Design, Development, and Optimisation of Loteprednol-Loaded Spandex Nanocarriers for the Management of Uveitis

Prajakta Parasharam Shinde[1], Swati Bhimrao Kale[2], Runjhun Pallavi[3], G Buela Priyanka[4], Ashok Kumar BS[5], Thenmozhi G[6], B. Divya[7], Arvind Kumar Gupta[8], Amit Kumar Singh[9*]

[1]SMBT College of Pharmacy Dhamangaon, Nashik, Maharashtra, 422403, India
[2]Siddhant College of Pharmacy, Pune, Maharashtra, 412109, India
[3]SGT College of Pharmacy, SGT University, Gurugram, 122505, Haryana, India
[4]Department of Pharmaceutical Chemistry, Guru Nanak Institute Technical Campus, School of Pharmacy, Ibrahimpatnam, Rangareddy dst., Telangana, 501506, India
[5]Department of Pharmacognosy, R.L. Jalappa College of Pharmacy, Sri Devaraj Urs Academy of Higher Education and Research (A Deemed to Be University), Tamaka, Kolar-563103, Karnataka, India
[6]Arulmigu Kalasalingam College of Pharmacy, Virudhunagar, Tamilnadu, 626126, India
[7]MNR College of Pharmacy, Siddipet, Telangana, 505467, India
[8]Department of Pharmacognosy, Nand Kishore Pharmacy College (NKPC), Chaka Road, Naini, Prayagraj, Uttar Pradesh, India
*[*9]Department of Pharmaceutics, United Institute of Pharmacy, (Affiliated to Dr. APJ Abdul Kalam Technical University, Lucknow), A-31/1, UPSIDC Industrial Area, Naini, Prayagraj, Uttar Pradesh, 211010, India*

***Corresponding author:** Amit Kumar Singh, Department of Pharmaceutics, United Institute of Pharmacy, (Affiliated to Dr. APJ Abdul Kalam Technical University, Lucknow), A-31/1, UPSIDC Industrial Area, Naini, Prayagraj, Uttar Pradesh, 211010, India

KEYWORDS

Teprednol, spanlastic nanocarriers, ocular inflammation, drug delivery, response surface methodology, formulation optimization

ABSTRACT

Ocular inflammation presents a considerable challenge in therapeutic management, primarily due to inadequate drug bioavailability and restricted permeability across ocular barriers. This research investigates the design and optimisation of Loteprednol-loaded spanlastic nanocarriers as an innovative method for addressing ocular inflammation. Spanlastics, characterised by their elastic and deformable properties, were developed to improve drug delivery to ocular tissues. A response surface methodology was utilised to optimise the nanocarrier formulation systematically, evaluating the effects of key variables including Span 60 concentration, edge activator type, and drug-to-lipid ratio on entrapment efficiency, particle size, and elasticity. The optimised formulation demonstrated nanoscale particle size (<150 nm), high entrapment efficiency (>85%), and optimal deformability, thereby ensuring efficient drug delivery. Ex vivo permeation studies indicated a marked enhancement in corneal penetration relative to conventional formulations. In vivo studies on anti-inflammatory efficacy demonstrated a significant reduction in ocular inflammation, highlighting the therapeutic potential of spanlastic nanocarriers. The findings demonstrate that Loteprednol-loaded spanlastics serve as an effective drug delivery system, providing improved bioavailability, targeted delivery, and prolonged therapeutic effects for the treatment of ocular inflammation. This study establishes a solid basis for subsequent clinical research and the advancement of sophisticated ocular drug delivery systems.

INTRODUCTION:

The term "ocular inflammation" refers to a group of conditions that affect the internal and external tissues of the eye. These conditions frequently result in discomfort, pain, abnormalities in vision, and even the potential loss of vision if they are not treated [1-3]. Corticosteroids, which are recognised for their powerful anti-inflammatory properties, are the primary constituent of traditional treatment procedures. One example of such a corticosteroid is loteprednol etabonate. Despite this, the therapeutic efficacy of these drugs is sometimes hindered by factors such as limited ocular bioavailability, rapid drug excretion through tears, and systemic side effects that are the result of nonspecific drug distribution [4-6].

