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*Review Article*

# ADVANCES IN LOPINAVIR FORMULATIONS: STRATEGIES TO OVERCOME SOLUBILITY, BIOAVAILABILITY, AND STABILITY CHALLENGES

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**ABSTRACT**

Lopinavir, a key protease inhibitor in antiretroviral therapy, faces significant challenges related to its poor solubility, low bioavailability, and low stability, which limit its therapeutic efficacy. This review explores a range of advanced formulation strategies developed to overcome these limitations, enhancing lopinavir's delivery and effectiveness. Nanoparticle-based systems such as solid lipid nanoparticles, nanostructured lipid carriers, and lipid-polymer hybrid systems demonstrate notable improvements in bioavailability, drug release, and lymphatic targeting. Additionally, solid dosage formulations like amorphous solid dispersions and proliposomes have been shown to significantly enhance solubility and stability, improving lopinavir's pharmacokinetic profile. By reviewing the preparation techniques, *in vivo* results, and comparative advantages of these innovative delivery systems, this article provides insight into their potential to optimize lopinavir-based therapies. Furthermore, the review discusses the role of these strategies in addressing adherence issues, ultimately improving patient outcomes. Continued research into the novel approaches is essential for advancing lopinavir delivery and enhancing its clinical efficacy in the treatment of HIV.

**KEYWORDS:** Antiretroviral therapy, Drug delivery systems, Bioavailability, HIV treatment, Pharmacokinetics

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## 1. Introduction

Lopinavir (LPV) is a synthetic peptide-like HIV protease inhibitor, designed to mimic natural substrates of the HIV-1 protease enzyme, allowing it to bind to the active site and inhibit enzyme function. Its full IUPAC name is (2*S*)-N-[(2*S*,4*S*,5*S*)-5-[2-(2,6-dimethylphenoxy) acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-(2-oxo-1,3-diazinan-1-yl) butanamide, with a molecular formula of $C_{37}H_{48}N_4O_5$ and a molecular weight of 628.81 g/mol. LPV's structure (Figure 1) is characterized by multiple stereocenters and functional groups crucial for pharmacological action. Key structural features include two phenyl rings, which enhance lipophilicity and improve membrane penetration. The hydroxyl at the 4-position increases binding affinity to HIV protease, stabilizing the drug-enzyme interaction. LPV also contains amide bonds that mimic HIV protease substrates, forming stable interactions with the enzyme's active site to inhibit viral replication. The dimethylphenoxy group further enhances lipophilicity, aiding cellular membrane passage. The functional groups — such as ester linkages, amide bonds, and the hydroxyl group — are essential for the drug's stability, substrate mimicry, and inhibitory effects on the HIV protease. LPV's stereochemistry, with chiral centers **at** positions 2, 4, 5, and 6, is vital for precise fit into the protease's active site. Any changes in stereochemistry would significantly reduce its efficacy, underlining the importance of this configuration for optimal antiviral action [1-6].

Lopinavir is an HIV-1 protease inhibitor that blocks the HIV-1 protease enzyme, essential for viral replication. This enzyme splits the Gag-Pol polyprotein into smaller, functional proteins needed to produce mature, infectious viral particles. By inhibiting this cleavage, LPV creates immature, non-infectious viral particles, significantly reducing viral replication [7, 8]. LPV is typically administered orally combined with ritonavir (RTV), which acts as a pharmacokinetic booster by inhibiting the CYP3A4 enzyme that metabolizes LPV, thereby increasing its BA and plasma concentration. Around 98–99% of LPV is bound

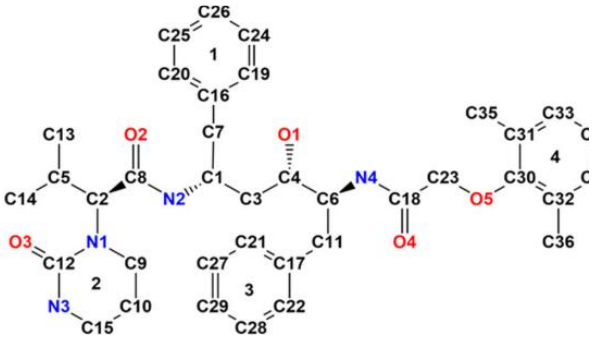


Fig 1. Chemical structure of lopinavir with carbon atom numbering system.

to plasma proteins, which limits the free drug available but allows the protein-bound form to act as a reservoir, prolonging its action. Metabolized extensively in the liver, LPV's half-life is extended to 5–6 hours when boosted with RTV, ensuring sustained therapeutic levels. LPV's antiviral effect is concentration-dependent, and its efficacy is typically measured by reductions in HIV viral load [9-12].

Lopinavir is associated with several side effects, including gastrointestinal disturbances like nausea and diarrhea, as well as dyslipidemia, hepatotoxicity, and hyperglycemia. Long-term use can lead to metabolic complications like fat redistribution and insulin resistance. This requires careful management, particularly in patients with pre-existing conditions like diabetes or cardiovascular disease [13, 14]. As LPV is metabolized via the CYP3A4 enzyme, it can interact with other medications processed by the same pathway, necessitating close monitoring and potential dose adjustments [15]. Resistance to LPV can develop over time due to mutations in the HIV protease enzyme, often resulting in cross-resistance with other protease inhibitors. This highlights the importance of combining LPV with other antiretroviral agents to overcome resistance [16, 17]. LPV remains a key component in combination therapies for HIV-1 treatment, especially in cases where resistance to other protease inhibitors has emerged, due to its high barrier to resistance and potent antiviral activity [18].

LPV is a vital component of antiretroviral therapy (ART), particularly in treating HIV-1 infection. A major strength of LPV is its high genetic barrier to resistance, making it particularly effective for patients who have developed resistance to other protease inhibitors, especially in second-line or salvage therapy [19, 20]. LPV is combined (cART) with other agents to achieve undetectable viral loads and prevent resistance. It is approved for pediatric use and recommended for pregnant women due to its safety and efficacy in preventing mother-to-child transmission [21, 22]. The World Health Organization (WHO) includes LPV/RTV in pediatric ART regimens, making it essential for managing resistant HIV strains in treatment-experienced patients [23].

## 2. Formulation challenges

Formulating LPV presents significant challenges because of its poor solubility, variable BA, and sensitivity to environmental conditions. Its low aqueous solubility limits gastrointestinal absorption, necessitating advanced strategies like solubilizing agents or lipid-based formulations to improve BA. The need for co-administration with RTV adds complexity to dosing and introduces potential drug interactions. LPV's stability is also a concern, particularly in regions with high temperatures and humidity, where degradation can compromise its efficacy. Taste masking is essential for pediatric formulations, as LPV's bitter taste complicates its use in children. Gastrointestinal side effects such as nausea and diarrhea further reduce adherence, highlighting the need for formulations that minimize these issues. Drug interactions, driven by CYP3A4 metabolism and RTV co-administration, add another layer of difficulty in managing multiple therapies. Pediatric and pregnant populations require tailored formulations that address dosing, safety, and ease of administration. Overcoming these challenges is crucial to enhancing LPV's therapeutic efficacy, reducing dependence on RTV, and ensuring improved patient adherence and outcomes in HIV treatment. Advanced formulations are necessary to address LPV's pharmacokinetic limitations, improve its BA, reduce the reliance on RTV, and enhance its stability. These innovations not only improve the drug's therapeutic efficacy but also simplify treatment regimens, leading to better adherence and ultimately contributing to improved results and quality of life for individuals with HIV [24, 25].

## 3. Nanoparticle-based delivery systems

### 3.1. Alginate-based nanoparticles

Angshuman et al. (2010) developed a sustained release method for the anti-HIV drug LPV using alginate-based nanoparticles. These nanoparticles were created through an *in situ* nanoemulsion-polymer crosslinking technique. Four different solvents were used, dichloromethane, n-hexane, 1,2-dichloroethane, and isopropyl alcohol, to encapsulate LPV. The loading of LPV into the nanoparticles was confirmed quantitatively using high-performance liquid chromatography (HPLC). The nanoparticles were characterized for their structure and surface morphology using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The hydrodynamic diameter of the nanoparticles was 127 nm with a Gaussian distribution. Studies on *in vitro* release revealed that the nanoparticles offered consistent drug release, with 30 to 80% of the loaded drug being released over 24 hours. The drug-to-polymer ratio influenced the release profile. The 1:6 ratio showed a more controlled release pattern. The drug release followed Higuchi kinetics, indicating a diffusion-controlled process rather than first-order kinetics [26].

### 3.2. Surface-stabilized nanoparticles

Jain et al. (2013) developed surface-stabilized nanoparticles to enhance the oral BA of LPV, an antiretroviral drug with poor aqueous solubility and extensive hepatic metabolism, eliminating the need for RTV. Surface-stabilized LPV nanoparticles (LPN) were made by antisolvent precipitation and high-pressure homogenization. Polyvinyl alcohol was used as a stabilizer. For stability and size, the nanoparticles were freeze-dried with mannitol. The nanoparticles had a particle size of 320 nm and a limited size distribution (polydispersity index <0.2). Oral freeze-dried stabilized LPN increased BA 3.11-fold in male Sprague-Dawley rats compared to free LPN with RTV. This indicates that the nanoparticle formulation significantly enhances the BA of LPV without the need for RTV. The study demonstrated

that surface-stabilized LPN substantially improved the oral BA of LPV, providing a promising alternative to conventional formulations that require RTV [27].

### 3.3. Pullulan acetate nanoparticles

Ravi et al. (2014) created pullulan acetate nanoparticles for oral LPV administration. FT-IR and NMR spectroscopy confirmed that pullulan was acetylated to pullulan acetate. LPV was encapsulated in nanoparticles utilizing emulsion-solvent-evaporation. Nanoparticles averaged 197 nm, showed great entrapment effectiveness (75%), and were monodisperse (PDI < 0.2). They were stable for 3 months. Nanoparticles have doubled the BA of free LPV in Wistar rats. Lymphoid organs like the liver, spleen, and lymph nodes, HIV reservoirs, received more nanoparticles. The study concluded that pullulan acetate nanoparticles could offer a superior delivery method for LPV, enhancing its BA and targeting viral reservoirs more effectively compared to free drug formulations [28].

### 3.4. Poly-ε-caprolactone (PCL)-based polymeric nanoparticles

Ravi et al. (2015) created PCL-based nanoparticles for oral LPV administration. Emulsion solvent evaporation produced LPV-loaded PCL nanoparticles with Box–Behnken design to optimize nanoparticle key features. Nanoparticles with 93.9% entrapment effectiveness were 195.3 nm in size. Nanoparticle LPV release *in vitro* was bi-phasic and persistent. LPV given via PCL nanoparticles had four times the oral BA of free LPV in male Wistar rats. The accumulation of nanoparticles in the liver and spleen suggested lymphatic absorption. Endocytosis was the main nanoparticle uptake mechanism in the rat-everted gut sac model. *In vitro* studies with rat microsomal metabolism revealed that the PCL nanoparticles provided metabolic protection to LPV, reducing its degradation. The study concluded that PCL-based nanoparticles are an effective system for increasing the oral BA of LPV and improving its pharmacokinetic profile, making them a valuable tool for drug delivery [29].

### 3.5. PLGA nanoparticles

Joshi et al. (2016) produced PLGA nanoparticles to improve LPV BA and intestinal transit. Nanoparticles were manufactured using nanoprecipitation and optimized using a 33-factor design, resulting in discrete spherical particles with an average size of 142.1 ± 2.13 nm and a high entrapment efficiency (EE) of 93.03 ± 1.27% No significant drug-polymer interactions were detected. Coumarin-loaded nanoparticles penetrated and absorbed in Caco-2 cells and the gut *in vitro*. Nanoparticles increased permeability 3.04-fold and BA 13.9-fold in rats compared to the free medication. The findings show that these nanoparticles could carry antiretroviral medicines to lymphatic reservoirs before systemic circulation [30].

Abou-El-Naga et al. (2017) explored the efficacy of LPV/RTV (L/R) in treating acute experimental toxoplasmosis, focusing on the character of PLGA nanoparticles in enhancing the drug's effects. The objective was to assess the effectiveness of LPV/RTV (L/R) and L/R loaded in poly lactic-co-glycolic acid (PLGA) nanoparticles against experimental acute toxoplasmosis. Swiss albino mice infected with the RH strain of *Toxoplasma gondii* were treated with either free L/R or L/R encapsulated in PLGA nanoparticles. The impact of treatments on mortality rate, parasite count, and parasitological improvements were evaluated. Ultrastructural changes in the tachyzoites were examined to understand the drug's mechanism of action. Both free L/R and L/R loaded in PLGA nanoparticles improved parasitological outcomes, including a reduction in mortality rate and parasite count. L/R in PLGA nanoparticles reduced parasite count in peritoneal fluid and liver and decreased parasite vitality and infectivity. Both forms of treatment prevented the egress of tachyzoites from host cells. L/R caused noticeable morphological distortions in tachyzoites. The treatments led to evidence of apoptosis and autophagy in tachyzoites. The nanotubular network inside the vacuole and the parasitophorous vacuole membrane were severely disrupted. The study demonstrated that free L/R and L/R encapsulated in PLGA nanoparticles significantly improved the outcomes of acute toxoplasmosis. The use of PLGA nanoparticles enhanced the drug's efficacy, allowing for a reduction in the effective dose needed. The findings suggest that HIV protease inhibitors like L/R could be repurposed as treatments for toxoplasmosis, offering potential new therapeutic options for patients, especially those with HIV who are at risk of opportunistic infections like toxoplasmosis [31].

