International Journal of Pharmacology

Int. J. Pharmacol. **2026**; 22(3): 50190
https://doi.org/10.31083/IJP50190

Research Article

Enhancement of Gemifloxacin Solubility and Bioavailability Using Hydroxypropyl-β-Cyclodextrin: *In Vitro* and *In Vivo* Evaluation

Jong Woo Kim[1,†], Jae Seon Kang[2,†], Ga Eun Park[1], Yeon Hee Kim[3,*], Kang Min Kim[4,*]

[1]Department of Pharmacy, Kyungsung University, 48434 Busan, Republic of Korea
[2]Brain Busan 21 Plus Research Project Group, Department of Pharmacy, Kyungsung University, 48434 Busan, Republic of Korea
[3]Formulation Team, Daejeon Research Institute, Kolon Pharm, 34302 Daejeon, Republic of Korea
[4]Department of Pharmaceutical Science and Technology, Kyungsung University, 48434 Busan, Republic of Korea
*Correspondence: dusgmlskfk@kolon.com (Yeon Hee Kim); kimkmks@ks.ac.kr (Kang Min Kim)
[†]These authors contributed equally.

Academic Editor: Mehmet Ozaslan
Submitted: 20 January 2026 Revised: 15 April 2026 Accepted: 17 April 2026 Published: 1 June 2026

Abstract

Background and Objective: Various solubilizing agents were used to improve the solubility of gemifloxacin, which has limited water solubility. Furthermore, the solubility enhancement and bioavailability of gemifloxacin were then assessed through *in vitro* solubility screening and *in vivo* pharmacokinetic studies in rats. **Materials and Methods**: Different solubilizing agents, including poloxamer 407, poloxamer 188, Soluplus®, Polyox N80, sodium lauryl sulfate, PEG 4000, L-arginine, L-lysine, β-cyclodextrin, and hydroxypropyl-β-cyclodextrin (HPCD), were evaluated for the associated solubilizing effects, and HPCD was selected as the optimal solubilizer. Gemifloxacin in samples collected from the *in vitro* and *in vivo* experiments was quantified using high-performance liquid chromatography (HPLC). **Results**: *In vitro* solubility screening showed that the solubility of gemifloxacin reached 69.37 $\pm$ 0.71 mg/mL with 4.1 g of HPCD, representing a 1.52-fold increase relative to the control group (45.68 $\pm$ 0.37 mg/mL). Further optimization revealed that 0.1 g of HPCD achieved a solubility of 66.27 $\pm$ 0.42 mg/mL, with minimal additional improvement at higher concentrations. The HPLC method exhibited excellent linearity (R^2 = 0.9998) over the range of 0.03–45 µg/mL. The *in vivo* pharmacokinetic study demonstrated that the area under the curve ($AUC_{0-\infty}$) of the gemifloxacin–HPCD formulation (Group A: 7.527 $\pm$ 0.60 µg·h/mL) increased approximately 1.53-fold compared with that of the gemifloxacin-alone group (4.928 $\pm$ 0.85 µg·h/mL), significantly improving the bioavailability of gemifloxacin. **Conclusion**: The solubilization strategy using HPCD can effectively improve the solubility and bioavailability of gemifloxacin, representing a promising approach for the development of the associated oral formulations.

Keywords: biological availability; gemifloxacin; 2-hydroxypropyl-beta-cyclodextrin; pharmacokinetics; solubility

1. Introduction

Pathogens such as *Streptococcus pneumoniae* (*S. pneumoniae*), which are easily transmitted in daily life, can cause community-acquired pneumonia (CAP). The risk of bacteremia and sepsis increases if CAP remains untreated; in severe cases, the long-term mortality risk remains high even after treatment [1]. These causative organisms survive and proliferate through type II topoisomerases (DNA gyrase) and topoisomerase IV, which are critical for bacterial DNA replication, transcription, repair, and recombination, thereby facilitating their easy transmission [2]. Therefore, the inhibition of DNA gyrase and topoisomerase IV is essential for the treatment of CAP and acute exacerbation of chronic bronchitis.

Gemifloxacin, moxifloxacin, and levofloxacin are new fluoroquinolone antibiotics. Gemifloxacin primarily targets and inhibits both DNA gyrase and topoisomerase IV, thereby inhibiting DNA synthesis and inducing cell death. Furthermore, gemifloxacin exhibits lower minimum inhibitory concentrations (MICs) against pathogens causing acute exacerbation of chronic bronchitis (AECB) than other fluoroquinolone antibiotics. Clinically, gemifloxacin reduces hospitalization duration and contributes to healthcare cost savings compared to ceftriaxone and clarithromycin. Gemifloxacin has demonstrated higher clinical success rates than levofloxacin over the long term, indicating that gemifloxacin offers multiple advantages over other fluoroquinolone antibiotics [3]. Pharmacokinetic results from previous studies have demonstrated that an average of 61 $\pm$ 9.5% of the dose was excreted in feces following oral administration to healthy subjects, whereas 36 $\pm$ 9.3% was eliminated in urine as an unchanged drug and metabolite. After repeated dosing of 320 mg, the mean renal clearance was approximately 11.6 $\pm$ 3.9 L/h (range 4.6–16 L/h), suggesting that active secretion is involved in the renal excretion of gemifloxacin [4].

Cyclodextrin (CD) is a cyclic oligosaccharide comprising glucose units linked by α-1,4-glycosidic bonds. Cyclodextrin is hydrophilic because of the presence of hydroxyl groups on its exterior, whereas its interior is relatively hydrophobic [5]. The application of β-CD in formu-

lation development is severely limited by both its low intrinsic aqueous solubility due to strong intramolecular hydrogen bonding and a strict acceptable daily intake (ADI) limit of 0.35 g for humans. In contrast, hydroxypropyl-β-cyclodextrin (HPCD) exhibits drastically improved aqueous solubility through hydroxypropyl substitution and superior oral tolerability (up to 8 g/day) with minimal gastrointestinal irritation [6]. Given these physicochemical and toxicological advantages, HPCD is considered a safer and more effective oral excipient than unmodified β-CD. In addition, previous studies have demonstrated that CD exhibits specific tissue distribution and clearance characteristics in rats, suggesting that it not only aids absorption but also influences pharmacokinetic changes [7].

Therefore, we identified solubilizing agents that affect the solubility of gemifloxacin, a respiratory antibiotic with multiple advantages. In addition, we used solubilizing agents to compare and evaluate the bioavailability and pharmacokinetics of a new gemifloxacin formulation developed using a reduced active ingredient content compared to a reference drug.

