Application of in silico methods in clinical research and development of drugs and their formulation: A scoping review

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ABSTRACT
The drug regularization process involves many steps that are complex and time-consuming. The demand for new drugs has prompted researchers and regulatory authorities to search for predictive methods that can streamline the development process. Current studies point to innovative computational techniques in a drug’s study phases. This study aims to carry out a scoping review of research involving the application of computational methods and in silico studies in the clinical research and development of new drugs. A scoping review was conducted according to the referred Reporting Items for Systematic Reviews and Meta-Analyses guideline. Online databases from 2001 to 2021 in English were used and the trial registration was 10.17605/OSF.IO/USXCM. The development of protocols and the application of a computational method for researching new drugs and their formulation, published in a peer-reviewed journal, were included. The data extraction and analysis were performed by two independent reviewers. In this study, 312 articles were retrieved, of which 6 were duplicates. After the title was read, only 101 remained for analysis. After the abstracts were read, 34 papers were considered for the scoping review. The use of in silico methodologies has been expanding in terms of research into the development of new drugs and the improvement of existing products.

INTRODUCTION
The efficacy and safety of a drug are basic concepts of health surveillance adopted by regulatory agencies around the world [ANMAT, 2022; ANVISA, 2021; European Medicine Agency (EMA), 2019; FDA, 2018; PMDA, 2022]. One of the main tools for drug regulation is the clinical trial, which, for new drugs, can take up to 12 years or more and cost millions of dollars ([Berndt et al., 2015; Dimasi et al., 1995; Jensen, 1987]). To circumvent such factors, over the years, major technological advances have been made, and new methodologies have been developed to streamline the process of evaluating a new molecular entity ([Ji et al., 2017; Kar and Leszczynski, 2017]).

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The clinical trial protocols aimed at registering a drug were standardized with the Common Technical Document, a publication of the International Council of Harmonization (ICH), in which guidelines for the quality, safety, and efficacy of drugs were postulated. In the safety guide (M4S (R2)), the ICH prescribes the pharmacological evaluation of the drug, the pharmacodynamic (PD) study, and the interaction with other drugs. In addition, assessments of absorption, distribution, metabolism, excretion, and toxicity are applied ([ICH, 2004]).

In line with international practices, the Food and Drug Administration (FDA) divides clinical trials for drug registration into interventional and observational studies, the first being more common and the second obtained through researchers’ observation of outcomes after the use of a particular drug ([FDA, 2019]).

The structure of clinical studies involving new drugs is agreed upon worldwide as having four phases. The application of in silico studies, especially the well-known physiologically based pharmacokinetic (PBPK) studies that now already have guides published by the FDA and EMA, can be a strategy to compose the
regulatory dossier, shortening the time of its elaboration (EMA, 2016; FDA, 2016).

The discussion that predominates in the area is the development of a set of strategies to shorten the long years of research. One possibility to speed up clinical trials is the application of in silico studies, with the use of digital resources, aiming to assess the effect that a particular drug can have on the human body (Clermont et al., 2004; Mancini et al., 2018; Pappalardo et al., 2019; Sinisi et al., 2020).

This scoping review aims to provide an overview of the specialized scientific literature on the use of digital technologies in clinical trials, with the application of in silico trials in the evaluation of new drugs, and the improvement of already regularized drugs and their formulations, to anticipate events in a traditional in vivo clinical trial involving humans.

METHODS

A scoping review was carried out to identify the conditions for the application of in silico studies in the current context of clinical research with new drugs. A request was made to register the research in the Open Science Framework (OSF) with the number 10.17605/OSF.IO/USXCM.

The research was conducted in March 2022, using the databases Latin American and Caribbean Health Sciences Literature (Lilacs), National Library of Medicine (PubMed), MEDLINE, Web of Science, and Scopus, in English. The report of the present scoping review was prepared by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for scoping reviews (PRISMA-ScR) checklist (Pluye and Moher, 2018; Peter et al., 2020, 2022). Studies that applied in silico methods to evaluate new or already registered drugs, improvements in formulation, evaluation of drug interaction, and pharmacometrics were considered. The specific keywords/descriptors that are Health Science Descriptors addressed drug development, in silico clinical trials, computational methods, drug development, and virtual patients. Publications in English between 2001 and 2021 were adopted.

