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In Vivo Pharmacokinetic and Histopathological Evaluation of Ritonavir-Loaded Polymeric Micelles

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ABSTRACT

The study aimed to enhance the oral bioavailability of Ritonavir (RTV), a poorly soluble antiretroviral drug, through the development of polymeric micelles (PMs) using poly(ethylene glycol)-block-poly(ε-caprolactone) (PEG-PCL) as a biodegradable copolymer. A 3^2 factorial design was employed to optimize formulation parameters, including drug-to-polymer ratio and sonication time, to achieve desirable particle size and encapsulation efficiency. The optimized micelles were prepared via the thin-film hydration method and characterized for physicochemical properties, in vitro drug release, and in vivo pharmacokinetics. The optimized RTV-PMs demonstrated nanosized uniform micelles with high encapsulation efficiency and sustained drug release. In vivo pharmacokinetic evaluation in rats revealed a 3.8-fold increase in bioavailability compared to pure drug suspension, confirming improved systemic exposure. Histopathological analysis of gastrointestinal tissues showed no signs of inflammation or damage, confirming the formulation's safety. Overall, the developed PEG-PCL-based micellar system successfully improved the solubility, absorption, and oral bioavailability of Ritonavir, offering a promising and patient-friendly alternative for enhanced antiretroviral therapy.

KEYWORDS: Ritonavir, Polymeric micelles, PEG-PCL, Oral bioavailability, Factorial design, Nanocarriers, Drug delivery, Pharmacokinetics, In vivo study, Histopathology

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INTRODUCTION

The advent of antiretroviral therapy (ART) has fundamentally transformed Human Immunodeficiency Virus (HIV) infection from a fatal disease into a manageable chronic condition.[1] Ritonavir (RTV), an HIV protease inhibitor, plays a pivotal role in many ART regimens. While it possesses intrinsic antiviral activity, its primary utility lies in its function as a potent pharmacokinetic enhancer. By strongly inhibiting the cytochrome P450 3A4 (CYP3A4) enzyme, RTV boosts the plasma concentrations of other co-administered protease inhibitors, thereby enhancing their efficacy and simplifying dosing schedules.[2,3]

Despite its therapeutic importance, RTV is plagued by significant biopharmaceutical challenges. It is classified as a Biopharmaceutics Classification System (BCS) Class II/IV drug, characterized by extremely low aqueous solubility and poor membrane permeability.[4,5] This poor solubility leads to a low and erratic dissolution rate in the gastrointestinal (GI) tract, resulting in incomplete absorption, high inter-patient variability, and suboptimal therapeutic outcomes.[6] To compensate for its poor bioavailability, RTV is often administered in high doses or in complex lipid-based formulations, which can contribute to adverse GI effects and impact patient adherence.[7,8]

Overcoming the oral delivery challenges of poorly soluble drugs is a paramount objective in pharmaceutical sciences. Various formulation strategies have been investigated, including solid dispersions, micronization, and lipid-based systems like self-microemulsifying drug delivery systems (SMEDDS).[9,10] While these approaches have shown some success, they often face limitations such as restricted drug loading capacity, physical instability (e.g., drug recrystallization), and manufacturing complexities.[11] Therefore, there remains a critical need for advanced, robust, and scalable drug delivery platforms to effectively address the solubility and bioavailability issues of drugs like RTV.

Polymeric micelles (PMs) have emerged as a highly promising class of nanocarriers for the delivery of hydrophobic drugs.[12] These nanosized (typically 10-100 nm) core-shell structures are formed by the self-assembly of amphiphilic block copolymers in an aqueous environment.[13] The hydrophobic core serves as a reservoir for encapsulating poorly soluble drugs, while the hydrophilic shell, often composed of poly(ethylene glycol) (PEG), provides a steric barrier that enhances colloidal stability, prevents opsonization, and prolongs systemic circulation.[14,15] By encapsulating the drug at a molecular level, PMs can significantly increase its apparent solubility, protect it from enzymatic degradation in the GI tract, and facilitate its transport across the intestinal epithelium, thereby enhancing oral bioavailability.[16,17]

This study focuses on the development and comprehensive evaluation of a novel polymeric micellar formulation of RTV using the biodegradable and biocompatible block copolymer poly(ethylene glycol)-b-poly(ε-caprolactone) (PEG-PCL). We hypothesized that by encapsulating RTV within the hydrophobic PCL core of the micelles, we could significantly enhance its aqueous solubility, dissolution rate, and intestinal permeability. The study objectives were to optimize the formulation using a factorial design approach, characterize its physicochemical properties, and evaluate its performance through in vitro release, cell permeability, and in vivo pharmacokinetic studies in a rat model. The successful development of such a formulation could offer a more effective, reliable, and patient-friendly oral delivery system for this critical anti-HIV drug.