2. Materials and Methods

2.1 Samples and Reagents

The gemifloxacin, moxifloxacin, and physiological saline were purchased from Sigma-Aldrich (St. Louis, MO, USA). Solubilizing agents, including HPCD, were obtained from Roquette (Lestrem, France), and polyethylene glycol 4000 (PEG 4000) was sourced from IC Chemical (Yeosu, Republic of Korea). β-Cyclodextrin and L-arginine were purchased from ES Food Ingredients Co., Ltd. (Gunpo, Republic of Korea), whereas L-lysine monohydrochloride was obtained from Saewon Mulsan Co., Ltd. (Seoul, Republic of Korea). Poloxamer 407, poloxamer 188, and Polyox N80 were acquired from Colorcon (Gunpo, Republic of Korea), and sodium lauryl sulfate (SLS) was purchased from Duksan (Ansan, Republic of Korea).

A Centrifuge 5804 R (Eppendorf; MA, USA), Evaporator Uuiequip Univapo 100H GA59 (UniEquip Laborgerätebau and Vertriebs GmbH; Munich, Germany), and Power Sonic 520 sonicator (Hwashin Tech; Seoul, Republic of Korea) were used to prepare the sample. High-performance liquid chromatography (HPLC) was performed using a Shimadzu system (Kyoto, Japan) equipped with an LC-20AD pump, SIL-20AC autosampler, CTO-20A column oven, and SPD-20A detector. Blood samples were collected in EDTA tubes (Vacuette; Kremsmünster, Austria). Analytical-grade trifluoroacetic acid (TFA) for mobile phase preparation was purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (ACN), methanol (MeOH), and monobasic sodium phosphate were obtained from Samchun Chemicals (Seoul, Republic of Korea). Formic acid was purchased from Junsei Chemical (Tokyo, Japan). All the solvents used were of extra-pure grade.

2.2 In Vitro Solubility Evaluation

In vitro solubility screening of gemifloxacin was performed using non-ionic surfactants, anionic surfactants, and solubilizing agents. The concentration of each excipient was determined by conducting preliminary solubility experiments and was set to the maximum concentration that could be completely dissolved in 4 mL of purified water. Accordingly, we used poloxamer 407 (0.37 g), poloxamer 188 (0.41 g), Soluplus® (0.41 g), Polyox N80 (0.25 g), sodium lauryl sulfate (0.34 g), PEG 4000 (2.44 g), L-arginine (0.58 g), L-lysine (0.44 g), β-cyclodextrin (0.03 g), and HPCD (4.1 g). Additional solubility experiments were conducted with 0.05, 0.10, and 0.20 g HPCD for further investigation. The control group was prepared using purified water without solubilizing agents, following the same experimental procedures.

Based on the reported saturation solubility of gemifloxacin (45.68 mg/mL at pH 7.0, 37 °C) [8], 0.5 g of gemifloxacin was added to ensure dissolution equilibrium. Next, the samples were vortex-mixed for 10 min at room temperature (25 $\pm$ 2 °C), followed by a 5-min rest period at room temperature to allow precipitation of undissolved drug. An aliquot of 0.1 mL of the supernatant was transferred to a 100 mL volumetric flask, dissolved with purified water, and filtered through a 0.45 µm regenerated cellulose (RC) filter (Sartorius AG; Goettingen, Germany). The filtered samples were analyzed according to the HPLC conditions, and the concentrations were calculated using the external standard method by applying the proportional relationship between the peak area and concentration of a single-concentration standard solution. All *in vitro* solubility experiments were performed five times for each condition (n = 5).

2.3 In Vivo Pharmacokinetic Study

Male Sprague-Dawley (SD) rats (237.6 $\pm$ 12.5 g) were used in this study. The study was approved by the Institutional Animal Care and Use Committee (IACUC) of Kyungsung University in accordance with the Animal Protection Act (Acts no. 4379 and no. 12681). Animals were obtained from Hyochang Science (Daegu, Korea) and maintained under controlled environmental conditions with room temperature at 22 $\pm$ 3 °C, relative humidity of 30–70%, and a 12-h light/dark cycle. All rats were fasted overnight before the experiment, with free access to water. Prior to each blood collection via the retro-orbital venous plexus, rats were briefly anesthetized by isoflurane inhalation (2.5–3% for induction; 1–2% for maintenance) using an anesthetic vaporizer with an induction chamber. At the end of the study, all animals were euthanized by carbon dioxide (CO_2) inhalation (100% CO_2; chamber fill rate 30–70% of chamber volume/min). The *in vivo* dose of gemifloxacin was calculated based on body weight proportion using a commercially available dose of 426.39 mg. The *in vitro* solubility evaluation results demonstrated maximum solubility enhancement at a gemifloxacin:HPCD weight ra-

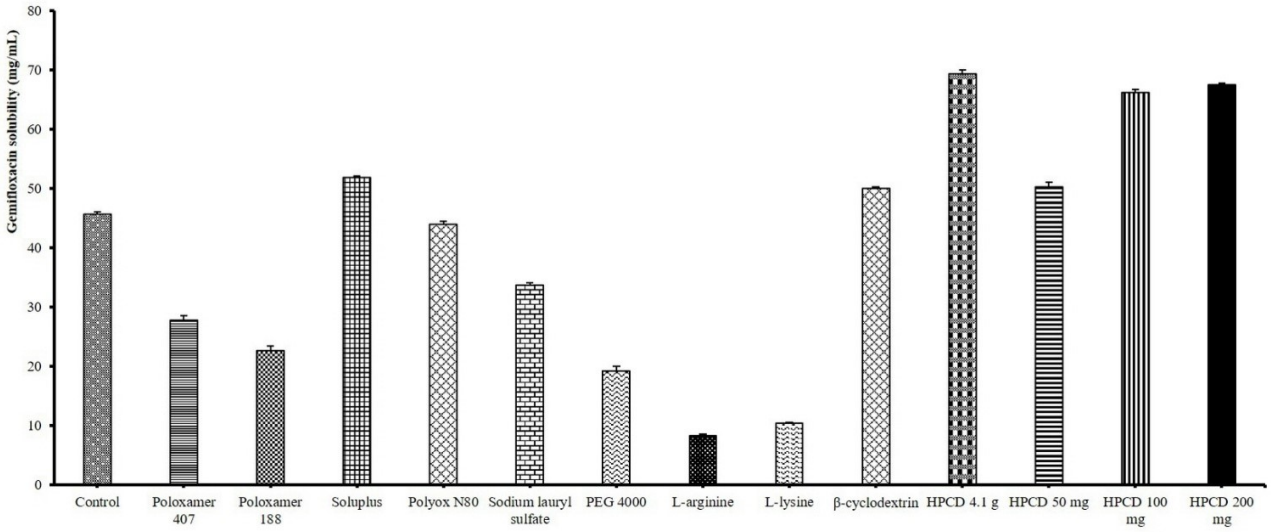

Fig. 1. Dosage and results of surfactants used in gemifloxacin solubility screening (*n* = 5 per group). All solubility analyses were determined by high-performance liquid chromatography (HPLC). The solubility of gemifloxacin ranged from 8.26 ± 0.25 mg/mL (L-arginine) to 69.37 ± 0.71 mg/mL (HPCD 4.1 g), and the control group (purified water) showed 45.68 ± 0.37 mg/mL. PEG, polyethylene glycol; HPCD, hydroxypropyl-β-cyclodextrin. Values are expressed as mean ± standard deviation (n = 5 per group).

