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Original Article

# Physiologically Based Pharmacokinetic Modeling of Regorafenib Monohydrate-Loaded PEGylated PLGA Nanoparticles Employing GastroPlus™ Software

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**Abstract**

Nanoparticles (NPs) have transformed drug delivery by altering the pharmacokinetics of small-molecule therapeutics by dramatically enhancing solubility, bioavailability and tumour specificity. By leveraging the enhanced permeability and retention EPR effect characteristics of tumour vasculature, nanoparticles exhibit preferential localisation within neoplastic tissues, enabling deep intratumoral penetration and the targeted delivery of therapeutic agents with high spatial precision. When wrapped in hydrophilic polymers like polyethylene glycol (PEG), these smart carriers evade immune detection, extending systemic circulation and amplifying drug accumulation at the disease site. Yet the very properties that make NPs so effective, such as their tunable size, shape, surface charge, and chemistry, also render their in vivo behaviour highly complex and difficult to predict from traditional in vitro assays. To bridge this gap, advanced modelling methodologiessuch as physiologically based pharmacokinetic modelling offer robust avenues for predicting the outcome, efficacy, and safety of these nanoengineered therapies. In these research findings, a robust rabbit physiologically based pharmacokinetic model or toxicokinetic model, has been calibrated and validated against reported literature to predict the pharmacokinetics of regorafenib monohydrate-loaded PEGylated PLGA nanoparticles. The model demonstrates a high degree of predictive reliability, with $C_{max}$ and AUC estimations achieving fold errors near unity, underscoring its potential as a high-value asset for preclinical simulation, risk assessment, and design optimisation of nano-formulation. This innovative modelling approach accelerates the path from bench to bedside, offering a powerful tool through which the intricate dance of nanoparticles within the body can be precisely understood.

***Keywords:*** *Nanoparticles; PBPK Modeling; Pegylated PLGA Nanoparticles; Pharmacokinetics; Regorafenib Monohydrate*

## Introduction

Cancer remains one of the leading global health challenges despite substantial advances in its diagnosis and treatment. It is currently the second most common cause of death worldwide, following cardiovascular diseases, and contributes significantly to morbidity and mortality in both developed and developing nations. In 2018, approximately 18.1 million new cancer cases were reported globally, with nearly 9.6 million cancer-related deaths. These numbers are projected to rise to 21.7 million new

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cases and 13 million deaths annually by 2030, underscoring the urgent need for more effective therapeutic strategies (Sung *et al.,* 2021; Sarkar *et al*., 2013; Thun *et al.,* 2010).

Conventional cancer treatment modalities include surgery, radiotherapy, chemotherapy, and targeted pharmacotherapy aimed at tumour eradication, regression, or growth inhibition. Recently, oral chemotherapy has gained considerable attention due to improved patient compliance and convenience, despite sharing comparable advantages and limitations with intravenous chemotherapy. Among oral anticancer agents, Tyrosine Kinase Inhibitors (TKIs) have emerged as a prominent therapeutic class for the management of leukaemia, lymphoma, and several solid tumours. The number of approved TKIs has increased substantially over the past decade, with more than 70 small-molecule protein kinase inhibitors currently employed in oral chemotherapy regimens (Mun *et al.,* 2018; Xu *et al.,* 2022).

Regorafenib monohydrate (RGF) is an orally administered, multi-kinase inhibitor with potent antineoplastic activity. It is clinically approved for the treatment of advanced gastrointestinal stromal tumours, hepatocellular carcinomas, and metastatic colorectal cancers. Despite its broad therapeutic utility, the clinical performance of RGF is hindered by poor in vivo exposure resulting from low aqueous solubility, extensive first-pass metabolism, and limited systemic circulation. These limitations not only reduce therapeutic efficacy but may also contribute to dose-related adverse effects, including hepatotoxicity (Rey *et al.,* 2015; Sartore-Bianchi *et al.,* 2014). RGF is categorised as a Biopharmaceutics Classification System (BCS) class II compound, exhibiting high permeability but low solubility. Although the USFDA-approved oral dose of 160 mg administered for 21 days in a 28-day treatment cycle is clinically effective, its marketed tablet formulation demonstrates only approximately 10% oral bioavailability.

PLGA-based nanotechnology drug delivery systems have emerged as a promising approach to address the biopharmaceutical limitations of poorly soluble anticancer agents. Nanoparticles (NPs) enhance drug solubility, improve pharmacokinetic profiles, and enable targeted delivery by exploiting the Enhanced Permeability and Retention (EPR) effect within tumour tissues. Additionally, PLGA nanoparticulate systems prolong systemic circulation, reduce off-target drug accumulation, minimise renal clearance, and improve therapeutic index and safety. Surface modification of nanoparticles with hydrophilic polymers such as polyethylene glycol (PEG) further imparts stealth characteristics by reducing opsonisation and subsequent clearance by the mononuclear phagocyte system, thereby facilitating enhanced tumour accumulation (Jimoh *et al.,* 2026; Karwasra *et al*., 2026).

In our earlier work, we successfully developed PEGylated PLGA nanoparticles encapsulating regorafenib monohydrate using a modified nanoprecipitation technique. This nano-formulation significantly improved the solubility, dissolution rate, and oral bioavailability of RGF compared to its conventional suspension, thereby enhancing its biopharmaceutical performance (Panigrahi *et al.,* 2021). However, nanoparticle-based formulations often exhibit complex and unique in vivo disposition profiles owing to variations in particle size, surface charge, morphology, and surface chemistry. As a result, direct extrapolation of in vivo pharmacokinetics and biodistribution from in vitro data alone remains challenging.

Physiologically based pharmacokinetic (PBPK) modelling offers a robust, mechanistic framework to characterise and predict the in vivo behaviour of both conventional and nanomedicine-based drug products. PBPK models integrate physiological parameters with drug-specific and formulation-specific properties to simulate absorption, distribution, metabolism, and excretion (ADME) processes under various biological conditions. These models are increasingly employed to predict systemic exposure, target-site concentrations, therapeutic outcomes, and potential toxicity of complex drug delivery systems, including nanotherapeutics (Kutumova *et al.,* 2022; Yuan *et al.,* 2022).

In pharmaceutical research, PBPK modelling plays a critical role in bridging the translational gap between preclinical animal studies and human clinical outcomes. Although animal models provide valuable insights into drug pharmacokinetics, efficacy, and safety, accurate prediction of human pharmacokinetics remains a significant challenge. PBPK modelling enables reliable extrapolation from

preclinical species to humans by incorporating species-specific physiological and biochemical parameters, thereby offering advantages over traditional allometric scaling approaches. Moreover, PBPK-based simulations reduce experimental costs and address ethical concerns associated with clinical testing in vulnerable populations (Dong *et al.,* 2015; Franz *et al.,* 2024).

