European Journal of Pharmaceutics and Biopharmaceutics 79 (2011) 382–391

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

The influence of dissolution conditions on the drug ADME phenomena

Sara Cascone, Felice De Santis, Gaetano Lamberti *, Giuseppe Titomanlio

Dipartimento di Ingegneria Industriale, Università degli Studi Salerno, Fisciano, Italy

ARTICLE INFO

Article history:
Received 3 February 2011
Accepted in revised form 8 April 2011
Available online 16 April 2011

Keywords:
Dissolution
Pharmacokinetic modeling
Enteric coated
ADME

ABSTRACT

In this work, a review of the apparatuses available to mimic what happens to a drug (or to foodstuffs) once ingested is presented. Similarly, a brief review of the models proposed to simulate the fate of a drug administered to a living body is reported. Then, the release kinetics of extended release of diclofenac from a commercial enteric-coated tablet was determined both in a conventional dissolution tester (USP Apparatus 2, Method A) as well as in an apparatus modified to reproduce a given pH evolution, closer to the real one than the one suggested by USP. The two experimental release profiles were reported and discussed; therefore, they were adopted as input functions for a previously proposed pharmacokinetic model. The obtained evolutions with time of plasma concentration were presented and used to assess the effectiveness of the commercial pharmaceutical products. The importance of a correct *in vitro* simulation for the design of pharmaceutical dosage systems was thus emphasized.

1. Introduction

The oral route is the most important drug administration route. Oral bioavailability is a fundamental requisite for any orally administrated drug to be effective. Solid drugs orally administrated are not immediately available to the biological system since they are absorbed only from a solution. During the development of the dosage form, *in vitro* tests serve as a guide in estimating the amount of drug released per unit time in a given dissolution medium. *In vitro* dissolution tests seem to be the most sensitive and reliable predictors of *in vivo* performances, and they offer a meaningful indication of physiological availability.

For most orally administered compounds, the process of drug reaching the systemic circulation can be broken down into two general steps: dissolution and absorption. Dissolution is defined as the process by which a solid substance enters in the solvent to yield a solution. To determine the dissolution rate of the drugs from solid dosage forms, several physicochemical processes in addition to the dissolution of pure chemical substances have to be considered, such as the wetting of the solid surface, the melting and the solvation of the solid, and the diffusion into bulk solution [1]. The physical characteristics of the dosage form, the wettability of the dosage unit, the penetration ability of the dissolution medium, the swelling process, and the disintegration and deaggregation of the dosage form are some of the factors that influence the dissolution features of the drug. Both dissolution and disintegration are key parameters in the product development strategy. Disintegration usually reflects the effects of formulation and manufacturing process parameters, whereas the dissolution from drug particles mainly reflects the effects of solubility and particle size, which are properties of the pharmaceutical raw material. The dissolution can also be influenced significantly by processing and formulation. Therefore, dissolution testing provides the means to evaluate the critical parameters, such as adequate bioavailability, and it provides information necessary to the formulator in the development of more effective dosage forms. Hence, dissolution analysis of pharmaceutical dosage forms has emerged as the most important test that will ensure the quality and the bioavailability of a product. The knowledge of critical operating variables for a dissolution testing device is important for product development, quality control, and research application.

In recent years, there has been a significant increase in available strategies for site-specific delivery in the gastrointestinal tract both to maximize the therapeutic response and to reduce side effects. One of the most effective drug release-controlling tools in targeted drug delivery is the enteric coating polymers. They are used in a wide range of delivery systems because of their pH-dependent effect to facilitate drug release in a specific site of the human body. Indeed, enteric-coated products are designed to remain intact in the stomach and then to release the active substance in the upper intestine. The main reasons for applying the enteric coat are to prevent irritation of the gastric mucosa or esophagus by the drug (e.g., aspirin), to protect the drug from destruction by gastric acid or enzymes (e.g., duloxetine), to target a drug to a specific region for a local effect, a high concentration or systemic absorption (e.g., colonic therapy of 5-acetylsalicylic acid), and to delay release for a delayed or a double-pulse effect (e.g., hydrocortisone for chrono-

* Corresponding author. Università degli Studi Salerno, Dipartimento di Ingegneria Industriale, via Ponte don Melillo, Fisciano (SA) 84084, Italy. Tel.: +39 089964077.
E-mail address: glamberti@unisa.it (G. Lamberti).

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doi:10.1016/j.ejpb.2011.04.003

therapy). Thus, the enteric coating technique could result in therapeutic benefits and cost saving since the efficacy is improved, the compliance of the patient is raised, and both the dose and the side effects are reduced. Furthermore, enteric coating is used as a research tool in studies to understand drug absorption [2].

The prediction of the drug concentration in blood, tissue, and organs is the goal of *in silico* pharmacokinetic modeling. The approaches to the modeling of the physiological phenomena can be different on the basis of the details used. The description of the phenomena taking place in the body is much closer to the real physiology if more details are considered. The processes, which a drug follows after the administration, are absorption, distribution, metabolism, and excretion (ADME); these processes determine the evolution of the drug concentration in the body. The route of administration, the physical and chemical properties of the substance, the characteristics of the dosage form, and the physiological conditions are the factors with the most important effect on the entity and on the rate of the ADME phenomena. Three different approaches can be followed to build an *in silico* model to predict the fate of administered drug: pure compartmental modeling, pure mathematical modeling, and physiologically based modeling. The compartmental approach is based on the schematization of the body by a system of interconnected volumes, the compartments, which can be easily identified as chemical reactors or as physical contacting units. The pure mathematical models are able to correlate the results of an *in vitro* dissolution tests to the *in vivo* drug concentration in the blood. Such correlations are commonly noted as IVIVC: *in vitro/in vivo* correlations. In the pure compartmental models, the compartments do not represent necessary anatomical units. In the physiologically based models, the compartments are representative of a tissue, an organ, or a group of organs, each with a specific function, and the interconnection between the compartments reproduces the effective one between tissue and organs.