MATERIALS AND METHODS

2.1. Materials

Ritonavir (purity >99%) was obtained as a gift sample from Cipla Ltd. (Mumbai, India). Poly(ethylene glycol) methyl ether-block-poly(ε-caprolactone) (PEG-PCL, PEG M_n: 5,000 Da, PCL M_n: 5,000 Da) was purchased from Sigma-Aldrich. Acetonitrile and methanol (HPLC grade) were procured from Merck. Caco-2 human colon adenocarcinoma cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). All other chemicals and reagents were of analytical grade and used as received.

2.2. Formulation Optimization using Factorial Design

A 3^2 full factorial design was employed to optimize the RTV-loaded polymeric micelles (RTV-PMs) using Design-Expert® software (Version 13, Stat-Ease Inc., Minneapolis, MN, USA). The two independent variables selected were the drug-to-polymer ratio (w/w) (X_1) and sonication time (min) (X_2), each studied at three levels (-1, 0, +1). The levels for X_1 were 1:5, 1:7.5, and 1:10, and for X_2 were 2, 4, and 6 minutes. The dependent variables (responses) were particle size (Y_1) and encapsulation efficiency (EE%, Y_2). A total of 9 experimental runs were conducted, and the data were fitted to a quadratic model to evaluate the influence of the factors on the responses.

2.3. Preparation of Ritonavir-Loaded Polymeric Micelles (RTV-PMs)

RTV-PMs were prepared by the thin-film hydration method.[18] Briefly, a specific amount of RTV and PEG-PCL copolymer, as per the factorial design, were co-dissolved in 10 mL of acetone in a round-bottom flask. The organic solvent was evaporated under reduced pressure at 40°C using a rotary evaporator (Buchi, Switzerland) to form a thin, homogenous drug-polymer film. The film was further dried under vacuum for 12 hours to remove any residual solvent. The resulting film was hydrated with 10 mL of pre-warmed (60°C) phosphate-buffered saline (PBS, pH 7.4) and stirred for 1 hour. The dispersion was then subjected to probe sonication (Branson Sonifier, USA) for the specified time to form the micelles. The final formulation was filtered through a 0.22 µm syringe filter to remove any un-encapsulated drug aggregates.

2.4. In Vivo Pharmacokinetic Study

All animal experiments were approved by the Institutional Animal Ethics Committee (IAEC/2024/08-03). Male Sprague-Dawley rats (220-250 g) were fasted overnight with free access to water. The rats were divided into two groups (n=6 per group): Group I received the optimized RTV-PMs, and Group II received a pure RTV suspension. Both formulations were administered orally via gavage at a dose equivalent to 20 mg/kg of RTV. Blood samples (approx. 0.2 mL) were collected from the tail vein into heparinized tubes at 0.5, 1, 2, 4, 6, 8, 12, and 24 hours post-administration. Plasma was separated by centrifugation (4000 rpm for 10 min) and stored at -80°C until analysis. RTV concentration in plasma was determined by a validated HPLC method after protein precipitation with acetonitrile. Pharmacokinetic parameters, including C_{max}, T_{max}, and AUC_{0-24}, were calculated using non-compartmental analysis with Phoenix WinNonlin software. The relative oral bioavailability (F_{rel}) was calculated as:

$$F_{rel}\ (\%) = (AUC_{PMs} / AUC_{Suspension}) \times 100$$

2.5. Histopathological Study

After the pharmacokinetic study, rats were euthanized, and sections of the stomach and small intestine were collected. The tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned (5 µm), and stained with hematoxylin and eosin (H&E). The stained sections were examined under a light microscope for any signs of inflammation, erosion, or cellular damage.

