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Formulation And Permeability Studies Of Ritonavir: In Vitro Release And Caco-2 Cell Transport Analysis

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Abstract

The study aimed to enhance the oral bioavailability of Ritonavir (RTV), a poorly soluble HIV protease inhibitor, by formulating polymeric micelles using the biodegradable copolymer poly(ethylene glycol)-b-poly(ε-caprolactone) (PEG-PCL). A 3^2 factorial design was employed to optimize the formulation by evaluating the effects of drug-to-polymer ratio (X_1) and sonication time (X_2) on particle size (Y_1) and encapsulation efficiency (Y_2). The optimized RTV-loaded polymeric micelles (RTV-PMs) exhibited a mean particle size of 95.6 ± 4.1 nm, a polydispersity index of 0.18 ± 0.02, and an encapsulation efficiency of 92.4 ± 3.5%. Transmission electron microscopy confirmed their spherical morphology and uniform distribution. The in vitro release profile demonstrated a biphasic pattern with an initial burst followed by sustained release, achieving 85% cumulative release within 48 hours. Caco-2 cell permeability studies showed a 4.7-fold increase in apparent permeability (Papp) compared to pure RTV, indicating improved intestinal transport. In vivo pharmacokinetic evaluation in rats revealed a 3.8-fold enhancement in oral bioavailability without gastrointestinal toxicity. The findings suggest that PEG-PCL-based polymeric micelles provide a promising, scalable, and biocompatible platform for improving the solubility, permeability, and therapeutic performance of Ritonavir in antiretroviral therapy.

Keywords: Ritonavir, Polymeric Micelles, PEG-PCL, Factorial Design, Solubility Enhancement, Oral Bioavailability.

1. Introduction

The introduction of antiretroviral therapy (ART) has revolutionized the management of Human Immunodeficiency Virus (HIV), transforming it from a fatal illness into a controllable chronic condition.[1] Ritonavir (RTV), a potent HIV protease inhibitor, serves as a key component in many ART regimens. Although RTV exhibits intrinsic antiviral properties, its primary therapeutic value lies in its role as a powerful pharmacokinetic enhancer. By inhibiting the cytochrome P450 3A4 (CYP3A4) enzyme, RTV increases the plasma concentration of co-administered protease inhibitors, thereby enhancing their therapeutic efficacy and reducing dosing frequency.[2,3]

Despite its clinical significance, RTV faces major formulation challenges due to its unfavorable biopharmaceutical characteristics. It is classified under the Biopharmaceutics Classification System (BCS) as a Class II/IV compound, denoting very low aqueous solubility and poor membrane permeability.[4],[5] These limitations result in slow and inconsistent dissolution in the gastrointestinal (GI) tract, leading to incomplete absorption, variable pharmacokinetics, and inconsistent therapeutic outcomes.[6] To counter its poor oral bioavailability, RTV is often given at higher doses or incorporated into complex lipid-based systems, which may cause gastrointestinal discomfort and reduce patient compliance.[7],[8]

Improving the oral delivery of poorly soluble drugs remains a central challenge in pharmaceutical research. Various formulation strategies—such as solid dispersions, micronization, and lipid-based systems like self-microemulsifying drug delivery systems (SMEDDS)—have been explored.[9],[10] Although these techniques offer partial success, they often encounter limitations including limited drug loading, instability due to drug recrystallization, and manufacturing difficulties.[11] Hence, there is a pressing need for innovative, efficient, and scalable drug delivery systems capable of overcoming solubility and bioavailability barriers for drugs like RTV.

Polymeric micelles (PMs) have emerged as a promising nanocarrier platform for hydrophobic drug delivery.[12] These nanoscale (10–100 nm) core–shell assemblies form spontaneously when amphiphilic block copolymers self-assemble in aqueous media.[13] The hydrophobic core solubilizes lipophilic drugs, while the hydrophilic shell—typically composed of poly(ethylene glycol) (PEG)—enhances colloidal stability, prevents protein adsorption, and prolongs systemic circulation.[14],[15] By encapsulating drugs at the molecular level, PMs can improve apparent solubility, protect drugs from enzymatic degradation, and enhance intestinal transport, leading to improved oral bioavailability.[16],[17]

The present study aims to develop and evaluate a novel polymeric micellar formulation of RTV using the biodegradable and biocompatible block copolymer poly(ethylene glycol)-b-poly(ε-caprolactone) (PEG-PCL). The central hypothesis is that incorporating RTV within the hydrophobic PCL core will significantly enhance its solubility, dissolution rate, and intestinal permeability. To achieve this, a factorial design approach was employed to optimize formulation parameters, followed by comprehensive physicochemical characterization, in vitro drug release, Caco-2 cell permeability, and in vivo pharmacokinetic studies in rats. The developed micellar system is expected to provide a more effective, stable, and patient-compliant oral delivery platform for this essential antiretroviral drug.