2. Aims of the work

In this work, the dissolution techniques and apparatuses in use (*in vitro* methods) are critically reviewed, as well as the common approaches followed to establish physiologically based pharmacokinetic models (*in silico* methods) were briefly analyzed and discussed. Another aim of this work was to test the effect of the pH evolution on the release kinetics from a commercial enteric tablet for the controlled release of diclofenac, as a first step in the development of a novel integrated platform to simulate the *in vivo* behavior of a drug after its administration. Also, the consequences of the dissolution conditions on the expected plasma profile were studied.

3. *In vitro* dissolution: state of the art

Many devices have been reported for determination of the dissolution rate, some of them accepted and classified by the pharmacopeias. The dissolution procedures differ in stirring rate rather than in basic principles. The apparatuses used for dissolution testing are the USP (United States Pharmacopeia) apparatuses 1, 2, 3, 4, 5, 6, and 7 [3]. All the apparatuses are temperature controlled.

In the USP Apparatus 1 (rotating basket apparatus), a wire-mesh basket is attached to a rotation shaft, which is then immersed into a dissolution vessel for the duration of the dissolution test. Since the dosage form is in direct contact with the basket, the physical dimensions and the motion of the basket can have a strong impact on the dissolution rate of the solid dosage unit. The basket height, the diameter of the basket opening, height of the open screen, and size of the mesh are specified in USP chapter ⟨711⟩. The advantages of this apparatus are the breadth of experience in its use, the possibility to obtain easily pH changes during a test, and the easiness of automation, which is important for routine investigations. On the other hand, the dosage form is subjected to dissolution/disintegration interactions, which could influence the rate of dissolution. Furthermore, other critical points are the presence of an hydrodynamic "dead zone" under the basket and the presence of a limited volume that does not ensures the perfect sink, especially for poorly soluble drugs.

In the USP Apparatus 2 (the paddle method), the vessels should be uniform with respect to their weight, inside diameter, and inside curvature. Statistically significant differences in dissolution rates have been reported when the same product was tested in different vessels. In this official method, the sample container itself serves as a liquid-stirring device. Under these conditions, there can be strong abrasion and wear of the sample due to mechanical impacts with the container surface. Instead of the simplicity of use, its high degree of standardization, and its robustness, this apparatus is characterized by difficulty in realizing pH changes with respect to the USP Apparatus 1, and the fluid dynamic is very complex and can vary with site of dosage form in the vessel and therefore may significantly affect drug dissolution.

The USP Apparatus 3 (the reciprocating cylinder) provides capability agitation and media composition changes during a run as well as full automation of the procedure. Furthermore, the reciprocating cylinder allows to obtain a good correlation for extended-release formulations because of the ease of sampling and because of the ease of pH changing during the test run which offers the advantage of mimicking the changes in physiological conditions. This apparatus was based on the recognition of the need to establish *in vitro*/*in vivo* correlation (IVIVC), since the dissolution results obtained with USP Apparatuses 1 and 2 may be affected by mechanical factors, such as shaft wobble, location, centering, deformation of the basket and paddles, and presence of bubbles in the dissolution medium. The deformation rates are higher than those of the apparatuses 2, but the Apparatus 3 is less diffused and used.

The USP Apparatus 4 (flow-through cell) is characterized by a continuous stream of dissolution medium through a small-volume cell containing the dosage form being tested. The dissolution medium flows through the cell from bottom to top. The pulsating movement of the piston pump supplies the need for further stirring or shaking elements. A filtration device at the top of the cell retains all the undissolved material. The flow-through cell system is usually operated as an open loop; therefore, new dissolution medium is continuously introduced into the system. In this way, all the drugs dissolved are immediately removed along the flow of the dissolution medium. This apparatus is reliable not only for the determination of the dissolution rate of tablets but also of capsules, semisolids, powders, and granules. Some advantages of the Apparatus 4 are the possibility of generating rapid pH changes during the test, continuous sampling, unlimited solvent volume, minimizing downtime between two tests, ability to adapt test parameters to physiological conditions, and retention of undissolved particles (without of additional step of filtration or centrifugation). Despite the fact that the apparatus is very versatile, the realized fluid dynamic is very far from the real one, it requires high volume of dissolution media, and deaeration is necessary.

The USP Method 5 (paddle over disk) or the USP Method 6 (rotating cylinder) is used for transdermal or patches testing. With paddle over disk, the transdermal patch is placed between a glass disk and an inert mesh. This is placed at the bottom of the vessel, with the meshing faced upwards, under a rotating paddle. Unlike dissolution testing, transdermal testing is carried out at 32 °C to reflect the lower temperature of the skin. Other variables such as the height of the paddle from the bottom of the vessel and sampling requirements are the same as dissolution of oral dosage form. The rotating cylinder is very similar to USP Method 1 (the rotating basket). With USP

Method 6, however, the basket assembly is replaced by a solid stainless steel cylinder. The cylinder consists of two parts that fit together: the main shaft/cylinder assembly plus an extension. The extension is used when the transdermal patch requires a larger area. The advantage of these apparatuses is the possibility to use a standard equipment (paddle), but conversely, the disk assembly restricts the patch size.