tio of 5:1. The samples for *in vivo* evaluation were prepared using this composition. The formulation was prepared freshly prior to use and administered immediately without storage. Fifteen rats were randomly assigned to three groups (n = 5 per group) for the gemifloxacin–HPCD complex study. The control group received 1.78 mg of gemifloxacin dissolved in 3 mL of physiological saline via oral administration. Group A received 1.0 mg of gemifloxacin with 0.2 mg of HPCD in 3 mL of physiological saline, and Group B received 0.5 mg of gemifloxacin with 0.2 mg of HPCD in 3 mL of physiological saline, both administered orally. For pharmacokinetic evaluation, 1 mL blood samples were collected from the retro-orbital venous plexus immediately before and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 4, and 6 h of oral administration of gemifloxacin. All blood samples were collected in EDTA tubes and centrifuged at 3000 rpm for 15 min at 4 °C to separate plasma. The separated plasma samples were stored at –70 °C until analysis. Pharmacokinetic parameters were calculated by non-compartmental analysis using BA-Calc 2007 version 1.0 (KFDA, Osong, Republic of Korea). The area under the plasma concentration–time curve from time zero to infinity ($AUC_{0-\infty}$) was calculated using the linear trapezoidal rule. In addition, other parameters, including maximum plasma concentration (Cmax), time to reach maximum concentration (Tmax), and elimination half-life ($t_{1/2}$) were determined.

2.4 Plasma Sample Preparation

An aliquot of 200 µL of the plasma sample was transferred to a microcentrifuge tube. To each sample, 10 µL of internal standard solution (IS; 120 µg/mL) and 420 µL of ACN containing 0.1% (v/v) formic acid were added. The tubes were sealed and vortex-mixed for 1 min, followed by centrifugation at 3000 rpm for 10 min at 4 °C. After centrifugation, 200 µL of the supernatant was transferred to another microcentrifuge tube and evaporated under reduced pressure using a speed vacuum at 40 °C. The residue was reconstituted with 200 µL of methanol and analyzed by HPLC.

2.5 Preparation of Gemifloxacin Standard Solutions, Quality Control (QC, and Internal Standard)

The diluent was prepared by mixing 80 mL of a solution containing 13.8 g of monobasic sodium phosphate dissolved in 100 mL of purified water with 720 mL of purified water and 200 mL of acetonitrile. This diluent was used to prepare a 0.36 mg/mL gemifloxacin stock solution and 120 µg/mL moxifloxacin (IS). The stock solution was diluted in a single step using the above diluent to achieve target concentrations of 0.03, 0.15, 0.3, 3, 6, 12, 24, and 45 µg/mL for plasma calibration curve preparation. Quality control (QC) samples were prepared at concentrations of 0.03, 0.3, 12, and 45 µg/mL.

2.6 Recovery

The recovery of gemifloxacin was evaluated using QC samples (0.03, 0.3, 12, and 45 µg/mL), with the requirement that the relative standard deviation (RSD) should not exceed 15%.

2.7 HPLC–UV Analysis Conditions

The HPLC–UV method was adapted from the USP method for gemifloxacin. A Vision HT C18-L column (4.6 $\times$ 250 mm, 5 µm particle size; Phenomenex, USA) was employed. The mobile phase consisted of ACN:distilled water (DW):trifluoroacetic acid at a ratio of 20:80:0.1 (v/v/v). The flow rate was set at 1 mL/min with an injection volume of 20 µL. Both column and autosampler temperatures were maintained at 25 °C, and detection was performed at 272 nm.

2.8 Statistical Analysis

All data are expressed as mean $\pm$ standard deviation (SD). Statistical comparisons between groups were performed using Student's *t*-test. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using BA-Calc 2007 (version 1.0, Biopharmaceutics Laboratory, College of Pharmacy, Kyung Hee University, Seoul, South Korea).

3. Results

3.1 In Vitro Solubility Studies

The *in vitro* solubility of gemifloxacin was screened using nonionic and anionic surfactants and solubility enhancers. Differences in gemifloxacin solubility were observed depending on the type of additive compared with the control group, and the results are presented in Fig. 1. The solubility of gemifloxacin was 69.37 $\pm$ 0.71 mg/mL using 4.1 g of HPCD, representing the greatest increase of 1.52-fold compared to the control group (45.68 $\pm$ 0.37 mg/mL). This was followed by the Soluplus® (51.82 $\pm$ 0.34 mg/mL) and β-cyclodextrin (50.04 $\pm$ 0.26 mg/mL) in descending order of solubility enhancement. Based on these findings, an additional screening was performed to determine the optimal HPCD concentration. The solubility of gemifloxacin was measured as 50.30 $\pm$ 0.81 mg/mL with 0.05 g of HPCD, 66.27 $\pm$ 0.42 mg/mL with 0.1 g of HPCD, and 67.52 $\pm$ 0.29 mg/mL with 0.2 g of HPCD. The increase in gemifloxacin solubility was limited under conditions with HPCD concentrations of 0.1 g or higher, suggesting a plateau effect. The phase solubility profile (Fig. 2) shows an initial increase in solubility followed by a plateau region, suggesting saturation of inclusion complex formation at higher HPCD concentrations.

3.2 Linearity, Accuracy, and Precision

The retention times of gemifloxacin and the IS were approximately 9.0 and 8.86 min, respectively. Plasma calibration curves were constructed using the same rat plasma collected on different working days. The linear regression equation over the concentration range of 0.03–45 µg/mL of gemifloxacin was determined to be y = 0.1009x – 0.0073 (R^2 = 0.9998, n = 5), demonstrating excellent linearity.

The intra- and inter-day accuracy and precision were evaluated by analyzing QC samples at four concentrations, each with five replicates. The accuracy was assessed by calculating the percentage deviation of the measured values from the theoretical concentrations, and the precision was evaluated as the coefficient of variation (CV) of repeated measurements. The accuracy and precision of the results are listed in Table 1. The intraday precision was confirmed to be within a maximum of 5.40%, and the accuracy was within $\pm$1.14%. Inter-day precision was below 5.71%, and accuracy was within $\pm$1.28%.