GastroPlus™ is a widely used commercial PBPK modelling platform that provides a mechanistic representation of the gastrointestinal tract and systemic disposition of drugs. Initially introduced in 1998, the software employs compartmentalised GI models to simulate drug dissolution, transit, and absorption across different intestinal regions. Over time, GastroPlus™ has evolved into a powerful predictive tool capable of forecasting clinical pharmacokinetic profiles using preclinical, in-vitro, and in-silico data with high accuracy (De Buck *et al.,* 2007; Kostewicz*et al.,* 2014; Shardlow *et al.,* 2013). The platform integrates physicochemical properties, formulation attributes, and physiological parameters, many of which can be computationally predicted using the built-in ADMET Predictor™ module (Kostewicz *et al.,* 2014).

In the present study, a PBPK model was developed using GastroPlus™ by integrating the physicochemical properties of regorafenib monohydrate, it's in vitro release characteristics, and pharmacokinetic data obtained from rabbit studies. The model was employed to simulate and compare the pharmacokinetic behaviour of regorafenib administered as a plain suspension (RGF SSP) and as PEGylated PLGA nanoparticles (RGF NPs). Model predictability was evaluated by comparing simulated and observed pharmacokinetic profiles. Overall, this study provides a mechanistic PBPK framework for predicting the in vivo performance of nanoparticulate regorafenib formulations and supports the rational design and development of advanced TKI-based nanotherapeutic strategies.

## Material and Methods

The physicochemical and biopharmaceutical input parameters for the PBPK modelling of RGF were obtained mainly from published literature, complemented by findings from our in-house research. In cases where relevant literature or proprietary data were unavailable, ADMET- predicted parameters were incorporated for PBPK model construction. The PBPK model development and validation were performed using GastroPlus® version 9.9.0011. Multiple GastroPlus modules, including Advanced Compartmental Absorption and Transit (ACAT™), ADMET® Predictor, PKPlus™, and Optimization™ tool, were employed throughout the modelling process.

RGF-loaded PEGylated PLGA nanoparticles (RGF NPs) were formulated using the nanoprecipitation technique, employing PEGylated PLGA as the polymer and Poloxamer 188 as the surfactant. Optimisation of the formulation was carried out using a Quality by Design (QbD) approach, specifically through a Box-Behnken design. The optimised and stable RGF NPs were produced by using 1.6% of PEGylated PLGA and 1% of Poloxamer 188 (Panigrahi *et al*., 2024).

### *Solubility Study*

The solubility of the drug was evaluated across various physiologically relevant buffer systems, including 0.1N HCl (pH 1.2), acetate buffer (pH 4.5), and phosphate buffers at pH 6.8 and 7.4. To ensure saturation, an excess quantity of each drug was introduced into a fixed volume of corresponding buffer solution. The suspensions were subsequently incubated in an orbital shaker maintained at 37 ± 0.5°C for 24 h. Post incubation, the mixtures were filtered, suitably diluted, and analysed to determine the drug concentration. UV-visible spectrophotometry was used for analysis, with detection wavelengths set at 261 nm for RGF to get the concentration of drug substance dissolved (Fujita *et al*., 2016).

### *In Vitro Drug Release*

An in vitro drug diffusion study was performed for both regorafenib monohydrate nanoparticles (RGF NPs) and the regorafenib monohydrate drug suspension (RGF SSP) using the dialysis sac method with a dialysis membrane. An equivalent dose of 25 mg of RGF was used in the study, with 100 mL of

pH 6.8 phosphate buffer containing 1% SLS (sodium lauryl sulphate) serving as the diffusion medium. The release study was conducted under controlled conditions, maintaining the temperature at 37 ± 0.5°C and agitation speed between 100 and 150 RPM. Quantitative analysis of drug concentration in the filtered samples was performed using a UV-visible spectrophotometer at an absorption wavelength of 261 nm (Panigrahi *et al*., 2024; Ma *et al*., 2021).

*Pharmacokinetic Study and Bioanalytical Method*

A selection of twelve male albino rabbits, exhibiting body weights between 1.5 and 2.5 kg, was made from the animal house stock for the purpose of this study. Each of the two groups contained six animals. The first group was given an oral suspension of the pure drug (RGF SSP), and the second group received enhanced RGF-loaded PEGylated PLGA nanoparticles by using an 8FG diameter Ryle's tube. The exact dose of RGF for the study was calculated by considering a 160 mg dose for humans, a weight factor for rabbits (2.5/1.5) and a 0.07 factor for each rabbit. The dose was calculated as 19 mg of RGF to be administered to the rabbit. The recommended dose for RGF SSP and RGF-loaded PEGylated PLGA nanoparticles is dispersed in 5 ml of distilled water and administered to six rabbits of groups A and B animals, respectively, as per the approved study design protocol.

A high-performance liquid chromatography (HPLC) method was developed and validated for the quantification of regorafenib in human plasma. The method is novel, stability-indicating, and designed to accurately monitor plasma levels of regorafenib, a multikinase inhibitor used in cancer therapy. Revaprazan was employed as an internal standard to ensure precision and accuracy during quantification. The method demonstrates the ability to separate regorafenib from its potential degradation products and endogenous plasma components, thereby confirming its stability-indicating nature. The validation process followed international guidelines (such as ICH and FDA bioanalytical method validation standards), assessing key parameters including linearity, precision, accuracy, selectivity, sensitivity, recovery, and stability under various conditions (e.g., short-term, long-term, freeze-thaw, and post-preparative stability). This method is suitable for application in pharmacokinetic studies, therapeutic drug monitoring, and bioequivalence assessments involving regorafenib (Kim *et al*., 2021). All the pharmacokinetic datasets, including time points, mean plasma concentrations, and standard deviations, were digitised to facilitate the development and validation of the PBPK model.

*PBPK Model Development and Simulation Approach*

GastroPlus® is an advanced simulation platform designed for mechanistic modelling of a drug's ADME and pharmacodynamic responses across various routes of administration, including intravenous, oral, ocular, inhalation, dermal, subcutaneous, and intramuscular in both human and preclinical species. By combining scientific accuracy with an easy-to-use interface, it facilitates more efficient and informed decision-making in drug development. The ADMET Predictor module enhances the functionality of GastroPlus® by allowing the prediction of key physicochemical properties, pharmacokinetic parameters, and CYP metabolism kinetics directly from a compound's chemical structure, supporting PBPK simulations within the GastroPlus platform.