USP Apparatus 7 (reciprocating disk apparatus) was initially introduced in the USP as small-volume apparatus for small transdermal patches but was later renamed the reciprocating holder apparatus with the adoption of four additional holders for transdermal system, osmotic pumps and other low dose delivery systems. With this apparatus, switching to different pH buffer is easy and provides deformation rates higher than Apparatus 2, but it is less used.

Different apparatuses (not USP approved) were built to overcome the deficiencies shown by the described apparatuses, such as poor agitation, absence of sink conditions, and inability to program progressive changes in the dissolution environment. The benefits of alternative dissolution approaches to the currently recommended USP methods applied to swellable/floatable delivery systems were shown by Pillay and Fassihi [4].

3.1. Alternative in vitro apparatuses

The Sartorius Absorption Model [5] mimics both the release from the dosage form in the gastrointestinal tract and drug absorption through the lipid barrier. This apparatus was composed of reservoirs for holding different media at 37 °C; typically, they are the simulated gastric fluid and simulated intestinal fluid. Furthermore, a diffusion cell with an artificial lipid barrier of known surface area is equipped, and a peristaltic pump that aids the transport of the solution or the media from the reservoir is connected with the diffusion cell. The drug substance under investigation is introduced, and its uptake in the diffusion cell is governed by its hydrophilic–lipophilic balance.

A simple apparatus to simulate the gastric emptying of digestive product composed of a fermentor regulated at 37 °C was proposed by Savalle et al. [6]. This method was based on the enzymatic hydrolysis of proteins with simultaneous dialysis of digested products to overcome the accumulation of digestion product and inhibition of proteolysis. The aim of this work was to simulate several of the most important phenomena observed *in vivo* in the stomach during digestion of milk proteins and to obtain an *in vitro* model for studying gastric digestion of proteins. Two peristaltic pumps, with variable flow rates, ensured the acidification of the medium and enzyme supply. The pH of the incubation medium was measured, but not controlled, during digestion. HCl was added at a constant flow rate together with diluted enzymes at a variable flow rate, and then variable emptying rates are requested to maintain a constant volume in the fermentor. The contents are shaken to homogenize the mixture and to ensure the formation of coagulum. The effluents were collected and immediately precipitated and centrifuged. The authors found that, at high acidification rate, the pH drop was too fast, whereas the acidification was incomplete with a low acidification rate. The choice of pH conditions is a very important factor affecting the enzymes activity and thus the composition of the evacuated products. The slow decrease in the pH is necessary to ensure emptying of intact proteins or large peptides. Finally, the authors, by means of biochemical techniques, followed and reproduced the kinetics of the gastric digestion of milk proteins.

A more complete and detailed *in vitro* model was developed by Minekus et al. [7]. This apparatus simulates the dynamic physiological processes that occur in the lumen of stomach and small intestine of men. This model is able to simulate the gastrointestinal transit, the pH, the bile salts concentrations, and the absorption of glucose. The model is composed by four successive chambers to simulate the stomach, the duodenum, the jejunum, and the ileum, respectively. Each compartment is formed by two connected units consisting of a glass jacket with a flexible wall inside. Water is pumped from a bath into the glass jacket around the flexible walls to control the temperature in the unit and to ensure also the mixing of the chime by alternate compression and relaxation of the flexible wall. In fact, the space between the rigid and the flexible wall of the chamber has been filled with a liquid or a gas under pressure, and as a result, the flexible wall is pinched. The mixture of the substances, which was present in the hose of the pressure chamber, will be driven out of this hose and forced through the intermediate piece into the unpinched hose of the pressure chamber. Then, the liquid is discharged through the outlet, and the same operation is repeated for the other chamber. In this way, the substances present inside the flexible wall will flow back again. With this continuous movement, the peristaltic movements of the stomach and the intestinal tract are simulated, and homogenization is ensured. The compartments are connected by peristaltic valve pumps. The pH values are controlled via computer by secreting acidic or basic solutions. The jejunal and ileal compartments are connected with hollow fibers to absorb digestion products and water from chyme and to modify electrolyte and bile salt concentration. The accuracy and reproducibility of the model were verified, as well as its ability to mimic the gastrointestinal transport of chyme. The model mimics gastrointestinal peristalsis, which results in physiological mixing. The pH and the enzymes and bile salts concentrations simulate the dynamic physiological patterns found *in vivo*. The hollow fiber devices appeared almost as efficient at absorbing glucose as the active process *in vivo*. The authors tested the model reliability to reproduce predetermined physiological parameters, such as meal size and duration, pH, gastric and intestinal secretions, gastrointestinal transit, and adsorption of digested product and water [7]. Water and metabolites were absorbed adequately through hollow fiber membranes inside the compartments. Experiments were performed to demonstrate that the short-chain fatty acid could be dialyzed efficiently and that their concentration could be maintained within physiological limits [8]. The stability of the *microflora* in the system was tested after inoculation with fresh fecal samples and after inoculation with a *microflora* that was maintained in a fermentor. This *in vitro* model was used for several studies, including the absorption of the products of digestion, the bioavailability of minerals, the survival rate of microorganism, the composition and enzymatic activities of the *microflora*, and the functionality of recombinant microorganism in the digestive environment [9]. This apparatus was also used to evaluate *in vitro*/*in vivo* correlations [10].