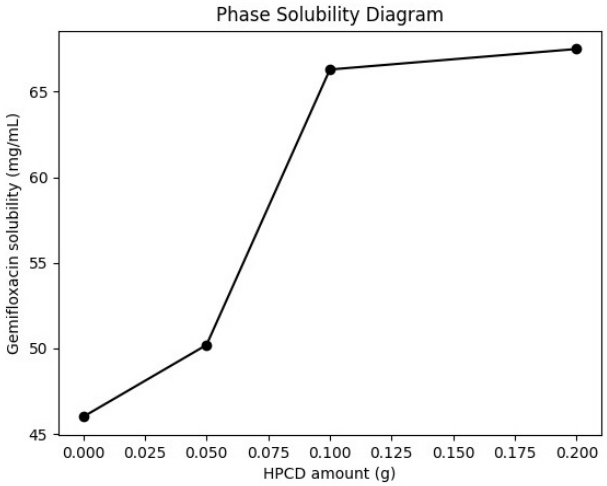

Fig. 2. Phase solubility diagram of gemifloxacin as a function of HPCD concentration. The solubility of gemifloxacin increased with increasing HPCD concentration up to 0.1 g, followed by a plateau at higher concentrations, indicating saturation of inclusion complex formation.

3.3 Recovery

The recovery of gemifloxacin from plasma samples was evaluated at QC sample concentrations of 0.03, 0.3, 12, and 45 µg/mL, resulting in recovery rates of 92.78%, 94.61%, 93.27%, and 97.89%, respectively. Each of the four QC concentrations was analyzed in five replicates, and the maximum relative standard deviation was 4.14%, indicating consistent and reliable extraction efficiency.

3.4 Pharmacokinetic Studies

Based on the solubility screening results of gemifloxacin, an *in vivo* study was conducted using a formulation containing HPCD. The pharmacokinetic parameters of the gemifloxacin–HPCD complex and gemifloxacin-alone formulations are summarized in Table 2. The mean plasma concentration–time profiles of gemifloxacin following oral administration to rats are shown in Fig. 3.

The Cmax value was higher in the control (1.199 $\pm$ 0.24 µg/mL) compared to Group A (1.175 $\pm$ 0.08 µg/mL) and Group B (0.931 $\pm$ 0.08 µg/mL). However, the $AUC_{0-\infty}$ values were significantly enhanced in Group A (7.527 $\pm$ 0.60 µg·h/mL) and Group B (5.582 $\pm$ 0.55 µg·h/mL), demonstrating increases of 1.53-fold and 1.13-

Table 1. Intra- and inter-day accuracy and precision of gemifloxacin in rat plasma (n = 5 per group).

	LLOQ (0.03 µg/mL)	Low QC (0.30 µg/mL)	Middle QC (12 µg/mL)	High QC (45 µg/mL)
Intra-day accuracy and precision				
Mean	0.03	0.30	12.13	44.83
SD	0.00162	0.0131	0.47	2.08
% CV	5.40	4.37	3.87	4.64
% Deviation	1.14	–0.87	1.08	–0.38
n	5	5	5	5
Inter-day accuracy and precision				
Mean	0.03	0.30	12.11	45.25
SD	0.00171	0.0146	0.59	2.41
% CV	5.71	4.87	4.87	5.33
% Deviation	–0.91	1.28	0.92	0.56
n	5	5	5	5

CV, coefficient of variation; SD, standard deviation; LLOQ, Lower Limit of Quantitation; QC, Quality Control.

Table 2. Pharmacokinetics of gemifloxacin in control, group A, and group B in rats (n = 5 each group).

Parameters	Control	Group A	Group B
C max (µg/mL)	1.199 ± 0.24	1.175 ± 0.08	0.931 ± 0.08
T max (h)	0.25	0.25	0.25
MRT (h)	3.91 ± 0.2	4.57 ± 0.1	4.27 ± 0.2
$t_{1/2}$ (h)	1.7 ± 0.2	2.2 ± 0.2	1.9 ± 0.2
AUC (0–6) (µg·h/mL)	2.778 ± 0.35	3.778 ± 0.15	2.867 ± 0.21
AUC (0–∞) (µg·h/mL)	4.928 ± 0.85	7.527 ± 0.60	5.582 ± 0.55

AUC, area under the curve; MRT, mean residence time. Values are expressed as mean ± standard deviation. Control group: 1.78 mg gemifloxacin in 3 mL saline; Group A: 1.0 mg gemifloxacin + 0.2 mg HPCD in 3 mL saline; Group B: 0.5 mg gemifloxacin + 0.2 mg HPCD in 3 mL saline.

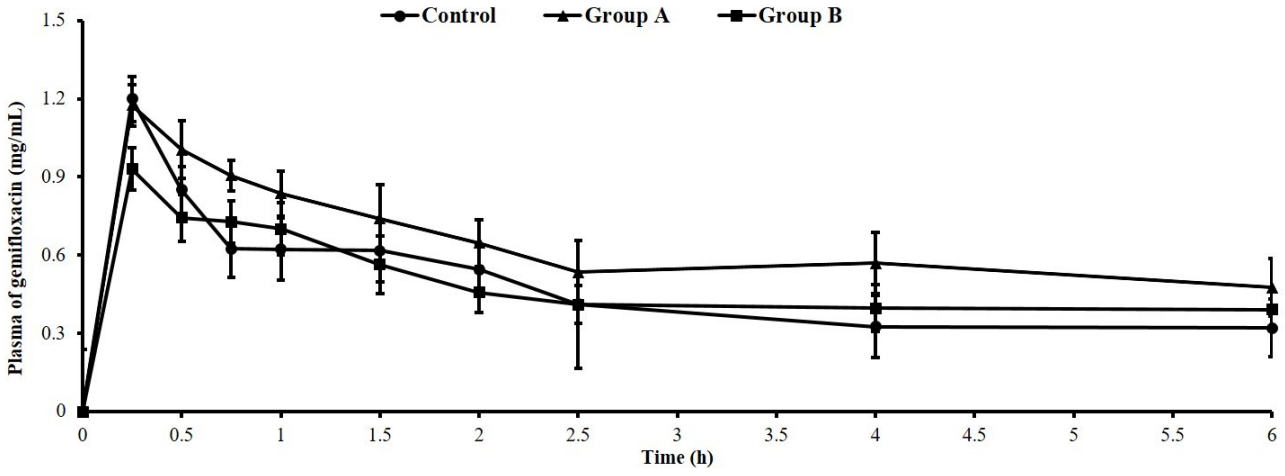

Fig. 3. Plasma concentration of gemifloxacin in rat plasma time curve (n = 5 each group). Gemifloxacin in plasma samples was analyzed by HPLC. Statistical significance between groups was determined using Student's t-test ($p < 0.05$). The groups are defined as follows: Control group: 1.78 mg gemifloxacin in 3 mL saline; Group A: 1.0 mg gemifloxacin + 0.2 mg HPCD in 3 mL saline; Group B: 0.5 mg gemifloxacin + 0.2 mg HPCD in 3 mL saline. Notably, Group A showed the highest $AUC_{0-\infty}$ (7.527 ± 0.60 µg·h/mL), which was significantly higher than that of the control group (4.928 ± 0.85 µg·h/mL). Values are expressed as mean ± standard deviation (n = 5 per group).

fold, respectively, compared to the control group (4.928 ± 0.85 μg·h/mL). In addition, both Groups A and B demonstrated an increasing trend in mean residence time (MRT) and elimination half-life ($t_{1/2}$) compared to the control group. These findings indicate that although the maximum plasma concentration was slightly lower in the HPCD formulations, the overall systemic exposure (as measured by AUC) was substantially improved, suggesting enhanced absorption and bioavailability.