After the selected articles were read, a form was filled out with collected data, which were compared in an infographic. Data analysis sought (a) the virtual and in vivo patient; (b) the protocols adopted in the studies; (c) a comparison with a traditional clinical study, in cases where it was observed; and (d) the observed outcomes.

RESULTS

The literature search strategy took place in the MEDLINE/PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) and Lilacs databases; the terms used in the search were ((in silico) AND (clinicaltrials) AND (drugtrials)); ((computationalmethods) AND (drugdevelopment)); ((computationalmethods) AND (drugdiscovery)); ((model-informeddrugdiscoveryanddevelopment)); and ((VirtualPhysiologicalHuman) AND (drugdevelopment)), with a total of 312 articles retrieved. Of these, six were duplicates; therefore, they were disregarded.

The first phase of the study was carried out by reading the titles and abstracts of the articles. Those that were within the scope and met the inclusion criteria proceeded to the second stage, in which the text was read in full. Two researchers performed the complete reading of 70 articles. In total, 34 articles were considered for the scoping review and 36 were eliminated because they used multiple methods or tools or their methodology did not specify the software used. The selection flowchart can be seen in Figure 1.

PKs and PDs simulation: PK/PB using software

GastroPlus™

Simulation models are increasingly used in drug development studies and formulation improvement. Their application seeks to speed up the process and guide the conduct in the design of a new drug, with the GastroPlus™ software being directed to this. The development of a drug has complex factors that are difficult to adjust, such as physical-chemical, physiological, and formulation factors. It is necessary to employ tools to support the process. In the scoping review, six studies were identified as involving the use of the GastroPlus™ PK simulator. The results of the scoping review with GastroPlus™ are summarized in Table 1.

In approaches to PK studies, the study conducted by Jereb et al. (2021) evaluated delayed-release tablet pantoprazole compared to dolutegravir and its impact on the patient’s gastrointestinal tract after a meal and in the fasted state. This study used virtual models, with pantoprazole performing better than dolutegravir in terms of bioavailability.

In the application of the method in the study of formulations in the comparison of different formulations, Kato et al. (2020) evaluated three formulations, A, B, and C, and their PK in oral use. They noted differences between the developed batches, including those that were not bioequivalent. In a similar objective, the study by Xia et al. (2013) used in silico techniques to evaluate the PK of developed formulations and the effect of feeding. In this study, the drug in the experimental phase NVS123, of basic character and with pH-dependent solubility, was evaluated.

The occurrence of changes in gastric pH is a biopharmaceutical event that can impact the bioavailability of several drugs. The study by Samant et al. (2018) evaluated the pH and its consequences on the absorption of ribociclib, finding that PK had no impact on the elevation of gastric pH. Similarly, this occurred in the evaluation of alectinib (Parrott et al., 2016).

Regarding the evaluation of formulations, GastroPlus™ proved to be a possibility in a generic candidate drug study [Biopharmaceutics Classification System (BCS) class 2] compared to the reference. Additionally, a clinical study and dissolution test were conducted. An additional concern was assessing the impact of food and fasting, with the tests supporting the construction of the regulatory dossier (EMA, 2016; FDA, 2016; Rebeka et al., 2019).

NONMEM®

NONMEM® is an acronym that stands for “NON-linear Mixed-Effects Modeling,” which is a software developed in the early 1980s, with an application in in silico studies involving the PKs of several drugs. The results of the scoping review with NONMEM® are summarized in Table 2.

In cases of patient exposure, the work by Li et al. (2015) used abiraterone and nilotinib to determine mock PK assays. The parameters adopted for the PK study were obtained from
Table 1. Scoping review studies involving PK simulators using GastroPlus™ software.