### 3.6. Solid drug nanoparticles (SDNs)

Liptrott et al. (2017) examined how LPV (LPV) solid drug nanoparticles (SDNs) interact with immune system cells, particularly human T cells and macrophages, which are HIV replication sites. The study aimed to assess the biocompatibility of LPV SDNs compared to aqueous solutions of LPV. The researchers found that LPV, in its non-nanoparticle form, significantly increased the secretion of inflammatory cytokines IL-1β and TNF-α from monocyte-derived macrophages, with levels ninefold and sixfold higher, respectively, than in untreated cells. This indicates a notable inflammatory response. However, LPV SDNs did not cause similar inflammatory reactions at comparable drug doses. LPV SDNs were immunologically inactive, meaning they did not influence human T cell and macrophage activity or phenotype. The study concluded that LPV SDNs exhibited comparable or even favorable biocompatibility compared to LPV in aqueous solution. This suggests that LPV SDNs might offer a safer alternative in terms of immune system interaction while retaining effective pharmacokinetic properties [32].

### 3.7. PLGA nanocapsules

Nassar et al. (2019) developed a unique drug delivery system to improve the oral BA and efficacy of LPV, an anti-AIDS medicine. Spray-dried biodegradable PLGA nanocapsules (NCs) with LPV were placed in MCPs. This formulation protected LPV from CYP3A pre-systemic metabolism and P-gp efflux. SEM verified NC formation and MCP entrapment. MCPs released LPV-loaded NCs and free LPV at pH 7.4 *in vitro*. The BA of LPV-loaded NCs embedded in MCPs was twice higher than Kaletra® in rats taken orally. This shows the formulation shields LPV against gut and liver breakdown, improving systemic absorption. LPV-treated rat serum dramatically inhibited HIV-1 replication in cultured SupT1 cells. LPV-NCs designs also reduced infectious HIV-1 generation more than Kaletra®, indicating better antiviral efficacy. The study demonstrates that embedding LPV in nanocapsules within

microparticles can enhance its oral BA and efficacy, providing a promising approach to improve treatment outcomes for HIV infection [33].

### 3.8. Eudragit RSPO-LPV nanoparticles

Katata-Seru et al. (2020) address the need for improved pediatric formulations of antiretroviral drugs to meet the UNAIDS 90-90-90 targets, which aim to increase access to treatment for children living with HIV. LPV, a protease inhibitor used in first-line HIV treatment, faces challenges such as low aqueous solubility, bitterness, and a short half-life, which result in limited dissolution and variable BA when taken orally. To overcome these challenges, the study developed and characterized a novel delivery system using Eudragit RSPO-LPV nanoparticles, which were incorporated into suppositories made from two different bases: fattibase and polyethylene glycol (PEG). Particle size, EE, zeta potential (ZP), and polydispersity index (PDI) were measured after nanoprecipitation. The nanoparticles averaged 191 nm, had a spherical shape, 79.0% EE, 0.224 PDI, and 25.87 mV ZP. FTIR, PXRD, SEM, and TGA showed that the medication was encapsulated without interaction. At 5°C and 25°C, the suppositories were tested for 12 weeks. Initial *in vitro* release studies conducted using HPLC indicated that suppositories formulated with PEG showed a better release profile, with higher drug concentrations being released compared to those made with fattibase. In conclusion, the study suggests that LPV-loaded Eudragit nanoparticles in rectal suppository form could provide a viable alternative for pediatric HIV treatment, offering an improved BA and overcoming issues associated with oral administration. Further investigation is desirable to fully establish the safety and efficacy of this delivery system [34].

## 4. Lipid-based formulations

### 4.1. Solid self-nannoemulsifying drug delivery systems (S-SNEDDS)

Patel et al. (2016) developed and optimized an S-SNEDDS to improve LPV dissolution rate and oral BA. Scheffe's mixture design found the best formulation of LPV's liquid SNEDDS (L-SNEDDS) using Capmul MCM C8, Cremophor RH 40, and propylene glycol. The L-SNEDDS with a drug loading of 160 ± 1.15 mg and globule size of 32.9 ± 1.45 nm produced over 95% drug release within 15 minutes. Adsorbing L-SNEDDS onto Neusilin US2 created S-SNEDDS. Solid-state investigations showed that crystalline LPV became amorphous, which may improve solubility. *In vivo* studies in Wistar rats demonstrated that S-SNEDDS significantly increased LPV BA, showing 2.97 times higher BA compared to pure LPV and 1.54 times higher compared to a marketed LPV/RTV formulation. The S-SNEDDS exhibited stability with a shelf life of approximately 2.85 years. The study concluded that the S-SNEDDS formulation significantly improves the dissolution rate and oral BA of LPV, presenting a capable method to enhance the delivery of poorly soluble drugs [35].

Khan et al. (2023) investigated LPV, an HIV-1 antiretroviral protease inhibitor, and its poor oral BA. High first-pass metabolism, low effectiveness, and poor water solubility limit LPV's BA. Researchers designed and optimized an LPV-specific SNEDDS to address these difficulties. The study prepared LPV-loaded SNEDDS via titration. They created six pseudo-ternary phase diagrams to find nanoemulsification optimum conditions. The formulations were selected for their droplet size (≤100 nm), PDI (≤0.5), good dispersibility (Grade A), and transmittance (>85%). For robustness, heating-cooling cycles, freeze-thaw cycles, and centrifugation trials examined formulation stability. The optimized formulation, LPV-SNEDDS (L-14), had a droplet size of 58.18 ± 0.62 nm, PDI of 0.326 ± 0.005, ZP of -22.08 ± 1.2 mV, and EE of 98.93 ± 1.18%. HRTEM showed that the droplets were always below 60 nm. In drug release studies, LPV-SNEDDS released approximately 99% of the LPV within 30 minutes, significantly outperforming the LPV-suspension in methylcellulose, which indicates a more efficient drug release profile. Furthermore, the Caco-2 cell uptake study revealed that LPV-SNEDDS (L-14) achieved significantly higher cellular uptake compared to free LPV in suspension, suggesting that the SNEDDS formulation enhances LPV's solubility and BA. This indicates the potential of SNEDDS as an efficacious platform for increasing the oral BA of poorly soluble drugs like LPV, thereby potentially enhancing therapeutic outcomes [36].

### 4.2. Solid self-nanoemulsifying oily formulations (S-SNEOFs)

Garg et al. (2016) developed and optimized S-SNEOFs to increase low oral BA and intestinal lymphatic uptake of HIV medication LPV. A patient-centric quality target product profile (QTPP) and formulation CQAs were defined to start the investigation. Failure mode and effect critical analysis (FMECA) was used to identify pharma product quality risks. The key material attributes (CMAs) for liquid SNEOFs (L-SNEOFs) were Maisine (a lipid), Tween 80 (an emulsifier), and Transcutol HP (a cosolvent) after preliminary solubility and phase titration investigations and factor screening. Using a D-optimal mixture design, the researchers optimized these CMAs to achieve the desired drug release and permeation characteristics. The optimal SNEOFs, with a globule size of approximately 53.16 nm, demonstrated excellent drug release and permeation *in vitro* and *ex vivo* studies. SNEOFs were adsorbed onto Aeroperl and crushed into tablets with MCC to improve stability and drug loading. *In situ,* single-pass intestinal perfusion (SPIP) experiments demonstrated better medication absorption than pure LPV. SNEOF-derived LPV was also absorbed into the lymphatic system by chylomicron flow block SPIP experiments. *In vivo* pharmacokinetic investigations in rats showed that SNEOFs had higher oral BA than the pure medication. Overall, the S-SNEOFs offered a comprehensive solution for managing HIV by improving the oral BA of LPV and enhancing its delivery to lymphatic sanctuary sites, where the virus is often sequestered [37].

### 4.3. Spray-dried liposomes

Maniyar and Kokare (2019) focused on developing and evaluating spray-dried liposomes of LPV for topical application. The study aimed to enhance the BA of LPV, an anti-HIV drug, by formulating it into spray-dried phospholipid vesicles and incorporating these into a topical cream with penetration enhancers. LPV-loaded spray-dried powder (L-SDP) was created using the spray-drying technique. The physical and chemical properties of these vesicles were characterized. It revealed that they had a globular shape with an average particle size of 270 nm and a PDI of 0.239, indicating moderate uniformity in particle size. The ZP was

−34.34 mV, suggesting good stability of the vesicles, while the EE was measured at 56.38% ± 1.24%. Various penetration enhancers (PEs) were tested to improve membrane fluidity. The L-SDP released most of the drug through a cellophane membrane after 3-4 hours, while liposomal formulations released it longer. Peppermint oil demonstrated the highest drug release, achieving 57.2%, tenfold greater than the drug cream's 5.91%. Olive oil also performed well, resulting in a drug release of 44.9%. These results highlight the significant impact of PEs on improving the drug's release from the formulation. The cream containing L-SDP and PEs showed superior drug deposition and release when tested on goat facial skins. The spray-dried liposomes of LPV, combined with effective penetration enhancers, significantly improved drug delivery through the skin. The formulation with peppermint oil demonstrated the highest drug release, suggesting that this approach could be effective for enhancing the topical application of LPV in HIV treatment [38].

4.4. **Niosomal gel**

Patel et al. (2012) formulated and evaluated a niosomal gel for the enhanced transdermal delivery of LPV. The researchers utilized the thin-film hydration to prepare niosomes with optimized molar ratios of Span 40 and cholesterol to achieve the desired characteristics. The resulting niosomal formulation was compared with ethosomal gel, another type of vesicular carrier, to assess their efficacy in transdermal drug delivery. Niosomes were formulated with Span 40 and cholesterol in a molar ratio of 1:0.9:0.6, which provided high drug EE and a small mean vesicular diameter. The performance of the niosomal gel was compared with ethosomal gel through several techniques. *Ex vivo* skin permeation showed that ethosomal carriers had better deposition in the skin, while niosomal carriers provided superior drug release. Fluorescence Microscopy and histopathology were used to evaluate the safety and distribution of the formulations. Histopathological studies indicated that niosomes had a better safety profile than ethosomes. *In vivo* BA study was done on male Wistar rats to measure the extent of absorption of LPV from the transdermal niosomal gel compared to its oral suspension. The results demonstrated that the niosomal gel significantly improved the systemic absorption of LPV, with a higher area under the curve (AUC0→∞) indicating better BA. The study concluded that the niosomal gel is a favored transdermal drug delivery system for LPV, offering improved drug release, safety, and systemic absorption compared to conventional oral formulations and ethosomal carriers [39].

Fayed et al. (2022) investigated the enhancement of intestinal absorption of LPV, an antiretroviral drug with low oral BA, by incorporating menthol into niosomal formulations. The researchers prepared niosomes using cholesterol, Span 60, and poloxamer 407, both with and without menthol, and evaluated these formulations for various characteristics. The niosomes, both with and without menthol, were spherical with average vesicle sizes of nearly 140.2 nm and 148.2 nm, correspondingly. The EE (EE%) was high, at 94.4% for standard niosomes and 96.3% for those containing menthol. Both types of niosomes exhibited slow release of LPV, with menthol accelerating the release but not exceeding 9%. The in situ rabbit intestinal absorption model showed significantly enhanced absorption of LPV from niosomes compared to a drug solution. Specifically, in the duodenum, the fraction absorbed was 24.15% for the solution, 73.09% for standard niosomes, and 83.23% for menthol-containing niosomes. In the jejuno-ileum, these values were 34.32%, 80.8%, and 86.56%, respectively. The research suggested that the absorption of LPV from niosomes was not dependent on drug release alone, indicating that intact vesicle absorption played a role in the enhanced BA. The study concluded that menthol-containing niosomes significantly improve the intestinal absorption of LPV, enhancing the oral BA of drugs with similar challenges [40].

**4.5. Solid lipid nanoparticles (SLNs)**

LPV was incorporated into glyceryl behenate-based SLNs by Alex et al. (2011) to improve oral BA and intestinal lymphatic channel distribution. The SLNs were created using heat homogenization and ultrasonication, yielding particles with a mean size of 230 nm, a PDI of less than 0.27, and a surface charge of about -27 mV. DSC, WAXS, and AFM confirmed the solid nanoparticles and homogenous drug distribution in the lipid matrix. Drug release was delayed at pH 6.8 phosphate buffer and pH 1.2 hydrochloric acid *in vitro*. SLNs secreted 4.91 times more LPV into the lymph than a standard drug solution in methyl cellulose in the intestinal lymphatic transport investigation. The area under the curve (AUC) showed 2.13 times higher BA for SLN than the standard solution. Accelerated stability testing exhibited no significant changes in particle size or PDI following storage at 25 ± 2 °C/ 60 ± 5% RH, and the optimized formulation had a 21.46-month shelf life [41].

Alex et al. (2011) studied how Compritol®-based SLNs could improve CNS LPV administration. Poor blood-brain and blood-cerebrospinal fluid barrier permeability makes LPV difficult to distribute to the CNS. The SLNs, incorporating Compritol® and coated with Poloxamer, showed enhanced properties for CNS delivery. The formulations achieved a maximum concentration ($C_{max}$) of 632.86 ± 81.61 ng/ml and a time to reach this concentration ($T_{max}$) of 25 ± 7.75 minutes, indicating improved BA compared to a free-drug suspension. There was a notable increase in LPV concentration in the cerebrospinal fluid with the SLN nanoparticle formulations. The nanoparticles were efficiently absorbed via the lymphatic system. They prolonged drug circulation in the blood and effectively targeted the CNS due to their lipophilicity and surface charge. The study concluded that Compritol®-based SLNs, because of their high biodegradability, biocompatibility, and non-toxicity, are a favored carrier system for enhancing the CNS delivery of LPV [42].