Tam and Anderson developed a method for assessing simulated biological dissolution of a pharmaceutical formulation and absorption of a pharmaceutically active compound [11]. According to the invention, this system comprises a dissolution chamber for determining the dissolution profile of the pharmaceutical formulation in a medium to be supplied to the apical surface of a cell monolayer, and a cell culture chamber wherein absorption may occur. Medium is provided from an apical medium source chamber to a dissolution chamber using a gradient and flow control. Parameters such as pH and osmolarity may be measured by the gradient and flow control system. The apical medium will include bile salts, lipid, or carbohydrates. The basal medium, which flows to the basal chamber of the cell culture chamber, may be of any type used to support cultured cells and may comprise growth media, sera, buffers, minerals, nutrients, hormones, growth factors, and antibiotics. An automatic flow control system controls the temperature and partial pressure of the gas. In the dissolution chamber, a dosage form is dissolved in the apical medium. The mixing rate in the dissolution chamber influences the unstirred water layer

surrounding the dosage form and thus is under automated control. Only a portion of the medium enters in the cell culture chamber to maintain a standard flow rate and to prevent excess shear stresses on the cell monolayer within the cell culture chamber. A filtration device may be incorporated into the dissolution chamber to control the size of the particles leaving the dissolution chamber. The basal surface of the cell monolayer is exposed to basal medium through the filter that is permeable to media. Transepithelial electrical resistance analysis means may be used to determine the viability or integrity of the cell monolayer. Rotation of the filter ensures good mixing and minimizes the unstirred water layer adjacent to the cell monolayer. According to the system, parameters such as mechanical stresses, intestinal distention, shear forces, and interspecies variability can be addressed by adjusting flow rates of the media through the cell culture chamber.

An *in vitro* model that may include different stages was proposed by Wickham and Faulks [12]. To ensure the reproducibility in size reduction, a precision cutting device was designed. The designed piece of the apparatus cuts food material into cubes of approximately 3 mm^3 dimensions, representing the approximate size of food produced by human chewing prior to swallowing. Food materials were first cut and then exposed to low levels of α-amylase at 37 °C (model *saliva*). First stage simulates the main body of the stomach (the *fundus*). This apparatus comprises an outer vessel and an inner digestion chamber comprising a rigid portion and a flexible portion. The fluids can be introduced and removed from the outer vessel whereas the foodstuff can be introduced into the internal rigid portion of the chamber. Reversible alternation of fluid pressure within the outer vessel causes reversible partial compression of the flexible portion for mixing foodstuff present therein. The mixing achieved in this stage is inhomogeneous. The contractions produced by the fluid in the outer vessel facilitate also the penetration of acid, enzyme, and digestive additives. Long after ingestion, the meal remains heterogeneous, so gastric secretions only penetrating around the outside of the food bolus, which is not diluted by secretions for an appreciable time. The inner chamber has a plurality of pH electrodes disposed within it, designed and positioned to minimally interfere with mixing. The additive will generally be one or more of acid, alkali, enzymes, phospholipids, and bile. Second stage of the model provides a simulation of the region of high shear (the *antrum*), mimicking both the rate and strength of contractions evaluated *in vivo*. This stage comprises a system of nested cylinders. An outer cylinder includes in a first end wall an entry port through which foodstuff can be introduced, and at the opposite end wall an exit port from which the foodstuff can be removed. The inner sliding cylinder mounted within the outer cylinder and including an aperture through which the foodstuff can be reversibly forced between the inner and the outer cylinders. A plunger is mounted within the inner cylinder for drawing the foodstuff through the entry port and the aperture into the inner cylinder. The aperture in the inner cylinder through which the foodstuff can be reversibly forced creates a shearing force. A piston defines the end wall of the chamber that remains at a predetermined distance. By repeated strokes of chamber forcing the foodstuff trough aperture, the foodstuff is subjected to sufficient shear to break the foodstuff down. When sufficiently broken down to mimic physiologic processes and foodstuff size, a valve (simulating the pylorus) is opened and the plunger is pushed upward so that the processed food can go out. Nutrient absorption and finally excretion may then be studied, for example, using the apparatus developed by Tam and Anderson previously described.

An artificial gut comprising two groups of hollow fibers was developed by Rozga and Demetriou [13]. The artificial gut comprises a first group of hollow fibers having an inner surface which is lined with at least a layer portion of a plurality of biological components (consisting essentially of enterocytes). Further, a second group of hollow fibers is adjacent to the first one. The enterocyte inner surface is perfused with a feeding solution containing nutrients, and the enterocytes absorb, process, and transport the nutrient across the wall of the first group of hollow fibers, toward the extra fiber space. The nutrients in the extra fiber space then diffuse into the second group of hollow fibers. A perfusate selected from the ensemble of culture medium, blood, and plasma can perfuse an inner surface of the second hollow fiber, whereby the perfusate is nourished by the nutrients.