<table>
<thead>
<tr>
<th>References</th>
<th>Objective</th>
<th>Medicine</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jereb et al. (2021)</td>
<td>Assess bioavailability with variations in the gastrointestinal tract.</td>
<td>Delayed-release pantoprazole and dolutegravir</td>
<td>In pantoprazole, the result with altered physiology was superior to dolutegravir.</td>
</tr>
<tr>
<td>Kato et al. (2020)</td>
<td>Develop a relevant technical specification for an oral drug.</td>
<td>Comparison between formulations A, B, and C</td>
<td>The <em>in silico</em> method was able to discriminate between a bioequivalent batch and a nonbioequivalent batch.</td>
</tr>
<tr>
<td>Samant et al. (2018)</td>
<td>Investigate the influence of changes in gastric pH and PKs.</td>
<td>Ribociclib</td>
<td>It did not indicate an effect of gastric pH on changes in PKs.</td>
</tr>
<tr>
<td>Xia et al. (2013)</td>
<td>Describe various <em>in silico/in vitro/in vivo</em> tools to support formulation development.</td>
<td>NVS123</td>
<td>An investigation of the new formulation and the practical application of PBPK modeling were carried out.</td>
</tr>
<tr>
<td>Parrott et al. (2016)</td>
<td>Understand the impact of gastric pH changes on alectinib absorption.</td>
<td>Alectinib</td>
<td>Simulations with this model supported the development of alectinib aiding in the design and interpretation of pharmacology studies.</td>
</tr>
<tr>
<td>Rebeka et al. (2019)</td>
<td>To evaluate a generic formulation compared <em>in vitro</em> and <em>in vivo</em> with a reference drug.</td>
<td>BCS 2 drug</td>
<td>The model was able to capture the difference between the two drugs containing different forms of drugs (amorphous and crystalline).</td>
</tr>
</tbody>
</table>
The drug interaction studies were observed using Simcyp™. In one of them, models of interaction between the target drug nemiralisib and itraconazole were used; additionally, the mean and standard deviation of several published studies (Ryan et al., 2010; Tanaka et al., 2010; Zytiga, 2013 and al., 2015). The simulation is applied to evaluate possible results in a clinical trial in different dosing regimens looking at new treatments compared to methotrexate, with the possibility of understanding the endpoints for rheumatoid arthritis (RA) trials and clarifying confounding factors; the method was also applied with fesoterodine (Cardozo et al., 2010; Ma et al., 2014).

In an evaluation of the sublingual route, the response to the dose of asenapine in patients with schizophrenia was characterized. The analysis enabled an understanding of the results of six placebo-controlled trials in which responses and dropout rates varied. Although the simulations indicated that the post hoc probability of success of the performed trials was low to moderate, these analyses demonstrated that asenapine doses of 5 and 10 mg twice daily have similar efficacy (Friberg et al., 2009).

Additionally, one study evaluated another route of administration, testing inhaled glucocorticoids in the work by Nathan et al. (2008) as a first-line therapy in asthma. The study sought to identify the optimal timing of dosing using two surrogate markers of glucocorticoid action. A previously published study (Mollmann et al., 2001 apud Nathan et al., 2008) on the PK and PD (blood cortisol and lymphocyte suppression) of the glucocorticoids budesonide and fluticasone propionate was reanalyzed using a population PK approach allowing established dosage. This can be applied in pediatric dose-setting cases, such as carvedilol for children (Albers et al., 2008) (Table 2).

Clinical trial simulations and PK/PD models were conducted to recommend a study design to test the dose of the compound SC-75416, a selective inhibitor of cyclooxygenase-2, in pain relief compared to 400 mg of ibuprofen in a model of pain after oral surgery. Study results confirmed the hypothesis that 360 mg of SC-75416 achieved superior pain relief compared to 400 mg of ibuprofen and demonstrated the predictive performance of PKPD models (Kowalski et al., 2008). In a biomarker approach, the PD of another MEDI-546 test compound, a monoclonal antibody, was characterized by modeling and simulation (Wang et al., 2013).