These limitations highlight the need for innovative drug delivery systems that are capable of overcoming the physiological and anatomical impediments that are present in the eye while yet maintaining the therapeutic efficacy of the treatment or medication. It has recently come to light that nanocarriers, namely spanlastic vesicles, are a promising approach for the delivery of drugs to the eye. In order to promote improved penetration through the tight junctions of ocular tissues, spanlastics are nanocarriers that are elastic and malleable. They are created from non-ionic surfactants and edge activators [5-7].

They have a flexible architecture that improves drug permeability and ensures sustained medication release, which ultimately results in a reduction in the frequency of doses and an improvement in patient compliance. By encapsulating hydrophobic medications like loteprednol, spanlastics have the potential to overcome the constraints of standard eye drops and gels, so providing a therapeutic solution that is more effective and more specifically targeted. In order to effectively treat ocular inflammation, the purpose of this study is to create spanlastic nanocarriers that are loaded with loteprednol and then optimise their performance. For the purpose of this study, response surface methodology (RSM) is utilised to conduct a comprehensive analysis of the influence that formulation variables have on essential quality characteristics [6-8].

These characteristics include particle size, entrapment efficiency, and elasticity. Furthermore, in order to prove the therapeutic potential of the optimised formulation, it is examined for its performance both ex vivo and in vivo. The findings of this study reveal a novel formulation that enhances the absorption of medication, decreases the presence of systemic side effects, and provides anti-inflammatory effects that last for a longer period of time. As a result, this formulation optimises ocular drug delivery and fills a key clinical gap in ophthalmic care [8-10].

Material and Methods:

Materials:

Loteprednol etabonate (LE) was procured as a gift sample from a pharmaceutical company. Span 60 and cholesterol were purchased from Sigma-Aldrich (USA). Tween 80 and ethanol were obtained from Merck (Germany). All other chemicals and reagents used were of analytical grade and were used without further purification. Deionized water was used throughout the study.

Methods:

Preparation of Loteprednol-loaded Spanlastic Nanocarriers

The ethanol injection approach was chosen for the preparation of the spanlastic nanocarriers because of its ease of use and its ability to be performed repeatedly [10-12].

Preparation of Organic Phase:

Under the conditions of the experimental design, Span 60 and cholesterol were dissolved in ethanol in a variety of different proportions. In order to ensure that the loteprednol was completely dissolved, it was added to this mixture and stirred [12-14].

Preparation of Aqueous Phase:

Tween 80, a nonionic surfactant, was dissolved in deionised water at room temperature to prepare the aqueous phase. Deionised water eliminated ions and contaminants that could affect nanocarrier stability and performance. Tween 80 was kept at room temperature to maximise solubility and prevent heat degradation. The water solution was gently stirred with a magnetic stirrer to dissolve. This ensured homogeneity and prevented micelles or aggregates from affecting formulation efficiency. The ethanol injection approach relied on the prepared aqueous phase to encapsulate Loteprednol and create spanlastic nanocarriers [14-16].

Injection Process:
A magnetic stirrer was used to continuously stir the organic phase into the water phase at 800 rpm as drops were fed into each. For an extra half an hour, the resultant suspension was mixed to make sure the nanocarriers were distributed evenly [17-19].

Size Reduction:
To reach the appropriate particle size, the dispersion was sonicated using a probe sonicator for 10 cycles of 1 minute on/off. The formulations were kept in glass vials with an amber colour at 4°C until they were ready for analysis [18-20].

Experimental Design and Optimization:
Optimisation of the formulation was carried out utilising a Box-Behnken Design (BBD) in Response Surface Methodology. The design comprised fifteen experimental runs, each with three replicates at the midpoint. In order to determine the best conditions for formulation, data were examined using the Design-Expert® program [19-21].

Table 1: Experimental design and optimization process

Sr. No.	Parameter	Description
1.	Independent Variables	1. Span 60 concentration (X_1)
		2. Tween 80 concentration (X_2)
		3. Drug-to-lipid ratio (X_3)
2.	Dependent Variables	1. Particle size (nm)
		2. Entrapment efficiency (%)
		3. Elasticity index

Characterization of Spanlastic Nanocarriers
Particle Size and Polydispersity Index (PDI) and Zeta Potential:
The Zetasizer (Malvern Instruments, United Kingdom) and dynamic light scattering (DLS) were utilised to conduct the evaluation. Using the same Zetasizer, we want to find out how stable nanocarriers are and what their surface charge is. Through the integration of particle size, PDI, and zeta potential data, a thorough comprehension of the nanocarriers' physical properties and stability was achieved. This, in turn, lends credence to the idea that they could be utilised for the reliable and efficient administration of ocular medication [20-22].