A comprehensive PBPK model is generally developed using a step-by-step approach to systematically define and refine the necessary parameters. In the initial phase of model construction, parameter values reflecting baseline physiological characteristics of the species are sourced from established literature, independent of the specific physicochemical or pharmacokinetic properties of the compound under investigation. In the next step, the model is tailored to a particular compound by incorporating its physicochemical properties. In the third stage, active processes such as transport and metabolism are defined based on data obtained from in vitro or in vivo studies. In instances where species-specific data are lacking, surrogate data from related species may be employed. Finally, if pharmacokinetic data for the compound in the target species are accessible, they can be used to refine and optimise the model

parameters (Mavroudis *et al*., 2018). A schematic overview of the model development process is presented in Figure 1.

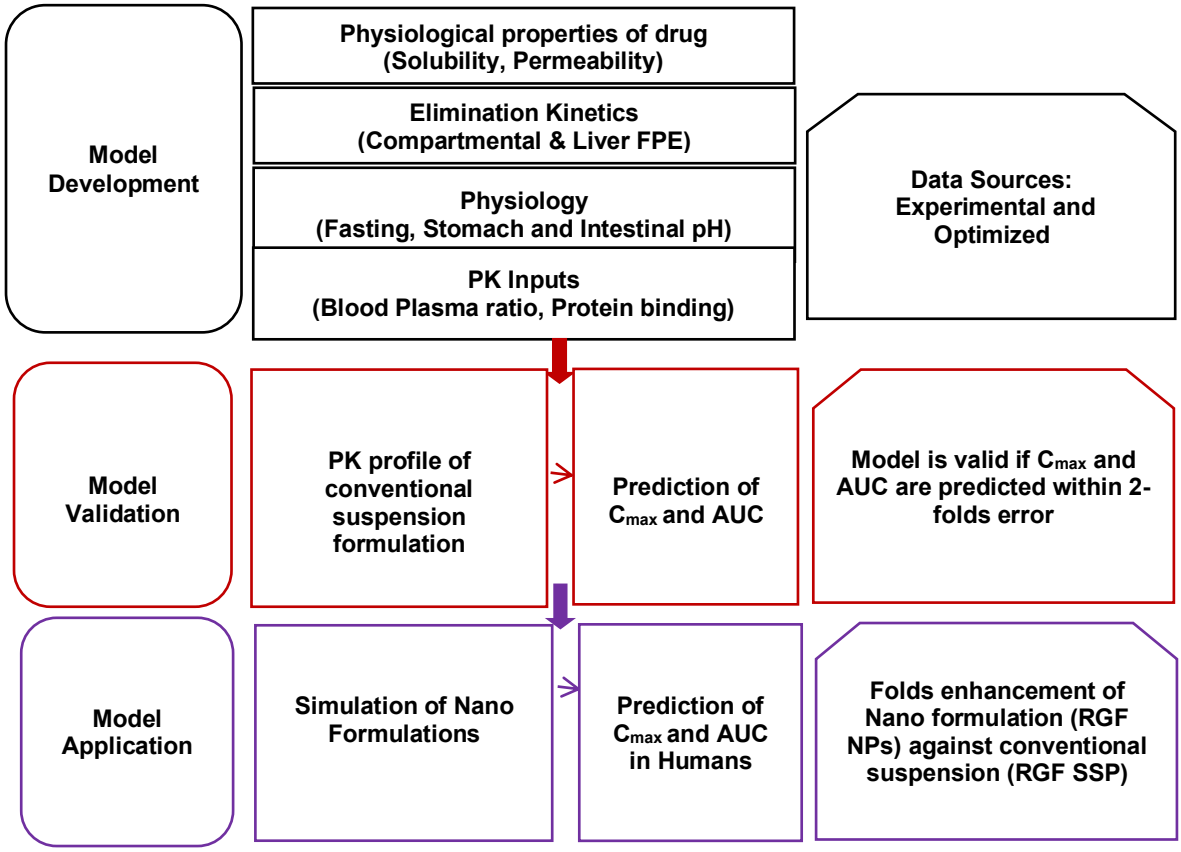


***Figure 1:****GastroPlus® Modeling Workflow for Simulation of PK Parameters Physicochemical Profiles and Biopharmaceutics Attributes*

The physicochemical and biopharmaceutical attributes used for developing the PBPK model of RGF are collected from literature (Gerisch *et al*., 2018) and presented in Table 1. Model development incorporated both the pH-dependent solubility profile and the in vitro release profile of the RGF SSP. Additionally, pharmacokinetic data from rabbit studies using RGF SSP were utilised for model calibration and simulation prior to extrapolating the findings to predict the pharmacokinetics of RGF NPs. For simulations under fasting conditions, a 5 mL water volume was used as the standard administration volume. The preclinical pharmacokinetic data obtained from rabbits for both RGF SSP and RGF NPs are summarized in subsequent sections.

***Table 1****:Gastroplus Model Inputs for Regorafenib Monohydrate [RGF]*

| Parameters | Regorafenib Monohydrate |
|---|---|
| Molecular weight | 500.83 g/mol |
| Solubility input (mg/mL, pH) | Refer Table 2 |
| Human effective permeability | 3.2 × 10-4 cm/s |
| Log P | 5 |
| PKa | 1.3, 12.0 |
| ABS Bioavailability (%) | 10 |
| Blood to plasma ratio (B/P) | 1.3 |
| Plasma protein binding (%) | 99.5 |
| Plasma protein binding (%Fup, adjusted %Fup) | 0.5, 0.124% |
| Drug Release | Refer Table 3 |
| Elimination kinetics | Refer Table 4 |
| Dosage form | CR Dispersed (nano-formulation and conventional) |
| Physiology | Fasting |

*Elimination Kinetics*

In physiological modelling, intravenous pharmacokinetic data are typically the most reliable source for determining elimination parameters. However, in the present case, intravenous data in rabbits is not available. Thus, the conventional suspension data was used to determine the elimination parameters. Further, for nano formulation, clearance was reduced to achieve the best fit. In the case of nano-formulations, low clearance is reported in the literature because of RES uptake and slow elimination. Thus, the optimisation of clearance for nano-formulation was found to be rational to best describe the pharmacokinetic behaviour (Table 4).