A new apparatus simulating the hydrodynamic and the mechanical condition in the gastrointestinal tract was developed by Garbacz et al. [14] to improve the predictability of dissolution testing. This apparatus is able to reproduce the pressure forces due to gut motility, the shear forces generated during the peristaltic movements in the intestine, and the loss of water contact when the dosage form is located in an intestinal air pocket. The device consists of a central pipe, with six steel netting spheres (chambers) of 35 mm diameter where the dosage forms reside. The central axle is coupled at one end to a pressure regulation unit by a rotating joint and on the other end to a stepping motor. Pressure waves are generated by a pulsatile inflation and deflation of a balloon inside the chambers that is tightly attached to the nozzle. The inflations are controlled by synchronized switching of solenoid valves. The pressure is regulated by a computer-controlled pressure-reducing device. The central axle is driven by a computer-controlled stepping motor. The composition of dissolution medium was based on the results of analytical characterization of the human gastrointestinal tract. Thus, the dissolution test device exposes the dosage form to an arbitrary sequence of movements, pressure waves, and phase of rest as occur under *in vivo* condition. During rotation, the spheres are immersed in the medium for the 50% of the time and for 50% of the time exposed to air. The medium was continuously homogenized. This device generates a discontinuous movement of the dosage form within the gastrointestinal tract to mimic the agitation caused by the gastrointestinal pressure waves as observed *in vivo*.

Recently, Bogataj et al. [15] developed a dissolution stirring device that simultaneously enables gentle mechanical contact of solid dosage form with grinding and its peristaltic movement due to the movement of the grinding. This apparatus comprises a temperature-controlled round-bottom vessel in which the stirring is performed by a cylindrical stirrer bar. The length of the stirring bar is about 8–10 mm shorter than the inner diameter of the vessel. At the bottom of the vessel, there are small beads that are mechanically pushed by the stirrer bar and thus provide peristaltic movements during stirring in a suitable medium. The preferred quantity of the beads used is the minimum amount that covers the bottom of the vessel in one layer; the maximum amount would be that which results in a height of about 400% of the stirring bar diameter. The beads are spherical and made of glass or other inert material that does not react with the vessel or with any substance in the vessel. Additionally, beads must not adhere on the surface of the dosage form, and they must have suitable density to settle on the bottom of the vessel. Dissolution profile obtained in pharmacopeial apparatus with paddle stirring element is compared with the profile obtained using the this device, working with commercially available tablets containing diclofenac sodium. The release kinetics observed in the novel apparatus is much faster than the one observed in the USP Apparatus 2.

4. In silico models: state of the art

To develop an *in silico* model, three different approaches could be followed: pure compartmental modeling, in which the body is described by a set of interconnected vessels; pure mathematical modeling, in which there is no body description but only a mathe-

matical description of the experimental data; and physiologically based pharmacokinetic modeling, in which the compartments correspond to a real structure of the body, each with a specific function.

There are several examples of pharmacokinetic compartmental models, one of the most important is the first whole body physiologically based pharmacokinetic (PBPK) model proposed by Jain et al. [16]. Mass transfer is modeled by a set of interconnected lumped compartments representing the various tissues. Each compartment is divided into three compartments representing the vascular, interstitial, and intracellular space, and drug concentration within each compartment is assumed to be uniform. The model is based on the schematization of the rat body into 21 compartments, each representative of an anatomical part. The mathematical structure of the model is made up by a system of 38 coupled ordinary differential equations, solved with the initial condition that all the concentrations except for the plasma are zero. The simultaneous resolution of the equations requires the knowledge of 98 different model parameters. An application of the model was carried out by the authors in a case of intravenous injections of zinc sulfate in rats, and a good agreement between the prediction of the model and the experimental data was found. In applying the model to zinc, the following assumption is made: There is instantaneous mixing of drug in the plasma after an intravenous injection, the effluent plasma concentration is equal to the drug concentration of the vascular subcompartment, and the capillary endothelial layer is more permeable than the cellular membrane. In spite of the fact that the model gives a complete description of the physiology and is very accurate, it is too difficult to be adopted for predictive purposes because of the high number of parameters which have to be fitted. Furthermore, the model concerns only the case of intravenous injection in rats, and then it cannot be used for the simulation of oral administration or for human physiology.

Yu et al. [17,18] developed a complete model, which is defined as the "Compartment Absorption and Transit" (CAT) model. The schematization of the gastrointestinal tract is made up by 10 compartment, and then the mathematical structure is made up by 10 ordinary differential equations which have to be solved with the initial conditions (on the fraction of the dose in the gastrointestinal tract and on the plasma concentration) to characterize the time profiles of the drug levels in each compartment. The knowledge of 18 parameters, of the absorption rates, of the degradation rate constants, of the elimination constant, and of the distribution volume is required to solve the equation. The CAT model was applied to simulate cases of oral drug administration (both in immediate and in controlled release formulations), and it showed a satisfying agreement between the model prediction and the experimental data. The CAT model is useful in the design of the controlled release delivery systems and has the potential to investigate the effects of physicochemical, physiological, and dosage form variables on the oral drug absorption. The main drawback of this model is that it can be applied only for oral administration. Furthermore, many other factors such as solubility, crystal form, blood flow, gastrointestinal pH, and dosage form factors have not been completely considered.