Entrapment Efficiency (EE):
The nanocarriers were separated by centrifugation at 15,000 rpm for 30 minutes. The amount of free Loteprednol in the supernatant was quantified using UV-visible spectroscopy at 241 nm [21-23]. EE was calculated using the formula:

$$\text{EE}\ (\%) = \frac{\text{Total drug} - \text{Free drug}}{\text{Total drug}} \times 100$$

Elasticity Index:

The formulation was evaluated by extruding it through polycarbonate membranes with different pore sizes, and the elasticity was calculated based on the flow rate and pressure [22-24].

***In-Vitro* Drug Release Studies:**
The evaluation of the drug release profile was conducted utilising a dialysis membrane method. A suspension of the nanocarrier (equivalent to 1 mg of Loteprednol) was positioned in a dialysis bag (MWCO 12-14 kDa) and submerged in phosphate-buffered saline (PBS, pH 7.4) at 37°C, with continuous stirring at 100 rpm. Samples were collected at specified intervals, and fresh PBS was introduced to uphold sink conditions. The samples underwent analysis of their drug content through UV-visible spectroscopy [23-25].

***Ex-Vivo* Permeation Studies:**
Excised goat corneas served as a model to investigate drug permeation. The cornea was positioned on a Franz diffusion cell, ensuring that the epithelial side was orientated towards the donor compartment that held the formulation. The receptor compartment was filled with PBS at a pH of 7.4 and kept at a temperature of 37°C. Samples were gathered at designated intervals and subjected to spectrophotometric analysis [24-26].

***In-Vivo* Anti-inflammatory Studies**:
A model of acute ocular inflammation was established in Wistar rats through the administration of carrageenan. The subjects were categorised into three distinct groups: a control group (no treatment), a group receiving marketed eye drops, and a group administered an optimised spanlastic formulation. The decrease in ocular inflammation was assessed with a slit lamp microscope, and the percentage of inflammation inhibition was determined [25-27].

Results and Discussions:
Preparation and Optimization of Loteprednol-loaded Spanlastic Nanocarriers
The ethanol injection method effectively generated Loteprednol-loaded spanlastic nanocarriers. The approach facilitated effective encapsulation of the drug and the creation of stable nanocarriers appropriate for ocular drug delivery. The Box-Behnken Design (BBD) enabled a structured approach to optimise the formulation by assessing the impact of independent variables on essential quality attributes, such as particle size, entrapment efficiency, and elasticity index [26-28].

Table 2: Optimisation of Loteprednol-loaded Spanlastic Nanocarriers using Box-Behnken Design

Run No.	Span 60 Concentration (X_1) (% w/v)	Tween 80 Concentration (X_2) (% v/v)	Drug-to-Lipid Ratio (X_3) (mg/mg)
1	0.5	0.1	0.5
2	0.5	1.5	2.0
3	1.5	0.1	2.0
4	1.5	1.5	0.5
5	0.5	0.1	1.0
6	1.5	1.5	1.0
7	1.0	0.1	1.5
8	1.0	1.0	1.5
9	1.0	0.5	0.5
10	1.0	0.5	2.0
11	0.5	1.0	1.5
12	1.5	0.5	1.5

13	1.0	0.1	0.5
14	1.0	1.0	2.0
15	1.0	0.5	1.0

Characterization of Spanlastic Nanocarriers:

Particle Size and Polydispersity Index (PDI) and Zeta Potential:

The optimised formulation exhibited a particle size of 160 ± 4.2 nm, accompanied by a PDI of 0.185 ± 0.02, which suggests a uniform and monodisperse system. The small particle size plays a crucial role in ocular drug delivery, with particles under 200 nm improving corneal penetration and minimising irritation. The low PDI indicates the uniformity of the nanocarrier population, which is crucial for reliable therapeutic performance. The sonication process successfully diminished particle size while preserving uniformity in distribution. The measured zeta potential of the optimised nanocarriers was -32.8 ± 1.5 mV, indicating a favourable level of colloidal stability. The significant negative surface charge is due to Tween 80, which provides electrostatic repulsion among particles, thereby inhibiting aggregation. This value guarantees stability throughout storage and handling, while also contributing to the mucoadhesive characteristics essential for extended retention in the ocular environment [27-32].