4. Discussion

Gemifloxacin is an amphoteric API possessing both carboxylic acid and amino functional groups, and exhibits pH-dependent limited aqueous solubility, which presents constraints in dissolution and absorption [9,10]. We compared different surfactants and solubility enhancers to improve the solubility of gemifloxacin. Poloxamers are non-ionic surfactants composed of hydrophilic (ethylene oxide, PEO)–hydrophobic (propylene oxide, PPO)–PEO block copolymers that form micelles in aqueous solutions, thereby reducing the interfacial tension between the drug and solvent and improving the wettability and dispersibility of drug particles [11]. However, as depicted in Fig. 1, the solubility values of poloxamer 407 and poloxamer 188 were 27.81 ± 0.23 mg/mL and 22.62 ± 0.73 mg/mL, respectively, which were lower than those of the control group. This limited enhancement was attributed to the fact that effective micellization is induced only above the critical micelle concentration (CMC); micelle formation is restricted at relatively low concentrations, preventing the solubility enhancement effect from being fully manifested [12].

In addition, SLS demonstrated a solubility of 33.71 ± 0.36 mg/mL, lower than that of the control group. This effect is likely due to the strong electrostatic interactions between the amphoteric drug gemifloxacin and SLS, resulting in the precipitation of a poorly soluble lauryl sulfate salt instead of micelle-mediated solubilization [13]. These results suggest that the application of surfactants to amphoteric drugs requires consideration of physical stability and drug–excipient interactions. Similarly, PEG 4000 exhibited a solubility of 19.19 ± 0.82 mg/mL. PEG functions simply as a solvent modifier and cannot effectively influence the polar or non-polar regions of the API, resulting in low solubility [14]. L-lysine and L-arginine exhibited very low solubilities of 10.37 ± 0.25 mg/mL and 8.26 ± 0.19 mg/mL, respectively. Although amino acids can enhance solubility via ionic and non-ionic interactions, the improvement was limited in this study. This suggests that the interaction between the drug and amino acids was insufficient to overcome the strong self-association of the drug molecules [15]. Soluplus® demonstrated a relatively high solubility increase of 51.82 ± 0.34 mg/mL, which was attributed to its function as a hydrophilic–hydrophobic graft copolymer that forms polymeric micelles and functions as a solid dispersion matrix, thereby enhancing the solubility of poorly soluble drugs. However, its effect has been reported to be limited by the critical micellization concentration and plateau concentration [16]. Polyox N80 displayed a solubility of 43.91 ± 0.52 mg/mL, similar to the control group. Polyox N80 is a high-molecular-weight polyethylene oxide primarily used as a viscosity enhancer and release matrix; it contributes to viscosity and release control rather than inclusion complex formation or strong micellar solubilization, resulting in relatively low solubility enhancement effects [17]. A comprehensive consideration of these results confirmed HPCD as the solubilizing agent exhibiting the greatest solubility enhancement effect in this study, and a solubility of 69.37 ± 0.71 mg/mL at 4.1 g. HPCD possesses high aqueous solubility due to hydroxypropyl substitution and significantly increases equilibrium solubility by forming inclusion complexes with drugs through its hydrophobic cavity. The plateau effect observed at HPCD concentrations ≥0.1 g can be explained by the phase solubility behavior of cyclodextrin inclusion complexes (Fig. 2). In general, drug–cyclodextrin systems exhibit a limited complexation capacity, where the increase in drug solubility is proportional to cyclodextrin concentration only up to a certain point [17]. Beyond this concentration, the system reaches a saturation state in which most of the drug molecules are already complexed, and further addition of HPCD does not significantly enhance solubility. Although the phase solubility profile suggests the formation of a drug–HPCD inclusion complex, this interpretation is based on indirect evidence. Advanced physicochemical characterization techniques such as differential scanning calorimetry (DSC), Fourier-transform infrared spectroscopy (FTIR), and nuclear magnetic resonance (NMR) were not employed in this study. Therefore, the molecular-level interactions between gemifloxacin and HPCD could not be directly confirmed. This behavior is consistent with the formation of a 1:1 inclusion complex and the transition from a linear phase to a plateau region in the phase solubility diagram. Additionally, the self-association of cyclodextrin molecules and the formation of aggregates at higher concentrations can lead to a deviation from ideal solubilization behavior, thereby limiting the solubilization efficiency [18]. Based on these results, HPCD was determined to be a highly applicable solubility enhancer in terms of physical stability and formulation design. Considering its applicability to tablet formulations, the HPCD concentration was increased in a stepwise manner. The highest solubility of 66.27 ± 0.42 mg/mL was observed at 0.1 g, suggesting that the solubility increase did not follow a simple dose-dependent pattern. Although a complete concentration–response curve was not established, the observed plateau in solubility above 0.1 g HPCD suggests that further increases in cyclodextrin concentration do not significantly enhance complexation efficiency under the tested conditions (Fig. 2). Previous studies have reported that cyclodextrin–drug complexes exhibit diverse phase–solubility curves and non-linear characteristics in the concentration–solubility relationship [19]. Therefore, 0.1 g

of HPCD was determined to be the optimal solubilization condition considering both the solubility enhancement effect and practical formulation applicability.