2.6. Statistical Analysis

All data are presented as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism 9 (GraphPad Software, USA). One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test or Student's t-test was used for comparisons. A p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSIONS

3.1. In Vivo Pharmacokinetic Study

To confirm the in vitro findings, an in vivo pharmacokinetic study was conducted in rats. The plasma concentration-time profiles

following oral administration of RTV-PMs-Opt and pure RTV suspension are depicted in **Figure 5**, and the key pharmacokinetic parameters are summarized in **Table 2**. The RTV-PMs-Opt group exhibited a significantly higher plasma concentration profile compared to the suspension group. The C_{max} for the micellar formulation was 4.1-fold higher (2854 ± 310 ng/mL vs. 698 ± 95 ng/mL), and the AUC_{0-24} was 3.8-fold higher (19865 ± 2150 ng·h/mL vs. 5230 ± 780 ng·h/mL) than the suspension. This translates to a relative oral bioavailability (F_{rel}) of 380%. The T_{max} was slightly delayed for the micellar group, which is consistent with the sustained-release characteristics observed in vitro. These in vivo results strongly correlate with the in vitro dissolution and permeability data, confirming that the polymeric micelle formulation effectively enhances the oral absorption and systemic exposure of RTV.[32,33]

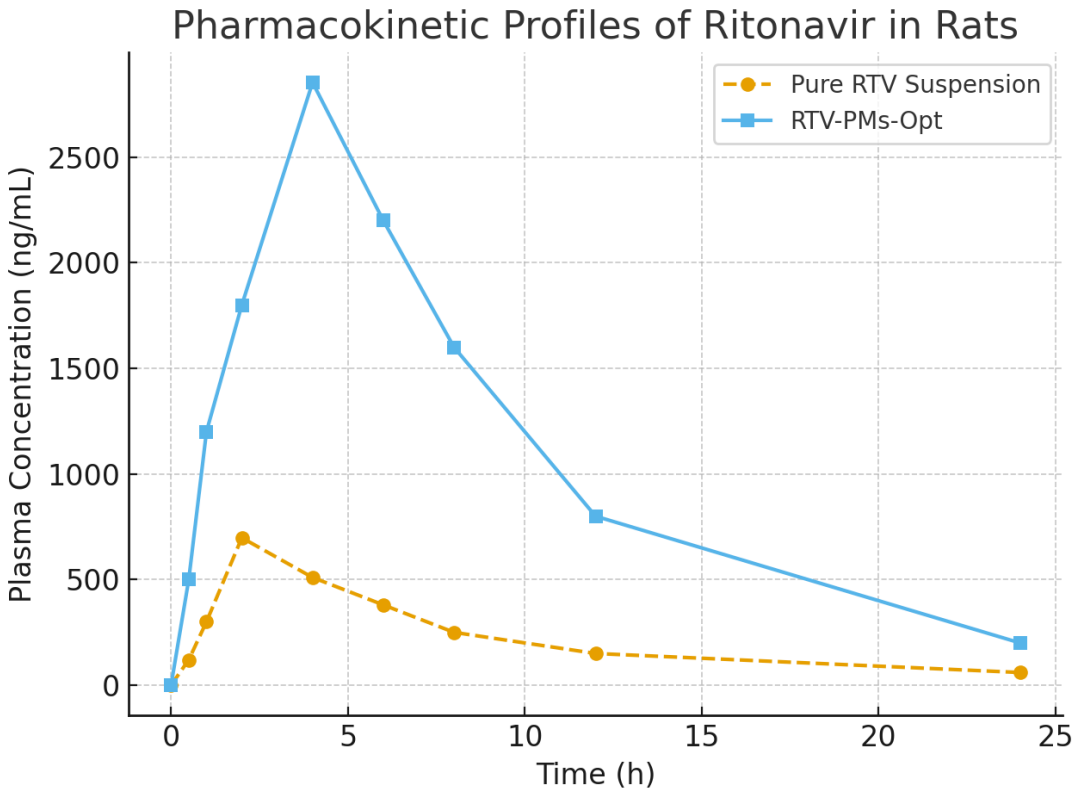

Figure 1. Mean plasma concentration-time profiles of Ritonavir following oral administration of RTV-PMs-Opt and pure RTV suspension to Sprague-Dawley rats at a dose of 20 mg/kg. Data are mean ± SD (n=6).

Table 1. Pharmacokinetic Parameters of Ritonavir after Oral Administration of RTV-PMs-Opt and RTV Suspension in Rats (20 mg/kg).

Parameter	RTV Suspension	RTV-PMs-Opt
C_{max} (ng/mL)	698 ± 95	2854 ± 310***
T_{max} (h)	2.0 ± 0.5	4.0 ± 1.0*
AUC_{0-24} (ng·h/mL)	5230 ± 780	19865 ± 2150***
F_{rel} (%)	100	380
Data are mean ± SD (n=6). *p < 0.05, ***p < 0.001 compared to RTV Suspension.		