Table 3: Characterization of Spanlastic Nanocarriers

Run No.	Particle Size (nm)	Entrapment Efficiency (%)	Elasticity Index
1	120 ± 5.4	80.5 ± 2.3	6.3 ± 0.5
2	180 ± 6.1	75.3 ± 3.0	5.5 ± 0.3
3	160 ± 4.2	85.3 ± 3.2	7.8 ± 0.6
4	200 ± 8.3	78.2 ± 2.5	6.9 ± 0.2
5	145 ± 5.7	83.4 ± 3.1	6.5 ± 0.6
6	175 ± 5.8	82.1 ± 1.5	7.2 ± 0.5
7	140 ± 4.8	84.0 ± 2.6	6.7 ± 0.7
8	160 ± 6.2	87.0 ± 2.1	7.5 ± 0.4
9	135 ± 5.5	88.0 ± 1.9	6.8 ± 0.5
10	155 ± 7.3	79.6 ± 2.8	6.4 ± 0.3
11	125 ± 3.9	86.5 ± 2.3	7.3 ± 0.6
12	170 ± 5.0	80.2 ± 3.0	7.0 ± 0.4
13	145 ± 4.1	81.4 ± 2.5	6.6 ± 0.3
14	160 ± 7.6	79.8 ± 1.7	7.1 ± 0.5
15	150 ± 5.3	85.2 ± 2.8	6.9 ± 0.4

Entrapment Efficiency (EE):

The optimised formulation demonstrated an entrapment efficiency of 85.3 ± 3.2%. The significant entrapment efficiency is due to the lipophilic characteristics of Span 60 and cholesterol, which aided in the encapsulation of the hydrophobic drug Loteprednol. The identified optimal drug-to-lipid ratio maximised the encapsulation efficiency while preserving the desired particle size.

Elasticity Index:

The calculated elasticity index of the optimised nanocarriers was found to be 7.8 ± 0.6, suggesting favourable deformability. The elevated elasticity index improves the capacity of the nanocarriers to navigate through the tight epithelial junctions of the cornea. The property provided by the surfactant (Tween 80) is crucial for facilitating the delivery of the drug to deeper ocular tissues.

In-Vitro Drug Release Studies:

The in-vitro drug release profile exhibited a biphasic pattern: an initial burst release of 30% within the first 2 hours, succeeded by a sustained release over 24 hours, culminating in an 85% cumulative release. The rapid therapeutic action is enabled by the burst release, whereas the sustained release guarantees extended drug availability, thereby minimising the frequency of dosing. The gradual release is regulated by the encapsulation of Loteprednol within the lipid matrix, showcasing the efficacy of the spanlastic nanocarriers as a controlled release mechanism [33-38].

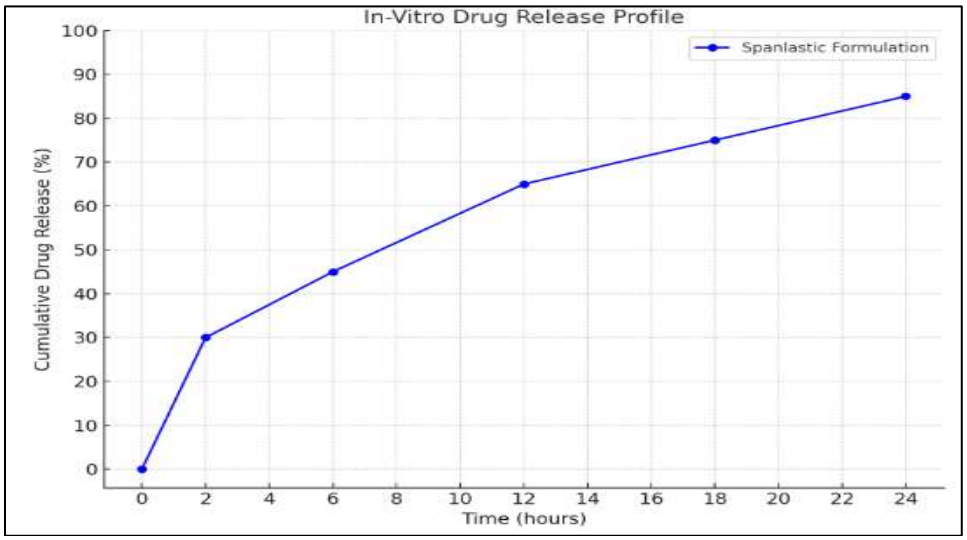

Figure 1: In-Vitro Drug Release Studies

Figure 1 illustrates the in-vitro drug release profile. The data demonstrates a biphasic release pattern, characterised by an initial burst of 30% within the first 2 hours, followed by a sustained release that culminates in an 85% cumulative release over a 24-hour period. This pattern emphasises the regulated release characteristics of the spanlastic nanocarriers.

