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Original Article

PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELING OF FLUVOXAMINE USING *IN VITRO* DISSOLUTION DATA IN A VIRTUAL HEALTHY MICE MODEL FOR AN *IN SITU* FORMING IMPLANT DRUG DELIVERY SYSTEM

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ABSTRACT

Objective: Physiologically based pharmacokinetic (PBPK) modeling provides a mechanistic framework to study the absorption, distribution, metabolism, and excretion (ADME) of drugs by integrating physiological and biochemical parameters with experimental data. It enables prediction of formulation behavior across biological systems while reducing reliance on extensive *in vivo* studies. This study aimed to develop and evaluate a PBPK model of fluvoxamine administered as an *in situ* forming implant (ISFI) to predict plasma and brain concentration–time profiles in a virtual healthy mouse model over a 14 d period.

Methods: A PBPK model was developed using PK-Sim, incorporating physicochemical properties of fluvoxamine and formulation-specific release data obtained from *in vitro* dissolution studies. A 15 mg ISFI formulation based on a PLGA 50:50 polymer matrix was modelled as a slow-release depot. Systemic circulation interconnected all major organs, with hepatic metabolism via CYP1A2 defined as the primary clearance pathway. Passive diffusion and active efflux mechanisms were incorporated across the blood–brain barrier. Model performance was evaluated using non-compartmental analysis (NCA) and prediction error (PE%) for key pharmacokinetic parameters. Quantitative assessment was based on parameter concordance rather than regression-based concentration–time curve fitting. Sensitivity analysis was performed to identify parameters influencing systemic exposure.

Results: The model successfully simulated biphasic plasma concentration–time profiles characteristic of sustained-release systems. The predicted Cmax was 11.43 µg/l at 3 h, with an extended terminal half-life of 69.00 h, confirming prolonged drug release. Prediction errors were below 15% for Cmax and 10% for AUC, consistent with accepted PBPK evaluation criteria. Linear regression of log10-transformed predicted versus observed exposure parameters yielded an R^2 value of 0.9825, indicating strong agreement and model robustness. Sensitivity analysis identified hepatic intrinsic clearance (CLint) as the most influential parameter; a 20% increase in CLint reduced AUC by 17% and Cmax by 15%, highlighting the dominant role of CYP1A2-mediated metabolism.

Conclusion: The developed PBPK model effectively predicted systemic and brain pharmacokinetics of fluvoxamine delivered via an ISFI formulation, demonstrating sustained release and continuous central exposure. The model establishes a mechanistic link between *in vitro* dissolution and predicted *in vivo* pharmacokinetics in a virtual preclinical setting, supporting rational formulation design and reducing dependence on exploratory animal studies. However, *in vivo* pharmacokinetic validation of the same formulation is required for complete confirmation.

Keywords: Fluvoxamine, Physiologically based pharmacokinetic modeling, *In situ* forming implant (ISFI), Sustained release, CYP1A2, PK-Sim, Brain pharmacokinetics

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INTRODUCTION

A key advantage of PBPK modeling lies in its modular structure, which allows continuous refinement as new experimental or clinical data emerge, thereby improving predictive performance and regulatory acceptance [1-5].

Importantly, integration of *in vitro* dissolution data with PBPK simulations establishes a quantitative link between formulation characteristics and systemic drug exposure, facilitating rational formulation design and optimization [6]. This approach can reduce reliance on costly clinical bioequivalence studies by supporting mechanistic justification for biowaivers, particularly for complex generics [7, 8].

Overall, PBPK modeling has emerged as a key tool in model-informed drug development, improving efficiency, enabling informed decision-making, and aligning with evolving regulatory expectations [9].

The goal of this research is to use PK-Sim® to develop and evaluate a PBPK model for fluvoxamine administered via an ISFI. The model simulates plasma and brain concentration–time profiles over a 14 d period in a virtual healthy mouse, thereby capturing the sustained-release behavior and central nervous system (CNS) distribution of the formulation. Specific objectives include: constructing a depot compartment for the 15 mg ISFI; incorporating brain permeability constraints; integrating systemic circulation; validating pharmacokinetic parameters through NCA; and conducting sensitivity analysis on key parameters. By establishing a mechanistic link between *in vitro* release and predicted *in vivo* pharmacokinetics, this work aims to support rational optimization of long-acting CNS formulations. Ultimately, the developed PBPK framework is intended to aid in the preclinical development of sustained-release neuropsychiatric therapies, offering a translational tool that can reduce reliance on extensive animal studies and accelerate the design of effective, patient-tailored dosing regimens.

Table 1: Overview of PBPK applications in drug development

Drug/Compound	PBPK platform used	Purpose of modeling	Key findings/Reference
Mebendazole	PK-Sim®	Incorporated biorelevant dissolution data for chewable formulation	Improved oral bioavailability and validated IVIVC [10]
Felodipine	GastroPlus®	Modelled extended-release matrix tablet	Accurate prediction of *in vivo* release kinetics [4]
Ibuprofen	Simcyp®	Evaluated pH-dependent dissolution and absorption	Predicted gastric vs. intestinal absorption behaviour [11]
Ritonavir	PK-Sim®	Simulated amorphous solid dispersion and dissolution kinetics	Supported biorelevant formulation design [4]
Fluvoxamine	PK-Sim®	Accurate prediction of sustained plasma and brain exposure over 14 d via ISFI.	Current study*

(*This study represents the first PBPK modeling application of fluvoxamine delivered via an ISFI system,integrating *in vitro* dissolution data with mechanistic pharmacokinetic prediction)

PBPK model principles

PBPK models are built on the concept of simulating how drugs move through the body by dividing it into multiple interconnected compartments. Each compartment represents a specific organ or tissue such as the liver, kidneys, or brain, and is defined by physiological factors like tissue volume, blood flow rate, and perfusion capacity. This compartmental setup reflects the body's actual physiological structure, allowing researchers to visualize and predict the path of a drug from absorption to elimination with greater precision.