The CAT model constituted the starting point for the development for the "Advanced Compartmental Absorption and Transit" (ACAT) model [19]. For drugs with low permeability and solubility, absorption may be not complete in the small intestine and the CAT model can be more accurate by treating the colon as an additional absorbing compartment. This model was implemented into a simulation software, *GastroPlus*. *GastroPlus* was tested as an *in silico* tool for the prediction of the effects of the physiological conditions on physicochemical parameters of some therapeutic substances. A basic assumption of ACAT model is that drug passing through the small intestine will have equal transit time in each of the compartments. The luminal barrier has been modified, respect to CAT model, by the addiction of compartments corresponding to the enterocytes and surrounding tissue. In addition, the ACAT model uses the concentration gradient across the apical and basolateral membranes to calculate the rate of drug transfer into and out of an enterocyte compartment for each gastrointestinal tract lumen compartment. Satisfying prediction of the absorption, distribution, metabolism, and excretion (ADME) phenomena was obtained, but this model can be used only after a lot of careful *in vitro* and *in vivo* measurements.

While several models and algorithms have been developed to predict bioavailability in an average person, efforts to accommodate intrinsic variability in the component processes are less common. An approach that incorporates such variability for human populations within a mechanistic framework is described, together with examples of its application to drug and formulation development by Jamei et al. [20]. The ADAM (Advanced Dissolution, Absorption and Metabolism) model is a development of a succession of representation of drug absorption from the human gastrointestinal tract ACAT model. It divides the gastrointestinal tract into nine anatomically defined segments from the stomach through the intestine to colon. Drug absorption from each segment is described as a function of release from the formulation, dissolution, precipitation, luminal degradation, permeability, metabolism, transport, and transit from one segment to another. It is assumed that absorption from the stomach is negligible, that the movements of solid and liquid drugs through each segment of the GIT (gastrointestinal tract) may be described by first-order kinetics, and that the metabolism in the colon is negligible. Finally, the transit time in each segment of the small intestine is assigned as a fraction of the total small intestine transit time, proportionally to the length of the segment. Furthermore, Jamei et al. [21] used a mechanistic approach implemented in the Simcyp Simulator to simulate complex absorption, distribution, metabolism, and excretion. The Simcyp Simulator can be used at different stage of drug discovery and development for a variety of purposes.

Di Muria et al. [22] proposed a PBPK model based on a simple representation of the body. It provides seven compartments, each of them representing an organ, a tissue, a fluid of the body, or a group of them.

The gastrointestinal tract is split into the stomach, the small intestine, and the large intestine; the gastrointestinal circulatory system, the liver block, the plasma block, and the tissue compartment describe the rest of the body. The PBPK model was conceived to be applied in the cases of both single and multiple administration. The initial conditions have to be diversified on the basis of the route of administration, the kind of pharmaceutical form, and the frequency of the administration. This model consists of the mass balance equations on the compartments (7, one for each compartment) and their initial conditions. Twenty-two physiological parameters are used in the model, but a very limited number of them (up to 5) have to be optimized. The model is able to predict the drug haematic levels for several administration routes. The capabilities of the model were confirmed by the comparison between the model predictions and the experimental data from literature for several case histories.

Models to predict the absorption from the human intestine have ranged in type and complexity from simple correlation to single experiment, to mathematical calculations using a variety of parameters, including mass balance approaches. A description of a physiologically based model of drug absorption in the human gastrointestinal tract was carried out by Grass [23]. The simulation combines a model of fluid movement in the gastrointestinal tract with a calculation of drug absorption (flux) in each intestinal segment (modeled as a separate compartment) over time. The evaluation of three parameters (solubility, surface area, and

permeability) taken from literature allows the calculation of the theoretical flux in each segment. The flux estimated in each compartment over time was combined to construct an absorption rate prediction.

Willmann et al. developed a physiologically based absorption model for orally administrated drugs in rats [24] and monkeys [25]. The gastrointestinal tract is modeled as a continuous tube with spatially varying properties. The mass transport through the intestinal lumen is described via an intestinal transit function. The only substance-specific input parameters of the model are the intestinal permeability coefficient and the solubility in the intestinal fluid. With this information, the complete temporal and spatial absorption profile can be calculated. The described model was used as a starting point for the development of a model simulating the gastrointestinal transit and passive absorption of a compound administered in solution in a fastened subject [26]. The overall performance of the model was investigated by calculating the fraction dose absorbed for a broad range of the input parameters.

Plusquellec et al. [27] built a pharmacokinetic model taking into account a discontinuous absorption along the gut, from n successive sites, a nonabsorbing intestinal segment being always in between two successive sites. An analytical expression of plasma concentration was obtained. This model is able to deal with areas under curve, bioavailability, and clearance and to point out the contribution of each site to the total absorption of the drug.

5. Materials and methods

5.1. Materials

Chemicals used are: (1) hydrochloric acid (HCl, reagent A, CAS 7647-01-0), 37% w/w solution, density 1200 kg m^{-3}, MM_A = 36.5 kg $kmol^{-1}$; (2) sodium hydroxide (NaOH, reagent B, CAS 1310-73-2), pure solid, MM_B = 40.0 kg $kmol^{-1}$; (3) sodium phosphate dodecahydrate ($Na_3PO_4{\cdot}12H_2O$, reagent S, CAS 10101-89-0), pure solid, MM_S = 380.12 kg $kmol^{-1}$.