Negi et al. (2013) used hot self-nanoemulsification (SNE) to create LPV-loaded SLNs to improve oral BA. The rapid cooling of poloxamer, stearic acid, and polyethylene glycol in hot water resulted in the formation of nanoparticles. A ternary phase diagram was used to analyze the self-nano-emulsification of the mixture. Optimized SLNs had 180.6 ± 2.32 nm particle size, 0.133 ± 0.001 PDI, and 91.5 ± 1.3% EE. The SLNs had a ZP of -13.4 ± 0.56 mV. Morphological tests employing TEM and AFM validated particle size and shape, while DSC and XRD proved solid-state properties of the SLNs. Due to improved lymphatic drug transport, LPV-loaded SLNs demonstrated greater oral BA than bulk LPV. The hot SNE approach can prepare SLNs with increased fatty acids, which could improve the oral administration of poorly

soluble medicines like LPV [43].

Ravi et al. (2014) developed LPV-loaded SLNs using stearic acid, polyvinyl alcohol, and mannitol, optimizing the formulation and comparing its pharmacokinetic performance with the marketed LPV/RTV coformulation. The main goal was to improve LPV oral BA and tissue distribution, especially in HIV reservoirs. LPV SLNs were created using hot melt emulsion and optimized using Plackett–Burman and Box–Behnken designs. The size and trapping efficiency of the nanoparticles were characterized. The optimized SLNs had 223 nm nanoparticles and 83% entrapment effectiveness. *In-vitro* drug release was biphasic and persistent, indicating regulated release. Wistar rats were used to compare the oral pharmacokinetics and tissue distribution of optimized SLNs and LPV/RTV coformulation. The SLNs increased oral LPV BA by 5 times compared to free LPV and 3.7 times when compared to the LPV/RTV coformulation. The marketed coformulation did not distribute LPV as well as SLNs in HIV reservoirs. Further *in vitro* experiments showed that SLNs increased LPV metabolic protection and endocytosis during absorption. This comprehensive evaluation suggests that SLNs not only improve the oral BA of LPV but also enhance its distribution to key HIV reservoirs, making them a promising alternative to traditional LPV/RTV formulations for better management of HIV [44].

Ravi and Vats (2017) investigated the pharmacokinetics of LPV and LPV-loaded SLNs in both normal and hepatic impaired rat models to address concerns about drug induced hepatotoxicity and hepatic metabolism. We evaluated the pharmacokinetics of free and LVP SLNs in a rat model with hepatic impairment. The liver was damaged by $CCl_4$ in rats. LPV and free SLNs (20 mg/kg) were orally fed to normal and hepatic impaired rats. Pharmacokinetics and tissue distribution were examined. The isolated perfused liver (IPL) model and cycloheximide-intervened lymphatic uptake were utilized to study LPV distribution. LPV SLNs had similar drug plasma profiles in normal and hepatic impaired rats, unlike free LPV, which had changed pharmacokinetics. The IPL model showed that the liver plays a minimal role in the disposition of LPV SLNs. Tissue distribution studies revealed a higher accumulation of LPV SLNs in lymphoid organs compared to free LPV. Cycloheximide pretreatment significantly reduced the AUC and $C_{max}$ of LPV SLNs, indicating that lymphatic uptake plays a role in the pharmacokinetics of SLNs. LPV SLNs maintain consistent pharmacokinetic profiles in both normal and hepatic-impaired rats, unlike free LPV, which is affected by hepatic metabolism. The study highlights the potential of LPV SLNs to bypass hepatic first-pass metabolism and achieve effective drug delivery, even in cases of liver impairment [45].

Ansari and Singh (2018) investigated a novel approach to increase the BA of LPV, an HIV protease inhibitor with limited oral BA due to extensive hepatic metabolism. The study aimed to develop a topical gel formulation of LPV using SLNs to overcome the limitations of oral administration and improve drug delivery. The SLNs were spherical after high-pressure homogenisation and SEM characterisation. DSC analysis confirmed LPV's SLN entrapment. A Franz diffusion cell was used to compare the topical gel to a pharmacological gel in *ex vivo* skin permeation investigations. An optimized SLN formulation with Compritol 888ATO (0.5%) as the lipid, Poloxamer 407 (0.25%) as the surfactant, and Labrasol (0.25%) as the co-surfactant had a drug EE of 69.78% with a mean particle size of 48.86 nm. In *ex vivo* experiments, the SLN-based gel released 71.197% of the medication over 12 hours, compared to 98.406% from the ordinary gel within 4 hours. *In vivo* studies in male Wistar rats revealed that the SLN-based gel provided a higher maximum concentration ($C_{max}$) of 20.3127 ± 0.6056 µg/mL and a greater area under the curve (AUC) compared to both the plain gel and oral suspension of the drug. The study concluded that the SLN-based topical gel formulation of LPV offers a modified drug release pattern and improved BA, suggesting it has the potential for effective HIV targeting through the topical route compared to oral administration [46].

**4.6. Nanostructured lipid carriers (NLCs)**

Garg et al. (2019) focused on developing and optimizing nanostructured lipidic carriers (NLCs) for delivering LPV to the brain, aiming to manage HIV-associated neurocognitive disorder. Using a Quality by Design framework, the study formulated NLCs of LPV. Compritol 888 and Maisine 35–1 were selected based on solubility evaluations. The formulation process involved identifying critical factors using Plackett-Burman design and optimizing these factors with Box-Behnken design. The optimized NLCs were analyzed for particle size, ZP, EE, and *in vitro* drug release. The NLCs showed significant improvements in cytotoxicity and uptake performance in Caco-2 cells and macrophages. Pharmacokinetic studies in rats revealed that LPV NLCs led to a 4.8-fold rise in peak plasma concentration and a 16.5-fold rise in the area under the curve compared to plain LPV. Additionally, the brain biodistribution of LPV improved by 2.8-fold with NLCs. The results demonstrated that NLCs significantly enhance the delivery and efficacy of LPV in targeting the brain, potentially reducing HIV-associated neurocognitive disorders by improving drug distribution and concentration in the brain, thus enhancing neurocognitive function [47].

Khan et al. (2019) created NLCs to improve the oral BA of LPV, an antiviral. High-shear homogenisation was used to prepare LPV-loaded NLCs, which were freeze-dried with trehalose for stability. The study characterised the optimized freeze-dried formulation (LPV-NLC-7-Tres). The formulation had a particle size of 286.8 ± 1.3 nm, polydispersity index of 0.413 ± 0.017, ZP of -48.6 ± 0.89 mV, and EE of 88.31 ± 2.04%. Transmission and SEM showed the particles were spherical, and DSC showed no drug-lipid interaction. LPV burst in simulated stomach and intestinal fluids *in vitro*. In Caco-2 cell lines, LPV-NLC-7-Tres had stronger cellular absorption than free LPV suspension. After six months of storage at 5 °C ± 3 °C, stability experiments showed negligible particle size changes and no significant changes in PDI, ZP, or drug content. BA tests in male Wistar rats showed LPV-NLC-7-Tres increased BA 6.98-fold over LPV suspension. This study concludes that NLCs are promising for enhancing the oral BA of LPV, offering potential improvements in drug delivery and efficacy [25].

Iontophoresis-loaded NLCs with LPV were developed by Moura et al. (2022). EPR spectroscopy and DSC were used to examine how electrical current affects NLC lipid

dynamics. A nanometric size of 179.0 ± 2.5 nm, a high drug load of 4.14%, and an EE of 80% was observed in the NLC-LPV. The formulation sustained chemical and physical stability under electric current, but EE dropped after 3 hours (67.21 ± 2.64%), resulting in quicker LPV release *in vitro*. EPR demonstrated that iontophoresis lowered NLC lipid dynamics, which was corroborated by DSC, which showed that electrical current caused polymorphic transition and drug solubilisation in the lipid matrix. LPV measurement in receptor media was enhanced by iontophoresis, with cathodic permitting 7.9 μg/cm$^2$ and anodic raising LPV levels by 1.8-fold compared to passive diffusion. These findings imply that NLC and iontophoresis can improve transdermal LPV distribution, potentially improving antiviral therapy [48].

### 4.7. Liposomes

Maniyar et al. (2020) aimed to enhance the efficacy of LPV, an HIV protease inhibitor, through an innovative drug delivery system. LPV-loaded phospholipid vesicles were spray-dried into a powder. This powder was added to peppermint and olive oil cream to improve medication delivery. The study examined how oils affect skin membrane fluidity, which is crucial for penetration improvement, using Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy. LPV-loaded spray-dried powder (L-SDP) cream formulation was optimized using a central composite design. *Ex-vivo* drug release experiments, skin deposition analysis, and cell proliferation assays using breast, lung, and skin melanoma cancer cell lines were performed. The MDA-MB-231 breast cancer cell line was also flow cytometrically analysed for DNA. The results revealed that the oils used in the cream acted effectively as penetration enhancers, significantly improving the drug deposition and permeability of the LPV-loaded cream. Fluorescence microscopy confirmed the cream's ability to penetrate effectively, while histopathological studies demonstrated the cream's safety and inertness. The *in-vivo* BA experiments exhibited a substantial increase in LPV BA. In conclusion, the study highlighted that the liposomal drug delivery system of LPV, when formulated as a cream with penetration enhancers, holds promise for enhanced drug delivery to systemic circulation and could be beneficial in treating conditions such as cancer [49].

## 5. Amorphous solid dispersions (ASDs)

### 5.1. ASDs using Eudragit® and MCC

Hamed et al. (2021) employed solvent evaporation to create amorphous solid dispersions (ASDs) to improve LPV solubility. The formulations were ternary combinations of Eudragit® E100, LPV, and Microcrystalline Cellulose (MCC) in various weight ratios. Solid-state characterization showed that just 3% of LPV in ASDs was crystalline. *In vitro* dissolution testing showed significantly enhanced drug release from the ASDs compared to pure LPV, with ASDs achieving 60.3–73.5% dissolution in the first 15 minutes, compared to less than 2% for pure LPV. Stability testing demonstrated that the ASDs were resistant to crystallization at 40 °C/75% humidity for one month. Chemometric models based on near-IR spectroscopy (NIR) and NIR-hyperspectroscopy (NIR-H) were used to quantify the crystalline fraction of LPV in the ASD, further validating the transformation. The findings suggest that ASDs effectively enhanced the dissolution and stability of LPV, providing a promising approach for improving the drug's BA [50].

Li and Taylor (2019) investigated the influence of microstructure on the dissolution performance of amorphous solid dispersions (ASDs) for poorly soluble drugs, focusing specifically on LPV. ASDs, where the drug is dispersed in a polymer matrix, are commonly used to improve the solubility of such drugs. However, ASDs can suffer from phase separation and crystallization issues, which can affect drug release performance. In this study, the researchers used a solvent-based process to prepare LPV ASDs and employed atomic force microscopy (AFM)-based nanoscale thermal analysis (nanoTA) to examine the microstructure at the submicron level. They discovered that the formation of heterogeneous domains within the ASDs improved the *in vitro* release of LPV, especially at drug loadings above 33% w/w. Key findings included that the composition and distribution of these phases, as well as the size and location of drug-rich domains, were critical to the observed changes in release kinetics. The study underscores the complexity of ASD microstructure and its significant impact on drug release, offering insights into optimizing ASD formulations for better performance [51].

Zi et al. (2019) aimed to enhance the solubility and BA of LPV, a Biopharmaceutical Classification System Class IV drug, known for its poor water solubility and permeability. The researchers established novel solid dispersions (SDs) of LPV using a polymeric surfactant, Soluplus, to enhance its BA. Solvent evaporation was initially employed for preliminary screening, and the optimized SD formulation was further improved using hot-melt extrusion (HME) to boost dissolution rates. Two matrix materials were explored: Kollidon VA 64 (VA64) and Soluplus. The study found that extrudates formed via HME exhibited significantly higher dissolution profiles compared to those prepared through solvent evaporation, attributed to stronger intermolecular interactions between LPV and the polymers during HME processing. Stability studies supported the robust nature of the extrudates over six months. Soluplus, due to its amphiphilic structure, not only formed hydrogen bonds with LPV but also created micelles, enhancing dissolution and BA. In addition, Soluplus inhibited P-glycoprotein (P-gp), thereby improving LPV permeability across rat intestines and Caco-2 cell monolayers. LPV had 1.70-fold higher BA in the Soluplus matrix than in VA64 and 3.70-fold higher than in crystalline LPV. These findings imply that Soluplus is a potential carrier to improve the BA of poorly soluble medicines, especially those with P-gp substrates [24].