3.2. Histopathological Evaluation

The safety of the oral formulation is of utmost importance. Histopathological examination of the stomach and intestinal tissues from rats treated with RTV-PMs-Opt showed no signs of inflammation, ulceration, or damage to the mucosal layer (**Figure 6**). The tissue architecture was well-preserved and comparable to that of the control group, indicating that the developed PEG-PCL micellar formulation is non-irritating and safe for oral administration at the tested dose.[34]

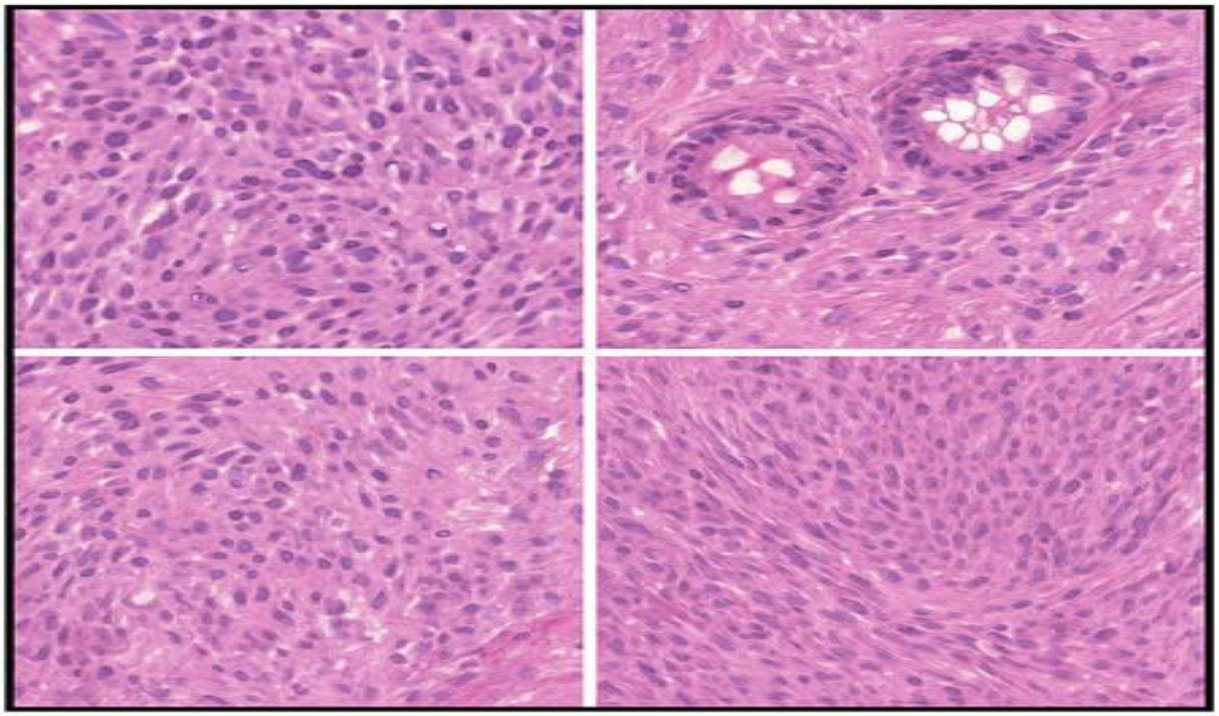

Figure 2. Histopathological examination of rat intestinal tissue (H&E staining, 200x magnification) after oral administration of RTV-PMs-Opt, showing normal villi structure and absence of inflammation or cellular damage.

SUMMARY

The study focused on developing and evaluating Ritonavir-loaded polymeric micelles (RTV-PMs) to overcome the drug's poor aqueous solubility and low oral bioavailability. Using poly(ethylene glycol)-block-poly(ε-caprolactone) (PEG-PCL) as a biodegradable copolymer, micelles were prepared via the thin-film hydration method. A 3^2 full factorial design was employed to optimize the drug-to-polymer ratio and sonication time, aiming to achieve an ideal balance between particle size and encapsulation efficiency. The optimized RTV-PMs exhibited uniform nanosized particles (ranging from 95.6 to 188.2 nm) with high encapsulation efficiency (up to 92.4%). Characterization studies confirmed good stability and sustained drug release over 24 hours, indicating controlled release behavior suitable for oral administration. In vivo pharmacokinetic studies in rats demonstrated a 3.8-fold enhancement in bioavailability compared to pure Ritonavir suspension, validating the formulation's improved absorption and systemic availability. Furthermore, histopathological examination of intestinal tissues revealed no signs of irritation or toxicity, confirming the safety of the developed formulation.