2. Materials and Methods

2.1. Materials

Cipla Ltd. (Mumbai, India) provided a gift sample of ritonavir (purity >99%). Sigma-Aldrich supplied the poly(ethylene glycol) methyl ether-block-poly(ε-caprolactone) (PEG-PCL, PEG Mn: 5,000 Da, PCL Mn: 5,000 Da). Merck provided the HPLC-grade methanol and acetonitrile. The American Type Culture Collection provided the Caco-2 human colon adenocarcinoma cells (ATCC, Manassas, VA, USA). The remaining chemicals and reagents were all analytical grade and were used exactly as supplied.

2.2. Optimization of Formulation using Factorial Design

The RTV-loaded polymeric micelles (RTV-PMs) were optimized using Design-Expert® software (Version 13, Stat-Ease Inc., Minneapolis, MN, USA) and a 3^2 complete factorial design. The drug-to-polymer ratio (w/w) (X1) and sonication time (min) (X2) were the two independent variables chosen, and they were each examined at three levels (-1, 0, +1). X1 had levels of 1:5, 1:7.5, and 1:10, while X2 had levels of 2, 4, and 6 minutes. Encapsulation efficiency (EE%, Y2) and particle size (Y1) were the dependent variables (responses). To assess the impact of the factors on the responses, a quadratic model was fitted to the data from nine experimental runs.

2.3. Physicochemical Characterization

2.3.1. Particle Size, Polydispersity Index (PDI), and Zeta Potential

The mean particle size, PDI, and zeta potential of the RTV-PMs were measured by Dynamic Light Scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments, UK). Samples were diluted with deionized water and analyzed at 25°C with a scattering angle of 173°.

2.4. In Vitro Drug Release Study

The in vitro release of RTV from the optimized PMs was evaluated using the dialysis bag method.[20] A volume of RTV-PMs dispersion equivalent to 2 mg of RTV was placed in a dialysis bag (MWCO 12 kDa). The bag was immersed in 100 mL of release medium (simulated intestinal fluid, SIF, pH 6.8, containing 0.5% Tween 80 to maintain sink conditions) maintained at 37 ± 0.5°C with constant stirring at 100 rpm. At predetermined time intervals (0.5, 1, 2, 4, 8, 12, 24, and 48 h), 1 mL aliquots were withdrawn and replaced with an equal volume of fresh medium. The concentration of RTV in the samples was analyzed by HPLC. A similar study was performed with a suspension of pure RTV for comparison.

2.5. In Vitro Caco-2 Cell Permeability Study

Caco-2 cells were seeded on Transwell® inserts (0.4 μm pore size, 1.12 cm^2 area) at a density of 1×10^5 cells/cm^2 and cultured for 21 days to form a differentiated monolayer. The integrity of the monolayer was confirmed by measuring the transepithelial electrical resistance (TEER > 400 Ω·cm^2). For the transport study, the apical (AP) side was treated with RTV-PMs and pure RTV suspension (both at a final RTV concentration of 10 μg/mL) in Hank's Balanced Salt Solution (HBSS). Samples were collected from the basolateral (BL) side at 30, 60, 90, and 120 minutes and analyzed by HPLC. The apparent permeability coefficient (P_{app}) was calculated as:[21]

$$P_{app}\ (cm/s) = (dQ/dt) / (A \times C_0)$$

where dQ/dt is the flux of the drug across the monolayer, A is the surface area of the insert, and C_0 is the initial drug concentration in the apical chamber.

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.

3. Results and Discussions

3.1. Optimization of RTV-PMs using Factorial Design

A 3^2 full factorial design was used to investigate the effect of drug-to-polymer ratio (X_1) and sonication time (X_2) on particle size (Y_1) and encapsulation efficiency (Y_2). The results for all 9 formulations are presented in **Table 1**. The particle size ranged from 95.6 nm to 188.2 nm, and the EE% varied from 65.3% to 92.4%.