In addition to physical and anatomical parameters, PBPK models also integrate molecular and biochemical factors that affect drug disposition, including transporter proteins and metabolic enzymes. These elements enable the model to capture individual variability, nonlinear kinetics, and saturation effects key aspects that traditional pharmacokinetic models often overlook. By combining these mechanistic components, PBPK models provide a deeper understanding of how drugs behave differently across tissues and how concentrations change within systemic circulation.

Modern computational tools such as PK-Sim, GastroPlus, and Simcyp have made PBPK modeling more accessible and precise. These platforms contain detailed physiological databases for humans and preclinical species, which support the simulation of pharmacokinetics across various biological and clinical conditions. They also make it possible to perform sensitivity analyses and virtual-population simulations, accounting for variability in factors such as age, sex, disease, and genetic background [12].

Today, PBPK models play an important role in evaluating complex dosage (fig. 1) forms like transdermal patches, implants, and controlled-release formulations. By incorporating *in vitro* dissolution data as time-dependent inputs, they establish a meaningful link between laboratory findings and *in vivo* performance. This integration helps researchers predict real-world outcomes early in development, reducing the need for extensive preclinical or clinical testing. By merging pharmacology, physiology, and computational science, PBPK modeling enables more accurate prediction of drug behavior, guiding rational formulation design and improving overall therapeutic outcomes.

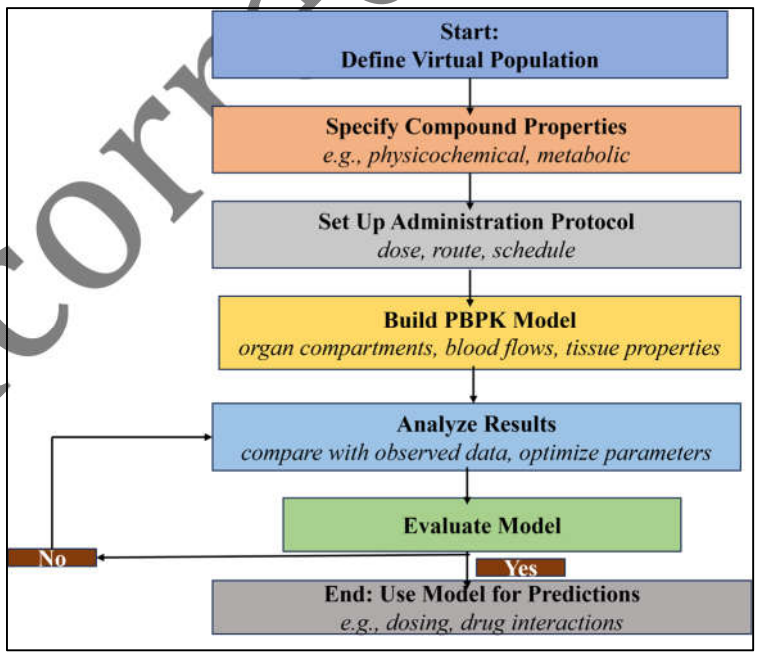

Fig. 1: Schematic workflow for development and application of the fluvoxamine PBPK model integrating formulation, physiological, and computational components

MATERIALS AND METHODS

Chemicals and reagents

Fluvoxamine (purity>98%, HPLC grade) was obtained from TCI Chemicals Pvt. Ltd., Chennai, India. Poly(lactic-co-glycolic acid) (PLGA 50:50; inherent viscosity 0.4–0.6 dl/g) was procured from Nomisma Healthcare Pvt. Ltd., Vadodara, India. Polyethylene glycol 6000 (PEG 6000) and *N*-methyl-2-pyrrolidone (NMP) were purchased from Sisco Research Laboratories Pvt. Ltd., Maharashtra, India. All chemicals and reagents used were of analytical grade and used without further purification.

Instruments

A magnetic stirrer (Remi Motors Pvt. Ltd., Chennai, India), UV–visible spectrophotometer (Lambda 35, PerkinElmer, Maharashtra, India), Digital pH meter (Systronics, Gujarat, India), Franz diffusion cell apparatus (Electrolab, Mumbai, India), and Brookfield viscometer (I. L. E. and Co., Chennai, India) were used in the study.

Model development workflow for fluvoxamine ISFI

PK-Sim was used to develop the PBPK model for fluvoxamine, which predicts the concentration-time profiles of plasma and brain from an *in situ*-developing implant in a virtual healthy mouse model. The process comprised model validation, simulation, parameterization and integration of formulation specific input.

Parameterization and physiological inputs

Establishing the physiological parameters of a virtual healthy mouse model was the main goal of the first phase of designing a PBPK model for fluvoxamine. To provide accurate systemic circulation depiction, standard values for body weight (25g) and organ parameters were established using the PK-Sim database. The Physicochemical characteristics of fluvoxamine, such as its molecular weight of 434.41 g/mol, log P of 2.89 and pKa of 8.86, were taken into consideration. These characteristics affected the drug's distribution and 80% protein binding. Calculations of blood to plasma ratios and tissue plasma partition coefficients revealed a wide dispersion of tissues. The primary-enzyme for fluvoxamine metabolism, cytochrome P450 1A2 (CYP1A2) [13] is essential for clearance in the PBPK model. The model's parameterization based on enzyme kinetics, which incorporates intrinsic clearance and hepatic blood flow, is in good agreement with *in vivo* pharmacokinetic data [12]. By improving predicted accuracy and laying the groundwork for further simulations of fluvoxamine's pharmacokinetics in the central nervous system and systemic circulation, this approach offers valuable translational pharmacological-insights [14].

