 cm^{-1} is associated with C=C bond stretching, often seen in aromatic rings or alkenes, reflecting the presence of double bonds within these structures. The aromatic ring stretching vibrations are evident at 1618.66 cm^{-1}, highlighting the presence of conjugated systems. The frequencies between 1406.82 cm^{-1} and 1225.89 cm^{-1} might be related to various C-H bending vibrations, with specific contributions to the overall vibrational pattern depending on the precise chemical environment. At 1002.42 cm^{-1}, of C-H out-of-plane bending vibrations, which are characteristic of aromatic compounds and help in understanding the orientation of hydrogen atoms relative to the ring plane. Aromatic C-H bending vibrations are also indicated at 825.75 cm^{-1}. Finally, lower frequencies around 700.86 cm^{-1} and 507.96 cm^{-1} often correspond to more complex bending and stretching vibrations, which can reveal intricate structural features within the molecule. This conclude EXE molecule. Peaks obtained are figure 1 mentioned below.

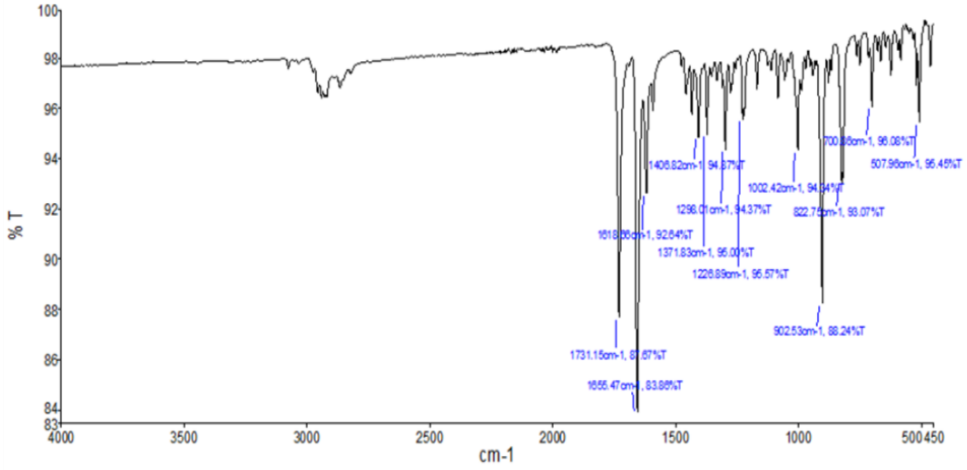

Figure 1: FTIR of EXE

Drug – Excipients compatibility study:
FTIR spectroscopy of physical mixture of EXE with excipients was carried out to access compatibility study. The spectra obtained for physical mixture comprising EXE (A), cetylpalmitate (B), almond oil (C) and cetyl alcohol (D) were reported that there were no any production and deletion of peak found in drug – excipients. Results were mentioned below in figure 2.