*In Vitro Drug Release Data Input*

The dissolution data of both conventional and nano-formulations is described through Weibull fitting after appropriate time scaling. Time scaling is a typically used approach to mathematically correct in vitro behaviour to in vivo to achieve observed Tmax appropriately. The dissolution profiles were characterised using the Weibull function as described in Equation 1. Time-scaled dissolution data integrated into the model are summarised in Table 3. The Weibull equation is represented in Eq-1 (Kollipara *et al*., 2023).

$$F = 100 \times (1 - e^{-\alpha t^{\beta}})$$

---------- Eq. - 1

*Physiology*

To simulate the in vivo pharmacokinetic behaviour of RGF under fasting conditions, the “Rabbit Physiology Fasting” module was employed. The absorption process was modelled using the Opt Log D Model SA/V 6.1 as the absorption scaling factor. Initially, permeability values predicted by the ADMET Predictor were used; these values were subsequently refined to better align with the observed in vivo oral pharmacokinetic data. The compartmental parameters (for rabbit physiology) used in GastroPlus are described inFigure 2.

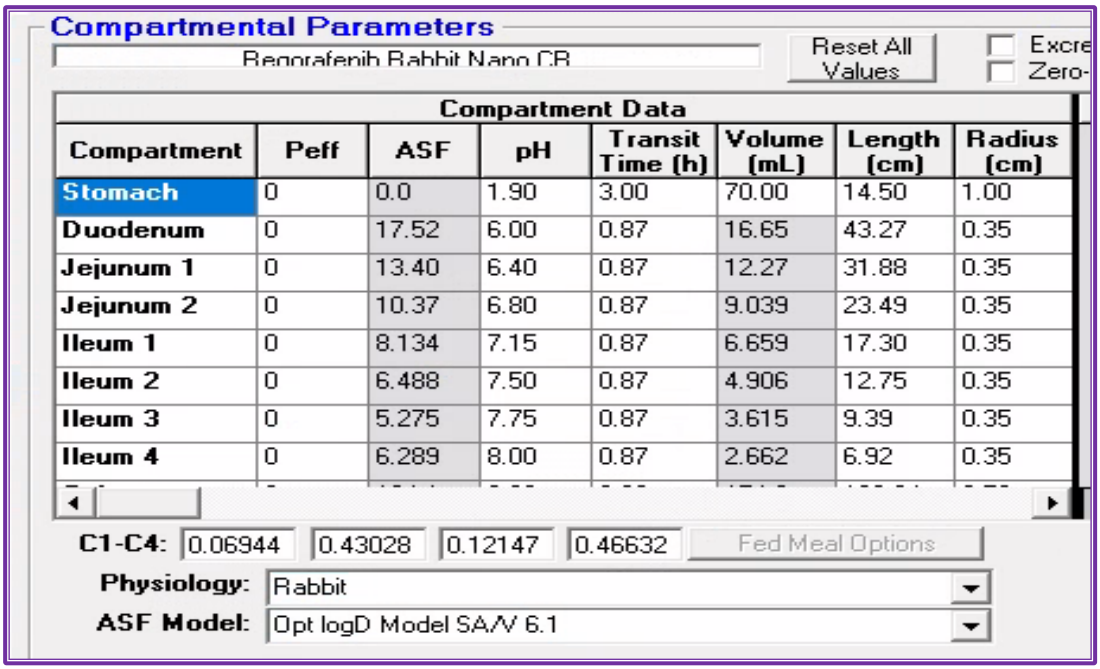


***Figure 2:****Compartmental Parameters (Rabbit Physiology) Used in Gastroplus Model Validation*

The PBPK model was constructed using input parameters such as the solubility profile and drug release characteristics of the RGF SSP formulation. The model was initially applied, tested, and verified in rabbits using plasma concentration data from preclinical studies with the RGF SSP. Subsequently, the same framework was validated using data from RGF NPs. Following this, the

model was used to simulate and predict pharmacokinetic parameters in humans. For model validation, Fold Error (FE) was calculated using standard equation 2. These metrics were employed to assess the reliability of the PBPK model, with acceptable model performance defined as fold differences between predicted and actual data confined within a two-fold range (0.5-2.0), indicating acceptable predictive performance (Kollipara *et al*., 2025; Ladumor *et al*., 2023).

$$\mathrm{FE} = 10 \log \frac{\text{Predicted value}}{\text{Observed value}} \ldots\ldots\ldots\ldots\ldots [\text{Eq. 2}]$$

*Model Application*

The established PBPK model for RGF was subsequently utilised to simulate the pharmacokinetic characteristics of RGF NPs. The calculated fold increase indicated a strong model fit, supporting its applicability for forecasting human pharmacokinetic parameters and further exploration in translational research using standard equations 3 and 4 (Kollipara *et al*., 2025; Ladumor *et al*., 2023).

$$\text{Folds increase (Observed)} = \frac{\text{Cmax Observed for RGF NPs}}{\text{Cmax Observed for RGF SSP}} \ldots\ldots\ldots\ldots\ldots [\text{Eq. 3}]$$

$$\text{Folds increase (Simulated)} = \frac{\text{Cmax Simulated for RGF NPs}}{\text{Cmax Simulated for RGF SSP}} \ldots\ldots\ldots\ldots\ldots [\text{Eq. 4}]$$

*Ethical Approval*

This study involved animal experimentation. The current study's protocol was approved before any of the animal experiments were completed. The Animal Care Committee Jeeva Life Sciences, Hyderabad, Institutional Animals Ethics (CPCSEA/IAEC/JAS/17/03/22/48, Approval No. 48) gave their approval for the pharmacokinetic investigation.

## Results

*Solubility*

The solubility of RGF was evaluated at 37°C across a range of physiological pH conditions (1.2 to 7.4). The study was conducted in triplicate, and the average solubility values are presented in Table 2. Results indicated that RGF exhibits poor solubility throughout the tested pH range.

***Table 2:*** *Solubility Study for RGF*

| pH | Solubility (mg/mL) at 24 h |
|---|---|
| pH 1.2 (0.1N HCl) | 0.113 |
| pH 4.5 Acetate Buffer | 0.152 |
| pH 6.8 Phosphate Buffer | 0.114 |
| pH 7.4 Phosphate Buffer | 0.109 |
| Purified Water | 0.112 |

*Critical Quality Attributes of RGF Nanoparticles:*

The formulation's Critical Quality Attributes (CQAs) were meticulously analysed to determine the influence of formulation variables on key performance indicators specifically minimising particle size, maintaining a PDI below 0.4, achieving a zeta potential near ±30 mV, and maximising entrapment efficiency. The optimised nanoparticle system, formulated with 1.6% PEGylated PLGA and 1% Poloxamer 188, showcased an impressive profile: an average particle size of 151.1 nm, a PDI of 0.398, and a ZP (Zeta potential) of -21.1 mV. In vitro drug diffusion studies revealed a marked improvement in drug release from RGF-loaded nanoparticles (RGF NPs) compared to the conventional RGF suspension (RGF SSP), highlighting the formulation's enhanced delivery potential.