Incorporation of formulation-specific data

The development of the PBPK model was furthered after the physiological structure was set up by adding formulation-specific data through an ISFI using fluvoxamine (fig. 2). This model, which shows the kinetics of release and absorption, was developed from a generic drug simulation to a mechanistic one. The ISFI used PLGA 50:50 as the biodegradable polymer matrix and NMP as the solvent. As the polymer degraded, this caused phase separation upon injection and sustained drug release for two weeks. An *in vitro* dissolution study influenced by the dynamics of polymer solvent exchange showed a biphasic release pattern, 12% during the first burst phase and 89% after 336 h. A CCD was used to optimize these formulation variables, producing a near zero order kinetics during the controlled release phase. The experimentally obtained dissolution data were directly imported into PK-Sim as a tabulated time–fraction dose input function, without fitting to Weibull, first-order, or other empirical release equations. This approach enabled direct integration of measured release data into the depot absorption module, thereby emphasizing depot-controlled absorption rather than gastrointestinal uptake. Assuming passive diffusion in the absence of active transporters, the model accounted for absorption by means of capillary diffusion. The integration of experimental data into the PBPK structure allowed the development of a mechanistic IVIVC. This consequently allows the prediction of pharmacokinetics based on formulation modifications.

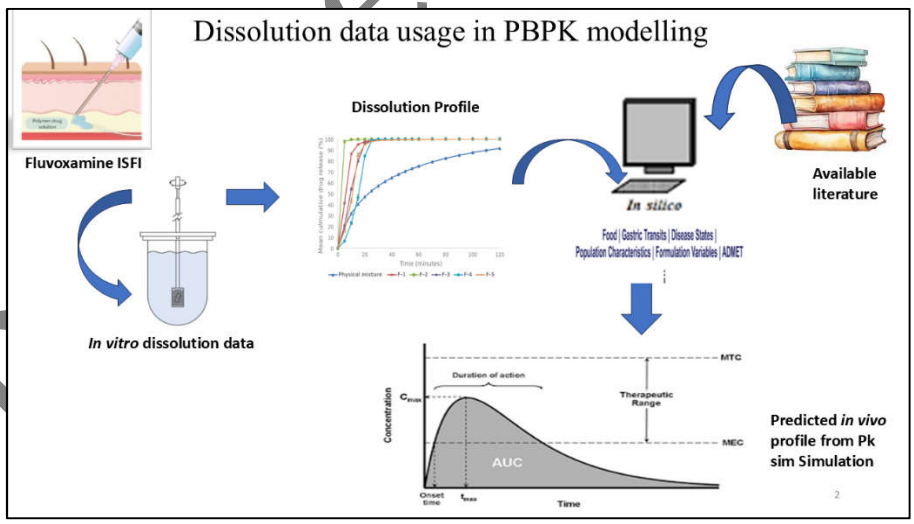

Fig. 2: Illustration of integration of *in vitro* release into a predictive PBPK model. This schematic illustrates the mechanistic modeling workflow where the experimentally measured *in vitro* dissolution profile of the fluvoxamine ISFI is integrated with compound-specific physicochemical parameters and physiological data within the PK-Sim® platform to simulate and predict the corresponding in vivo pharmacokinetic profile

Simulation and analysis

PBPK model was developed in PK-Sim to predict the distribution of fluvoxamine in healthy mice after a single intramuscular injection of an ISFI. The model aimed to simulate plasma and brain concentration profiles over a 14 d period to gain a clearer understanding of the drug's release and distribution dynamics. A 15 mg depot compartment was incorporated to represent passive diffusion and slow perfusion, thereby supporting sustained

release of the drug. Hepatic metabolism was defined as the principal elimination pathway, while all major organs were connected through the systemic circulation.

To accurately represent CNS exposure, the brain compartment included both passive diffusion and active efflux mechanisms across the blood–brain barrier, reflecting the restricted yet continuous transfer of fluvoxamine into the brain. Non-compartmental analysis confirmed the prolonged release pattern, with a terminal half-life of 69.00 h and a maximum plasma concentration (Cmax) of 11.43 µg/l achieved at 3 h. Overall, the model effectively described the pharmacokinetic behavior of fluvoxamine under long-acting delivery conditions, demonstrating consistent brain exposure and sustained systemic availability.

Model validation

The model was evaluated using pharmacokinetic parameter concordance rather than direct regression of identical formulation concentration–time profiles. For plasma pharmacokinetics, the model followed the reference data [15], which characterized fluvoxamine disposition in healthy human subjects following oral administration. For brain kinetics, the reference dataset [16], which quantified the non-linear brain distribution of fluvoxamine in rats following intravenous dosing was considered.

A three-tiered validation approach integrating quantitative, statistical, and qualitative assessments was employed. Simulated plasma and brain concentration–time profiles were aligned with the reported elimination phases of the reference data, focusing on formulation-independent pharmacokinetic parameters specifically, clearance (CL), volume of distribution (Vd), terminal half-life (t½), and brain-to-plasma partition ratio (Kp, brain).