The medium used in the first stage of USP method (ACID stage) was 750 mL of 0.1 N HCl, and it was obtained diluting 6.3 mL of reagent A to 750 mL. The medium used in the second stage of USP method (buffer stage) was obtained adding to the medium used in first stage 250 mL of 0.2 M sodium phosphate tribasic. To obtain this, 19.0 g of reagent S was dissolved in 250 mL of distilled water.

The buffer to start the controlled pH test (pH 4.8) was prepared (V = 0.5 L) by mixing reagent A and reagent S at concentrations C_A = 0.4 mol/L and C_S = 0.2 mol/L. This buffer was obtained mixing 38 g of reagent S and 16.6 mL of reagent A and adding distilled water to volume. The solutions to be used to control the pH were obtained as follows: (1) Acid solution (HCl 2 M): 83 mL of the reagent A diluted to 0.5 L with distilled water; (2) Basic solution (NaOH 2 M): 40 g of the reagent B dissolved in 0.5 L of distilled water; the sudden increase in pH expected after 2 h in the controlled pH test was accelerated by adding 0.4 g NaOH/25 mL distilled water, as an aid to the dosage of basic solution commanded by the pump.

The tests were carried out on commercial tablets (Modified Release), enteric coated (Diclofenac EG, ATC M01AB05, Eurogenerici, Milan, Italy). Each tablet contains 100 mg of the active molecule (Diclofenac Sodium, DS). Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID) taken to reduce inflammation and as an analgesic reducing pain in condition, such as arthritis or acute injury. Diclofenac is available in stomach acid-resistant formulations, fast disintegrating oral formulations, slow and controlled-release forms, suppositories, and injectable forms. To be effective the drug, the drug contained in the coated tablet must target the *mucosa* of the terminal *ileum* and colon for localized release. Release of drug in the stomach and upper small intestine is undesirable as this will lead to premature absorption and consequent drug wastage as well as possible systemic side effects. The tablet used is coated to resist the low pH in the stomach and release in the neutral environment such as the intestinal one.

5.2. Methods

The dissolution tests were simultaneously performed with the conventional method (using an USP Apparatus 2) according to the USP guides and with a new *in vitro* apparatus which is able to simulate the real pH changes in the gastrointestinal tract. The USP method A was used to evaluate the drug released as the conventional dissolution test. The drug content was assayed spectroscopically at λ = 273 nm.

To control the pH in the dissolution medium into one of the Apparatus 2 vessel, two peristaltic pumps were used to dose the acidic and the basic solutions, respectively. The pump action was obtained by a gear motor (12 V, 8 rpm, supplied by RS-Instruments, Italy, cod. 255-9649). To permit the pumping, a peristaltic pump head (WM101 supplied by RS-Instruments, Italy) was coupled with the gear motor and a silicon tube was housed inside the roller. The tube is trapped by two rollers, and when they are activated by the control system, the solution contained within the tube is forced to discharge. The pH is measured by using a glass pH probe (supplied by RS-Instruments, Italy, cod. 205-075) which provides the data to evaluate the acidic or basic solution flow rate to reach the desired pH value in the dissolution medium. A data acquisition board (DAQ NI PCI – 6031 supplied by the National Instruments) is used to measure and collect the sensor signals. A dedicated software was developed to provide the control of the pH and the temperature evolution in the *ad hoc* device.

6. Results and discussion

6.1. In vitro results

Dissolution tests were carried out on a commercial coated tablet for the delayed release of 100 mg of diclofenac. Conventionally, the dissolution for orally administrated drugs is studied following the US Pharmacopeia rules, as described in the USP chapter ⟨711⟩. In this chapter, the methodologies to perform the dissolution of different kind of drug formulation (immediate or delayed release) in different USP accepted apparatuses are specified. For the enteric-coated dosage forms, USP defines two different procedures for testing the release, both of them comprising two steps: an acid stage and a neutral stage. The temperature is kept constant during all the dissolution tests at 37 °C, the physiological temperature. The pH is initially maintained on 1.0 to simulate the gastric environment, and then it is quickly raised up to 6.8 to simulate the intestinal environment. Thus, according to the conventional method, the pH evolution followed by the tablet is reported (as dotted line) in Fig. 1. To evaluate the amount of drug released, spectrometer analysis of the dissolution medium to evaluate the drug-released concentration is performed automatically, after a proper calibration of the analytical method. Because of the volume of the dissolution medium during the test is kept constant and the initial value is known, the released mass of drug could be easily calculated. The release pattern obtained is reported in Fig. 2 (full circles are the experimental data). The pharmaceutical dosage form seems to be fully enteric, and only the 8% of the drug contained in the tablet is released in the acidic environment during the first 120 min (the total content is 100 mg of drug in the tablet). The re-

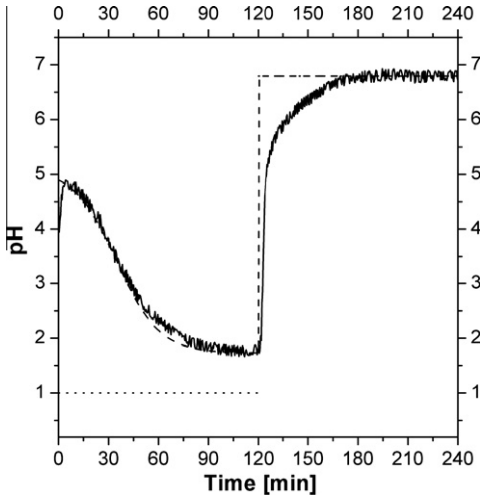

Fig. 1. The pH histories in the conventional method, dotted line (USP method A, pH 1.0 for the first two hours, then pH 6.8); a real pH evolution [28], dashed line (pH decreasing from 4.8 to 2.0 in the first two hours, then pH 6.8); and the pH evolution realized in the modified apparatus, continuous line (as obtained by the control system programmed to follow the real pH evolution).