### 5.2. Lipid-polymer hybrid ASDs

Kasbaum et al. (2023) developed hot-melt extruded lipid-polymer hybrid solid dispersions containing poorly soluble LPV. The study selected miscibility and compatibility-based lipid and polymeric adjuvants to improve LPV solubility and dissolution. Preformulation studies recommended Soluplus®, PVPVA, polyethylene glycol 400, Kolliphor® HS15, phosphatidylcholine, and sodium taurodeoxycholate. HME processing significantly enhanced EE, especially in Soluplus®-based extrudates (93.8% compared to 19.8% in the physical mixture), without degrading LPV. The Soluplus®-based extrudates demonstrated a 24.3-fold improvement in LPV dissolution

compared to pure LPV, and 2.8 times higher dissolution than the PVPVA-based extrudates. This enhanced dissolution was attributed to the effects of HME on the size and solid-state properties of LPV in the Soluplus®-based formulation. The study concluded that HME is a promising method to improve the BA of LPV through polymer-lipid hybrid formulations [52].

### 5.3. Amorphous lopinavir printlets

The study by Kayalar et al. (2024) explored the development of amorphous LPV "printlets" using the Selective Laser Sintering (SLS) 3D printing method to increase the BA of this poorly soluble drug. The research focused on the effects of formulation variables such as disintegrants (magnesium aluminum silicate and microcrystalline cellulose) and polymer (Kollicoat® IR) concentrations while keeping printing parameters constant. Analytical techniques, including DSC and XRD, confirmed the successful transformation of crystalline LPV into an amorphous form. The study found that increasing disintegrant concentration significantly improved drug dissolution, with release rates ranging from 71.1% to 99.3% within 120 minutes. A pharmacokinetic (PK) study in rabbits demonstrated that the printlets provided faster and more extensive absorption compared to traditional compressed tablets. Specifically, the $T_{max}$ was four times faster, while $C_{max}$ and AUC were 2.5 and 1.7 times higher, respectively. The findings indicate that SLS printing effectively creates amorphous drug delivery systems that enhance the BA of poorly soluble drugs like LPV [53].

### 5.4. Co-crystals

Fayed et al. (2022) developed and evaluated LPV-menthol co-crystals to enhance the dissolution rate and intestinal absorption of LPV. The limited bioavailability of LPV is attributed to its poor dissolution, extensive pre-systemic metabolism, and significant efflux by P-glycoprotein in the intestines. To address these issues, the researchers co-processed LPV with menthol using wet co-grinding to form co-crystals. Various molar ratios of LPV to menthol were tested, and the optimum ratio was found to be 1:2. Characterization techniques, including FTIR, DSC, and XRD, confirmed the formation of LPV-menthol co-crystals at the 1:2 ratio. Additional menthol led to phase separation due to self-association, indicating that excess menthol is not beneficial. The co-crystal formulation significantly improved the dissolution rate of LPV, increasing its dissolution efficiency from 24.96% for the unprocessed drug to 91.43% for the optimized co-crystal formulation. Furthermore, the co-crystals enhanced LPV's intestinal permeability, as shown by increased absorption in the intestinal segments compared to the pure drug. The study highlights menthol's effectiveness as a co-crystal co-former in improving the dissolution and absorption of LPV, potentially addressing the challenges associated with its oral BA [54].

### 5.5. Amorphous solid dispersions for fixed-dose combination therapies

The study by Yun et al. (2023) focused on developing an amorphous solid dispersion (ASD) to enhance the solubility and BA of LPV and RTV, two poorly soluble drugs used in HIV treatment. The ASD was created by dispersing LPV and RTV in a polymer solution and adsorbing this mixture onto Florite PS-10, an adsorbent that enhanced powder fluidity. The physical and chemical characteristics of the ASD were studied using various methods, including DSC and powder XRD. The results showed that the ASD significantly improved the solubility of LPV and RTV, with increases of 5.71 and 4.38 times, respectively, compared to their raw forms. Copovidone, a polymer used in the formulation, maintained solubility over 10 days, preventing precipitation, which occurred in ASDs without copovidone. Pharmacokinetic studies in rats demonstrated an 1186% increase in relative BA for LPV equated to the raw drug and a 2.1-fold improvement over commercial products. The ASD did not show increased cytotoxicity, indicating its potential as an effective and safe oral delivery system for LPV/RTV in HIV therapy [55].

## 6. Combination and fixed-dose paediatric formulations

Pham et al. (2016) created a child-friendly, flexible solid dosage form of poorly water-soluble medicines for pediatric use utilizing a fixed-dose combination of LPV and RTV. The researchers developed a nanotechnology that manufactures in situ self-assembly nanoparticles (ISNPs) using granules and water. This method was utilized to make LPV ISNP and LPV/RTV ISNP fixed-dose granules. Drug-loaded ISNPs showed particle sizes < 158 nm, monodispersed distribution, over 95% EE for both medicines, and stability for over 8 hours under simulated physiological circumstances. The granules were stable at room temperature for 6 months and pleasant. Compared to the standard LPV/RTV tablet (Kaletra®), the LPV/RTV ISNP granules increased BA by 2.56-fold and LPV concentrations in tissues, including HIV sanctuary locations, in rat models. The study found that ISNP technology can develop appealing, stable, and flexible pediatric granules for fixed-dose combinations of weakly water-soluble medications, improving drug BA and child administration. This represents a novel advancement in pediatric drug delivery systems [56].

## 7. Prodrugs and hybrid systems

### 7.1. Lipophilic ester prodrugs

Else et al. (2012) investigated the pharmacokinetics of LPV in pregnant women, comparing the oral BA and the efficacy of two formulations: the standard soft-gel capsule (SGC) and a melt-extruded tablet. The primary aim was to determine if the improved BA of the tablet formulation could better maintain LPV plasma concentrations throughout pregnancy, particularly during the third trimester when drug exposure often decreases. In the study, 19 HIV-infected pregnant women were divided into two groups: 8 received the SGC formulation, and 11 received the tablet formulation, both dosed at 400/100 mg twice daily. Pharmacokinetic data were collected at various points during the second and third trimesters, as well as postpartum. The researchers measured LPV concentrations using high-pressure liquid chromatography-tandem mass spectrometry. Results showed that total LPV exposures decreased in the third trimester compared to the second trimester, with reductions of 35% for the SGC group and 28% for the tablet group. However, the tablet formulation demonstrated a 15% higher area under the concentration-time curve (AUC) and a 25% higher maximum concentration compared to the SGC in the third trimester. Notably, all patients receiving the tablet had LPV concentrations

above 1,000 ng/mL, whereas one patient receiving the SGC had concentrations below this threshold. In terms of unbound LPV, the tablet group showed a higher percentage of AUC being unbound during the second and third trimesters compared to postpartum, though these differences were not statistically significant. Importantly, 17 out of 19 patients achieved an undetectable viral load at delivery, with no cases of HIV transmission reported. The study concluded that while LPV exposure was reduced during the third trimester, the tablet formulation's improved oral BA partially offset this reduction. This suggests that the melt-extruded tablet could be a more effective option for maintaining adequate LPV levels throughout pregnancy compared to the standard SGC formulation [57].

Qin et al. (2021) explored a novel strategy to enhance the delivery of LPV to HIV reservoirs in the mesenteric lymphatic system using a lipophilic ester prodrug approach. Traditional antiretroviral therapies (cART) are ineffective at penetrating HIV reservoirs in the mesenteric lymphatic system, including mesenteric lymph nodes (MLNs). The study developed lipophilic ester prodrugs designed to target the mesenteric lymphatic system more effectively. *In silco* models were used to predict prodrug affinity to chylomicrons (CMs), leading to the synthesis of several prodrug candidates. The prodrugs were tested *in vitro* and *ex vivo* to evaluate their potential for targeting mesenteric lymph and conversion to active LPV. When tested in rats, oral administration of the lipophilic prodrug approach combined with lipid-based formulations significantly improved the delivery of LPV to mesenteric lymph and MLNs. The activated ester prodrug showed the highest efficiency, with LPV levels in mesenteric lymph being up to 16.9 times the protein binding-adjusted IC90 (PA-IC90) for HIV-1. LPV levels in MLNs were up to 7.2 times the PA-IC90 following administration of the activated ester prodrug. The activated ester prodrug approach also substantially increased the systemic exposure of LPV compared to unmodified LPV. The study concludes that using a lipophilic ester prodrug strategy, especially when combined with lipid-based formulations, can significantly enhance the delivery of LPV to HIV reservoirs and potentially reduce the size of these reservoirs. This approach shows promise for improving the effectiveness of antiretroviral therapies against HIV [58].

### 7.2. Proliposomes

Patel GM et al. (2017) developed proliposomes using Quality by Design (QbD) to improve the oral BA of LPV. The researchers first defined a QTPP focused on patient-centric outcomes and identified CQAs that are crucial for the product's performance. This involved determining which attributes of the proliposome formulation would be critical for ensuring its effectiveness and safety. A risk assessment was conducted to identify potential risks that could impact the CQAs. Based on preliminary studies, the lipid-to-drug ratio and the amount of carrier were selected as critical material attributes (CMAs) for optimization. Using a face-centered central composite design, the researchers optimized these CMAs. They focused on achieving optimal liposome vesicle size, high drug EE, and effective drug release within 60 minutes. Mathematical relationships between CQAs and CMAs were derived using multiple linear regression analysis. The optimal formulation demonstrated excellent performance with over 90% drug EE and more than 95% drug release in 60 minutes. The vesicle size of the optimized proliposome was 659.7 ± 23.1 nm. Solid-state characterization techniques, including DSC, SEM, and XRD, showed that the drug transformed from a crystalline to an amorphous form, which is often associated with improved solubility. Oral BA studies conducted in Wistar rats revealed that the LPV proliposome formulation had 2.24 times higher BA compared to pure LPV. Additionally, it exhibited 1.16 times higher BA than a commercially available LPV/RTV formulation. This indicates a significant improvement in the drug's absorption and systemic availability. Stability testing according to International Conference on Harmonisation (ICH) guidelines showed that the optimized proliposome formulation remained stable for 6 months, ensuring its viability for use. Overall, this study demonstrated that proliposomes, developed with a QbD approach, offer significant advantages in enhancing the oral BA of poorly soluble LPV, potentially improving its therapeutic efficacy in treating HIV [59].

### 7.3. Microspheres

Madgulkar et al. (2018) focused on enhancing the oral BA of LPV, a BCS Class IV drug known for its poor BA because of P-glycoprotein (P-gp) efflux and limited permeation. The researchers aimed to improve the BA of LPV without the need for RTV co-administration by formulating microspheres using thiolated xyloglucan (TH-MPs) as a carrier. To create the thiomeric microspheres, the researchers utilized an ionotropic gelation method involving alginic acid and calcium ions. The microspheres were characterized through various techniques, including FTIR for interaction studies, and assessed for EE, drug release characteristics, surface morphology, and mucoadhesion. A $3^2$ factorial design was employed to optimize the formulation. The optimized thiomeric microspheres exhibited a high EE of 93.12% and a time for 80% drug release (T80) of 358.1 minutes. The microspheres demonstrated 88% mucoadhesion after one hour. *Ex vivo* studies using everted chick intestines showed that the permeation of LPV from the microspheres was enhanced approximately 3.15 times compared to free drugs. *In vivo,* studies in a rat model revealed a more than 3.22-fold increase in the relative BA of LPV when using the microspheres compared to the traditional combination of LPV and RTV. Overall, the study concluded that thiolated xyloglucan microspheres significantly improved the oral BA of LPV, providing a potential alternative to RTV co-administration for better therapeutic efficacy [60].

## 8. Other novel delivery systems

### 8.1. Metered-dose transdermal spray

Patel et al. (2015) created a LPV metered-dose transdermal spray (MDTS) using chemical and physical approaches to improve skin drug penetration. Varying Kollidon® VA 64 concentration optimized MDTS sprayability and volatilization, with 5% w/v being the optimum. In *ex vivo* experiments, the optimized formulation showed a powerful drug permeation enhancement ratio of 1.77 and a steady-state transdermal flow of 52.5 μg/cm²/h through micropore pig ear skin. *In vivo* assessment showed a three-fold increase in MDTS relative BA (291.15%) via micropore skin compared to oral tablet suspension ($AUC_{0-\infty}$ = 45.94 h*μg/mL), demonstrating

the transdermal route's efficiency in increasing drug availability. The formulation is safe, efficacious, and stable, suggesting further clinical study. The MDTS appears to be a promising transdermal LPV delivery and BA option [61].

**8.2. Cyclodextrin complexation**

Adeoye et al. (2020) explored complexation using cyclodextrins (CyDs) for enhancing the solubility and delivery of LPV, an antiretroviral drug with limited oral BA. The researchers utilized *in silico* methods to predict the most effective cyclodextrin for complexing with LPV. Their analysis identified a highly substituted (2-hydroxy) propyl-gamma-cyclodextrin (HP17-γ-CyD) as the optimal candidate. HP17-γ-CyD was successfully synthesized and compared with gamma-cyclodextrin (γ-CyD) and commercially available hydroxypropyl-gamma-cyclodextrin (HP-γ-CyD). The study prepared LPV complexes using two methods: supercritical assisted spray drying (SASD) and co-evaporation (CoEva), at molar ratios of 1:1 and 1:2. The results showed that HP17-γ-CyD significantly improved the amorphization and solubilization of LPV compared to other cyclodextrins. Additionally, the SASD technique was found to be more effective in enhancing the solubilization and release of LPV from the complexes. This study underscores the utility of *in silico* approaches in selecting the most suitable cyclodextrin for drug delivery systems, offering a promising method for enhancing the oral BA of LPV [62].