*Drug Release Study*

The in vitro drug diffusion study for both RGF NPs and RGF SSP was performed using the dialysis sac method, with samples collected according to scheduled time points. UV spectrophotometric analysis at 261 nm (ʎmax) was performed to measure drug concentrations in the collected samples. The optimised RGF NPs demonstrated approximately 99% drug release, significantly higher than the 60% release observed with the RGF SSP. For modelling purposes, time scaling was applied to align with the observed Tmax and to achieve an optimal fit for the simulation. The drug release profile with time scaling is presented in Table 3.

***Table 3:****Drug Release Profile (With Time Scaling) For RGF SSP and RGF Nps*

| Original Time Points (Hr) | Time Scaled (5x Reduced) (Hr) | Drug Release for RGF SSP (%) | Drug Release for RGF NPs of Optimized Batch (%) |
|---|---|---|---|
| 0 | 0 | 0 | 0 |
| 6 | 1.2 | 30.53 | 72.6 |
| 8 | 1.6 | 36.17 | 78.2 |
| 12 | 2.4 | 60.17 | 99.8 |

*Elimination Kinetics*

The elimination kinetics of both the nano-formulation and the conventional suspension formulation after optimisation of clearance are presented in Table 4.

***Table 4:*** *Elimination Kinetics Used for Model Development*

| Parameters (units) | RGF SSP | RGF NPs |
|---|---|---|
| Cl (L/h) | 0.03 | 0.01 |
| Vd (L/Kg) | 0.7 | 0.5 |
| Bw (Kg) | 2.5 | 2.5 |
| Permeability (cm/s) | 3.26 x 10-4 | |

*Animal Study PK Results*

The PK data (serum drug concentration vs. time) obtained from the study conducted on Rabbits for both RGF SSP and RGF NPs is described in Table 5.

***Table 5:****Serum-Drug Concentration vs. Time for Optimized RGF Nps Vs RGF SSP*

| Time (hr) | Serum Concentration (ng/mL) | |
|---|---|---|
| | Optimized RGF NPs (ng/mL) | RGF SSP (ng/mL) |
| 0 | 0 | 0 |
| 2 | 651.85 ± 189 | 231.09 ± 204 |
| 4 | 1906.48 ± 413 | 689.65 ± 457 |
| 6 | 3025.64 ± 879 | 2369.012 ± 617 |
| 8 | 8369.1 ± 591 | 8759.301 ± 1303 |
| 12 | 18236.35 ± 1020 | 13023.06 ± 1111 |
| 18 | 23363.97 ± 502 | 9363.105 ± 603 |
| 24 | 6935.6 ± 207 | 4320.69 ± 387 |

*(Mean ± SD), n = 6, n is the number of observations*

*PBPK Simulations*

The study involved the development of a PBPK model aimed at predicting critical pharmacokinetic parameters, particularly Cmax and AUC. The model was constructed by integrating physicochemical characteristics, pharmacokinetic data, and relevant physiological parameters. The resulting simulations successfully predicted Cmax and AUC values within a two-fold error range (predicted-to-observed ratio between 0.5 and 2), demonstrating the model's reliability and accuracy (Kollipara *et al*., 2025; Ladumor *et al*., 2023).

*Model Development for RGF*

Using the physicochemical, pharmacokinetic and physiological data summarised in Table 1, together with standard solubility and drug release information from Table 2 and Table 3, the foundational PBPK model for RGF was established. Elimination kinetics derived from oral administration data are modified based on bioavailability and first-pass metabolism for model construction to characterise drug clearance and presented in Table 4. The Weibull equation was utilised to establish a relationship between in vitro and *in vivo* drug release profiles (Kollipara *et al.,* 2023). During model development, physiological parameters specific to rabbits were incorporated. Time scaling was applied to the dissolution data to align with the observed Tmax, and permeability was adjusted accordingly to achieve a better fit. Initially, the model was calibrated using data from the RGF SSP and subsequently validated using data from RGF NPs incorporating optimised clearance values. The Weibull fits for both RGF SSP and RGF NPs are presented in Figure 3 and Figure 4, respectively.