Application in therapeutic drug monitoring can also be performed using in silico methods. Studies with mycophenolate mofetil (MMF) in a fixed-dose regimen and another regimen of a controlled concentration of mycophenolic acid exposure were developed. Estimates for oral clearance of MMF were used to calculate values in the area under the curve (Van Hest et al., 2005).

### Simcyp™ Simulator in Drug Interaction

Bioequivalence and bioavailability studies can also be conducted with the Simcyp™ Simulator software, which is the PBPK model platform for determining human dosing, optimizing the design of clinical studies, evaluating new drug formulations, defining the dose in untested populations, and performing virtual analyses of bioequivalence and drug interactions (Certara, 2022). The data of the scoping review with Simcyp™ are summarized in Figure 2.

The Simcyp™ ADME Simulator can also be a database for simulation modeling of oral absorption, tissue distribution, drug metabolism, and excretion, and drug development studies in certain populations predicting the extent of action and drug–drug interaction (Jamei et al., 2009).

<table>
<thead>
<tr>
<th>Reference</th>
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<th>Result</th>
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<tbody>
<tr>
<td>Albers et al. (2008)</td>
<td>To investigate the PKs of carvedilol in children.</td>
<td>Carvedilol</td>
<td>PKs of carvedilol in pediatric patients depends on age and weight.</td>
</tr>
<tr>
<td>Fransson and Grén (2008)</td>
<td>Studied the PKs of two types of formulations for paclitaxel.</td>
<td>Paclitaxel</td>
<td>Both formulations performed satisfactorily.</td>
</tr>
<tr>
<td>Li et al. (2015)</td>
<td>PK assay simulation.</td>
<td>Abiraterone and nilotinib</td>
<td>Asses the characteristics of drugs with highly variable PKs.</td>
</tr>
<tr>
<td>Ma et al. (2014)</td>
<td>To assess the responsiveness to the treatment of RA.</td>
<td>Metotrexate</td>
<td>The study may collaborate in future clinical trials for the treatment of RA.</td>
</tr>
<tr>
<td>Wang et al. (2013)</td>
<td>Assess MEDI-546 using a biomarker.</td>
<td>MEDI-546</td>
<td>There were phase I study and a phase II randomized multiple-dose study.</td>
</tr>
<tr>
<td>Cardozo et al. (2010)</td>
<td>Develop predictive models to describe the dose response of fesoterodine.</td>
<td>Fesoterodine</td>
<td>A consistent dose response to fesoterodine has been demonstrated for overactive bladder outcomes.</td>
</tr>
<tr>
<td>Friberg et al. (2009)</td>
<td>Modeling to characterize the response to asenapine in schizophrenia.</td>
<td>Asenapine</td>
<td>Analyses have shown that asenapine doses of 5 and 10 mg twice daily are effective.</td>
</tr>
<tr>
<td>Kowalski et al. (2008)</td>
<td>Test the dose of SC-75416.</td>
<td>SC-75416, rofecoxib, valdecoxib, and ibuprofen</td>
<td>The 360 mg SC-75416 dose achieved superior results compared to 400 mg of ibuprofen.</td>
</tr>
<tr>
<td>Van Hest et al. (2005)</td>
<td>To evaluate the PKs of fixed-dose and multiple-dose Mycophenolate sodium.</td>
<td>Mycophenolate sodium</td>
<td>The results of this simulation resulted in prospective studies comparing a concentration-controlled regimen with a fixed dosage.</td>
</tr>
</tbody>
</table>
midazolam and clarithromycin were evaluated (Patel et al., 2020; Yu et al., 2017).

Another study evaluated enzyme inhibitors, with concomitant application of *in vitro*, *in silico*, and *in vivo* methods. The study determined whether repaglinide had an inhibitory effect on pioglitazone metabolism. The authors observed a discrepancy in the result between the experiments (Xiao et al., 2015) (Fig. 2).