**8.3. Cyclodextrin polymers**

The study by Adeoye et al. (2020) focused on synthesizing soluble cyclodextrin (CD) polymers crosslinked with pyromellitic dianhydride (PMDA) and evaluating their efficacy as carriers for the antiretroviral drug LPV. Water-soluble cyclodextrin polymers were successfully synthesized by crosslinking methyl-ß-cyclodextrin (MßCD) and (2-hydroxy)propyl-ß-cyclodextrin (HPßCD) with PMDA. LPV was loaded into these CD polymers, creating sub-micron sized particles. The physicochemical characterization confirmed successful synthesis, with a 12–14-fold increase in LPV solubility. When loaded with LPV, pHPßCD showed a typical dose-dependent anti-HIV-1 activity consistent with LPV's known profile. The LPV-loaded pMßCD demonstrated a synergistic anti-HIV-1 effect, with a concentration-independent antiviral activity, achieving a maximum percentage inhibition of 91%. Cytotoxicity assays showed that both pHPßCD and pMßCD improved the safety profile of LPV. The viral infectivity assays discovered that while both polymers initially had a dose-independent anti-HIV-1 effect, the LPV-loaded pHPßCD reverted to a dose-dependent activity, whereas pMßCD maintained its enhanced antiviral effect even with LPV loading. In summary, the study demonstrated that CD polymers crosslinked with PMDA could effectively enhance LPV solubility and BA, with the potential for improved and synergistic anti-HIV-1 activity, especially with pMßCD [63].

**8.4. Vitamin E-TPGS micelles**

Mahajan and Patil (2020) investigated LPV-loaded Vitamin E-TPGS micelles to improve its oral BA, an HIV-1 protease inhibitor with poor aqueous solubility. The researchers made micelles *via* thin-film hydration and optimized the formulation using a central composite design based on the TPGS-to-drug ratio and rotary evaporator rotational speed. The optimization method produced 91.71 nm micelles, 0.129 polydispersity index, and -24.8 mV ZP. The micelles had 99.36% EE and 20.83% drug loading. DSC and powder XRD measurements demonstrated drug encapsulation in micelles, while TEM pictures showed their spherical shape. Micelles increased LPV dissolution *in vitro*. Oral LPV micelles increased relative BA 3.17-fold compared to suspensions. The study concludes that Vitamin E-TPGS micelles effectively improve the solubility and BA of LPV, potentially addressing some of the limitations associated with current highly active antiretroviral therapy (HAART) [64].

**8.5. Polymeric micelles**

Chaudhari and Handge (2020) formulated and evaluated LPV-loaded polymeric micelles, to improve the drug's solubility and BA, and to avoid the need for combining LPV with RTV. LPV, a BCS class IV drug used in HIV-1 treatment, is known for its poor solubility and BA, which this study sought to address. To achieve these objectives, various formulations of polymeric micelles were prepared using different Pluronic copolymers (F188 and F127) and a co-solvent (Tween80). The formulations were characterized by several parameters including critical micelle concentration (CMC), micelle size, DSC, XRD, drug loading efficiency, and stability. The results indicated that the mixed micelles, utilizing both hydrophobic and hydrophilic Pluronic F68 in combination with the co-solvent, achieved the highest EE of 29%. These micelles had a vesicle size of 0.156 µm. The DSC, FTIR, and XRD studies confirmed the effective encapsulation and stability of LPV in the optimized formulation. The study concluded the use of Pluronic F68 with a co-solvent resulted in higher EE and drug loading capacity compared to other Pluronic combinations, making it a favorable approach for enhancing the delivery and efficacy of LPV in HIV treatment [65].

**8.6. Polyethylene glycol (PEG) succinate conjugate**

Aremu et al. (2020) investigated the development and evaluation of a polyethylene glycol (PEG) succinate conjugate of LPV aimed at improving the drug's solubility and reducing its toxicity. LPV, a common treatment for HIV, suffers from poor aqueous solubility, which limits its oral BA and shortens its plasma half-life. To address these issues, the researchers synthesized a PEG (5,000) succinate (PEG–Suc–LPV) conjugate of LPV using an esterification method. The successful formation of this conjugate was confirmed through various analytical techniques. FTIR revealed the disappearance of the O-H stretch band in PEG–Suc, indicating the development of an ester linkage between PEG and LPV. The conjugate was further analyzed using ^1H NMR, XRD, and DSC, all of which confirmed the amorphous nature of the conjugate, in contrast to the crystalline nature of the pure components. The PEG–Suc–LPV conjugate demonstrated significantly improved solubility compared to pure LPV, increasing from 80 ppm to 318 ppm. Additionally, toxicity tests conducted using the *Danio rerio* (zebrafish) model indicated that the conjugate was less toxic than pure LPV, with a lower LC50 value (60.8 ppm for PEG–Suc–LPV compared to 6.42 ppm for pure LPV). Overall, the PEG-Suc conjugate of LPV was shown to enhance the drug's hydrophilicity and reduce toxicity, making it a promising carrier for enhancing the therapeutic profile of LPV [66].

### 8.7. Milk-based formulations

Salim et al. (2023) explored the solubilization behavior of the antiretroviral drugs LPV and RTV when co-administered in milk-based formulations during digestion. Using small-angle X-ray scattering (SAXS), the research examined how these drugs behave in the gastrointestinal (GI) tract post-ingestion, focusing on drug–drug interactions in combination therapy. The study revealed that RTV improved LPV's solubilization during digestion, while LPV reduced RTV's solubilization. This interaction highlights the complex dynamics of drug solubilization in combination therapies. The findings emphasize the importance of understanding individual drug solubilization in lipid-based formulations, as this behavior can influence drug absorption, metabolism, and the overall efficacy of antiretroviral therapy. Additionally, the results suggest potential food effects on drug exposure, reinforcing the need for further investigation into formulation approaches for optimal HIV treatment in children [67].

## 9. Long-acting injectable formulations

The study by Tanaudommongkon et al. (2021) focuses on developing in situ self-assembly nanoparticles (ISNPs) for the long-acting subcutaneous injection of LPV and RTV to improve patient adherence in HIV treatment and prevention. ISNP was developed to improve medication pharmacokinetics and prolong release. The ISNPs had a homogeneous size distribution with an average particle size of 167.8 nm and a polydispersity value of less than 0.35. With 25% LPV and 6.3% RTV drug loadings, both medicines had > 98% entrapment efficiency. The gradual release of LPV *in vitro* showed 20% release by day 5 and sustained release beyond 14 days. RTV released faster than LPV in the first 5 days but slowly subsequently. Pharmacokinetic studies revealed that LPV and RTV remained at therapeutic concentrations (above 160 ng/mL for LPV and 50 ng/mL for RTV) 6 days post-injection. The study concluded that ISNPs provided sustained drug release, highlighting their potential as a long-acting injection formulation for improving patient adherence to HIV therapy [68].

Cattaneo et al. (2018) developed a three-in-one, long-acting nanosuspension reformulation of off-patent antiretroviral drugs, specifically targeting low-income and middle-income countries. The researchers aimed to enhance patient adherence to antiretroviral therapy, which is crucial for the successful treatment and prevention of HIV/AIDS. The nanosuspension contains a combination of LPV, efavirenz, and tenofovir, designed to be administered via a single subcutaneous injection. This formulation provided prolonged systemic concentrations of the drugs, maintaining effective drug levels for at least two weeks in non-human primates. The nanosuspension exhibited higher drug retention within lymph node cells, suggesting improved targeting of HIV reservoirs. The authors noted that this approach could potentially reduce the dosing frequency and offer a more cost-effective and accessible option for resource-limited settings, thereby improving adherence and reducing treatment gaps in low-income and middle-income countries [69].

For HIV treatment, McConnachie et al. (2018) examined a long-acting 4-in-1 nanosuspension formulation of LPV, RTV, tenofovir (TFV), and lamivudine (3TC). Poor patient adherence and suboptimal medication exposure in residual HIV tissues were addressed. The nanosuspension was subcutaneously administered to four macaques and drug levels were measured in plasma, LNMCs, and PBMCs over five weeks. The results demonstrated that plasma and PBMC levels of LPV, TFV, and 3TC were sustained for five weeks, with PBMC drug concentrations 12 times, 16 times, and 42 times higher for LPV, RTV, and 3TC, respectively. The plasma half-lives of LPV, TFV, and 3TC were 219.1, 63.1, and 136.3 hours, respectively, and much longer in PBMCs. LNMCs had higher LPV, TFV, and 3TC concentrations than PBMCs and plasma. This work demonstrates that a single dose of the 4-drug nanosuspension could sustain drug levels in important tissues for up to five weeks, giving a promising long-acting HIV treatment that targets viral reservoirs and increases patient adherence [70].

Koehn et al. (2018) conducted a study on a three-in-one drug-combination nanoparticle (DcNP) formulation including LPV, efavirenz (EFV), and tenofovir (TFV), intended for long-acting HIV therapy. The objective was to assess how this novel nanoformulation could sustain drug levels in plasma and cells, particularly in HIV-host tissues. Two weeks were spent measuring plasma, PBMC, and LNMC drug concentrations after a single subcutaneous injection of DcNP in two macaques. Over two weeks, LNMCs had 57- to 228-fold higher drug concentrations than plasma for all three medications, while PBMCs had equivalent or higher drug concentrations. This suggests that the DcNP formulation significantly enhances the persistence and penetration of the drugs in critical HIV-host cells, potentially improving therapeutic outcomes by targeting the virus more effectively within key tissues. The study concludes that this three-in-one long-acting nanosuspension could enhance the efficacy of established HIV therapies [71].

## 10. Recent developments in LPV formulations

Perazzolo et al. (2024) developed and validated a Physiologically Based Pharmacokinetic (PBPK) model for Drug-Combination Nanoparticles (DcNP), an innovative delivery system intended for the simultaneous administration of multiple drugs in a prolonged, targeted fashion. The DcNP system allows for drugs, such as LPV, RTV, and tenofovir, to be co-delivered from the injection site to the lymphatic system before reaching the systemic circulation. The study distinguishes two classes of long-acting injectable products: Class I, which involves sustained drug release at the injection site, and Class II, which involves the uptake and retention of the drug-carrier complex in the lymphatic system. The PBPK model validation was performed using data from three nonhuman primate studies with nine pharmacokinetic datasets, involving different dosing regimens and fixed-dose ratios. The PBPK model passed validation in 8 out of 9 cases. Specifically, the model successfully predicted pharmacokinetics for RTV and tenofovir across various scenarios. However, for LPV, the model did not pass validation in one study due to delayed drug accumulation caused by RTV's CYP3A inhibition, leading to a mechanism-based drug-drug interaction (DDI). The study concluded that the validated PBPK model can effectively predict the pharmacokinetics of drugs in DcNP systems and account for potential DDIs in long-acting combination therapies [72].

**11. Discussion of patents**

The patent US9370578B2 outlines an innovative oral dosage form combining crystalline LPV and crystalline RTV, designed to enhance the dissolution rate, BA, while maintaining excellent content uniformity. The formulation also strives for stability under harsh storage conditions typical of climate zones III and IV (high temperature and humidity). The crystalline LPV is mixed with a brittle vehicle, which helps maintain the drug in its crystalline form and enhances the stability of the formulation. The brittle vehicle stabilizes the crystalline form of LPV and may include substances like microcrystalline cellulose or inorganic substances such as silicates. The brittle vehicle is essential in achieving a good dissolution profile and mechanical stability of the tablets. The formulation is made by co-milling crystalline LPV with the brittle vehicle, ensuring that LPV remains in its crystalline form. The crystalline RTV is added later in the manufacturing process, which can include further blending and granulation steps before the final tablet or capsule formation. The formulation shows superior in-vitro dissolution profiles, especially within the first 45 minutes of dissolution. This rapid dissolution is expected to result in better *in-vivo* plasma levels of both LPV and RTV. The dosage form is designed to be stable even under harsh storage conditions, minimizing decomposition and ensuring long-term effectiveness. This patent represents an advancement in drug delivery systems for antiretroviral therapy by improving the dissolution rate, BA, and stability of the combination of LPV and RTV, which are critical in HIV treatment [73].

The patent application WO2013034927A1 outlines a novel formulation approach for antiretroviral drugs, particularly focusing on improving the BA and efficacy of LPV. This formulation incorporates a blend of excipients to improve the solubility and intestinal absorption of LPV, which is often limited because of its low aqueous solubility. The patent details methods to prepare stable, amorphous forms of the drug using specific polymers and solvents that inhibit crystallization and increase the drug's dissolution rate. Additionally, the formulation aims to optimize the release profile and metabolic stability of LPV, potentially decreasing the frequency of dosing and improving patient compliance. This could offer significant therapeutic advantages in the treatment of HIV, improving the effectiveness of LPV in suppressing viral loads [74].

The patent application WO2019224779A1 describes a technique for enhancing the solubility and BA of poorly water-soluble drugs, particularly LPV. The invention focuses on developing lipid-based formulations, such as self-emulsifying drug delivery systems (SEDDS), and solid dispersions to increase drug dissolution and absorption in the gastrointestinal tract. The formulation utilizes a combination of oils, surfactants, and cosolvents to form a stable emulsion when in contact with gastrointestinal fluids, enhancing the solubilization of the drug and improving its BA. This method is aimed at addressing the challenge of poor drug solubility, which limits the efficacy of oral drug administration. Additionally, the patent claims the ability to decrease the drug's dose needed for therapeutic efficacy by optimizing its absorption profile through these novel formulations. The invention also suggests that these formulations can be adapted for various poorly soluble drugs beyond LPV, broadening its potential applications in drug delivery [75].