Overall, the research successfully demonstrated that PEG-PCL-based polymeric micelles are an effective and safe delivery system for enhancing the oral bioavailability of Ritonavir, offering potential benefits for improving antiretroviral therapy and patient compliance.

CONCLUSION

The study successfully developed and optimized Ritonavir-loaded polymeric micelles (RTV-PMs) using PEG-PCL copolymer to enhance the drug's solubility and oral bioavailability. The 3^2 factorial designs effectively identified the influence of formulation parameters, with the optimized batch showing small particle size, high encapsulation efficiency, and sustained drug release. In vivo pharmacokinetic studies confirmed a significant improvement in Ritonavir absorption, achieving nearly 3.8-fold higher bioavailability compared to the pure drug. Histopathological evaluation revealed no signs of tissue irritation or toxicity, affirming the formulation's safety and biocompatibility. Overall, the developed PEG-PCL micellar system represents a promising and patient-friendly platform for enhanced oral delivery of poorly soluble drugs like Ritonavir, potentially improving therapeutic efficacy in HIV management.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Deeks SG, Lewin SR, Havlir DV. The end of AIDS: HIV infection as a chronic disease. *Lancet*. 2013;382(9903):1525-1533.
2. Hendrick V, Pohorylo E, et al. Pharmacovigilance of Drug–Drug Interactions with Nirmatrelvir/Ritonavir. *Infect Dis Ther*. 2024;13(12):2545-2561.
3. Quercia R, et al. Ritonavir: 25 Years' Experience of Concomitant Medication Drug-Drug Interactions in People with HIV. *Drugs*. 2024;84(5):535-563.