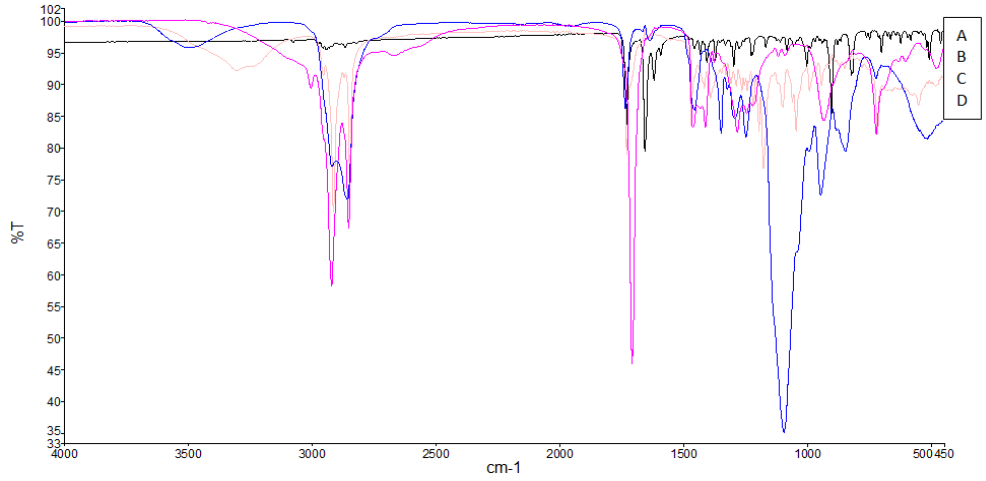

Figure 2: FTIR of physical mixture

Ultraviolet spectroscopy:

UV spectroscopy of EXE has taken in phosphate buffer solution by analyzing prepared working dilutions in ascending order. Linearity was found with equation y=0.185x+0.093; R^2=0.996. Result shown in table 2 & figure 3.

Table 2: Absorbances found in UV

Concentration (µg/ml)	Absorbance at 216 nm
0.2	0.134
0.4	0.165
0.6	0.199
0.8	0.241
1	0.281

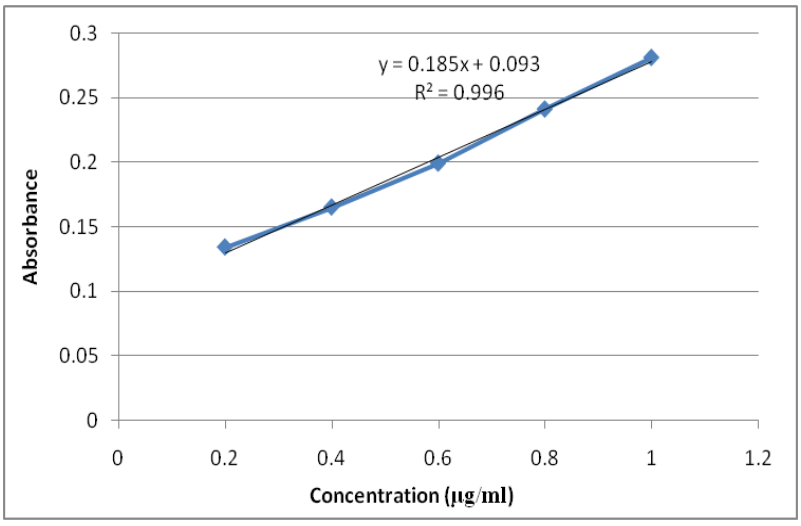

Figure 3: UV spectroscopy of EXE

Particle size & PI, Zeta potential, % Drug entrapment:

All NLC formulations appear milky white and have a weakly acidic pH of 4–5. Among the batches particle sizes are 79.90 - 694.00 nm, polydispersity index (PI) found as 0.148 - 0.741, zeta potential -25.58 to -36.55 and % drug entrapment was found in the range of 59.64 - 80.82%

Among all NLCEXE2 have a slightly larger particle size (103.30 nm) than NLCEXE 1 & or 3 and a higher PI of 0.409, indicating a broader size distribution. Its zeta potential of -32.54 mV reflects better stability, and it achieves the highest drug entrapment at 80.82%. Hence it is considered as best optimized one.

Table 3: Results of Particle size & PI, Zeta potential, % Drug entrapment

Batch	Particle size (nm)	PI	Zeta potential (mV)	% Drug entrapment
NLCEXE1	79.90	0.148	-25.58	75.61
NLCEXE2	103.30	0.409	-32.54	80.82
NLCEXE3	96.90	0.471	-29.37	78.21
NLCEXE4	207.30	0.741	-36.46	62.19
NLCEXE5	566.40	0.575	-36.55	68.42
NLCEXE6	694.00	0.709	-31.18	63.04
NLCEXE7	169.40	0.271	-26.76	59.64