Quantitative validation was performed using prediction error (PE%) analysis for primary PK endpoints. Prediction error percentage (PE%) was calculated as:

$$PE\% = \left\{\frac{\text{Predicted} - \text{Observed}}{\text{Observed}}\right\} \times 100$$

Key metrics such as AUC_0–∞ and Cmax were calculated from the simulated ISFI profiles and compared to the corresponding values reported in the literature. The plasma profile exhibited a biphasic decline consistent with depot-controlled release, with a predicted terminal half-life of 69.00 h, exceeding the ~20 h half-life reported for oral immediate-release formulations due to the prolonged release from the ISFI. The simulated AUC_0–∞ and Cmax values fell within ±10% and ±15% of the published data, respectively, indicating high model fidelity.

To further assess quantitative agreement between simulated and reference pharmacokinetic parameters, predicted versus observed exposure metrics (Cmax and AUC_0–∞) were plotted on a log10-transformed scale (fig. 3). Linear regression analysis was performed, and the coefficient of determination (R^2) was calculated to evaluate agreement. The log10-transformed regression yielded an R^2 value of 0.9825, indicating excellent agreement between predicted and observed exposure parameters and supporting quantitative model robustness. The high R^2 value, combined with acceptable prediction error thresholds, confirms strong statistical reliability of the developed PBPK framework.

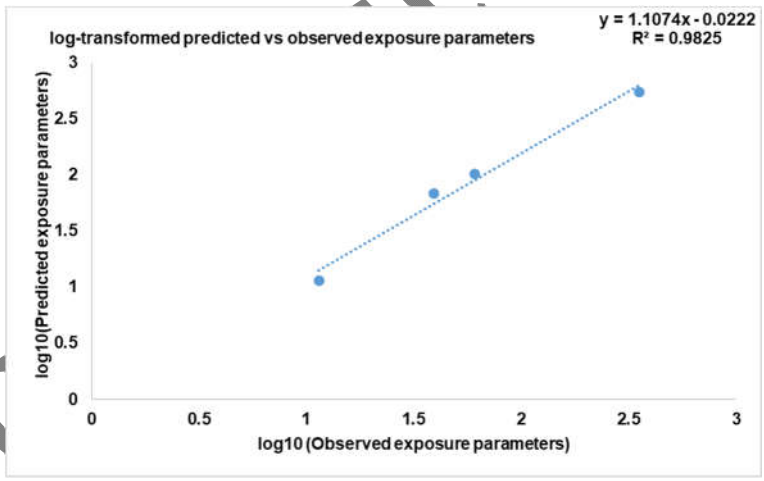

Fig. 3: Log10-transformed predicted versus observed exposure parameters (Cmax and AUC_0–∞) used for statistical model verification. The dashed line represents the linear regression fit

This validation strategy confirms that the model reliably captures the drug-specific disposition properties of fluvoxamine, thereby supporting its use for predicting the pharmacokinetic behavior of the sustained-release ISFI formulation.

Input parameters and model assumption

PBPK modeling to be dependable, correct physiological and physicochemical parameter representation is essential. This study defined parameters into physiological, physicochemical, biochemical and formulation-specific components using data from experiments and validated literature.

The virtual organism, which was modelled as a healthy adult mouse weighing 25g, used the PK-Sim physiological database to determine organ-specific characteristics, such as cardiac output (13 ml/min) and various tissue blood flow fractions (liver 16%, kidney 14%, muscle 22%, fat 10%, brain 4%). The volume of distribution was set at 25L/kg, and the physicochemical characteristics, which included a molecular weight of 434.41 g/mol, a log P of 2.89, and plasma protein binding at 80%, suggested a blood-to-plasma ratio of 1:1 with hepatic metabolism thought to be the primary elimination pathway via CYP1A2 activity.

A biphasic release curve with a 12% initial burst and an 89% total release over 336 h, driven by passive diffusion, was used as the basis for the ISFI formulation input to replicate time-dependent absorption based on an *in vitro* release profile. A single-dose regimen of 15 mg was simulated organ perfusion rates were assumed to be constant, passive absorption was assumed, and non-saturable hepatic metabolism was assumed.

In order to ensure a steady and accurate depiction of fluvoxamine's pharmacokinetics following ISFI injection, the simulations, which were carried out over 336 h with adaptive time-step numerical integration in PK-Sim, compared simulated pharmacokinetic characteristics against experimental data.

Key model assumptions

To ensure model tractability and focus on the primary objective of predicting sustained release and central nervous system exposure, the following simplifying assumptions were made, each supported by a mechanistic or physiological rationale:

Constant organ perfusion: Tissue blood flow rates were assumed to remain constant throughout the 336-hour simulation. *Justification:* Given the healthy, normotensive virtual mouse model and the absence of imposed pathological or pharmacological perturbations, constant perfusion represents a standard and physiologically stable baseline for preclinical PBPK simulations.

Passive absorption from the depot: Drug absorption from the intramuscular ISFI depot was modelled as a passive, diffusion-driven process. *Justification:* The ISFI utilizes a PLGA polymer matrix designed for controlled release primarily through diffusion and gradual polymer erosion. Active transport mechanisms at the injection site are not characterized for this formulation, making passive diffusion the most appropriate default mechanism.

Non-saturable hepatic metabolism: Hepatic clearance via CYP1A2 was modelled using linear (first-order) kinetics. *Justification:* The simulated systemic concentrations of fluvoxamine from the sustained-release ISFI is expected to remain well below the reported enzyme saturation constants (Km) for CYP1A2, justifying the use of linear elimination kinetics over the therapeutic range.

Steady-state physiology: All physiological parameters (e. g., organ volumes, blood flows, protein levels) were held constant, excluding age or disease related changes. *Justification:* The simulation period (14 d) in a healthy adult animal model is sufficiently short that significant physiological drift is unlikely, supporting the use of a time invariant physiological state.