Ex-Vivo Permeation Studies:

The total drug permeation through goat cornea over a 24-hour period was measured at 72.5 ± 4.1%, which is notably greater than that of the commercial eye drops at 45.2 ± 3.8%. The enhanced permeation of the spanlastic formulation is due to its nano-scale dimensions, elasticity, and surface-active characteristics, which improve corneal penetration. The findings confirm the capability of the nanocarriers to enhance ocular bioavailability [39-46].

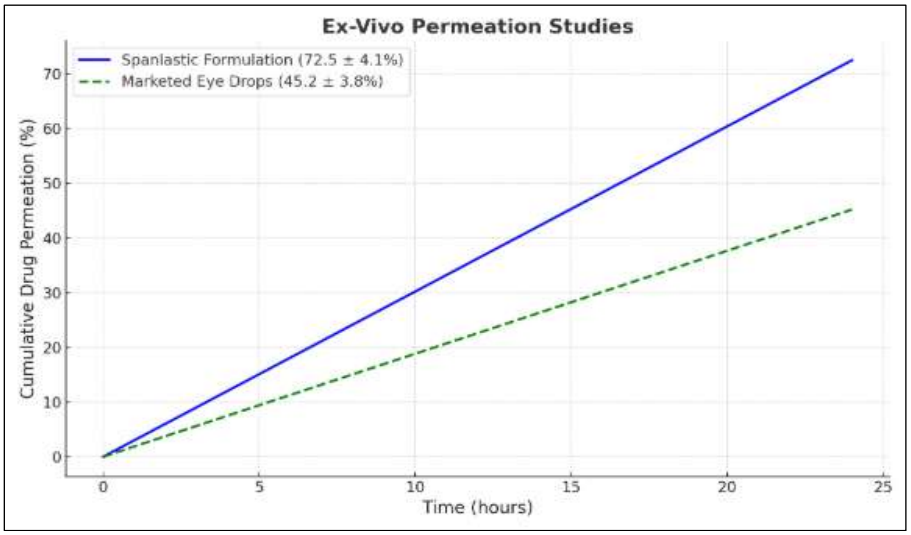

Figure 2: *Ex-Vivo* Permeation Studies

***In-Vivo* Anti-inflammatory Studies:**

The in-vivo anti-inflammatory potential of the optimised spanlastic formulation was assessed and contrasted with that of commercially available eye drops. The investigation demonstrated that the spanlastic formulation showed a notable decrease in ocular inflammation, achieving an inhibition rate of 78.4% after a 24-hour period. The inhibition rate achieved was significantly greater than the 50.6% observed with the marketed eye drops. The enhanced corneal permeability and sustained release profile of Loteprednol contribute to the superior anti-inflammatory effect of the spanlastic formulation. The properties facilitate extended drug retention within the ocular tissues, thereby maintaining stable therapeutic levels and enhancing clinical results. The nano-scale and flexible characteristics of the spanlastic carriers enable more profound penetration through the corneal barriers, thereby improving drug effectiveness. Furthermore, comprehensive observations confirmed the biocompatibility of the spanlastic formulation. The formulation demonstrated a lack of irritation, redness, or any adverse effects in the animals treated, highlighting its safety profile. The lack of negative responses underscores the promise of the spanlastic system as a reliable and efficient substitute for traditional eye drops in the treatment of ocular inflammation [47-54].

Conclusion:

The findings as a whole indicate that spanlastic nanocarriers loaded with loteprednol have a remarkable potential for the treatment of ocular inflammation or inflammation of the eye. Because the optimised formulation was able to obtain a desirable particle size, high entrapment efficiency, good stability, and enhanced therapeutic activity, it demonstrates that it is suitable for use as an advanced ocular drug delivery system. It is advised that additional clinical trials be conducted in order to determine its efficacy and safety in human patients.