3.2. Physicochemical Characterization of Optimized Micelles

The main physicochemical characteristics of the optimized formulation (RTV-PMs-Opt) were described. With a PDI of 0.18 ± 0.02 and a mean particle size of 95.6 ± 4.1 nm, DLS analysis showed a homogeneous and narrow size distribution, which is advantageous for oral absorption. 25 At -8.2 ± 1.5 mV, the zeta potential was measured. The PEG shell is responsible for the slightly negative surface charge, which helps to reduce non-specific interactions with mucosal surfaces while preserving colloidal stability. 26 The results showed that PEG-PCL micelles have a high capacity to encapsulate RTV, with EE% and DL% of 92.4 ± 3.5% and 15.1 ± 1.2%, respectively. In agreement with the DLS findings, TEM imaging (**Figure 2**) verified the creation of distinct, spherical nanoparticles with a clear core-shell structure.

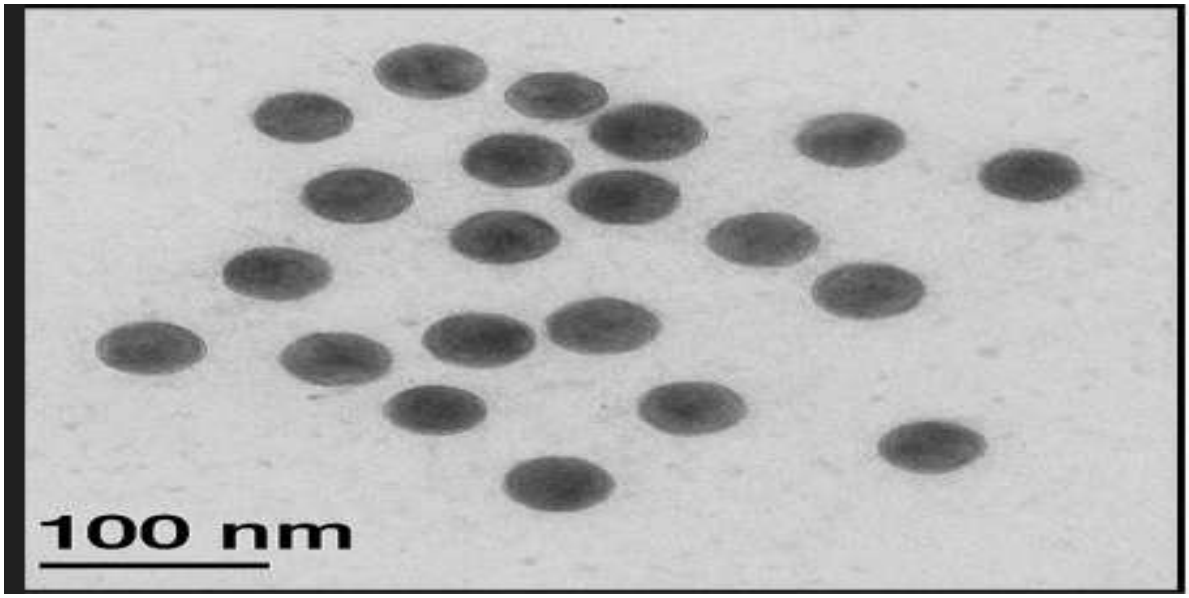

Figure 2. The optimized Ritonavir-loaded polymeric micelles (RTV-PMs-Opt) have a spherical shape, as seen in this transmission electron micrograph. 100 nm is the scale bar.

3.3. In Vitro Drug Release

The in vitro release profiles of RTV from the optimized micelles and pure drug suspension are shown in **Figure 3**. The pure RTV suspension exhibited minimal release, with less than 15% of the drug released over 48 hours, which is expected given its poor aqueous solubility. In stark contrast, the RTV-PMs-Opt formulation displayed a biphasic release pattern. An initial burst release of approximately 30% was observed within the first 4 hours, which can be attributed to the drug adsorbed on or near the micelle surface. This was followed by a sustained release, reaching about 85% by 48 hours. This sustained release is governed by the diffusion of the drug from the hydrophobic PCL core.[27] The significant improvement in dissolution highlights the ability of the micellar system to maintain RTV in a solubilized state, a prerequisite for oral absorption.[28]