These diverse formulation strategies highlight the ongoing efforts to improve the delivery of LPV, addressing its solubility, BA, stability, and targeted delivery, particularly for HIV treatment.

**12. Advancing LPV delivery**

The development of novel approaches for the transfer of LPV has the potential to significantly enhance its efficacy and BA, addressing key challenges in current antiretroviral therapy. Despite its crucial role in the treatment of HIV, LPV's poor solubility, variable pharmacokinetics, and dependence on RTV as a booster agent limit its overall therapeutic potential. By investigating the novel strategies, we can address the current limitations in LPV-based treatments and improve outcomes for individuals living with HIV.

**12.1. Stimuli-Responsive Nanoparticles**

Pharm et al. (2020) highlighted the importance of Stimuli-responsive nanoparticles intended to release drugs in response to specific environmental triggers like pH, temperature, or the existence of specific enzymes in the GI tract or target tissues, like the lymphatic system or central nervous system (CNS). By tailoring LPV release to the biological environment, these systems could improve BA and target delivery to HIV reservoirs, minimizing systemic side effects. This approach offers a promising solution for precise drug delivery and sustained release of LPV at the site of action [76].

**12.2. Exosome-based delivery**

Koh et al. (2023) investigated the potential of exosomes — naturally occurring vesicles that facilitate cell-to-cell communication — as biocompatible carriers. By engineering exosomes for targeted delivery to HIV reservoirs, including the central nervous system (CNS) and lymphatic system, this method aims to improve the precision of drug delivery and reduce unnecessary systemic exposure. This innovative strategy could significantly enhance the effectiveness of LPV while minimizing associated toxicity [77].

**12.3. CRISPR-Cas9-enhanced drug delivery**

Asmamaw et al. (2021) examine the CRISPR/Cas-9 genome-editing system, known for its precision and effectiveness in modifying genomes. The system consists of guide RNA (gRNA) and Cas-9 protein, and its process includes recognition of target sequences, cleavage to induce double-stranded breaks, and repair via cellular mechanisms. CRISPR/Cas-9 has diverse applications in medicine, agriculture, and biotechnology, facilitating advancements in crop nutrition and potential treatments for conditions like cancer, HIV, and genetic disorders. Although CRISPR-Cas9 is primarily a gene-editing tool, its application in drug delivery could revolutionize targeting mechanisms for LPV. CRISPR-based systems could modulate viral reservoirs, making them more susceptible to LPV or reducing viral resistance. This strategy has the potential to increase the drug's effectiveness in hard-to-reach HIV reservoirs, enhancing treatment outcomes [78].

**12.4. Ultra-long-acting injectable formulations**

Kovarova et al. presented an innovative ultra-long-

acting (LA) removable drug delivery system for HIV treatment and prevention, addressing the critical issue of medication non-adherence in chronic conditions. Unlike current LA antiretroviral (ARV) formulations, which cannot be removed once administered, this system allows for safe removal after up to nine months of drug delivery. Using pre-clinical models, including non-human primates and humanized BLT mice, the researchers demonstrated that a single subcutaneous dose of ultra-LA dolutegravir effectively suppressed viral load and provided protection against multiple high-dose vaginal HIV exposures in BLT mice. This approach highlights a promising strategy for enhancing adherence and offers various therapeutic applications in HIV management. Ultra-long-acting injectables, could provide sustained LPV release over weeks or even months, drastically improving patient adherence by decreasing the frequency of dosing. These preparations would keep therapeutic drug levels over extended periods, minimizing the need for daily administration and increasing treatment efficacy [79].

**12.5. Peptide-based drug carriers**

Khairkhah et al. (2023) reviewed the application of cell-penetrating peptides (CPPs) as promising carriers for drug delivery in combating viral infections. CPPs have garnered interest for their ability to enhance the delivery of therapeutic agents, including peptide/protein-based and nucleic acid-based vaccines, into cells with minimal toxicity. Peptide-based drug carriers, designed to encapsulate LPV, could enhance the drug's cellular uptake by mimicking viral or cellular peptides. This method would improve the targeting of immune cells and HIV reservoirs, leading to more efficient drug delivery and potentially increasing LPV's antiviral activity within the body [80].

**12.6. Theranostic nanoparticles**

Kevadiya et al. (2020) developed rod-shaped theranostic nanoparticles to improve antiretroviral drug (ARV) delivery for HIV. They created rilpivirine (RPV) nanoparticles with lutetium-177 in bismuth sulfide nanorods (177LuBSNRs) and evaluated their properties. The nanoparticles showed rapid uptake and distribution in macrophages without adverse effects, particularly in lymphoid tissues. The study highlights 177LuBSNRs as effective theranostic tools for assessing long-acting ARV pharmacokinetics, offering the potential for enhanced HIV treatment. By incorporating imaging techniques, such as MRI, into the nanoparticle design, clinicians can personalize LPV treatment, adjusting dosages based on real-time data, which could enhance treatment precision and outcomes [81].

**12.7. Artificial Intelligence (AI)-designed formulations**

Jiang et al. (2022) highlight the use of Artificial Intelligence (AI) in developing solid dosage forms, such as tablets and capsules, as a more efficient alternative to traditional trial-and-error methods. The review covers AI's role in pharmaceutical sciences, regulatory guidance, strategies for building databases, data processing, and comparisons of various AI algorithms. It also discusses case studies showcasing AI's effectiveness in formulation development, particularly through deep learning-based image analytics. The authors conclude that AI technologies can significantly enhance the understanding and prediction of drug formulation properties, streamlining the development process. AI could also optimize drug solubility, stability, and release profiles, offering a fast and efficient way to develop improved LPV formulations with enhanced BA [82].

**12.8. Biopolymer-based hydrogels**

Hama et al. (2023) explored recent advancements in biopolymer-based hydrogels for tissue engineering, highlighting their potential for tissue regeneration. The study emphasizes how hydrogels can be tailored to meet specific requirements for various tissues by adjusting their mechanical properties, degradation rates, and cell affinity through molecular design. The review also discusses high-functional hydrogels that incorporate drug delivery systems (DDSs), allowing for the controlled release of drugs or bioactive substances. Overall, the authors provide insights into the molecular design and clinical applications of these innovative hydrogels in regenerative medicine. Biopolymer-based hydrogels could encapsulate LPV and release the drug in response to specific physiological stimuli, like inflammatory markers or enzymes found in HIV-infected tissues. This bioresponsive delivery system targets drug release more effectively, improving LPV's therapeutic action and reducing unnecessary systemic exposure [83].

**12.9. Nanobots for targeted delivery**

Hu et al. (2020) explored the potential of micro/nanorobots in targeted drug delivery, surgical procedures, and disease diagnosis. Unlike traditional methods that rely on blood circulation, these autonomous robots can navigate directly to hard-to-reach areas, enhancing drug delivery precision. Powered by external sources (magnetic fields, light, etc.) or chemical reactions, they include both cell-based robots and DNA origami, which lack autonomous movement. While promising, research is mostly limited to *in vitro* studies, with *in vivo* applications still in early development. The authors call for further biological research to validate micro/nanorobot efficacy in living organisms and discuss current challenges and future directions. These microscale or nanoscale robots could be engineered to transport LPV to specific sites in the body, such as lymph nodes or the CNS. By actively navigating to target areas, nanobots could minimize off-target effects and deliver LPV precisely where it is needed most [84].

**12.10. Gene therapy-linked drug delivery**

Pan et al. (2021) highlighted the advancements in gene therapy drug delivery systems for treating genetic diseases. Gene therapies, including siRNA, shRNA, antisense oligonucleotides, CRISPR/Cas9, plasmid DNA, and miRNA, hold significant promise for biomedical applications. However, effective delivery systems are essential to prevent drug degradation and ensure the targeting of tissues, cells, and organelles. Viral vectors remain the most common delivery method due to their efficiency, but nanocarriers are emerging as a superior alternative with enhanced performance. The study explores current gene therapy approaches, and delivery systems, and discusses future challenges and opportunities in nano-delivery system development. Gene therapy could be used to enhance the expression of specific drug transporters or enzymes in tissues that aid in LPV absorption or reduce its metabolism. This strategy

could improve LPV BA in challenging areas, such as the CNS, by increasing local drug uptake or reducing drug degradation, potentially overcoming existing barriers to effective HIV treatment [85].

## 13. Conclusion

Innovative delivery systems for LPV have shown significant promise in enhancing its solubility, bioavailability, and targeted drug delivery. Nanoparticle-based systems, such as alginate nanoparticles, demonstrate sustained release but require *in vivo* validation. Surface-stabilized nanoparticles markedly improve bioavailability without necessitating RTV co-administration, while pullulan acetate and PCL-based nanoparticles further enhance lymphatic uptake and provide sustained release profiles. PLGA nanoparticles offer the potential for repurposing in opportunistic infections by improving oral bioavailability and targeting viral reservoirs. LPV solid drug nanoparticles improve immune compatibility, reducing inflammatory responses. However, challenges remain regarding long-term stability, scalability, and clinical translation, necessitating further *in vivo* studies and clinical validation trials. Lipid-based delivery systems, including solid self-nanoemulsifying drug delivery systems and self-nanoemulsifying oily formulations, also exhibit significant improvements in dissolution rates and bioavailability and enhance lymphatic absorption. Additionally, spray-dried liposomes and niosomal gels improve drug deposition and release for topical and transdermal applications, while solid lipid nanoparticles and nanostructured lipid carriers show sustained release profiles and targeted delivery to the central nervous system, indicating the potential for managing HIV-associated neurocognitive disorders. Amorphous solid dispersions effectively address LPV's solubility challenges, with various formulations demonstrating enhanced dissolution and stability. Notably, in situ self-assembly nanoparticles for fixed-dose combinations of LPV and RTV exhibit high encapsulation efficiency and significant bioavailability improvements, particularly in pediatric HIV treatment. Prodrug and hybrid systems, including lipophilic ester prodrugs and proliposomes, enhance LPV levels in challenging reservoirs by targeting the mesenteric lymphatic system and improving stability. Other innovative approaches, such as metered-dose transdermal sprays and cyclodextrin complexation, further optimize LPV delivery while addressing solubility and P-glycoprotein (P-gp) efflux limitations. Collectively, these advancements underscore the versatility of novel delivery systems in overcoming LPV's pharmacokinetic challenges. Strategies like stimuli-responsive nanoparticles for targeted release, exosome-based carriers for precise delivery to HIV reservoirs, and CRISPR-Cas9-enhanced systems for modulating viral reservoirs present promising avenues for improving LPV's efficacy. Additionally, ultra-long-acting injectables may enhance patient adherence by reducing dosing frequency, while peptide-based carriers could improve cellular uptake. Innovations such as theranostic nanoparticles for real-time monitoring, AI-designed formulations for optimization, biopolymer-based hydrogels for responsive release, nanobots for targeted delivery, and gene therapy-linked strategies for enhanced absorption all aim to overcome current limitations in LPV treatments, ultimately leading to better outcomes for individuals living with HIV.

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## References

1. Aronson, J.K. Lopinavir. In *Meyler's Side Effects of Drugs* (Sixteenth Edition), Elsevier, **2016**; pp. 669-675.
2. Eckhardt, B.J.; Gulick, R.M. Drugs for HIV Infection. In *Opal, Infectious Diseases*, 4th ed.; Jonathan, C., William, G.; Eds.; Elsevier, **2017**: pp.1293-1308.
3. Muralidharan, N.; Sakthivel, R.; Velmurugan, D.; Gromiha, M. M. Computational studies of drug repurposing and synergism of lopinavir, oseltamivir and ritonavir binding with SARS-CoV-2 protease against COVID-19. *J. Biomol. Struct. Dyn.* **2021**, *39(7),* 2673-2678.
4. Eche, S.; Kumar, A.; Sonela, N.; Gordon, M.L. Acquired HIV-1 protease conformational flexibility associated with lopinavir failure may shape the outcome of darunavir therapy after antiretroviral therapy switch. *Biomolecules* **2021**, *11(4),* Art. No: 489. DOI: 10.3390/biom11040489
5. Mahdi, M.; Mótyán, J.A.; Szojka, Z.I.; Golda, M.; Miczi, M.; Tőzsér, J. Analysis of the efficacy of HIV protease inhibitors against SARS-CoV-2′ s main protease. *Virol. J.* **2020**, *17(190),* 1-8. DOI: 10.1186/s12985-020-01457-0
6. Ghosh, A.K.; Osswald, H.L.; Prato, G. Recent Progress in the Development of HIV-1 Protease Inhibitors for the Treatment of HIV/AIDS. *J. Med. Chem.* **2016**, *59(11*), 5172-5208. DOI: 10.1021/acs.jmedchem.5b01697
7. Von der Helm, K. Retroviral proteases: Structure, function and inhibition-from a non-anticipated viral enzyme to the target of a most promising HIV therapy. *Biol. Chem.* **1996**, *377(12),* 765-774. DOI: 10.1515/bchm3.1996.377.12.765
8. Ho, W.S.; Zhang, R.; Tan, Y.L.; Chai, C.L. COVID-19 and the promise of small molecule therapeutics: Are there lessons to be learnt? *Pharmacol. Res.* **2022**, *179*, Art. No: 106201. DOI: 10.1016/j.phrs.2022.106201
9. Marin, R.C.; Behl, T.; Negrut, N.; Bungau, S. Management of antiretroviral therapy with boosted protease inhibitors—darunavir/ritonavir or darunavir/cobicistat. *Biomedicines* **2021**, *9(3),* Art. No: 313. DOI: 10.3390/biomedicines9030313
10. ter Heine, R.; Van Waterschoot, R.A.; Keizer, R.J.; Beijnen, J.H.; Schinkel, A.H.; Huitema, A.D. An integrated pharmacokinetic model for the influence of CYP3A4 expression on the *in vivo* disposition of lopinavir and its modulation by ritonavir. *J. Pharm. Sci.* **2011**, *100(6*), 2508-2515. DOI: 10.1002/jps.22457
11. Kandel, S.E.; Lampe, J.N. Inhibition of CYP3A7 DHEA-S oxidation by lopinavir and ritonavir: an alternative mechanism for adrenal impairment in HIV antiretroviral-treated neonates. *Chem. Res. Toxicol.* **2021**, *34(4),* 1150-1160. DOI: 10.1021/acs.chemrestox.1c00028
12. Hul, M.W.; Montaner, J.S. Ritonavir-boosted protease inhibitors in HIV therapy. *Ann. Med.* **2011**, *43(5),* 375-388. DOI: 10.3109/07853890.2011.572905