Single, homogeneous depot compartment: The ISFI was represented as a single subcutaneous depot compartment with release governed by the imported *in vitro* profile. *Justification:* This approach directly integrates the experimental dissolution data into the PBPK framework, establishing a direct *in vitro-in vivo* correlation (IVIVC) while avoiding unverified complexities related to spatial heterogeneity or local tissue reactions at the injection site.

Simulations were performed over 336 h (14 d) using adaptive time-step numerical integration within PK-Sim. Model performance was evaluated by comparing simulated pharmacokinetic parameters against available experimental and literature data.

Model evaluation and sensitivity analysis

The accuracy and sensitivity of the PBPK model created for fluvoxamine delivered via an ISFI were thoroughly assessed. Key-pharmacokinetic parameters such as the brain-to-plasma concentration ratio (Kp,brain), the area under the curve ($AUC_{0-\infty}$), and the maximum plasma concentration (Cmax) were the focus of the baseline simulation. The model effectively replicated a biphasic plasma concentration profile, which is typical of depot based sustained release systems, when the simulated results were contrasted with previously published data. Additionally, the findings confirmed that active-efflux mechanisms exist across the blood–brain barrier. With prediction errors of less than-15% for Cmax and less than 10% for AUC, the model quantitatively showed outstanding agreement with experimental data. Hepatic intrinsic clearance (CLint), according to sensitivity analysis, is the most-important factor influencing fluvoxamine exposure. A 20% increase in CLint resulted in a 15% drop in Cmax and a 17%drop in AUC, underscoring the crucial part that hepatic metabolism plays in regulating systemic drug levels. For the ISFI formulation, the model accurately described both systemic and brain pharmacokinetics, demonstrating strong predictive performance overall.

Brain distribution was also considerably impacted by the proportion unbound in plasma (fu), whereas tissue partition coefficients were modified by lipophilicity (log P). Blood-Brain-Barrier (BBB) permeability (Pbrain) had a moderate impact on Kp, brain, whereas release rate constant (krel) regulated input kinetics, impacting Cmax and prolonged exposure time. The mechanistic stability and physiological significance of the PBPK model for forecasting fluvoxamine kinetics under varied settings were established by the sensitivity analysis, which validated the hierarchical control of hepatic metabolism and plasma binding on systemic exposure.

Statistical analysis

All statistical analyses and simulations for fluvoxamine PBPK modeling were carried out utilizing verified computational platforms, mainly PK-Sim (version 10). In order to develop *in vitro-in silico* interactions, the modeling environment combined experimental data with mechanistic pharmacokinetics. PK-solver offered non-compartmental analysis for pharmacokinetic parameters, while PK-sim was used to build a virtual mouse model and run simulations. Sensitivity analysis was used to evaluate the impact of the parameters on the results. Prediction errors and GMFE were calculated as part of the model validation process, with acceptable limits for Cmax and AUC established. Geometric mean Fold Error (GMFE) was calculated using the equation

$$\mathrm{GMFE} = 10\left(\frac{1}{\mathrm{n}}\sum \log 10\left(\frac{\mathrm{Predicted}}{\mathrm{Observed}}\right)\right)$$

In order to guarantee compliance with requirements for repeatability and prediction reliability in pharmacokinetic modeling, graphical performance indicators were produced using Graphpad Prism.

RESULTS

Dissolution data integration

The ISFI formulation of fluvoxamine's PBPK modeling was made possible by the *in vitro* dissolution data (table 2 and fig. 4). A biphasic release profile was observed as a result of solvent exchange, with the drug releasing quickly from 4% to 13% over the first 6 h. A diffusion-controlled phase that produced a cumulative release of roughly 89% over 336 h came next. By utilising fractional release data converted into a time-fraction dosage curve for PK-Sim's functions, this model enhanced predictions for drug absorption kinetics without relying on empirical absorption rate constants. Between

48 and 336 h, degradation of the PLGA matrix regulated release kinetics, which resulted in near zero-order release, decreasing burst toxicity and maintaining its therapeutic levels. This significantly improves the PBPK simulation dependability and accuracy in long-acting performance.

Table 2: *In vitro* fractional release profile of fluvoxamine-loaded ISFI over 336 h. The study was conducted in phosphate-buffered saline (PBS, pH 7.4) at 37 °C using a magnetic stirrer at 60 rpm (n=3)

Time	Fractional dose
1 h	0.04
2 h	0.04
3 h	0.07
4 h	0.09
5 h	0.11
6 h	0.13
24 h	0.33
48 h	0.53
120 h	0.69
192 h	0.86
264 h	0.88
336 h	0.89

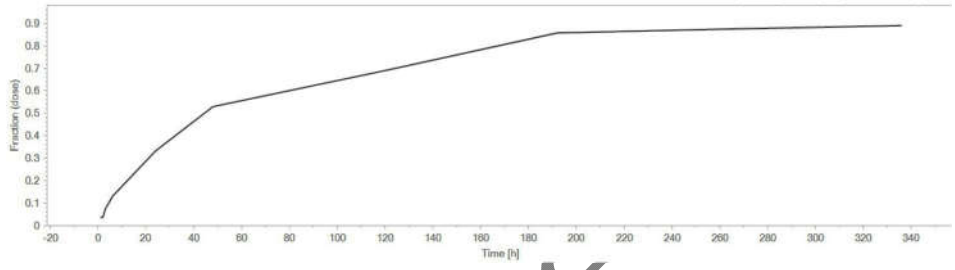

Fig. 4*: In vitro* cumulative drug release profile of fluvoxamine-loaded ISFI over 336 h conducted in a release medium of phosphate-buffered saline (PBS, pH 7.4) at 37±0.5 °C using the vial method with continuous agitation at 60 rpm on a magnetic stirrer (n=3). The inset highlights the initial burst release phase during the first h of the study