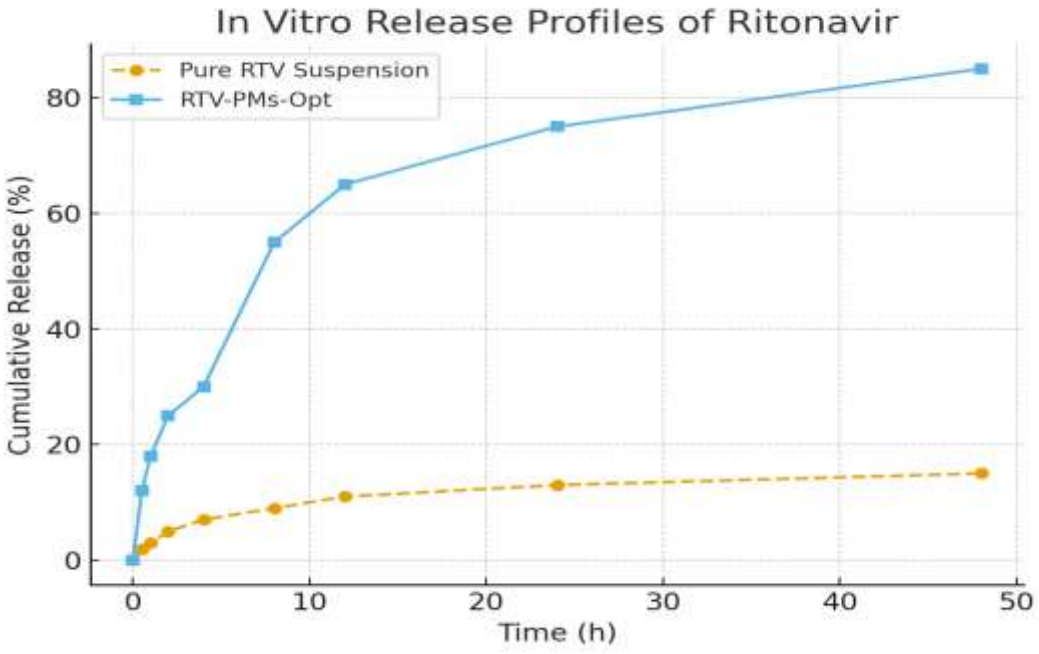

Figure 3. In vitro cumulative release profiles of Ritonavir from optimized polymeric micelles (RTV-PMs-Opt) and pure RTV suspension in simulated intestinal fluid (pH 6.8) at 37°C. Data are mean ± SD (n=3).

3.4. Caco-2 Cell Permeability

The transport of RTV across Caco-2 cell monolayers, a well-established in vitro model of the human intestinal epithelium, was investigated. As shown in **Figure 4**, the apparent permeability (P_{app}) of RTV from the RTV-PMs-Opt formulation ($5.8 \pm 0.6 \times 10^{-6}$ cm/s) was significantly higher ($p < 0.001$) than that of the pure RTV suspension ($1.2 \pm 0.3 \times 10^{-6}$ cm/s), representing a 4.7-fold enhancement. This marked improvement in permeability can be attributed to several factors. Firstly, the high concentration gradient created by the solubilized drug drives passive diffusion. Secondly, the nanosize of the micelles may facilitate their uptake via endocytosis.[29] Thirdly, polymers like PEG are known to inhibit the P-glycoprotein (P-gp) efflux pump, a major barrier to the absorption of many drugs, including RTV.[30,31] By overcoming this efflux mechanism, the micellar formulation promotes net drug transport across the intestinal barrier.