13. Juan, C.; Jiang, X.; Qiang, Z. Side effects and tolerability of post-exposure prophylaxis with zidovudine, lamivudine, and lopinavir/ritonavir: a comparative study with HIV/AIDS patients. *Chin. Med. J.* **2014**, *127(14),* 2632-2636. DOI: 10.3760/cma.j.issn.0366-6999.20140538
14. Lepage, M.A.; Rozza, N.; Kremer, R.; Grunbaum, A. Safety and effectiveness concerns of lopinavir/ritonavir in COVID-19 affected patients: A retrospective series. *Clin. Toxicol*. **2021**, 59(7), 644-647. DOI: 10.1080/15563650.2020.1842882
15. Badowski, M.E.; Burton, B.; Shaeer, K.M.; Dicristofano, J. Oral oncolytic and antiretroviral therapy administration: dose adjustments, drug interactions, and other considerations for clinical use. *Drugs. Context*. **2019**, *8*, 1-31. DOI: 10.7573/dic.212550
16. Giacomelli, A.; Pezzati, L.; Rusconi, S. The crosstalk between antiretrovirals pharmacology and HIV drug resistance. *Expert. Rev. Clin. Pharmacol*. **2020**, *13(7),* 739-760. DOI: 10.1080/17512433.2020.1782737
17. Nastri, B.M.; Pagliano, P.; Zannella, C.; Folliero, V.; Masullo, A.; Rinaldi, L.; Galdiero, M.; Franci, G. HIV and drug-resistant subtypes. *Microorganisms* **2023**, 11(1), Art. No: 221. DOI: 10.3390/microorganisms11010221
18. Wensing, A.M.; van Maarseveen, N.M.; Nijhuis, M. Fifteen years of HIV Protease Inhibitors: raising the barrier to resistance. *Antiviral. Res.* **2010**, *85(1),* 59-74. DOI: 10.1016/j.antiviral.2009.10.003
19. Su, B.; Wang, Y.; Zhou, R.; Jiang, T.; Zhang, H.; Li, Z.; Liu, A.; Shao, Y.; Hua, W.; Zhang, T.; Wu, H. Efficacy and tolerability of lopinavir/ritonavir-and efavirenz-based initial antiretroviral therapy in HIV-1-infected patients in a tertiary care hospital in Beijing, China. *Front. Pharmacol*. **2019**, *10*, Art. No: 1472. DOI: 10.3389/fphar.2019.01472
20. Gandhi, R.T.; Bedimo, R.; Hoy, J.F.; Landovitz, R.J.; Smith, D.M.; Eaton, E.F.; Lehmann, C.; Springer, S.A.; Sax, P.E.; Thompson, M.A.; Benson, C.A.; Buchbinder, S.P.; Del Rio, C.; Eron Jr, J.J.; Günthard, H.F.; Molina, J.M.; Jacobsen, D.M.; Saag, M.S. Antiretroviral drugs for treatment and prevention of HIV infection in adults: 2022 recommendations of the International Antiviral Society–USA panel. *JAMA* **2023**, *329(1),* 63-84. DOI: 10.1001/jama.2022.22246
21. Pasley, M.V.; Martinez, M.; Hermes, A.; d'Amico, R.; Nilius, A. Safety and efficacy of lopinavir/ritonavir during pregnancy: a systematic review. *AIDS. Rev.* **2013**, *15(1),* 38-48. DOI: 10.1080/15284336.2018.1459343
22. Eggleton, J.S.; Nagalli, S. Highly Active Antiretroviral Therapy (HAART). In: StatPearls [Internet], Eds.; StatPearls: Treasure Island (FL), **2024**. Available online: https://www.ncbi.nlm.nih.gov/books/NBK554533/ (accessed 23 September 2024)
23. Hamers, R.L.; de Wit, T.F.; Holmes, C.B. HIV drug resistance in low-income and middle-income countries. *Lancet HIV* **2018**, *5(10),* e588-e596. DOI: 10.1016/S2352-3018(18)30173-5
24. Zi, P.; Zhang, C.; Ju, C.; Su, Z.; Bao, Y.; Gao, J.; Sun, J.; Lu, J.; Zhang, C. Solubility and bioavailability enhancement study of lopinavir solid dispersion matrixed with a polymeric surfactant-Soluplus. *Eur. J. Pharm. Sci*. **2019**, *134*, 233-245. DOI: 10.1016/j.ejps.2019.04.022
25. Khan, A.A.; Mudassir, J.; Akhtar, S.; Murugaiyah, V.; Darwis, Y. Freeze-dried lopinavir-loaded nanostructured lipid carriers for enhanced cellular uptake and bioavailability: statistical optimization, *in vitro* and *in vivo* evaluations. *Pharmaceutics* **2019**, *11(2),* Art. No: 97. DOI: 10.3390/pharmaceutics11020097
26. Angshuman, B.; Bhattacharjee, S.K.; Mahanta, R.; Biswanth, M.; Bandyopadhaya, S.K. Alginate based nanoparticulate drug delivery for anti-HIV drug lopinavir. *J. Global. Pharm. Tech*. **2010**, *2(3),* 126-132.
27. Jain, S.; Sharma, J.M.; Jain, A.K.; Mahajan, R.R. Surface-stabilized lopinavir nanoparticles enhance oral bioavailability without coadministration of ritonavir. *Nanomedicine* **2013**, *8(10),* 1639-1655. DOI: 10.2217/nnm.12.181
28. Ravi, P.R.; Vats, R.; Balija, J.; Adapa, S.P.; Aditya, N. Modified pullulan nanoparticles for oral delivery of lopinavir: Formulation and pharmacokinetic evaluation. *Carbohydr. Polym*. **2014**, *110*, 320-328. DOI: 10.1016/j.carbpol.2014.03.099
29. Ravi, P.R.; Vats, R.; Dalal, V.; Gadekar, N.; Adoitya, N. Design, optimization and evaluation of poly-ε-caprolactone (PCL) based polymeric nanoparticles for oral delivery of lopinavir. *Drug. Dev. Ind. Pharm*. **2015**, *41(1),* 131-140. DOI: 10.3109/03639045.2013.850710
30. Joshi, G.; Kumar, A.; Sawant, K. Bioavailability enhancement, Caco-2 cells uptake and intestinal transport of orally administered lopinavir-loaded PLGA nanoparticles. *Drug. Deliv*. **2016**, *23(9),* 3492-3504. DOI: 10.1080/10717544.2016.1199605.
31. Abou-El-Naga, I.F.; El Kerdany, E.D.; Mady, R.F.; Shalaby, T.I.; Zaytoun, E.M. The effect of lopinavir/ritonavir and lopinavir/ritonavir loaded PLGA nanoparticles on experimental toxoplasmosis. *Parasitol. Int*. **2017**, *66(6),* 735-747. DOI: 10.1016/j.parint.2017.08.007
32. Liptrott, N.J.; Giardiello, M.; McDonald, T.O.; Rannard, S.P.; Owen, A. Lack of interaction of lopinavir solid drug nanoparticles with cells of the immune system. *Nanomedicine* **2017**, *12(17),* 2043-2054. DOI: 10.2217/nnm-2017-0095
33. Nassar, T.; Rohald, A.; Naraykin, N.; Barasch, D.; Amsalem, O.; Prabhu, P.; Kotler, M.; Benita, S. Nanocapsules embedded in microparticles for enhanced oral bioavailability and efficacy of Lopinavir as an anti-AIDS drug. *J. Drug. Target*. **2019**, *27(5-6),* 590-600. DOI: 10.1080/1061186X.2018.1552275
34. Katata-Seru, L.; Ojo, B.M.; Okubanjo, O.; Soremekun, R.; Aremu, O.S. Nanoformulated Eudragit lopinavir and preliminary release of its loaded suppositories. *Heliyon* **2020**, *6(5),* Art. No: e03890. DOI: 10.1016/j.heliyon.2020.e03890
35. Patel, G.; Shelat, P.; Lalwani, A. Statistical modeling, optimization and characterization of solid self-nanoemulsifying drug delivery system of lopinavir using design of experiment. *Drug. Deliv*. **2016**, *23(8),* 3027-3042. DOI: 10.3109/10717544.2016.1141260
36. Khan, A.A.; Akhtar, S.; Yadav, Y.; Atiya, A.; Alelwani, W.; Bannunah, A.M.; Mahmood, S. Lopinavir-Loaded Self-Nanoemulsifying Drug Delivery System for Enhanced Solubility: Development, Characterisation and Caco-2 Cell Uptake. *Curr. Drug. Deliv*. **2023**, *20(10),* 1474-1486. DOI: 10.2174/1567201819666220817111054
37. Garg, B.; Katare, O.P.; Beg, S.; Lohan, S.; Singh, B. Systematic development of solid self-nanoemulsifying oily formulations (S-SNEOFs) for enhancing the oral

bioavailability and intestinal lymphatic uptake of Lopinavir. *Colloids. Surf. B: Biointerfaces* **2016**, *141*, 611-622. DOI: 10.1016/j.colsurfb.2016.02.012