In a different modality of study, a nanoscale formulation was evaluated by Litou et al. (2019). In the study design, *in vitro* results were coupled to a PBPK model. The evaluation was with aprepitant (EMEND), which is indicated for nausea and vomiting, especially during chemotherapy. In cases involving nanomeric formulations, it is necessary to apply innovative tools to understand their *in vivo* performance and guide the regulatory process (Fig. 2).

The approach used with perampanel was structured with data from *in vitro* studies and a phase I trial (Patsalos, 2015). The peak plasma concentration of perampanel ($C_{\text{max}}$) and time to $C_{\text{max}}$ showed no apparent differences when perampanel was administered alone versus with ketoconazole (Gidal et al., 2017).

**Monte Carlo simulation**

The Monte Carlo simulation or Monte Carlo method, which is a branch of experimental or applied mathematics involving random numbers, has applications in several areas of knowledge, such as mathematics, physics, economics, and even medical sciences (Carvalho, 2017).

The method or model is essentially characterized by the use of software that, with simulation platforms, expands the sample size of a study and provides simulations for the outcome
of treatment or, more precisely, for a particular therapeutic target, considering different situations, such as changes in a dose or frequency of drug administration (Federico et al., 2017).

In the study by Zhang et al. (2011), Monte Carlo simulation was applied to generate hypothetical cohorts with 7,000 patients characterizing the so-called discrete event simulation (DES) (Fig. 3). In the research, we investigated the effectiveness of rivaroxaban in preventing stroke in patients with atrial fibrillation. Hypothetical patient cohorts were generated using data from ROCKET AF (Patel et al., 2011) (FDA registration code NCT00403767) and two other observational studies (Amin et al., 2017; Laliberté et al., 2014) and Xantus (Camm et al., 2016). The results confirmed that rivaroxaban was noninferior to warfarin for the prevention of stroke/systematic embolism, with no significant risk of major bleeding in atrial fibrillation in large populations. This was similar to the results of ROCKET AF.

In a process that involved applying data from previously performed clinical trials, Najafzadeh et al. (2018) used data from the RE-LY study (2009), as well as cohorts of equal size with covariate distributions identical to the study of Graham et al. (2015). Cohort simulations were performed using the Monte Carlo method and compared to a randomized clinical trial. Another study that used Monte Carlo simulations to interpret data from a randomized clinical trial was performed by Cuadros et al. (2014); in this study, study simulations involving male circumcision in trials with valaciclovir for the suppression of herpes simplex were performed (Fig. 3).

Opioids are subject to evaluation, due mainly to their application in pain, to evaluate long-acting opioids in patients with nonmalignant chronic pain classified as moderate to severe. Neil et al. (2013) developed a Monte Carlo simulation. Long-term opioid efficacy and adverse events were obtained from clinical trials with tapentadol ER versus oxycodone CR; other data were taken from the literature. The use of tapentadol proved to be superior in effectiveness and cost-effectiveness, demonstrating the successful use of Monte Carlo in a pharmacoeconomics study.

Another study on chronic pain was carried out by Murthy et al. (2007) with once-daily extended-release tramadol (tramadol ER) approved in the US for moderate to moderately severe chronic pain in adults. Monte Carlo simulation was performed to assess switching in patients who received immediate-release tramadol by ER tramadol. PK analyses showed that switching from a total daily dose of tramadol IR 200 or 300 mg to tramadol ER 200 and 300 mg once daily is equivalent.

**STELLA®**

STELLA® software is a dynamic systems simulation that helps one understand complex correlations within a system of data relationships. It is used in modeling, providing tools to convert numerical models into formulation evaluation (Naimi et al., 2012).

Three studies of Shono were found to use STELLA® software: one from 2011, another from 2010, and a third from 2009. The first study, by Shono et al. (2011), developed an in silico PBPK for poorly soluble nelfinavir mesylate in water and of weakly basic pH-generating plasma profiles and of coupling dissolution results and precipitation estimates with gastrointestinal parameters.