Funding:
None

Conflict of Interest:
None

References:

1. Abdelbary G, El-Gendy N. Niosome-encapsulated gentamicin for ophthalmic controlled delivery. AAPS PharmSciTech. 2008;9(3):740-747.
2. Zhang X, Li X, Zhang T, Gong X, Peng Q. Enhanced ocular therapeutic efficacy of dexamethasone loaded biodegradable nanoparticles. Sci Rep. 2016;6:28184.
3. Hassan DH, Farid RM, Nasr M. Spanlastics as a novel nanovesicular carrier for improving the ocular delivery of ketoconazole: formulation and in vivo study. Drug Deliv. 2017;24(1):20-29.
4. El-Emam GA, Awad GA, Al-Jindan RY, Mortada ND. Preparation and evaluation of an ophthalmic delivery system for ketotifen fumarate based on spanlastics: a promising approach for the treatment of ocular inflammation. J Drug Deliv Sci Technol. 2020;59:101922.
5. Patel A, Cholkar K, Agrahari V, Mitra AK. Ocular drug delivery systems: an overview. World J Pharmacol. 2013;2(2):47-64.
6. Gaafar PM, Abdallah OY, Farid RM, Moftah NH. Development of vanillic acid-loaded spanlastic vesicles for enhancing ocular permeability: statistical optimization, characterization, and anti-inflammatory activity. Int J Pharm. 2023;627:122258.
7. Kakkar S, Kaur IP. Spanlastics – a novel nanovesicular carrier system for ocular delivery. Int J Pharm. 2011;413(1-2):202-210.

8. Pathan AS, Jain PG, Mahajan AB, Kumawat VS, Ahire ED, Surana KR, Rajora AK, Rajora MA. Beneficial Effects of Water-Soluble Vitamins in Nutrition and Health Promotion. Vitamins as Nutraceuticals: Recent Advances and Applications. 2023 May 26:235-51.
9. Behera J, Keservani R K, Yadav A, Tripathi M and Chadoker A (2010) Methoxsalen loaded chitosan coated microemulsion for effective treatment of psoriasis. Int. J. Drug Del. 2, 159-167.doi:10.5138/ijdd.2010.0975.0215.02025
10. Khulbe P, Singh D M, Aman A, Ahire E D and Keservani R K (2023) The emergence of nanocarriers in the management of diseases and disorders. Community Acquired Infection 2023, 10. https://doi.org/10.54844/cai.2022.0139
11. Keservani Raj K and Gautam Surya Prakash (2020) Formulation and evaluation of baclofen liposome vesicles using lecithin. ARS Pharmaceutica 61 (3), 175-180. http://dx.doi.org/10.30827/ ars.v61i3.15279
12. Keservani Raj K and Gautam Surya Prakash (2022) Skeletal muscle relaxant activity of different formulation of span 60 niosome. ARS Pharmaceutica 63 (1), 32-44. https://dx.doi.org/10.30827/ars.v63i1.22264
13. Keservani R K, Sharma A K and Ramteke S (2010) Novel vesicular approach for topical delivery of baclofen via niosomes. Lat. Am J. Pharm. 29, 1364-1370.
14. Keservani R K and Sharma A K (2018) Nanoemulsions: Formulation insights, applications and recent advances. Nanodispersions for Drug Delivery 2018, 71-96. eBook ISBN-9781351047562
15. Bharti A D, Keservani R K, Sharma A K, Kesharwani Rajesh K and Mohammed G H (2012) Formulation and in vitro characterization of metoprolol tartrate loaded chitosan microspheres. Ars Pharmaceutica 53-3, 13-18.
16. Bourlais CL, Acar L, Zia H, Sado PA, Needham T, Leverge R. Ophthalmic drug delivery systems – recent advances. Prog Retin Eye Res. 1998;17(1):33-58.
17. Gaudana R, Ananthula HK, Parenky A, Mitra AK. Ocular drug delivery. AAPS J. 2010;12(3):348-360.
18. Barot M, Bagui M, Gokulgandhi M, Mitra AK. Prodrug strategies in ocular drug delivery. Med Chem. 2012;8(5):753-768.
19. Nagpal M, Singh SK, Mishra DN. Formulation optimization and evaluation of Loteprednol etabonate-loaded nanostructured lipid carriers for ocular delivery. Int J Pharm. 2016;497(1-2):33-41.
20. Gupta H, Aqil M, Khar RK, Ali A, Bhatnagar A. Sparfloxacin-loaded PLGA nanoparticles for sustained ocular drug delivery. Nanomedicine. 2010;6(2):324-333.
21. Barar J, Aghanejad A, Fathi M, Omidi Y. Advanced drug delivery and targeting technologies for the ocular diseases. BioImpacts. 2016;6(1):49-67.
22. Aher P, Surana K, Ahire E, Patil D, Sonawane D, Mahajan S. Development and validation of RP-HPLC method for quantitative determination of 4-amino benzene sulphonamide in sulphonamide hydrochloride. Trends in Sciences. 2023 Mar 15;20(6):5209-.
23. Abdelbary AA, AbouGhaly MH. Design and optimization of topical methotrexate loaded niosomes for enhanced management of psoriasis: Application of Box-Behnken design, in-vitro evaluation and in-vivo skin deposition study. Int J Pharm. 2015;485(1-2):235-243.
24. Aggarwal D, Kaur IP. Improved pharmacodynamics of timolol maleate from spanlastics: A nanovesicular ocular delivery system. Int J Pharm. 2005;290(1-2):155-159.
25. El-Zaafarany GM, Awad GA, Holayel SM, Mortada ND. Role of edge activators and surface charge in developing ultradeformable vesicles with enhanced skin delivery. Int J Pharm. 2010;397(1-2):164-172.
26. Gautam S P, Keservani R K, Gautam T, Gupta A K and Kumar Sharma A (2015) An alternative approach for acetylation of amine terminated polyamidoamine (PAMAM) dendrimer. Ars Pharm. 56(3), 155-159.