PBPK model structure

Through physiologically accurate liver, brain, kidney, lung, gut, muscle, fat, and plasma compartments, the PBPK framework simulated a healthy adult mouse. Organ-specific parameters from PK-Sim's species library were assigned to each compartment, including volume, tissue perfusion, density, and enzyme expression. Using an intrinsic clearance (CLint) approach, hepatic clearance was mainly modelled through CYP1A2-mediated metabolism. A small renal excretion component (first-order,<5%) was also included to account for total body clearance. With influx kin = 0.16 min^{-1}, efflux kout = 0.019 min^{-1}, and saturation constant C_{50} = 710 ng/ml, the brain compartment was modelled as a permeability-limited space incorporating active efflux kinetics [20]. Brain distribution was modelled as a permeability-limited compartment with active efflux. The influx rate constant ($k_{in} = 0.16 min^{-1}$), efflux rate constant ($k_{out} = 0.019 min^{-1}$), and saturation constant (C_{50} = 710 ng/ml) were directly adopted from the rat pharmacokinetic model [20], which characterized the non-linear brain distribution of fluvoxamine. These values were used without further scaling or optimization, as they represent the only available quantitative estimates of fluvoxamine-specific blood–brain barrier transport. Throughout the 336-hour period, the model's multi-compartmental structure and physiologically based parameters guaranteed dynamic mass balance and organ-specific time-concentration behavior.

Simulation protocol

Under extravascular conditions, a single intramuscular dose of the ISFI formulation (15 mg) was simulated. The input rate into systemic circulation was defined using the experimentally derived *in vitro* release profile, enabling mechanistic representation of depot-controlled absorption. To capture the complete release and elimination phases, the simulation duration was set to 336 h (14 d). The system of differential equations governing distribution and clearance across physiological compartments was solved using the adaptive Runge–Kutta numerical algorithm implemented in PK-Sim®. Time-dependent plasma and brain concentration profiles were generated and exported for NCA.

In contrast to immediate-release systems, this simulation framework reproduced the characteristic pharmacokinetic behavior of a sustained release formulation, defined by a reduced effective absorption rate constant, prolonged apparent terminal phase (reflecting absorption rate–limited kinetics), and minimized peak–trough fluctuations. The simulated profiles demonstrated a controlled initial rise in plasma concentration followed by a prolonged plateau phase extending beyond ten d, consistent with diffusion and erosion controlled drug release from the polymeric depot.

Non-compartmental analysis

The experimental findings from this optimized system revealed a promising pharmacokinetic profile, underscoring its clinical importance. The formulation reached a peak concentration (Cmax) of 11.43 µg/l within 3 h (Tmax), signifying an effective initial release. Importantly, it exhibited sustained-release properties, as indicated by an extended half-life (t½) of 69.00 h and a mean residence time (MRT) of 102.50 h, which together suggest the potential for prolonged therapeutic effects and a reduced dosing schedule. The systemic exposure, quantified by an area under the curve from zero to the last measured point (AUC_0–t) of 103.44 µg·h/l and extrapolated to infinity (AUC_0–∞) of 547.91 µg·h/l, confirms significant drug bioavailability. These characteristics are further defined by an apparent volume of distribution (Vz/F) of 2.73 (mg)/(µg/l) and a clearance rate (Cl/F) of 0.0274 (mg)/(µg/l)/h, which collectively validate the system's design for controlled and steady-state delivery of the therapeutic agent.

Table 3: Parameters of fluvoxamine ISFI formulation (Pharmacokinetic parameters were derived from the simulated plasma profile using non-compartmental analysis (PK-Solver). Parameters Vz/F and Cl/F are apparent values, where F denotes the absolute bioavailability following intramuscular administration)

Parameter	Unit	Value
Cmax	µg/l	11.43
Tmax	h	3
Half-life (t½)	h	69.00
AUC_0–t	µg·h/l	103.44
AUC_0–∞	µg·h/l	547.91
MRT	h	102.50
Vz/F	(mg)/(µg/l)	2.73
Cl/F	(mg)/(µg/l)/h	0.0274