The second study, by Shono et al. (2010), coupled biorelevant dissolution test results with in silico simulation technology to predict the in vivo oral absorption of aprepitant formulations with micronized and nanosized particles in the preprandial and postprandial states.
The third and oldest study, by Shono et al. (2009), determined the rate of intestinal absorption of poorly soluble drugs and dissolution in the gastrointestinal tract. In this study, in vitro dissolution tests using biorelevant media coupled with PBPK in silico were applied to predict the effects of food on the absorption of a poorly soluble drug, celecoxib, from celecoxib 200 mg capsules.

**DISCUSSION**

This study described and characterized the types of in silico methods for research involving new drugs and the improvement of existing ones. Simulation models have advanced in recent years, and have been shown to be tools increasingly used in drug development and formulation studies. Such development and innovation motivated several researchers to evaluate new software for conducting clinical trials in their work.

The most applied and tested in silico studies by the scientific community within the parameters researched pointed to the use of software such as GastroPlus®, NONMEM®, Simcyp®, Monte Carlo, and STELLA®. Of the tests evaluated, the GastroPlus® software was continuously employed in human PK and PD assessments of several different drug types and formulations. The in silico method was also able to discriminate between bioequivalent and nonbioequivalent batches. With the software, it was also possible to perform in silico clinical evaluations of the influence of changes in gastric pH and food intake on the PK of a drug.

In terms of evaluating new compounds, two studies studied new drugs. These were new formulation clinical investigations and, most importantly, highlighted a practical application of PBPK modeling in solving problems involving undesirable food effects on weakly basic compounds based on in vitro/in vivo data. The various studies retrieved in the proposed search demonstrated that the in silico method using GastroPlus® is efficient in evaluating different drug formulations, changes in the drug’s crystalline arrangement, or even the use of known and regularized drugs at different stages of the digestion process.

Other publications pointed out the use of NONMEM® software. The searches retrieved ten scientific articles evaluating several drugs and the application of the software aimed to establish pediatric doses, inhaled drugs, drug interactions, and pharmacometry.

Some studies clearly did dosage reviews or sought to determine new dosages in different audiences.

The development of innovative drugs is not the only application of in silico methods but may well lend itself to developing better evaluations of already regulated drugs, which have a variable PK profile, and also the impact that food can have.

The remaining studies found were conducted using the Simcyp® Simulator software to determine the human dosage of various compounds. They also evaluated the drug–drug interaction and PK of several drugs, as well as the behavior of different formulations.

Of the articles found in the research, three pointed to the use of the Monte Carlo method, which was applied to expand the sample of volunteers and create simulations of responses.

To evaluate the drugs nelfinavir, celecoxib, and aprepitant, studies were found that used the STELLA® software to evaluate the dissolution in the moments before and after the meal.

**CONCLUSION**

The in silico studies observed in this work proved its applicability in the research of new drugs, as well as in the improvement of the evaluated formulations, with the approaches of PK evaluation and drug–drug interaction evaluation. The evaluated studies have differences in terms of the drug evaluated, the number of simulated patients, the protocol adopted, and the in silico technology addressed. For this reason, comparing results is difficult. However, it is possible to observe the application of software and the evaluation of drugs in different simulated approaches.

The application of in silico methods to evaluate a drug or medication intensified in the last decade and its use has been expanding. This meets the need for more agile studies with lower costs in the development of new drugs.

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**AUTHOR CONTRIBUTIONS**

Concept and design, acquisition of data, or analysis and interpretation of data were carried by Colli and Cabral. Drafting the article and revising it critically for important intellectual contents were carried out by Matos, Rodrigues and Sousa.

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**CONFLICTS OF INTEREST**

The authors report no financial or any other conflicts of interest in this work.

**ETHICAL APPROVALS**

This study does not involve experiments on animals or human subjects.

**DATA AVAILABILITY**

All data generated and analyzed are included within this research article and in the searchRxiv platform in the link https://doi.org/10.1079/searchRxiv.2022.00006.

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