The bioanalytical method was validated according to the ICH M10 guidelines before conducting *in vivo* experiments to ensure the reliability and validity of the analytical method for the quantitative analysis of gemifloxacin in plasma. The linear regression equation of the calibration curve established in rat plasma over the range of 0.03–45 μg/mL was y = 0.1009x – 0.0073 (R^2 = 0.9998, n = 5), demonstrating appropriate linearity. The lower limit of quantification (LLOQ) of gemifloxacin was set at 0.03 μg/mL; the accuracy was within ±1.14%, and precision was up to 5.71% at this concentration, meeting the criteria (≤20%) presented in the ICH M10 guidelines. The three QC sample concentrations excluding LLOQ demonstrated accuracy within ±1.28% and precision up to 5.33%, all meeting the acceptance criteria (±15%) presented in the ICH M10 guidelines [20]. Thus, the analytical method established in this study has sufficient reliability and reproducibility for quantitative analysis of gemifloxacin in rat plasma. *In vitro* solubility evaluation results confirmed that HPCD significantly improved gemifloxacin solubility. Pharmacokinetic experiments were conducted in rats to evaluate whether this *in vitro* solubility enhancement translates into improved *in vivo* absorption. Although the absolute Cmax values were higher in the control group than in Groups A and B, the dose-corrected Cmax/dose values of Group A (1.175 ± 0.08 μg/mL) and Group B (1.862 ± 0.08 μg/mL) increased by 1.75-fold and 2.77-fold, respectively, compared to the control group (0.673 ± 0.13 μg/mL). To clarify the calculation of dose-normalized exposure, the Cmax values were normalized by the administered dose (Cmax/dose). The calculated values were 0.673, 1.175, and 1.862 for the control, Group A, and Group B, respectively. Accordingly, the dose-normalized Cmax values in Group A and Group B were increased by 1.75-fold and 2.77-fold, respectively, compared to the control group. The $AUC_{0-\infty}$ values of Group A (7.527 ± 0.60 μg·h/mL) and Group B (5.582 ± 0.55 μg·h/mL) increased by 1.53-fold and 1.13-fold, respectively, compared to the control group (4.928 ± 0.85 μg·h/mL). Notably, Group B received half the dose of gemifloxacin compared to Group A, which was intentionally designed to evaluate whether the HPCD formulation could maintain or enhance drug absorption efficiency at reduced doses. Despite the lower administered dose, Group B exhibited a higher dose-normalized Cmax compared to both the control and Group A. This indicates that the HPCD complex improved the absorption efficiency of gemifloxacin, rather than simply increasing systemic exposure due to higher dosing. These findings suggest that the formulation may enable dose reduction while maintaining effective drug absorption. Despite the low administered dose, the observed improvement in absorption cannot be explained solely by an increase in solubility. Rather, the formation of an inclusion complex with HPCD likely inhibited the crystallization of gemifloxacin, thereby reducing precipitation and maintaining a supersaturated state within the intestinal lumen [21]. In particular, to overcome the unstirred water layer (UWL), which is recognized as a primary absorption barrier for poorly water-soluble drugs, the inclusion complex maintains a steep concentration gradient across the entire UWL. As a result, the inclusion complex efficiently transports the drug across the UWL to the lipophilic epithelial surface. Upon reaching the membrane surface, the complex reversibly dissociates, releasing free drug molecules and thereby enhancing mucosal drug permeation [22]. This suggests that the increased solubility of the gemifloxacin–HPCD complex increased the drug concentration in the intestinal lumen, resulting in enhanced absorption by passive diffusion [23]. The interaction between HPCD and phospholipids increases acyl chain disorder, leading to enhanced membrane fluidity and structural perturbation, which in turn increases membrane permeability [24]. This increase in membrane permeability may have partially contributed to the enhanced gemifloxacin absorption observed in this study. These results were consistent with those of previous studies demonstrating that the bioavailability of poorly soluble drugs can be improved by HPCD inclusion complex [25]. HPCD is a widely used pharmaceutical excipient with a well-established safety profile. Previous studies have reported a no-observed-adverse-effect level (NOAEL) of approximately 600 mg/kg/day in rats and low acute toxicity with oral LD_{50} values exceeding 2000 mg/kg. In the present study, the administered amount of HPCD was substantially lower than these safety thresholds, suggesting minimal risk of toxicity [26]. The increased bioavailability of gemifloxacin is attributed to its improved solubility and enhanced intestinal absorption induced by complexation with HPCD. The plasma concentration of gemifloxacin was the highest at the first sampling time point (1.199 ± 0.24 μg/mL) and decreased by approximately 48.04% by the third sampling time point (0.623 ± 0.09 μg/mL) in the control group. Thereafter, no change was observed in the concentration magnitude from the fourth sampling time point (0.621 ± 0.11 μg/mL), and a biphasic decline pattern was observed where the rate of decrease gradually became more gradual (Fig. 3). This pattern is consistent with that mentioned in previous reports, suggesting that the experimental design and data derived in this study are highly reliable [27]. Plasma concentrations were also the highest at the first sampling time point, at 1.175 ± 0.08 μg/mL and 0.931 ± 0.08 μg/mL, respectively, in Groups A and B. The plasma concentrations of Group A (0.905 ± 0.06 μg/mL) and Group B (0.727 ± 0.08 μg/mL) decreased by 23% and 22%, respectively, at the third sampling time point, and thereafter demonstrated a gradual decline pattern from subsequent sampling points (Fig. 3). Unlike the rapid plasma concentration decline observed in the control group, the gradual and sustained decline pattern observed in Groups A and B was consistent with that reported in previous pharmacokinetic studies, demonstrating

an increased systemic exposure time to drugs in inclusion complexes using HPCD [28]. Various solubilization strategies have been explored to improve the bioavailability of poorly soluble fluoroquinolone antibiotics. However, previously reported approaches have often faced technical limitations. For example, lipid–polymer hybrid nanoparticles have exhibited formulation failure or physical instability due to uncontrolled electrostatic interactions [29]. In addition, polymeric micelle systems that require complex multistep preparation processes and the use of organic solvents have shown only limited improvements in bioavailability, with increases of approximately 1.6-fold [30]. In contrast, the HPCD inclusion strategy employed in the present study significantly improved the Cmax/dose by up to 2.77-fold compared with the control group using a simple preparation process without organic solvents. These results suggest that HPCD complexation represents a highly effective, practical, and promising strategy for improving the oral delivery of gemifloxacin. The improvement of gemifloxacin bioavailability observed in this study is expected to reduce the required amount of API, ultimately enhancing dosing safety and significantly improving patient compliance through smaller tablet sizes [31,32]. In addition, the reduced API usage may contribute to lowering the drug cost.

These results suggest that HPCD complexation can improve the absorption rate of gemifloxacin, demonstrating its potential to effectively improve the oral delivery of gemifloxacin.

5. Strengths and Limitations of This Study

This study has several strengths. First, a systematic and comprehensive screening of ten structurally diverse solubilizing agents including non-ionic surfactants, an anionic surfactant, amino acids, and cyclodextrins was performed under identical experimental conditions, enabling a direct and objective comparison of solubilization capacity for gemifloxacin. Second, the selected HPCD-based formulation was evaluated through both *in vitro* solubility studies and *in vivo* pharmacokinetic studies in rats, providing an integrated assessment that bridges bench-level observations with physiologically relevant outcomes. Third, the bioanalytical HPLC method was rigorously validated in accordance with ICH M10 guidelines, demonstrating excellent linearity (R^2 = 0.9998), precision (CV $\leq$5.71%), and accuracy (within $\pm$ 1.28%), which supports the reliability of the pharmacokinetic data reported herein. However, several limitations should be acknowledged. First, the *in vivo* pharmacokinetic study was conducted using a small sample size (n = 5 per group), which may limit the statistical power and generalizability of the pharmacokinetic findings; future studies with larger cohorts are warranted to confirm these results. Second, the study was restricted to male Sprague-Dawley rats, and it remains unknown whether the observed solubility and bioavailability improvements would translate to the same extent in female animals or in human subjects; further preclinical and clinical studies are needed to evaluate species- and sex-dependent differences. Third, long-term stability testing of the HPCD formulation under various storage conditions was not conducted, and its feasibility for scale-up manufacturing into a solid dosage form (e.g., tablet) remains to be demonstrated. Another limitation of this study is the lack of direct physicochemical characterization of the gemifloxacin–HPCD inclusion complex. Techniques such as DSC, FTIR, NMR, and determination of stability (binding) constants were not performed, which limits the ability to conclusively confirm inclusion complex formation and quantify the strength of interaction. Therefore, the proposed mechanism of solubility enhancement is based on indirect evidence, including phase solubility analysis and pharmacokinetic outcomes. Future studies are warranted to perform detailed structural and thermodynamic characterization of the complex.