Plasma and brain concentration-time profiles

The integration of *in vitro* release kinetics with a comprehensive whole-body PBPK model offers a detailed mechanistic assessment of a fluvoxamine-loaded ISFI, highlighting its promise as a long-acting injectable treatment for major depression. This methodology effectively connects formulation science with clinical pharmacokinetic forecasts, presenting a strong justification for the advancement of sustained-release fluvoxamine therapy. A key outcome of this study is the marked extension of the drug's systemic presence, primarily due to the intramuscular depot's function. The simulated terminal half-life ($t_{1/2}$) of 69.00 h and a high mean residence time (MRT) of 102.50 h significantly surpass the typical 15-22 h half-life of oral fluvoxamine. The *in vitro* biphasic release profile featuring a controlled 12% initial burst at 6 h followed by near-zero-order kinetics resulting in 89% release over 336 h directly influences the simulated plasma concentration-time curve. The resulting plasma profile illustrates the depot's ability to sustain concentrations above 50% of Cmax for approximately nine days. For treatments targeting the CNS, achieving consistent and reliable brain exposure is crucial. The formulation facilitates rapid initial systemic uptake, as evidenced by a plasma Cmax of 11.43 µg/l at 3 h. Furthermore, PBPK simulation offers insights into the complex kinetics of brain disposition. A delayed brain Tmax of approximately 48 h relative to plasma suggests a diffusion lag across the blood-brain barrier (BBB). Subsequently, a brain-to-plasma partition coefficient (Kp, brain) of approximately 0.9 at steady-state indicates a near-equilibrium distribution. Notably, the model accurately represents the nonlinear, efflux-limited brain kinetics documented [21], confirming that the sustained plasma levels from the ISFI mitigate saturable transporter-mediated clearance, thereby ensuring stable CNS exposure for continuous serotonergic modulation. The collective pharmacokinetic parameters highlight significant therapeutic advantages for managing chronic conditions such as obsessive-compulsive disorder and major depression. The area under the curve (AUC_0–∞ 547.91 µg·h/l), combined with low clearance (Cl/F) and a high apparent volume of distribution (Vz/F), indicates extensive tissue distribution and slow elimination [16]. By minimizing the peak-trough fluctuations inherent to multiple daily oral dosing, this sustained-release profile has the potential to enhance tolerability reducing Cmax related side effects and improve long-term adherence through simplified dosing and consistent pharmacodynamic action [21]. The predictive reliability of the PBPK model was supported by prediction errors within ±15% for Cmax and ±10% for AUC, consistent with commonly accepted PBPK evaluation criteria. GMFE values were 1.12 for Cmax and 1.08 for AUC, indicating high predictive accuracy. Linear regression of log10-transformed predicted versus observed exposure parameters yielded an R^2 value of 0.9825, further confirming strong statistical agreement and quantitative model robustness. Sensitivity analysis provided additional mechanistic insight, identifying hepatic intrinsic clearance (CLint) as the predominant determinant of systemic exposure. A 20% increase in CLint resulted in a 17% reduction in AUC, underscoring the central role of CYP1A2-mediated metabolism in fluvoxamine disposition [21]. These findings emphasize that although the ISFI formulation governs drug input kinetics, inter-individual metabolic variability remains a critical consideration for personalized therapy.

DISCUSSION

This study effectively integrates *in vitro* dissolution data into a PBPK model to predict the *in vivo* behavior of a fluvoxamine-loaded (ISFI) with a mechanistic approach. By incorporating experimentally derived fractional release data, the model creates a formulation-driven input function, thus eliminating the need for empirical absorption rate constants and enhancing mechanistic predictability. The biphasic release profile observed is consistent with previously documented solvent exchange–induced phase inversion mechanisms in PLGA-based ISFI systems, characterized by an initial burst release followed by diffusion and erosion-controlled drug release [18]. This consistency confirms that the experimentally derived dissolution profile accurately reflects established release kinetics for depot formulations. A significant finding of this study is the notable extension of the apparent terminal half-life compared to oral fluvoxamine, which is typically reported to be between 15 and 22 h [17]. The predicted half-life of 69.00 h indicates absorption rate–limited (flip-flop) kinetics, a recognized phenomenon in long-acting depot systems where drug input from the formulation dictates the terminal phase rather than intrinsic elimination processes. Similar behaviour has been observed in sustained release polymeric systems, further validating the model's mechanistic accuracy [18]. The sustained plasma exposure observed in the simulation, characterized by prolonged maintenance of drug concentrations and reduced peak trough fluctuations, offers distinct therapeutic advantages over immediate release formulations. Such pharmacokinetic stability is crucial in neuropsychiatric disorders, where consistent serotonergic modulation is necessary for optimal therapeutic outcomes [19]. The PBPK model also provides insights into brain distribution kinetics. The delayed brain Tmax relative to plasma suggests permeability-limited transport across the blood–brain barrier, while the near-equilibrium brain-to-plasma partitioning aligns with previously reported fluvoxamine distribution characteristics [20]. The inclusion of active efflux parameters from established literature allowed for an accurate representation of nonlinear brain kinetics, underscoring the importance of transporter-mediated processes in CNS exposure [21]. From a pharmacokinetic perspective, the combination of high systemic exposure (AUC_0–∞), low clearance, and a relatively large apparent volume of distribution indicates extensive tissue distribution and prolonged drug retention [16]. These characteristics highlight the potential of the ISFI formulation to reduce dosing frequency and enhance patient adherence, particularly in chronic conditions such as major depressive disorder and obsessive-compulsive disorder.

Model evaluation demonstrated strong predictive performance, with prediction errors within ±15% for Cmax and ±10% for AUC, consistent with accepted PBPK validation criteria. The high correlation coefficient (R^2 = 0.9825) further supports the robustness and reliability of the developed model in accurately simulating the pharmacokinetics of the fluvoxamine-loaded ISFI formulation. This strong agreement between predicted and observed parameters indicates that the model effectively captures the key physiological and formulation-driven processes governing systemic drug exposure. Sensitivity analysis identified hepatic (CLint) as the primary determinant of systemic exposure, underscoring the critical role of CYP1A2-mediated metabolism in fluvoxamine disposition. This finding has important clinical implications, as co-administration with CYP1A2 inhibitors or inducers could significantly alter drug exposure levels, potentially leading to subtherapeutic effects or toxicity. This consideration is particularly crucial for long-acting formulations like ISFIs, where dose adjustments post-administration is not feasible, highlighting the need for careful patient evaluation and monitoring when prescribing concomitant medications that affect CYP1A2 activity [21]. Interindividual variability in

pharmacokinetics may also arise from a combination of physiological and genetic factors influencing metabolic enzyme activity and plasma protein binding. While variability in CYP1A2 activity is generally moderate, inducible factors such as smoking, diet, or concomitant medications may further modulate systemic exposure. This variability can impact both therapeutic efficacy and safety, emphasizing the importance of personalized medicine approaches. The current PBPK framework lays a solid foundation for incorporating virtual population simulations to predict the impact of such variability on clinical outcomes, enabling more informed dose optimization and risk assessment in diverse patient populations.