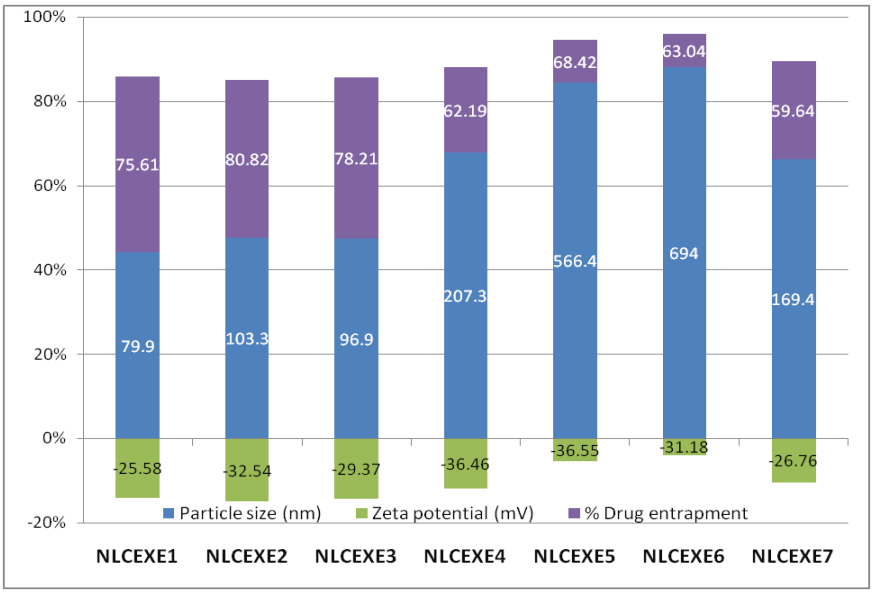

Figure 4: Graphical presentation of Particle size, Zeta potential & % Drug entrapment

% *In vitro* diffusion:

The *in vitro* diffusion study shows that different samples (NLCEXE1 through NLCEXE7) release EXE at varying rates over time. Initially, diffusion is minimal. After one hour, NLCEXE1 has the highest diffusion (10.16%), and NLCEXE7 the lowest (6.31%). By two hours, NLCEXE1 remains the highest (22.36%), while NLCEXE7 is still the lowest (10.15%). At four hours, NLCEXE1 and NLCEXE2 have the highest values (36.9% and 34.48%), whereas NLCEXE7 has the lowest (16.81%). After six hours, NLCEXE1 and NLCEXE2 continue to lead (44.8% and 42.91%), and NLCEXE7 is at 22.12%. By eight hours, diffusion rates are nearing equilibrium, with NLCEXE2 at 59.81% and NLCEXE7 at 35.87%. At twelve and twenty-four hours, diffusion stabilizes, with NLCEXE2 and NLCEXE1 showing the highest rates (70.97% and 85.34%, respectively), and NLCEXE7 the lowest (60.14%). Overall, NLCEXE2 exhibits the highest diffusion efficiency, while NLCEXE7 is the least effective. Results are mentioned in table 4 and figure 5.

Table 4: % *In vitro* diffusion

Time (Hr)	NLCEXE1	NLCEXE2	NLCEXE3	NLCEXE4	NLCEXE5	NLCEXE6	NLCEXE7
0	0	0	0	0.00	0.00	0	0
1	10.16	8.48	8.26	7.92	7.83	7.22	6.31
2	22.36	21.25	13.04	14.12	13.54	12.4	10.15
4	36.9	34.48	25.95	25.53	23.89	18.43	16.81
6	44.8	42.91	32.22	35.80	34.35	26.91	22.12
8	57.29	59.81	42.04	48.96	47.65	35.15	35.87
12	68.36	70.97	62.95	60.30	58.10	47.48	42.98
24	85.34	88.42	81.19	79.75	72.85	68.04	60.14