6. Conclusion

This study successfully demonstrated that HPCD effectively enhanced the solubility and bioavailability of gemifloxacin through a systematic evaluation combining *in vitro* solubility screening and *in vivo* pharmacokinetic studies. Among the different solubilizing agents evaluated, including non-ionic surfactants (poloxamers, PEG 4000, Soluplus®, Polyox N80), anionic surfactants (SLS), amino acids (L-arginine, L-lysine), and cyclodextrins (β-cyclodextrin, HPCD), HPCD exhibited superior solubility enhancement, achieving a maximum solubility of 69.37 $\pm$ 0.71 mg/mL at a high concentration (4.1 g), representing a 1.52-fold increase over the control. The optimized HPCD concentration of 0.1 g achieved 66.27 $\pm$ 0.42 mg/mL solubility, demonstrating its practical applicability for tablet formulations. The validated HPLC method provided reliable quantification with excellent linearity (R^2 = 0.9998), precision (CV $\leq$5.71%), and accuracy (within $\pm$1.28%), meeting the ICH M10 guidelines. *In vivo* pharmacokinetic studies revealed that HPCD formulations significantly improved bioavailability, with $AUC_{0-\infty}$ increasing by 1.53-fold (Group A) and 1.13-fold (Group B) compared to gemifloxacin alone. The dose-normalized Cmax/dose values increased by 1.75-fold and 2.77-fold, respectively, indicating enhanced absorption efficiency. The sustained plasma concentration profiles observed for the HPCD formulations, characterized by a gradual rather than rapid decline, suggest prolonged systemic exposure and improved pharmacokinetic profiles. These results established HPCD-based solubilization as a promising and practical strategy for the development of improved oral gemifloxacin formulations, potentially leading to enhanced therapeutic outcomes, improved patient compliance, and reduced dosing requirements for clinical applications. Future investigations are warranted to clinically validate, optimize manufacturing processes for commercial development, and explore the underlying molecular mechanisms of drug-CD interactions.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from corresponding author on reasonable request.

Author Contributions

JWK, GEP, JSK, YHK and KMK conceptualized and designed the study and performed the experiments. YHK, KMK and JSK supervised the study and performed data acquisition, analysis, and interpretation. GEP and JWK wrote the manuscript and prepared the figures and tables. YHK and KMK performed the reference search and confirmed the authenticity of all data. All authors contributed to editorial revisions of the manuscript. All authors read and approved the final manuscript and have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The study was approved by the Institutional Animal Care and Use Committee of Kyungsung University (approval number: study-2025-001A).

Acknowledgment

Not applicable.

Funding

This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea [grant number: RS-2020-KH088726 (HR20C0026)].

Conflicts of Interest

The authors declare no conflicts of interest. We confirm that YHK's affiliation with Kolon Pharm did not influence data interpretation, the writing of the manuscript, or the scientific judgments made in this study.

References

[1] Kim TW, Lee SU, Park B, Jeon K, Park S, Suh GY, *et al*. Clinical effects of bacteremia in sepsis patients with community-acquired pneumonia. BMC Infectious Diseases. 2023; 23: 887. https://doi.org/10.1186/s12879-023-08887-5.

[2] Bush NG, Diez-Santos I, Abbott LR, Maxwell A. Quinolones: mechanism, lethality and their contributions to antibiotic resistance. Molecules. 2020; 25: 5662. https://doi.org/10.3390/molecules25235662.

[3] Jivcu C, Gotfried M. Gemifloxacin use in the treatment of acute bacterial exacerbation of chronic bronchitis. International Journal of Chronic Obstructive Pulmonary Disease. 2009; 4: 291–300. https://doi.org/10.2147/copd.s3903.

[4] Drugbank. Gemifloxacin. 2005. Available at: https://go.drugbank.com/drugs/DB01155 (Accessed: 28 November 2025).

[5] Dsugi NFA, Elbashir AA, Suliman FEO. Supramolecular interaction of gemifloxacin and hydroxyl propyl β-cyclodextrin spectroscopic characterization, molecular modeling and analytical application. Spectrochimica Acta. Part A, Molecular and Biomolecular Spectroscopy. 2015; 151: 360–367. https://doi.org/10.1016/j.saa.2015.06.031.

[6] Brewster ME, Loftsson T. Cyclodextrins as pharmaceutical solubilizers. Advanced Drug Delivery Reviews. 2007; 59: 645–666. https://doi.org/10.1016/j.addr.2007.05.012.

[7] Mu K, Jiang K, Wang Y, Zhao Z, Cang S, Bi K, *et al*. The biological fate of pharmaceutical excipient β-Cyclodextrin: pharmacokinetics, tissue distribution, excretion, and metabolism of β-Cyclodextrin in rats. Molecules. 2022; 27: 1138. https://doi.org/10.3390/molecules27031138.

[8] LG Chem, Ltd. FACTIVE® (gemifloxacin) tablets, for oral use. 2019. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2003/021158lbl.pdf (Accessed: 28 December 2025).

[9] Ishafie HS, Sadeek SA, Camele I, Mohamed AA. Biochemical characterization of new gemifloxacin schiff base (GMFX-o-phdn) metal complexes and evaluation of their antimicrobial activity against some phyto- or human pathogens. International Journal of Molecular Sciences. 2022; 23: 2110. https://doi.org/10.3390/ijms23042110.

[10] Lungu IA, Moldovan OL, Biriș V, Rusu A. Fluoroquinolones hybrid molecules as promising antibacterial agents in the fight against antibacterial resistance. Pharmaceutics. 2022; 14: 1749. https://doi.org/10.3390/pharmaceutics14081749.

[11] Munir R, Hadi A, Khan SUD, Asghar S, Irfan M, Khan IU, *et al*. Solubility and Dissolution Enhancement of Dexibuprofen with Hydroxypropylbetacyclodextrin (HPβCD) and Poloxamers (188/407) Inclusion Complexes: Preparation and In Vitro Characterization. Polymers. 2022; 14: 579. https://doi.org/10.3390/polym14030579.