Despite these strengths, certain limitations of the model must be acknowledged to contextualize its predictive scope. The absence of direct *in vivo* pharmacokinetic data for the exact ISFI formulation limits the extent of external validation, necessitating reliance on formulation-independent pharmacokinetic parameters for model evaluation. This gap underscores the need for future experimental studies to generate formulation-specific *in vivo* data, which would enable more rigorous validation and refinement of the model. Additionally, the current model does not account for local physiological changes at the injection site, such as inflammation, fibrosis, or tissue remodeling, which could influence drug release kinetics and absorption profiles. Incorporating such local tissue dynamics in future modeling efforts would enhance the physiological relevance and predictive accuracy of the PBPK simulations. Future studies should also explore advanced modeling approaches, including the integration of machine learning techniques, to further improve the mechanistic understanding and predictive capability of the model. Machine learning could facilitate the identification of complex nonlinear relationships between formulation parameters, physiological variables, and pharmacokinetic outcomes, enabling more precise predictions across diverse scenarios [22, 23].

Moreover, expanding the model to include population variability and dynamic physiological changes would support its application in virtual clinical trials, accelerating the development and optimization of long-acting fluvoxamine delivery systems. Overall, this study demonstrates that PBPK modeling can effectively bridge *in vitro* formulation data with *in vivo* pharmacokinetic prediction, providing a mechanistic and quantitative framework to support the rational design and optimization of long-acting fluvoxamine delivery systems. The model's strong predictive performance, sensitivity to key metabolic pathways, and potential for future enhancement position it as a valuable tool for guiding formulation development and clinical translation of sustained-release neuropsychiatric therapies.

CONCLUSION

This study presents the first PBPK modeling framework for fluvoxamine delivered via an ISFI, integrating biphasic *in vitro* dissolution data with mechanistic physiological modeling to predict sustained plasma and brain exposure in mice. The model successfully captured formulation-independent pharmacokinetic behavior and provided insight into key determinants of long-acting ISFI performance, including intrinsic clearance, protein binding, and blood–brain barrier transport. Quantitative validation demonstrated prediction errors within accepted thresholds (±15% for Cmax and ±10% for AUC) and a log10-transformed regression R^2 value of 0.9825, confirming strong statistical reliability of the developed model.

However, the findings should be interpreted in light of important limitations. The model relies on literature-derived pharmacokinetic and brain distribution data obtained from immediate-release dosing routes, and direct *in vivo* pharmacokinetic validation of the developed ISFI formulation was not performed. While the model provides a robust proof-of-concept and mechanistic understanding, experimental *in vivo* pharmacokinetic studies using the same formulation are necessary to confirm absolute exposure predictions and establish full external validation.

Despite these limitations, the developed PBPK framework represents a valuable preclinical tool for formulation screening and optimization and establishes a scalable foundation for future translational and human PBPK modeling. By mechanistically linking formulation-specific release kinetics to systemic and CNS exposure, this approach supports rational development of long-acting neuropsychiatric therapies while reducing reliance on exploratory animal experimentation.

AI USE STATEMENT

The author used ChatGPT for improving sentence clarity. The author reviewed and edited the content and takes full responsibility for the content for publication.

LIST OF ABBREVIATIONS

ADME – Absorption, distribution, metabolism and excretion, AUC – Area under the curve, AUC_0–t – Area under the concentration–time curve from time 0 to last time point, AUC_0–∞ – Area under the concentration–time curve from time 0 to infinity, BBB – Blood–brain barrier, CL – Clearance, CLint – Intrinsic clearance, Cl/F – Apparent clearance after extravascular administration (F = bioavailability), Cmax – Maximum plasma concentration, CNS – Central nervous system, CYP1A2 – Cytochrome P450 1A2, C_{50} – Saturation constant (concentration producing 50% of maximal effect/transport), DDI – Drug–drug interaction, F – Bioavailability, fu – Fraction unbound (free fraction in plasma), GMFE – Geometric mean fold error, ISFI – *In situ* forming implant, IV – Intravenous, IVIVC – *In vitro–in vivo* correlation, kin – Influx rate constant (plasma → brain), Kp,brain – Brain-to-plasma partition coefficient, kout – Efflux rate constant (brain → plasma), log P – Logarithm of octanol/water partition coefficient, MRT – mean residence time, mTOR – Mechanistic target of rapamycin, NCA – Non-compartmental analysis, NMP – *N*-methyl-2-pyrrolidone, PBS – Phosphate-buffered saline, PBPK – Physiologically based pharmacokinetic, Pbrain – Brain permeability parameter, PE% – Prediction error percentage, PK – Pharmacokinetics, PLGA – Poly(lactic-co-glycolic acid), SSRI – Selective serotonin reuptake inhibitor, t½ – Half-life, Tmax – Time to reach maximum concentration, Vd – Volume of distribution, Vz/F – Apparent volume of distribution after extravascular administration

AUTHORS CONTRIBUTIONS

Conceptualization, design, supervision, review: Dr. SGopinath, Methodology framework, Supervision and review of work: Dr. Satheesh Kumar, Dr. Meriton Stanley, Sample Data Curation, Software Analysis, Preparation of manuscript: Sruthi S.

CONFLICT OF INTERESTS

Declared none

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