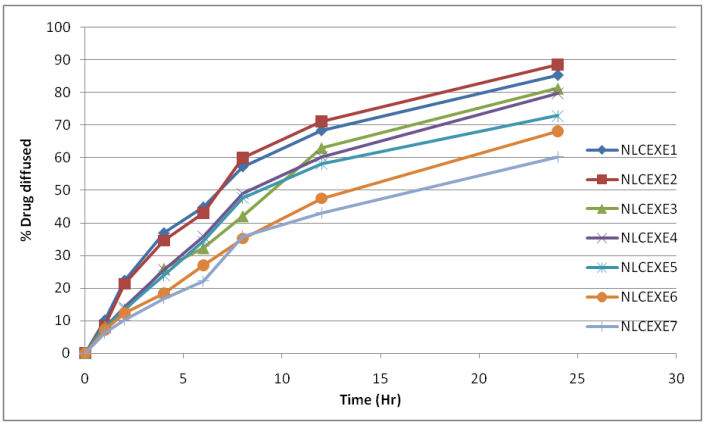

Figure 5: % *In vitro* diffusion

Stability study:

The stability study results indicate that the formulation remained visually consistent throughout the 6-month period, retaining its milky white appearance without any changes. The pH of the formulation stayed steady at 4.16, showing no fluctuations over time. Drug entrapment efficiency saw a slight decrease from 80.82% initially to 79.94% after 6 months, suggesting a small loss in drug entrapment over time. Similarly, in vitro diffusion of the EXE at 24 hours decreased marginally from 88.42% to 87.55% over the same period. Overall, the formulation demonstrated good stability, with only minor variations which is in drug entrapment and diffusion efficiency however these are considered insignificant as per ICH guidelines.

Table 5: Stability study

Period	**Initial**	**After 1 month**	**After 3 months**	**After 6 months**
Appearance	Milky white	No change	No change	No change
pH	4.16	No change	No change	No change
% Entrapment efficiency	80.82	80.73	80.16	79.94
% *In vitro* diffusion at 24th hr	88.42	88.25	87.98	87.55

CONCLUSION:

Executed research concludes, the potential use of NLCs as an advanced drug delivery system for poorly soluble drugs like exemestane. The successful development and optimization of NLCs in this study could significantly enhance the delivery and efficacy of anticancer therapies, contributing to more effective and patient-friendly treatment options. Further investigations and clinical evaluations will be essential to translate these findings into practical therapeutic solutions.

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REFERENCES:

1. Amaral C, Correia-da-Silva G, Almeida CF, Valente MJ, Varela C, Tavares-da-Silva E, Vinggaard AM, Teixeira N, Roleira FMF. An exemestane derivative, oxymestane-D1, as a new multi-target steroidal aromatase inhibitor for estrogen receptor-positive (ER+) breast cancer: effects on sensitive and resistant cell lines. Molecules. 2023;28:789. https://doi.org/10.3390/molecules28020789
2. Exemestane drug profile [Internet]. Available from: https://go.drugbank.com/drugs/DB00990
3. Rizwanullah M, Perwez A, Alam M, Ahmad S, Mir S, Rizvi M, Amin S. Polymer-lipid hybrid nanoparticles of exemestane for improved oral bioavailability and anti-tumor efficacy: an extensive preclinical investigation. Int J Pharm. 2023;642:123136.