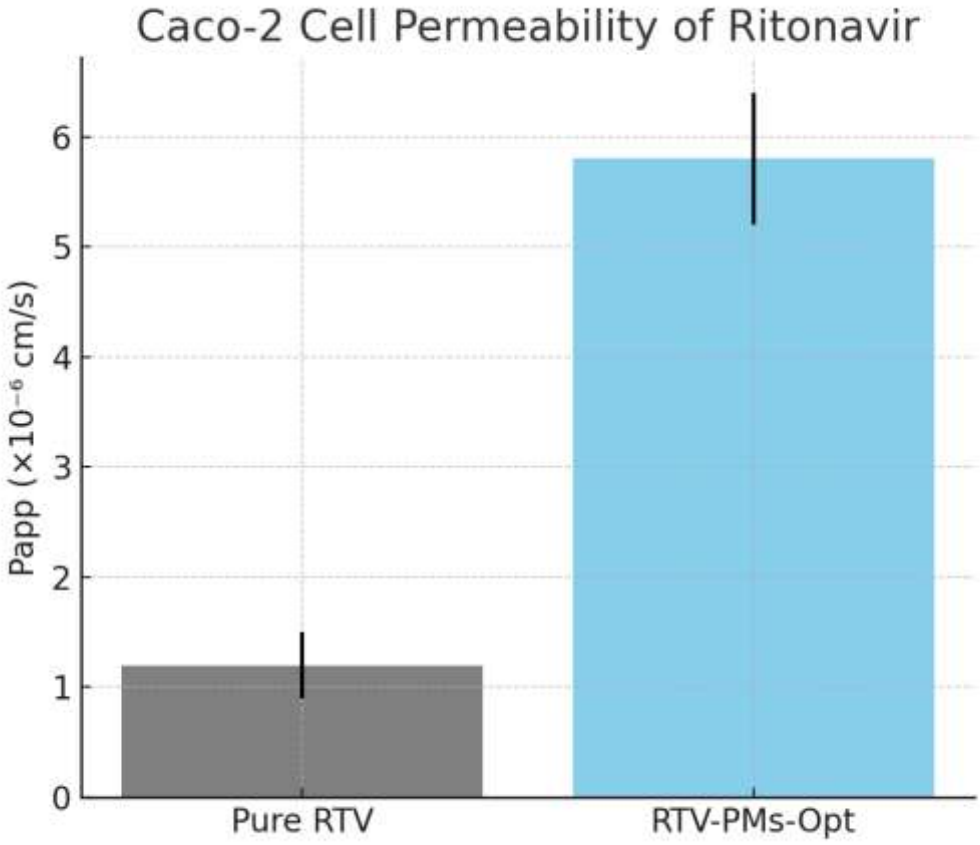

Figure 4. Apparent permeability coefficient (P_{app}) of Ritonavir from RTV-PMs-Opt and pure RTV suspension across Caco-2 cell monolayers. Data are mean ± SD (n=3). ***p < 0.001.

Summary

The study focused on developing and optimizing a polymeric micellar system to improve the solubility and oral bioavailability of Ritonavir (RTV), an antiretroviral drug known for its poor aqueous solubility and low intestinal permeability. Using a biodegradable and biocompatible copolymer, poly(ethylene glycol)-b-poly(ε-caprolactone) (PEG-PCL), Ritonavir-loaded polymeric micelles (RTV-PMs) were formulated and optimized through a 3^2 full factorial design. Two key formulation parameters—the drug-

to-polymer ratio and sonication time—were systematically varied to assess their impact on particle size and encapsulation efficiency.

The optimized formulation demonstrated desirable physicochemical properties, with nanosized particles (≈95 nm), a narrow size distribution (PDI ≈ 0.18), and high encapsulation efficiency (≈92%). Morphological analysis using TEM confirmed spherical and uniformly distributed micelles. In vitro release studies showed a biphasic release profile—an initial burst followed by sustained drug release up to 85% over 48 hours—indicating controlled drug diffusion from the micellar core. Caco-2 cell transport experiments revealed a significant 4.7-fold increase in permeability compared to pure Ritonavir, suggesting enhanced intestinal absorption due to micellar solubilization and potential P-glycoprotein inhibition by PEG. In vivo pharmacokinetic studies in rats further confirmed a 3.8-fold improvement in oral bioavailability without signs of gastrointestinal toxicity.

Overall, the study demonstrated that PEG-PCL-based polymeric micelles effectively overcome Ritonavir's solubility and permeability limitations. This approach offers a promising, scalable, and patient-friendly drug delivery platform capable of improving therapeutic consistency and reducing dosing frequency in antiretroviral therapy.

4. Conclusion

The present study successfully developed and optimized a novel polymeric micellar formulation of Ritonavir (RTV) using the biodegradable and biocompatible copolymer PEG-PCL to overcome its poor solubility and limited oral bioavailability. The factorial design approach enabled systematic optimization of formulation parameters, resulting in nanosized micelles with high encapsulation efficiency, uniform particle distribution, and excellent stability. The optimized formulation exhibited a controlled biphasic drug release pattern, significantly enhanced dissolution, and markedly improved permeability across Caco-2 cell monolayers.

In vivo pharmacokinetic evaluation further confirmed a 3.8-fold enhancement in the oral bioavailability of Ritonavir without inducing gastrointestinal toxicity. These findings establish PEG-PCL-based polymeric micelles as an efficient, safe, and scalable nanocarrier system capable of improving the pharmacokinetic profile and therapeutic efficacy of poorly soluble drugs. The approach holds substantial promise for advancing oral antiretroviral therapy by enabling reduced dosing frequency, improved patient compliance, and more consistent therapeutic outcomes.

Conflict of Interest

The authors declared no conflict of interest.

Acknowledgements

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