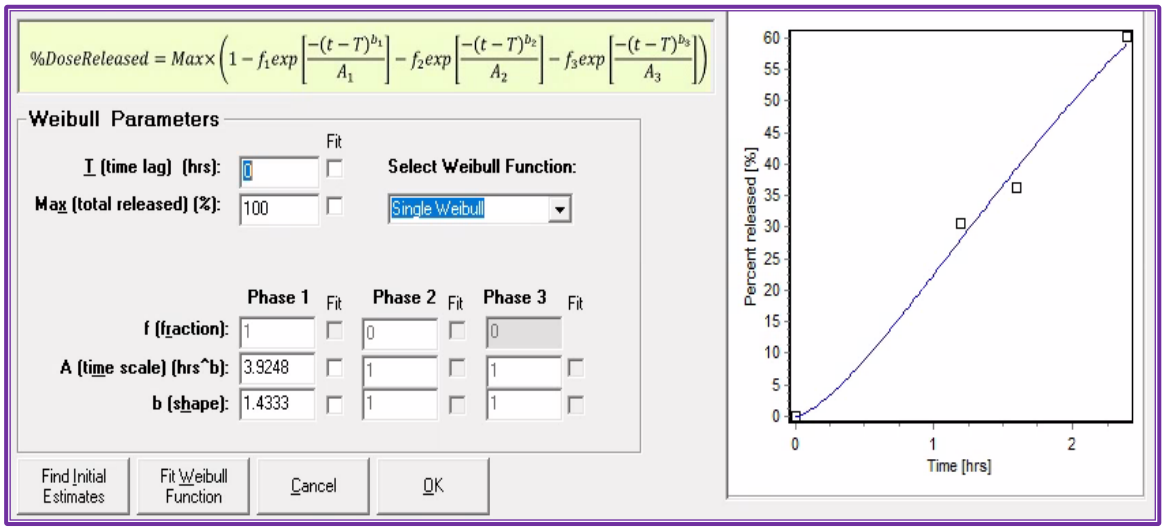


***Figure 3:****Comparison of In Vitro Drug Release and Adjusted Weibull Input Drug Release for RGF SSP*

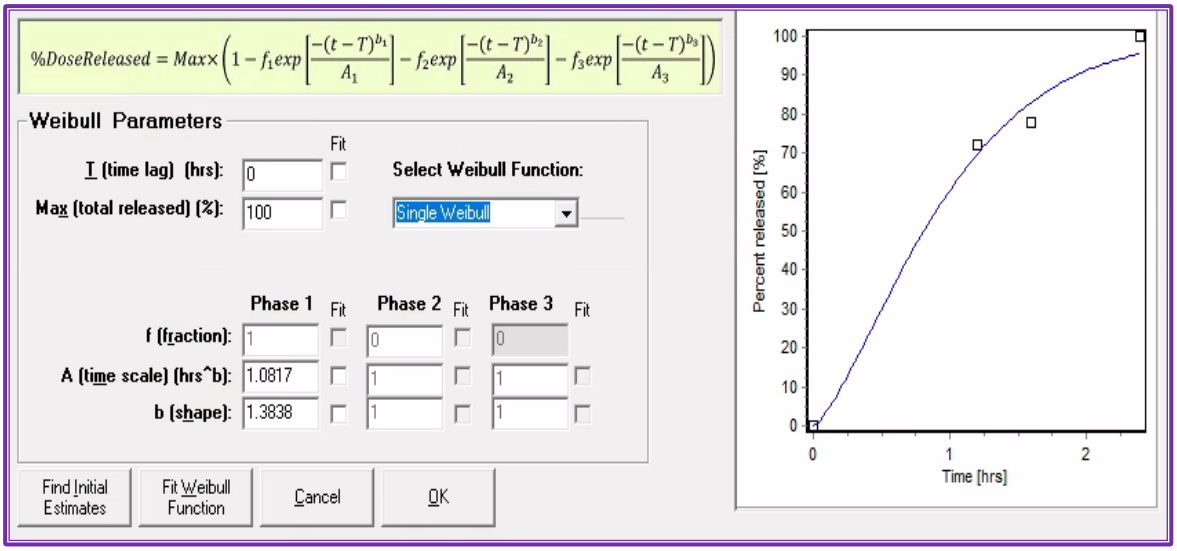


***Figure 4:****Comparison of In Vitro Drug Release and Adjusted Weibull Input Drug Release for RGF Nps*

*Model Validation*

Following the successful development of the model for RGF SSP, comprehensive validation was conducted using data from RGF NPs. The validation results are presented in Table 6 and Figs. 5 and 6. As shown in Fig. 4, there is strong concordance demonstrated between the measured and

simulated plasma concentration time profiles for RGF SSP, with predictions aligning within the observed variability. Table 6 further demonstrates that Fold Errors (FEs) were close to 1, reinforcing the model's reliability. Table 7 and Fig. 6 confirm that predicted $C_{max}$ and AUC values for RGF NPs remain within acceptable fold ranges compared to observed data. Overall, most observed-to-predicted ratios approached unity, and nearly all results were within a two-fold difference, validating the robustness of the developed PBPK model.

***Table 6:****Observed Vs Model Simulated Data for RGF SSPAnd RGF Pegylated PLGA Nanoparticles*

| Parameters | RGF SSP | | | Optimized RGF NPs | | |
|---|---|---|---|---|---|---|
| | Observed | Simulated | FE | Observed | Simulated | FE |
| Cmax (ng/mL) | 13020 | 9084.8 | | 23360 | 13480 | 0.58 |
| Tmax (h) | 12 | 9.76 | 0.8 | 18 | 11.6 | 0.64 |
| AUC 0-t (ng.h/mL) | 173000 | 177800 | 1.02 | 288400 | 281400 | 0.98 |

***Table 7:****Folds Increase in PK Parameters Observed Vs Predicted*

| Parameters | Observed Folds Increase | Prediction Folds Increase |
|---|---|---|
| Cmax (ng/mL) | 1.79 | 1.48 |
| AUC 0-t (ng.h/mL) | 1.67 | 1.58 |

Simulated AUC and Cmax values were within a two-fold range (0.5–2.0) of observed data, consistent with accepted PK modeling criteria. The model demonstrated close agreement between predicted and observed pharmacokinetic parameters for both formulations. For RGF SSP, fold error (FE) values were 0.70 for Cmax and 1.02 for AUC. Correspondingly, RGF NPs exhibited FE values of 0.58 for Cmax and 0.98 for AUC. All FE values met the predefined acceptance criteria, with AUC predictions approaching unity, indicating strong concordance between simulated and observed systemic exposure.