[12] Mondal L, Mukherjee B, Chakraborty S, Bhattacharya S, Ehsan I, Sengupta S, *et al*. Comparison of enhanced solubility profiles, analysis of thermodynamic parameters and pharmacokinetic profile related to tamoxifen citrate solubilisation. Novel Approaches in Drug Design & Development. 2018; 3: 555624. https://doi.org/10.19080/NAPDD.2018.03.555624.

[13] Guo Y, Sun CC. Pharmaceutical Lauryl Sulfate Salts: Prevalence, Formation Rules, and Formulation Implications. Molecular Pharmaceutics. 2022; 19: 432–439. https://doi.org/10.1021/acs.molpharmaceut.1c00690.

[14] Merck. Liquid formulation solubility enhancement. Pharmaceutical & biopharmaceutical manufacturing. 2025. Available at: https://www.sigmaaldrich.com/KR/ko/technical-documents/technical-article/pharmaceutical-and-biopharmaceutical-manufacturing/classical-pharma-manufacturing/liquid-formulation-solubility-enhancement (Accessed: 26 December 2025).

[15] ElShaer A, Ouyang D, Hanson P, Mohammed AR. Preparation and evaluation of amino acid based salt forms of model zwitterionic drug ciprofloxacin. Journal of Pharmaceutics & Drug Delivery Research. 2013; 2: 1. http://dx.doi.org/10.4172/2325-9604.1000111.

[16] Pignatello R, Corsaro R, Bonaccorso A, Zingale E, Carbone C, Musumeci T. Soluplus® polymeric nanomicelles improve solubility of BCS-class II drugs. Drug Delivery and Translational Research. 2022; 12: 1991–2006. https://doi.org/10.1007/s13346-022-01182-x.

[17] Muhamad H, Bashir AB, Charlton-Harrison J, Abdulhussain R, Mawla N, Patel K, *et al*. Hot-melt extruded-FDM 3D-printed polyethylene oxide tablets: Dissolution imaging analysis of swelling and drug release. European Journal of Pharmaceutics and Biopharmaceutics. 2025; 208: 114636. https://doi.org/10.1016/j.ejpb.2025.114636.

[18] Loftsson T, Másson M, Brewster ME. Self-association of cy-

clodextrins and cyclodextrin complexes. Journal of Pharmaceutical Sciences. 2004; 93: 1091–1099. https://doi.org/10.1002/jps.20047.

[19] Saokham P, Muankaew C, Jansook P, Loftsson T. Solubility of Cyclodextrins and Drug/Cyclodextrin Complexes. Molecules. 2018; 23: 1161. https://doi.org/10.3390/molecules23051161.

[20] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). ICH guideline M10 on bioanalytical method validation and study sample analysis. European Medicines Agency. 2022. Available at: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-m10-bioanalytical-method-validation-step-5_en.pdf (Accessed: 31 December 2025).

[21] Liu M, Higashi K, Ueda K, Moribe K. Supersaturation maintenance of carvedilol and chlorthalidone by cyclodextrin derivatives: Pronounced crystallization inhibition ability of methylated cyclodextrin. International Journal of Pharmaceutics. 2023; 637: 122876. https://doi.org/10.1016/j.ijpharm.2023.122876.

[22] Loftsson T. Drug permeation through biomembranes: cyclodextrins and the unstirred water layer. Die Pharmazie. 2012; 67: 363–370. https://doi.org/10.1691/ph.2012.1698.

[23] Sugano K, Kansy M, Artursson P, Avdeef A, Bendels S, Di L, *et al*. Coexistence of passive and carrier-mediated processes in drug transport. Nature Reviews Drug Discovery. 2010; 9: 597–614. https://doi.org/10.1038/nrd3187.

[24] Gharib R, Fourmentin S, Charcosset C, Greige-Gerges H. Effect of hydroxypropyl-β–cyclodextrin on lipid membrane fluidity, stability and freeze-drying of liposomes. Journal of Drug Delivery Science and Technology. 2018; 44: 101–107. https://doi.org/10.1016/j.jddst.2017.12.009.

[25] Lima BS, Campos CA, Santos ACRS, Santos VCN, Trindade GGG, Pereira EWM, *et al*. Development of morin/hydroxypropyl-β-cyclodextrin inclusion complex: Enhancement of bioavailability, antihyperalgesic and anti-inflammatory effects. Food and Chemical Toxicology. 2019; 126: 15–24. https://doi.org/10.1016/j.fct.2019.01.038.

[26] Chandrama Singh S, Choudhary M, Mourya A, Khatri DK, Singh PK, Madan J, *et al*. Acute and Subacute Toxicity Assessment of Andrographolide-2-hydroxypropyl-β-cyclodextrin Complex via Oral and Inhalation Route of Administration in Sprague-Dawley Rats. The Scientific World Journal. 2022; 2022: 6224107. https://doi.org/10.1155/2022/6224107.

[27] Allen A, Bygate E, Oliver S, Johnson M, Ward C, Cheon AJ, *et al*. Pharmacokinetics and tolerability of gemifloxacin (SB-265805) after administration of single oral doses to healthy volunteers. Antimicrobial Agents and Chemotherapy. 2000; 44: 1604–1608. https://doi.org/10.1128/AAC.44.6.1604-1608.2000.

[28] Su J, Zhang X, Cao S, Liu C, Fu X, Zhang R, *et al*. Pharmacokinetic studies of hyperoside-2-hydroxypropyl-β-cyclodextrin inclusion complex and ameliorated DSS-induced colitis in mice. Bioscience Reports. 2023; 43: BSR20230003. https://doi.org/10.1042/BSR20230003.

[29] Cheow WS, Hadinoto K. Factors affecting drug encapsulation and stability of lipid-polymer hybrid nanoparticles. Colloids and Surfaces. B, Biointerfaces. 2011; 85: 214–220. https://doi.org/10.1016/j.colsurfb.2011.02.033.

[30] Sun Y, Mao Y, He X, Zhao X. Development and evaluation of mPEG-PLLA polymeric micelles encapsulating enrofloxacin for enhanced solubility, bioavailability, and antibacterial performance. Frontiers in Veterinary Science. 2025; 12: 1595137. https://doi.org/10.3389/fvets.2025.1595137.

[31] Morales D, Pacurariu A, Slattery J, Pinheiro L, McGettigan P, Kurz X. Association Between Peripheral Neuropathy and Exposure to Oral Fluoroquinolone or Amoxicillin-Clavulanate Therapy. JAMA Neurology. 2019; 76: 827–833. https://doi.org/10.1001/jamaneurol.2019.0887.

[32] Schiele JT, Quinzler R, Klimm HD, Pruszydlo MG, Haefeli WE. Difficulties swallowing solid oral dosage forms in a general practice population: prevalence, causes, and relationship to dosage forms. European Journal of Clinical Pharmacology. 2013; 69: 937–948. https://doi.org/10.1007/s00228-012-1417-0.