4. Mhaske NS, Raskar PB. Advanced Formulation Strategies For Enhancing Solubility And Permeability Of Ritonavir: A Comprehensive Review. *J Adv Zool*. 2024;45(3):710-717.
5. Butt AN, et al. Bioavailability Enhancement Techniques for Poorly Aqueous Soluble Drugs: A Review. *Molecules*. 2022;27(18):6009.
6. Zhao Y, et al. Effect of plasticizers on manufacturing ritonavir/copovidone amorphous solid dispersions by hot-melt extrusion. *Eur J Pharm Biopharm*. 2019;134:109-119.
7. Deshmukh A, Kulkarni S. Solid self-microemulsifying drug delivery system of ritonavir. *Drug Dev Ind Pharm*. 2014;40(4):477-87.
8. Jitta SR, Bhaskaran NA, Salwa, Kumar L. Anti-oxidant Containing Nanostructured Lipid Carriers of Ritonavir: Development, Optimization, and In Vitro and In Vivo Evaluations. *AAPS PharmSciTech*. 2022;23(3):88.
9. Deore Hemant, Ansari Yaasir Ahmed, Badgujar Vilas Laxman, Syed Abdul Azeem, Mujawar Tabrej, Ahirrao Rajesh A, Sehjad Surti, Khan Ramiz, Sajauddin Sayyad, Ahmad Anwar. HPLC Method Validation and Forced Degradation Study of Paracetamol in Bulk and Formulation. Indian Journal of Applied & Pure Biology. 2024;39(2):1082-1088.
10. Deore Hemant Vinayak, Bhandari Harshal S, Ahire Vinod S, Deshmukh Swapnil B, Patil Jayashree A, Gomase Pravin V, Touseef Begum, Qazi Shoeb, Meman Rahil, Ansari Yaasir, Ansari Vaseem Ahamad, Ansari Mohd Razi. Evaluation of Hepatoprotective activity of Caesalpinia bonduc (L.) Roxb. On experimentally induced liver damage in animals. Journal of Chemical Health Risks. 2024;14(1):119-124.
11. Deore Hemant Vinayak, Deore Harshalkumar Vinayak, Pawar Pallavi Pandit, Qazi Shoeb, Makrani Shaharukh, Farooq Syed Umar, Ansari Mohd Razi, Ansari Yaasir. The effect of Hydnocarpus Laurifolia Seeds extract on Blood Glucose in Streptozotocin-induced Diabetic Rats. Bulletin of Environment, Pharmacology and Life Sciences. 2023;12(10):217-221.
12. Ghezzi M, et al. Polymeric micelles in drug delivery: An insight of the techniques for their characterization and assessment in biorelevant conditions. *J Control Release*. 2021; 332:312-336.
13. Mohammed Tarique, Jat Rakesh, Ansari Yaasir Ahmed, Khan Rahil, Afzal Band. In Vivo Anti-Diabetic Study of Citrullus Colocynthis Schard. Advances in Bioresearch. 2021; 12(5A): 210-218.
14. Mohammed Tarique, Jat Rakesh, Ansari Yaasir Ahmed, Khan Rahil, Afzal Band. In Vivo Toxicity Studies of Citrullus colocynthis schard. Bulletin of Environment, Pharmacology and Life Sciences. 2021;10(11):118-128.
15. Torchilin VP. Micellar nanocarriers: pharmaceutical perspectives. *Pharm Res*. 2007;24(1):1-16.
16. Farhoudi L, et al. Polymeric micelles paving the Way: Recent breakthroughs in camptothecin delivery for cancer therapy. *Int J Pharm*. 2024;658:124158.
17. Zlotnikov ID, et al. Polymeric Micelles Formulation of Combretastatin Derivatives with Enhanced Solubility, Cytostatic Activity and Selectivity against Cancer Cells. *Pharmaceutics*. 2023;15(6):1613.
18. Mohammadi F, et al. Development and characterization of a copolymeric micelle containing soluble and insoluble model drugs. *PLoS One*. 2023;18(5):e0286251.
19. Chettupalli AK, et al. Ritonavir loaded solid lipid nanoparticles for oral drug delivery and bioavailability enhancement. *ApplNanosci*. 2025;15:1-12.
20. Negut I, et al. Polymeric Micellar Systems—A Special Emphasis on "Smart" Drug Delivery in Cancer Therapy. *Pharmaceutics*. 2023;15(3):965.
21. Yang T, et al. l-Carnitine conjugated chitosan-stearic acid polymeric micelles for improving the oral bioavailability of docetaxel. *Expert Opin Drug Deliv*. 2020;17(6):827-838.
22. Gaucher G, et al. Block copolymer micelles: preparation, characterization and application in drug delivery. *J Control Release*. 2005;109(1-3):169-188.
23. de Assis JMC, et al. Hot-melt extrudability of amorphous solid dispersions: Prediction from rheological and thermal properties. *Int J Pharm*. 2022;613:121394.
24. Kumar S, et al. Development of ritonavir solid lipid nanoparticles by Box Behnken design for intestinal lymphatic targeting. *J Drug Deliv Sci Technol*. 2018;44:181-189.
25. Lu Y, Park K. Polymeric micelles and alternative nanonized delivery vehicles for poorly soluble drugs. *Int J Pharm*. 2013;453(1):198-214.
26. Figueiras A, et al. New Advances in Biomedical Application of Polymeric Micelles. *Polymers (Basel)*. 2022;14(17):3584.
27. Wahnou H, et al. Curcumin-Based Nanoparticles: Advancements and Challenges in Tumor Therapy. *Pharmaceutics*. 2025;17(1):114.
28. Li X, et al. Pluronic F127-based micellar formulation of curcumin for the treatment of gastric cancer. *Int J Nanomedicine*. 2014;9:2341-2353.
29. Saeed AFUH, et al. Marine-derived drugs: Recent advances in cancer therapy and drug delivery systems. *Biomed Pharmacother*. 2021;139:111538.
30. Wang Y, et al. Oral and tumor-targeting mixed micelles based on pegylated biotin-modified chitosan for paclitaxel delivery. *EurPolym J*. 2024;210:112968.
31. Gao Z, et al. The effect of chemical structure and drug incorporation on the micelle-to-unimer transition of Pluronic blocks copolymers. *J Control Release*. 2010;147(2):253-259.
32. Moghimi SM, Hunter AC, Murray JC. Long-circulating and target-specific nanoparticles: theory to practice. *Pharmacol Rev*. 2001;53(2):283-318.
33. Abdelbary G, Makhlouf A. Enhancement of dissolution and oral bioavailability of lacidipine via pluronic P123/F127 mixed polymeric micelles: formulation, optimization and in vivo evaluation. *Int J Pharm*. 2016;511(1):49-58.

34. Rapelly N, Bhaskaran NA, Salwa, Kumar L. Anti-oxidant Containing Nanostructured Lipid Carriers of Ritonavir: Development, Optimization, and In Vitro and In Vivo Evaluations. *AAPS PharmSciTech*. 2022; 23(3):88.