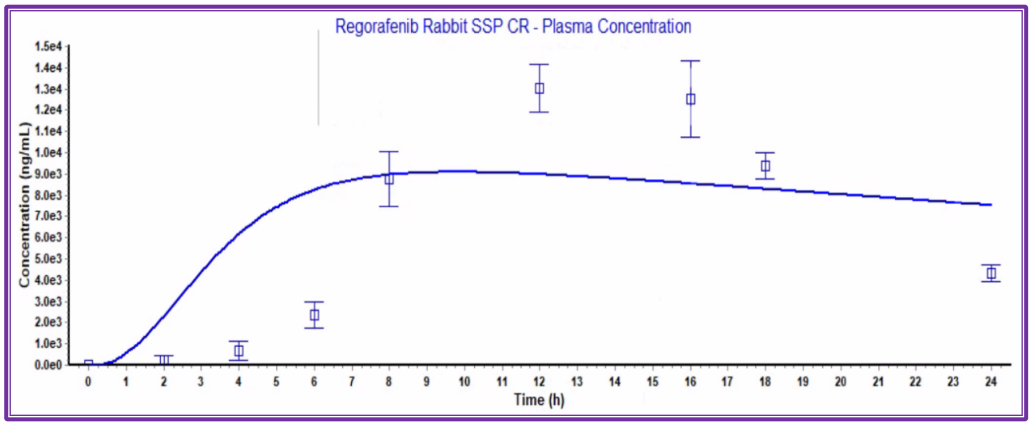


***Figure 5:****Simulated Serum Concentration-Time Profile for RGF SSP*

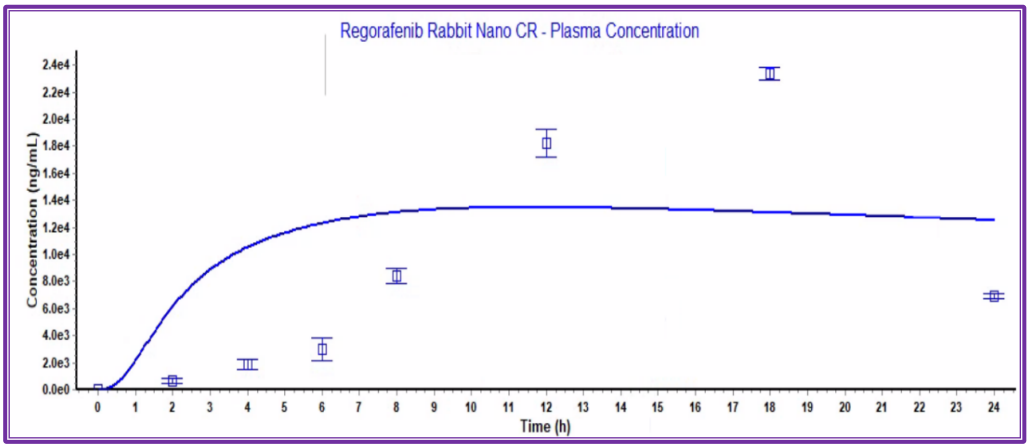


***Figure 6:****Simulated Serum Concentration-Time Profile for RGF Optimized RGF NPs*

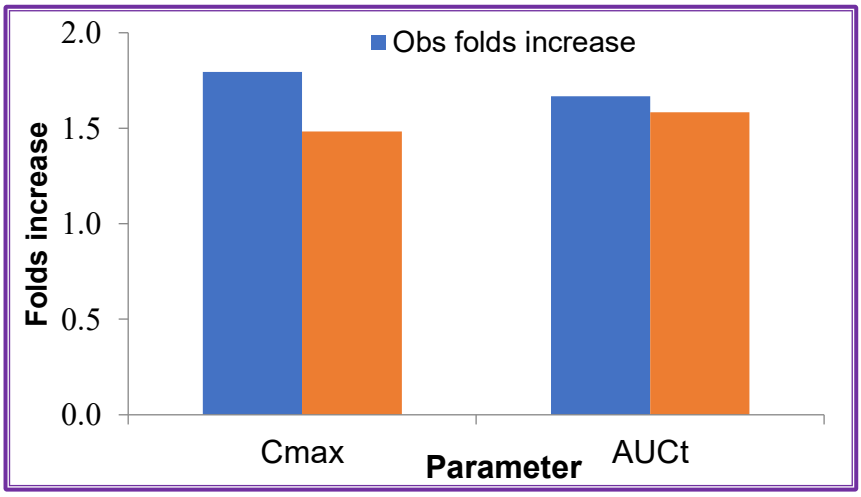


***Figure 7:****Observed Fold Increased Vs. Predicted Fold Increased*

**Discussion**

Regorafenib monohydrate is characterised by negligible solubility across the pH range of 1.2 to 7.4, representative physiological conditions, and classified as a BCS class II compound having limited bioavailability due to poor aqueous solubility (Xia *et al*., 2013). To boost aqueous solubility, enhance bioavailability, and prolong systemic circulation, a sophisticated nanoparticle system was engineered using PEGylated PLGA (Alharbi &Alhazmi, 2026). The incorporation of PEG not only stabilises the formulation but also fine-tunes the drug release kinetics from the PLGA matrix, offering the potential for a controlled and sustained therapeutic effect (Jimoh *et al*., 2026; Karwasra *et al*., 2026). The formulation was meticulously engineered via the nanoprecipitation technique, harnessing PEGylated PLGA as the core polymer and Poloxamer 188 as the dynamic surfactant. To fine-tune the composition and ensure optimal stability, a Box-Behnken design was strategically employed, enabling the development of a refined and robust nanoparticle system (Panigrahi *et al*., 2021).

The animal pharmacokinetic (PK) study showcased a striking enhancement in the absorption of RGF from the nanoparticle formulation (RGF NPs), with markedly superior PK parameters compared to the pure RGF suspension (RGF SSP). This dramatic improvement can be credited to the formulation's ability to elevate drug solubility and facilitate efficient permeation across the gastrointestinal barrier, culminating in significantly boosted bioavailability. Before a drug makes its debut in human studies, precise prediction of PK parameters from in vitro or preclinical in vivo data is a cornerstone of modern drug development. These insights are instrumental in crafting optimal dosing regimens, ensuring a balance of safety and efficacy and unlocking key biomarkers, setting the stage for a seamless transition from bench to bedside (Bassani *et al*., 2024). However, the complex behaviour of nanoparticles within systemic circulation, coupled with substantial physiological differences between animals and humans, makes it challenging to directly extrapolate animal data for human applications. An effective approach to expedite this process involves utilising advanced modelling techniques. These tools can offer early insights into the behaviour of carriers, including evaluations of their effectiveness and potential toxicities (da Silva *et al*., 2025).

PBPK models are commonly constructed using data from animal studies to estimate the distribution and concentration of chemicals in humans. However, variations in physiology and exposure pathways between animals and humans can influence the accuracy and interpretation of these models. Reliable predictions often depend on the availability of detailed data to inform model parameters, which may necessitate chemical-specific modifications to the input values (Bassani *et al*., 2024).

PBPK modelling rapidly emerges as a powerful asset in risk assessment, offering the ability to simulate how compounds distribute across different tissues with precision. By integrating essential ADME processes in target species, it effectively links in vitro data to in vivo outcomes, establishing itself as a crucial tool in pharmacokinetic and toxicological assessment (Mavroudis *et al*., 2018).PBPK

models bring drug behaviour to life by blending physiological details like organ volumes, blood flow and enzymes or transporter level with drug-specific traits such as clearance and tissue partitioning. Together, these inputs map the drug's pharmacokinetic behaviour and its interactions with physiological systems. Often, the drug-specific data is scaled up from the in vitro studies, transforming lab-based insights into predictions of real-world pharmacokinetics (Loisios-Konstantinidis *et al*., 2025; Yuan *et al*., 2019).

This manuscript presents the development of a PBPK model utilising the physicochemical properties of RGF along with the drug release and pharmacokinetic data from the animal study of RGF SSP. The model was subsequently refined using the input parameters specific to RGF NPs to enable prediction of their pharmacokinetic behaviours. The simulated outcomes were then compared with the experimental data to validate the model's predictive accuracy.