4. Khan S, Sharma A, Jain V. An overview of nanostructured lipid carriers and its application in drug delivery through different routes. Adv Pharm Bull. 2023 Jul;13(3):446-60.
5. Garg J, Pathania K, Sah SP, et al. Nanostructured lipid carriers: a promising drug carrier for targeting brain tumours. Futur J Pharm Sci. 2022;8:25. https://doi.org/10.1186/s43094-022-00414-8
6. Kanojia N, Sharma, Gupta N, Singh S. Applications of nanostructured lipid carriers: recent advancements and patent review. Biointerface Res Appl Chem. 2022;12:638-52.
7. Kassem A, Abbas L, Coutinho O, Opara S, Najaf H, Kasperek D, Pokhrel K, Li X, Tiquia-Arashiro S. Applications of Fourier transform-infrared spectroscopy in microbial cell biology and environmental microbiology: advances, challenges, and future perspectives. Front Microbiol. 2023 Nov 21;14:1304081.
8. Venkata Ramana P, Rama Krishna Y, Chandra Mouli K. Experimental FT-IR and UV–Vis spectroscopic studies and molecular docking analysis of anti-cancer drugs exemestane and pazopanib. J Mol Struct. 2022;1263:133051. https://doi.org/10.1016/j.molstruc.2022.133051
9. Kim MH, Kim KT, Sohn SY, Lee JY, Lee CH, Yang H, Lee BK, Lee KW, Kim DD. Formulation and evaluation of nanostructured lipid carriers (NLCs) of 20(S)-protopanaxadiol (PPD) by Box-Behnken design. Int J Nanomedicine. 2019 Oct 25;14:8509-20.
10. Salunkhe SS, Bhatia NM, Pokharkar VB, Thorat JD, Bhatia MS. Topical delivery of idebenone using nanostructured lipid carriers: evaluations of sun-protection and anti-oxidant effects. J Pharm Investig. 2013;43(4):287-303.
11. Ebtavanny T, Soeratri W, Rosita N. Effect of lipid composition on nanostructured lipid carrier (NLC) on ubiquinone effectiveness as an anti-aging cosmetic. Int J Drug Delivery Technol. 2018;8(10):252-58.
12. Bondì ML, Craparo EF, Giammona G, Cervello M, Azzolina A, Diana P, Cirrincione G. Nanostructured lipid carriers-containing anticancer compounds: preparation, characterization, and cytotoxicity studies. Drug Deliv. 2007;14(2):61-7. https://doi.org/10.1080/10717540600739914
13. Moin A, Deb TK, Osmani RA, Bhosale RR, Hani U. Fabrication, characterization, and evaluation of microsponge delivery system for facilitated fungal therapy. J Basic Clin Pharm. 2016 Mar;7(2):39-48.
14. Orlu M, Cevher E, Araman A. Design and evaluation of colon-specific drug delivery system containing flurbiprofen microsponges. Int J Pharm. 2006;318:103-17.
15. Pawar AP, Gholap AP, Kuchekar AB, Bothiraja C, Mali AJ. Formulation and evaluation of optimized oxybenzone microsponge gel for topical delivery. J Drug Deliv. 2015;2015:261068.
16. Zaki Rizkalla CM, Latif Aziz R, Soliman II. In vitro and in vivo evaluation of hydroxyzine hydrochloride microsponges for topical delivery. AAPS PharmSciTech. 2011;12:989-1001.
17. Kumari K, Sachan AK, Singh S, Kapoor A. Formulation and evaluation of nanostructured lipid carrier for the management of psoriasis. Int J Pharm Sci & Res. 2021;12(6):3381-90.
18. Nerli GM, Gonçalves LMD, Cirri M, Almeida AJ, Maestrelli F, Mennini N, Mura PA. Design, evaluation, and comparison of nanostructured lipid carriers and chitosan nanoparticles as carriers of poorly soluble drugs to develop oral liquid formulations suitable for pediatric use. Pharmaceutics. 2023;15:1305.
19. Wissing SA, Kayser O, Muller RH. Solid lipid nanoparticles for parenteral drug delivery. Adv Drug Deliv Rev. 2004;56:1257-72.
20. Mehnert W, Mader K. Solid lipid nanoparticles: production, characterization and applications. Adv Drug Deliv Rev. 2001;47:165-96.
21. ICH Guidelines. ICH Q1A(R2): Stability Testing of New Drug Substances and Products. International Conference on Harmonisation; 2003. Available from: ICH Official Website.
22. Jenning V, Thunemann AF, Gohla SH. Characterization of a novel solid lipid nanoparticle carrier system based on binary mixtures of liquid and solid lipids. Int J Pharm. 2000;199:167-77.