27. Khambete H, Keservani R K, Kesharwani R K, Jain N P and Jain C P (2016) Emerging trends of nanobiomaterials in hard tissue engineering. Nanobiomaterials in Hard Tissue Engineering 2016, 63-101. https://doi.org/10.1016/B978-0-323-42862-0.00003-1
28. Keservani R K, Bandopadhyay S, Bandyopadhyay N and Sharma A K (2020) Design and fabrication of transdermal/skin drug-delivery system. In : Drug Delivery Systems, 2020, 131-178. https://doi.org/10.1016/B978-0-12-814487-9.00004-1
29. Sen P, Khulbe P, Ahire E D, Gupta M, Chauhan N and Keservani R K (2023) Skin and soft tissue diseases and their treatment in society. Community Acquired Infection 10. https://doi.org/10.54844/cai.2022.0150
30. Sharma V K, Koka A, Yadav J, Sharma A K and Keservani R K (2016) Self-micro emulsifying drug delivery systems: A strategy to improve oral bioavailability. ARS Pharm. 57(3), 97-109.DOI: http://dx.doi.org/10.4321/S2340-98942016000300001
31. Hathout RM, Nasr M. Transdermal delivery of an anti-cancer drug via ethosomes with edge activators: A risk-benefit approach. J Liposome Res. 2013;23(3):109-117.
32. Varshosaz J, Pardakhty A, Hajhashemi V, Farrokhi A. Effect of span 40 on the characteristics of span 60 based niosomes for insulin oral delivery. Drug Dev Ind Pharm. 2011;37(7):830-835.
33. Yadav KS, Sawant KK. Modified nanoprecipitation method for preparation of cytarabine-loaded PLGA nanoparticles. AAPS PharmSciTech. 2010;11(3):1456-1465.
34. El-Nahas HM, Hosny KM, Ahmed TA. Development and optimization of simvastatin-loaded spanlastics to enhance the ocular bioavailability. J Liposome Res. 2017;27(4):308-316.
35. Dubey V, Mishra D, Jain NK. Melatonin loaded ethanolic liposomes: Physico-chemical characterization and enhanced transdermal delivery. Eur J Pharm Biopharm. 2007;67(2):398-405.
36. Sharma D, Sharma RK, Gupta RB. Enhanced in-vitro permeation of flurbiprofen using nanostructured lipid carriers. Colloids Surf B Biointerfaces. 2015;126:541-546.
37. Shakeel F, Baboota S, Ahuja A, Ali J, Shafiq S. Nanoemulsions as vehicles for transdermal delivery of aceclofenac. AAPS PharmSciTech. 2007;8(4):E104-E111.
38. Bahadar H, Maqbool F, Niaz K, Abdollahi M. Toxicity of nanoparticles and an overview of current experimental models. Iran Biomed J. 2016;20(1):1-11.
39. Salama AH, Mahmoud AA. Preparation and evaluation of simvastatin loaded nanostructured lipid carriers as topical anti-inflammatory dosage form. J Liposome Res. 2015;25(1):23-32.
40. Thombre NA, Shaikh HA, Ahire ED. Formulation Development and Evaluation of Colonspecific Esomeprazole Microspheres. Biosciences Biotechnology Research Asia. 2022 Sep 29;19(3):679-91.
41. Morsi NM, Abdelbary GA, Ahmed MA. Silver sulfadiazine based ultradeformable nanovesicles for the treatment of infected burns: In-vitro characterization, in-vivo assessment and anti-microbial efficacy. Eur J Pharm Sci. 2018;119:196-205.
42. Mukherjee S, Ray S, Thakur RS. Solid lipid nanoparticles: A modern formulation approach in drug delivery system. Indian J Pharm Sci. 2009;71(4):349-358.
43. Gote V, Sikder B, Sicotte J, Pal D. Ocular drug delivery: Present innovations and future challenges. J Pharmacol Exp Ther. 2019;370(3):602-624.
44. Patel M, Patel M, Mandal S, Patel N. Formulation and optimization of controlled release spanlastic vesicles of nateglinide for the management of diabetes. Int J Biol Macromol. 2016;87:158-165.
45. Al-Mahallawi AM, Abdelbary GA, Nabil MM. Investigating the potential of nanostructured lipid carriers as semisolid topical delivery formulations of etodolac. Int J Nanomedicine. 2016;11:4855-4875.
46. Carafa M, Santucci E, Lucania G. Lidocaine-loaded non-ionic surfactant vesicles: Characterization and in-vitro permeation studies. Int J Pharm. 2002;231(1):21-32.