38. Maniyar, M.G.; Kokare, C.R. Formulation and evaluation of spray dried liposomes of lopinavir for topical application. *J. Pharm. Investig.* **2019**, *49*, 259-270. DOI: 10.1007/s40005-018-0403-7
39. Patel, K.K.; Kumar, P.; Thakkar, H.P. Formulation of niosomal gel for enhanced transdermal lopinavir delivery and its comparative evaluation with ethosomal gel. *AAPS. Pharm. Sci. Tech.* **2012**, *13*, 1502-1510. DOI: 10.1208/s12249-012-9871-7
40. Fayed, N.D.; Essa, E.A.; El Maghraby, G.M. Menthol augmented niosomes for enhanced intestinal absorption of lopinavir. *Pharm. Dev. Technol.* **2022**, *27(9),* 956-964. DOI: 10.1080/10837450.2022.2136195
41. Alex, M.A.; Chacko, A.J.; Jose, S.; Souto, E.B. Lopinavir loaded solid lipid nanoparticles (SLN) for intestinal lymphatic targeting. *Eur. J. Pharm. Sci.* **2011**, *42(1-2*), 11-18. DOI:10.1016/j.ejps.2010.10.002
42. Alex, A.; Paul, W.; Chacko, A.J.; Sharma, C.P. Enhanced delivery of lopinavir to the CNS using Compritol®-based solid lipid nanoparticles. *Ther. Deliv.* **2011**, *2(1),* 25-35. Doi: 10.4155/tde.10.96
43. Negi, J.S.; Chattopadhyay, P.; Sharma, A.K.; Ram, V. Development of solid lipid nanoparticles (SLNs) of lopinavir using hot self nano-emulsification (SNE) technique. *Eur. J. Pharm. Sci.* **2013**, *48(1-2*), 231-239. DOI: 10.1016/j.ejps.2012.10.022
44. Ravi, P.R.; Vats, R.; Dalal, V.; Murthy, A.N. A hybrid design to optimize preparation of lopinavir loaded solid lipid nanoparticles and comparative pharmacokinetic evaluation with marketed lopinavir/ritonavir coformulation. *J. Pharm. Pharmacol.* **2014**, *66(7)*, 912-926. DOI: 10.1111/jphp.12217
45. Ravi, P.R.; Vats, R. Comparative pharmacokinetic evaluation of lopinavir and lopinavir-loaded solid lipid nanoparticles in hepatic impaired rat model. *J. Pharm. Pharmacol.* **2017**, *69(7),* 823-833. DOI: 10.1111/jphp.12716
46. Ansari, H.; Singh, P. Formulation and in-vivo evaluation of novel topical gel of lopinavir for targeting HIV. *Curr. HIV. Res.* **2018**, *16(4)*, 270-279. DOI: 10.2174/1570162X16666180924101650
47. Garg, B.; Beg, S.; Kumar, R.; Katare, O.P.; Singh, B. Nanostructured lipidic carriers of lopinavir for effective management of HIV-associated neurocognitive disorder. *J. Drug. Deliv. Sci. Tech.* **2019**, *53*, Art. No: 101220. DOI: 10.1016/j.jddst.2019.101220
48. Moura, R.B.; Andrade, L.M.; Alonso, L.; Alonso, A.; Marreto, R.N.; Taveira, S.F. Combination of lipid nanoparticles and iontophoresis for enhanced lopinavir skin permeation: Impact of electric current on lipid dynamics. *Eur. J. Pharm. Sci.* **2022**, *168*, Art. No: 106048. DOI: 10.1016/j.ejps.2021.106048
49. Maniyar, M.; Chandak, A.; Kokare, C. Lopinavir loaded spray dried liposomes with penetration enhancers for cytotoxic activity. *Infect. Disord. Drug. Targets* **2020**, *20(5),* 724-736. DOI: 10.2174/1871526519666191008112207
50. Hamed, R.; Mohamed, E.M.; Sediri, K.; Khan, M.A.; Rahman, Z. Development of stable amorphous solid dispersion and quantification of crystalline fraction of lopinavir by spectroscopic-chemometric methods. *Int. J. Pharm.* **2021**, *602*, Art. No: 120657. DOI: 10.1016/j.ijpharm.2021.120657
51. Li, N.; Taylor, L.S. Microstructure formation for improved dissolution performance of lopinavir amorphous solid dispersions. *Mol. Pharm.* **2019**, *16(4),* 1751-1765. DOI: 10.1021/acs.molpharmaceut.9b00117
52. Kasbaum, F.E.; de Carvalho, D.M.; de Jesus Rodrigues, L.; Cardoso, G.; Pinho, L.A.; Martins, F.T.; Cunha-Filho, M.; Taveira, S.F.; Marreto, R.N. Development of lipid polymer hybrid drug delivery systems prepared by hot-melt extrusion. *AAPS. Pharm. Sci. Tech.* **2023**, *24(6),* Art. No: 156. DOI: 10.1208/s12249-023-02610-y
53. Kayalar, C.; Helal, N.; Mohamed, E.M.; Dharani, S.; Khuroo, T.; Kuttolamadom, M.A.; Rahman, Z.; Khan, M.A. *In Vitro* and *In Vivo* testing of 3D-Printed Amorphous Lopinavir Printlets by Selective Laser Sinitering: Improved Bioavailability of a Poorly Soluble Drug. *AAPS. Pharm. Sci. Tech.* **2024,** *25(1)*, Art. No: 20. DOI: 10.1208/s12249-023-02729-y
54. Fayed, N.D.; Arafa, M.F.; Essa, E.A.; El Maghraby, G.M. Lopinavir-menthol co-crystals for enhanced dissolution rate and intestinal absorption. *J. Drug. Deliv. Sci. Technol.* **2022**, *74*, Art. No: 103587. DOI: 10.1016/j.jddst.2022.103587
55. Yun, T.S.; Jung, M.; Bang, K.H.; Lee, H.K.; Jin, M.; Yoo, H.; Won, J.H.; Song, B.; Hwang, Y.R.; Baek, J.S.; Cho, C.W. An economically advantageous amorphous solid dispersion of the fixed combination of lopinavir and ritonavir. *J. Pharm. Investig.* **2023**, *53(4),* 549-561. DOI: 10.1007/s40005-023-00623-0
56. Pham, K.; Li, D.; Guo, S.; Penzak, S.; Dong, X. Development and *in vivo* evaluation of child-friendly lopinavir/ritonavir pediatric granules utilizing novel in situ self-assembly nanoparticles. J. *Control. Release.* **2016**, *226*, 88-97. DOI: 10.1016/j.jconrel.2016.02.001
57. Else, L.J.; Douglas, M.; Dickinson, L.; Back, D.J.; Khoo, S.H.; Taylor, G.P. Improved oral bioavailability of lopinavir in melt-extruded tablet formulation reduces impact of third trimester on lopinavir plasma concentrations. *Antimicrob. Agents Chemother.* **2012**, *56(2),* 816-824. DOI: 10.1128/AAC.05186-11
58. Qin, C.; Chu, Y.; Feng, W.; Fromont, C.; He, S.; Ali, J.; Lee, J.B.; Zgair, A.; Berton, M.; Bettonte, S.; Liu, R. Targeted delivery of lopinavir to HIV reservoirs in the mesenteric lymphatic system by lipophilic ester prodrug approach. *J. Control. Release* **2021,** *329*, 1077-1089. DOI: 10.1016/j.jconrel.2020.10.036
59. Patel, G.M.; Shelat, P.K.; Lalwani, A.N. QbD based development of proliposome of lopinavir for improved oral bioavailability. *Eur. J. Pharm. Sci.* **2017**, *108*, 50-61. DOI: 10.1016/j.ejps.2016.08.057
60. Madgulkar, A.R.; Bhalekar, M.R.; Kadam, A.A. Improvement of oral bioavailability of lopinavir without co-administration of ritonavir using microspheres of thiolated xyloglucan. *AAPS. Pharm. Sci. Tech.* **2018**, *19(1),* 293-302. DOI: 10.1208/s12249-017-0834-x
61. Patel, D.; Kumar, P.; Thakkar, H.P. Lopinavir metered-dose transdermal spray through microporated skin: Permeation enhancement to achieve therapeutic needs. *J. Drug. Deliv. Sci. Tech.* **2015**, *29*, 173-180. DOI: 10.1016/j.jddst.2015.07.004

62. Adeoye, O.; Conceição, J.; Serra, P.A.; da Silva, A.B.; Duarte, N.; Guedes, R.C.; Corvo, M.C.; Aguiar-Ricardo, A.; Jicsinszky, L.; Casimiro, T.; Cabral-Marques, H. Cyclodextrin solubilization and complexation of antiretroviral drug lopinavir: In silico prediction; Effects of derivatization, molar ratio and preparation method. *Carbohydr. Polym.* **2020**, *227*, Art. No: 115287. DOI: 10.1016/j.carbpol.2019.115287
63. Adeoye, O.; Bártolo, I.; Conceição, J.; da Silva, A.B.; Duarte, N.; Francisco, A.P.; Taveira, N.; Cabral-Marques, H. Pyromellitic dianhydride crosslinked soluble cyclodextrin polymers: Synthesis, lopinavir release from sub-micron sized particles and anti-HIV-1 activity. *Int. J. Pharm.* **2020**, *583*, Art. No: 119356. DOI: 10.1016/j.ijpharm.2020.119356
64. Mahajan, H.S.; Patil, P.H. Central composite design-based optimization of lopinavir vitamin E-TPGS micelle: *In vitro* characterization and *in vivo* pharmacokinetic study. *Colloids. Surf. B. Biointerfaces* **2020**, *194*, Art. No: 111149. DOI: 10.1016/j.colsurfb.2020.111149
65. Chaudhari, S.P.; Handge, N.M. Formulation, development and evaluation of lopinavir loaded polymeric micelles. *J. Sci. Tech.* **2020**, *5(4),* 173-187. DOI: 10.46243/jst.2020.v5.i4.pp173-187
66. Aremu, O.S.; Katata-Seru, L.; Mkhize, Z.; Botha, T.L.; Wepener, V. Polyethylene glycol (5,000) succinate conjugate of lopinavir and its associated toxicity using Danio rerio as a model organism. *Sci. Rep.* **2020**, *10(1),* Art. No: 11789. DOI: 10.1038/s41598-020-68666-z
67. Salim, M.; Ramirez, G.; Clulow, A.J.; Hawley, A.; Boyd, B.J. Implications of the Digestion of Milk-Based Formulations for the Solubilization of Lopinavir/ Ritonavir in a Combination Therapy. *Mol. Pharmaceutics* **2023**, *20(4),* 2256-2265. DOI: 10.1021/acs.molpharmaceut.3c00072
68. Tanaudommongkon, I.; Tanaudommongkon, A.; Dong, X. Development of in situ self-assembly nanoparticles to encapsulate lopinavir and ritonavir for long-acting subcutaneous injection. *Pharmaceutics* **2021**, *13(6),* Art. No: 904. DOI: 10.3390/pharmaceutics13060904
69. Cattaneo, D.; Perno, C.F.; Rizzardini, G. A three-in-one, long-acting, nanosuspension reformulation of off-patent antiretrovirals for low-income and middle-income countries. *AIDS* **2018**, *32(17),* 2625-2627. DOI:10.1097/QAD.0000000000002011
70. McConnachie, L.A.; Kinman, L.M.; Koehn, J.; Kraft, J.C.; Lane, S.; Lee, W.; Collier, A.C.; Ho, R.J. Long-acting profile of 4 drugs in 1 anti-HIV nanosuspension in nonhuman primates for 5 weeks after a single subcutaneous injection. *J. Pharm. Sci.* **2018**, *107(7),* 1787-1790. DOI: 10.1016/j.xphs.2018.03.005
71. Koehn, J.; Iwamoto, J.F.; Kraft, J.C.; McConnachie, L.A.; Collier, A.C.; Ho, R.J. Extended cell and plasma drug levels after one dose of a three-in-one nanosuspension containing lopinavir, efavirenz, and tenofovir in nonhuman primates. *AIDS* **2018**, *32(17),* 2463-2467. DOI: 10.1097/QAD.0000000000001969
72. Perazzolo, S.; Shen, D.D.; Scott, A.M.; Ho, R.J. Physiologically based pharmacokinetic model validated to enable predictions of multiple drugs in a long-acting drug-combination nano-particles (DCNP): confirmation with 3 HIV drugs, lopinavir, ritonavir, and tenofovir in DCNP products. *J. Pharm. Sci.* **2024**, *113(6),* 1653-1663. DOI: 10.1016/j.xphs.2024.02.018
73. Dominique, M.; Konstantin, H. (2016). Dosage form comprising lopinavir and ritonavir. U.S. Patent No. 9,370,578 B2. Washington, DC: U.S. Patent and Trademark Office.
74. Giardiello, M.N.; McDonald, T.O.; Owen, A.; Rannard, S.P. Compositions of lopinavir and ritonavir. International Publication number WO 2013/034927 A1.
75. Hampson, I.; Hampson, L. Lopinavir and ritonavir for the treatment of cervix disoprders. International Publication number WO 2019/224779 A1.
76. Pham, S.H; Choi, Y.; Choi, J. Stimuli-Responsive Nanomaterials for Application in Antitumor Therapy and Drug Delivery. *Pharmaceutics* **2020**, *12(7),* Art. No: 630. DOI: 10.3390/pharmaceutics12070630
77. Koh, H.B.; Kim, H.J.; Kang, S.W.; Yoo, T.H. Exosome-Based Drug Delivery: Translation from Bench to Clinic. *Pharmaceutics* **2023**, *15(8),* Art. No: 2042. DOI: 10.3390/pharmaceutics15082042
78. Asmamaw, M.; Zawdie, B. Mechanism and Applications of CRISPR/Cas-9-Mediated Genome Editing. *Biologics* **2021**, *15*, 353-361. DOI: 10.2147/BTT.S326422
79. Kovarova, M.; Benhabbour, S.R.; Massud, I.; Spagnuolo, R.A.; Skinner, B.; Baker, C.E.; Sykes, C.; Mollan, K.R.; Kashuba, A. D. M.; García-Lerma, J. G.; Mumper, R. J.; Garcia, J. V. Ultra-long-acting removable drug delivery system for HIV treatment and prevention. *Nat. Commun.* **2018**, *9(1),* Art. No: 4156. DOI: 10.1038/s41467-018-06490-w
80. Khairkhah, N.; Namvar, A.; Bolhassani, A. Application of Cell Penetrating Peptides as a Promising Drug Carrier to Combat Viral Infections. *Mol. Biotechnol.* **2023**, *65(9),* 1387-1402. DOI: 10.1007/s12033-023-00679-1
81. Kevadiya, B.D.; Ottemann, B.; Mukadam, I. Z.; Castellanos, L.; Sikora, K.; Hilaire, J. R.; Machhi, J.; Herskovitz, J.; Soni, D.; Hasan, M.; Zhang, W.; Anandakumar, S.; Garrison, J.; McMillan, J.; Edagwa, B.; Mosley, R. L.; Vachet, R. W.; Gendelman, H. E. Rod-shape theranostic nanoparticles facilitate antiretroviral drug biodistribution and activity in human immunodeficiency virus susceptible cells and tissues. *Theranostics* **2020**, *10(2*), 630-656. DOI: 10.7150/thno.39847
82. Jiang, J.; Ma, X.; Ouyang, D.; Williams, R. O. 3rd. Emerging Artificial Intelligence (AI) Technologies Used in the Development of Solid Dosage Forms. *Pharmaceutics* **2022**, *14(11),* Art. No: 2257. DOI: 10.3390/pharmaceutics14112257.
83. Hama, R.; Ulziibayar, A.; Reinhardt, J.W.; Watanabe, T.; Kelly. J.; Shinoka. T. Recent Developments in Biopolymer-Based Hydrogels for Tissue Engineering Applications. *Biomolecules* **2023**, *13(2),* Art. No: 280. DOI: 10.3390/biom13020280
84. Hu, M.; Ge, X.; Chen, X.; Mao, W.; Qian, X.; Yuan, W. E. Micro/Nanorobot: A Promising Targeted Drug Delivery System. *Pharmaceutics*. **2020**, *12(7),* Art. No: 665. DOI: 10.3390/pharmaceutics12070665
85. Pan, X.; Veroniaina, H.; Su, N.; Sha, K.; Jiang, F.; Wu, Z.; Qi, X. Applications and developments of gene therapy drug delivery systems for genetic diseases. *Asian J. Pharm Sci.* 2021, *16(6),* 687-703. DOI: 10.1016/j.ajps.2021.05.003