To unlock the full potential of advanced formulations like nanoparticles, understanding how in vitro drug release translates to in vivo performance is key to predicting drug absorption into the bloodstream. In this study, the Weibull equation was harnessed through the GastroPlus® optimisation module to fine-tune dissolution parameters (Table 3) and reveal the most biologically relevant profiles. After correcting for bioavailability, the elimination parameters of RGF were established. The corrected elimination kinetics is given in Table 4. These refined profiles were then embedded into the powerful ACAT model within GastroPlus, enabling dynamic and accurate simulation of systemic drug availability, bridging the gap between lab-based data and real-world therapeutic outcomes (Chou *et al*., 2025; Xia *et al*., 2013). The in vitro drug release data were quantitatively described using Weibull release functions, producing refined dissolution profiles that were seamlessly integrated into the pharmacokinetic model. As depicted in Fig. 3 and 4, the Weibull equations served as the predictive tool for simulating the in vivo dissolution dynamics of RGF SSP and RGF NPs, enhancing the model's physiological relevance and predictive power.

After incorporating rabbit-specific physiological parameters and drug release data for RGF SSP into the PBPK model, its predictive performance was rigorously evaluated using pharmacokinetic data from RGF NPs. This validation phase served not only to refine model accuracy through re-estimation of key parameters and assessment of predictive improvement but also to explore the sensitivity of physiological variables under definite dosing scenarios. Attention was given to the intricate clearance mechanisms of nanoparticles, enabling the identification of any model observation discrepancies as potential artefacts of clearance dynamics. For RGF, no distinct metabolic or transport differences were observed between rabbits and other species. However, the PBPK framework allows for the flexible incorporation of interspecies variability, such as species transporters, through targeted adjustments of compound-related parameters (e.g., partition coefficients), enhancing the model's translatability and mechanistic insight (Mavroudis *et al*., 2018).

The model exhibited robust predictive capability when validated against oral pharmacokinetic data, with fold errors (FE) well within established acceptable ranges. As shown in Figs. 5 and 6, the validation results affirm the model's accuracy and reliability. Notably, fold errors remained close to unity (1), indicative of a high level of precision with no systematic tendency towards over- or underprediction (Bassani *et al*., 2024). Model validation was deemed successful when the simulated pharmacokinetic parameters, particularly the AUCand $C_{max,}$fell within a 2-fold range (specifically between 0.5 and2 -fold) of the observed data. This criterion reflects a widely accepted standard for establishing predictive reliability in PK modelling (Li *et al*., 2025; Ladumor *et al*., 2023). As detailed in Table 6, the model demonstrated commendable predictive accuracy across both formulations. Importantly, all FE values fell within the acceptable range of 0.5-1, and the FE values for AUC were notably close to 1, underscoring the model's strong capacity to accurately predict PK behaviour. Nevertheless, model-based predictions are inevitably influenced by physiological variability inherent in living systems. As clinical data from human subjects becomes available, this real-world variability can be seamlessly incorporated into the model, refining its predictive power and enhancing the accuracy of future simulations (da Silva *et al*., 2025).

The experimentally determined PSD and dissolution inputs were directlyintegrated into the PBPK model to describe the absorption process and to predict in vivo pharmacokinetics. Incorporation of nanoparticle PSD enabled the model to capture dissolution and absorption behaviour, and the resulting simulations were found to be predictive of observed in vivo data. This agreement establishes a quantitative link between in vitro nanoparticle attributes and in vivo performance and thereby demonstrates that the model is accountable for IVIVE rather than relying solely on empirical fitting.Furthermore, the PBPK model was validated using independent in vivo data, and the successful prediction of pharmacokinetic profiles supports the scientific credibility of the IVIVE framework applied for the nano-formulation.

Overall, the work performed in this article provides a way forward to model nano-formulations of TKIs, such as regorafenib. The nano-formulations are typically characterised by enhanced dissolution rate and lower particle size, and in the present case, dissolution rate was used to predict in vivo behaviour. Apart from these two parameters, the in vivo behaviour is further governed by enhanced cellular uptake through the RES system and slow elimination. To enhance the research in this domain, further experiments can be planned wherein the tissue distribution behaviour of nano-formulations can be studied. Integration of plasma and tissue distribution modelling substantially advances the mechanistic insight into nanotherapeutic formulations, and the aspect will be addressed in forthcoming publications. The findings presented herein aim to support the optimisation of TKI therapies through innovative formulation strategies, ultimately improving therapeutic efficacy and patient quality of life.

*Limitations*

While the study demonstrates robust predictive accuracy for regorafenib monohydrate nanoparticle formulations using PBPK modelling in GastroPlus, a few limitations warrant acknowledgement. Rabbit-specific parameters drive simulations, yet marked interspecies gaps in GI physiology, enzyme/transporter expressions, and PK variability hinder human extrapolation. Additionally, the nanoparticle disposition mechanism is not investigated in the current study.

*FutureScope of Research*

Future work should prioritise human translation via model refinement with emerging clinical PK data and interspecies scaling factors (e.g., allometric adjustments for clearance). This framework can extend to other BCS class II TKIs, assessing synergies in scale-up manufacturing, physiological stability, and adaptive PBPK for personalised dosing. The nanoparticle disposition mechanism can be investigated further by using tumour-bearing animal species.

## Conclusion

In the present study, a robust PBPK model was meticulously developed using animal data to forecast the in vivo behaviour of regorafenib monohydrate-loaded PEGylated PLGA nanoparticles (RGF NPs). The model delivered highly accurate pharmacokinetic predictions and was successfully validated, positioning it as a robust framework for extending PBPK modelling across multiple species. This model-based approach holds significant promise in drug development, streamlining formulation design, minimising costly trial-and-error processes, and reducing reliance on extensive human clinical trials, ultimately accelerating the path from laboratory to clinic. PBPK modelling has rapidly evolved into a powerful mainstream tool across the pharmaceutical industry and academia, widely leveraged for insightful prediction, analysis, and interpretation of pharmacokinetic (PK) data. The integration of advanced technologies such as Artificial Intelligence and Machine Learning (AI/ML), in vitro–in vivo extrapolation (IVIVE), and advanced PBPK frameworks holds the potential to revolutionise drug development. When strategically integrated to suit specific project needs, these innovations can dramatically elevate the precision and efficiency of PK predictions for small molecules, streamlining both preclinical and clinical phases of drug discovery.

## Conflicts of Interest

No financial or other conflicts of interest are disclosed by the authors.

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