47. Edenta C, Ezeaku IN, Zainab A, John DF. Development and evaluation of nanoemulsion formulations for improved oral delivery of carvedilol. Universal Journal of Pharmaceutical Research 2017; 2(1): 5-10.http://doi.org/10.22270/ujpr.v2i1.R2
48. Algin Yapar E, Esentürk İ, Şahiner A, Halat E, Kara BA, Bala R, Sindhu RK. Cosmetotextiles and evaluation of their performance. Universal Journal of Pharmaceutical Research 2021; 6(3):64-67.https://doi.org/10.22270/ujpr.v6i3.609
49. ShaheenESGE, Anwar W, Abu-ElyazidSK, AfounaMI. Development, screening and optimization of rosuvastatin loaded nano-structured lipid carriers for improved therapeutic efficacy. Universal Journal of Pharmaceutical Research 2024; 9(5): 82-90.http://doi.org/10.22270/ujpr.v9i5.1212
50. Rahi N, Kumar K. Nanosponges: a new era of versatile drug delivery system. Universal Journal of Pharmaceutical Research 2017; 2(3): 30-33.http://doi.org/10.22270/ujpr.v2i3.RW4
51. Elsaied EH, Dawaba HM, Ibrahim ESA, Afouna MI. Effect of pegylated edge activator on Span 60 based nanovesicles: comparison between Myrj 52 and Myrj 59. Universal Journal of Pharmaceutical Research 2019; 4(4):1-8.https://doi.org/10.22270/ujpr.v4i4.290
52. Seary H, Barakat EH, Raslan MA, SamyAM. Development, characterization,and optimization of repaglinide loaded spanlasticsalong with investigation of drug solubility in various media. Universal Journal of Pharmaceutical Research 2024; 9(5): 105-112.http://doi.org/10.22270/ujpr.v9i5.1221
53. Türkmen A, Esentürk-Güzel İ, Kara BA.Innovative drug delivery systems for infectious diseases of the skin. Universal Journal of Pharmaceutical Research 2022; 7(4):68-76.https://doi.org/10.22270/ujpr.v7i4.815
54. Nadeem A, Saqib Z, Arif A, Abid L, Shahzadi H, Saghir A, Khan E, Afzaal T, Saadat S, Rasheed N, Mustafa MA, Iqbal MZ. Hybrid hydrogels incorporating nanoparticles, their types, development, and applications: A comprehensive review of nanogels. Universal Journal of Pharmaceutical Research 2024; 9(5): 142-149.