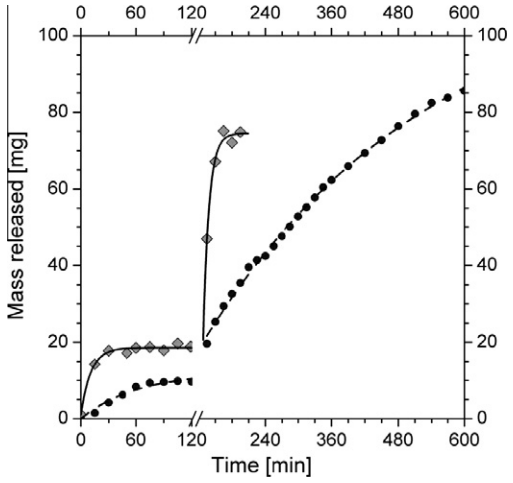

Fig. 2. The release kinetics obtained by the conventional method (USP Method A, full circles and dashed line) and by the novel technique (diamonds and continuous line). Symbols are experimental data, and lines are fitting equation to be used in pharmacokinetic simulations.

lease in the buffer stage is well controlled (after 8 h, less than 90 mg was released).

In spite of the USP recommendations, it was demonstrated that the pH in the stomach increases during the ingestion of food, depending on the pH and the buffer capacity of the food, and subsequently decreases due to acid secretions [2]. Thus, the pH in the stomach is not 1.0, but just before the meal, the pH is about 4.8, and it starts to decrease after the meal [28] (physiological pH evolution is shown in Fig. 1, as a dashed line). Its value reaches 2.0 after two hours (the exact value is variable individually, and it depends on the meal nature and quantity). To reproduce a given pH evolution, a program was developed to provide the control of the pH and the temperature evolution in the new device using the LabVIEW environment, developed by the National Instruments. The pH evolution measured in the modified apparatus is reported as a continuous line in Fig. 1. The physiological pH changes are well approximated in the device, except for the late change which derives from the fast neutralization of the medium after 120 min. To evaluate the relevance of the pH evolution on the release pattern, the same tablet previously analyzed according to the conventional method was then analyzed in the new device in which pH of the dissolution medium was adjusted to follow the physiological pH evolution. To determine the concentration of the drug released during the experiments, samples of dissolution medium were withdrawn every 15 min and analyzed spectrometrically. Because the volumetric flow rate of the pump, the sample volume withdrawn for the analysis, and the volume of acid and basic solution added to adjust the pH in the medium were known, it was possible to calculate the volume changes in the dissolution vessel and then the mass of drug released was evaluated. The experimental release pattern obtained in the new device is reported in Fig. 2 (as diamonds). Under these conditions, the release pattern is completely different: During the acid stage, the drug released is higher (more than 20 mg), and the remaining drug is released during the buffer stage very quickly (less than one hour to release about 80 mg).

Comparing the two release patterns, it could be noticed that the release in the real stomach (which is very similar to the release in the pH variable technique) would be more than twice the release in the conventional *in vitro* test (USP). The release in the real intestine would occur in a very short time (in the conventional *in vitro* test, it takes more than 8 h).

Therefore, with the aim of ensuring an enteric behavior (limited release in gastric environment) and a controlled release thereafter, two remarks could be done:

i. The coating is generally insufficient because the occasionally high values of pH can dissolve the coating layer, starting the release in an unexpected moment.
ii. The polymers used in the formulation should be pH resistant by themselves (such as the polymers proposed recently [29]), since the dissolution of the coating layer leaves the matrix unprotected and easily degradable (the acid attack caused the damage of the matrix and then the fast release observed in the intestine).

These observations clarify how the availability of such a modified dissolution test should cause the manufacturer to change the materials used and the preparation processes of the pharmaceuticals.

6.2. In silico results

The *in vitro* results obtained were subsequently used to build an *in silico* model able to take into account the pH changes experienced by a drug once ingested.

A simplified version of the model proposed by Di Muria et al. [22] was used to simulate the plasma profile of orally administrated diclofenac. A schematic of the model used is reported in Fig. 3. Each block represents an organ, a fluid, or a group of them: the gastrointestinal lumen, the gastrointestinal circulatory system, the liver, and the plasma. The continuous arrows represent the mass flow rates between the compartments. The dashed arrows represent the drug inlets after administration by intravenous or oral routes. In this work, only the oral route of administration is considered. The model consists of mass balance equations on the compartments (coupled with their initial conditions). The assumptions made to solve the mass balances are that the blocks can be considered continuous stirred reactors and that the mechanism of drug transport across biological membranes is passive diffusion. In order to evaluate the plasma concentration of the drug, for this application, the compartment that represents the scarcely perfused tissue and organs is not considered. Furthermore, focusing on the drug release in the small intestine, the compartment simulating the colon is neglected. Thus, the parameters that have to be taken

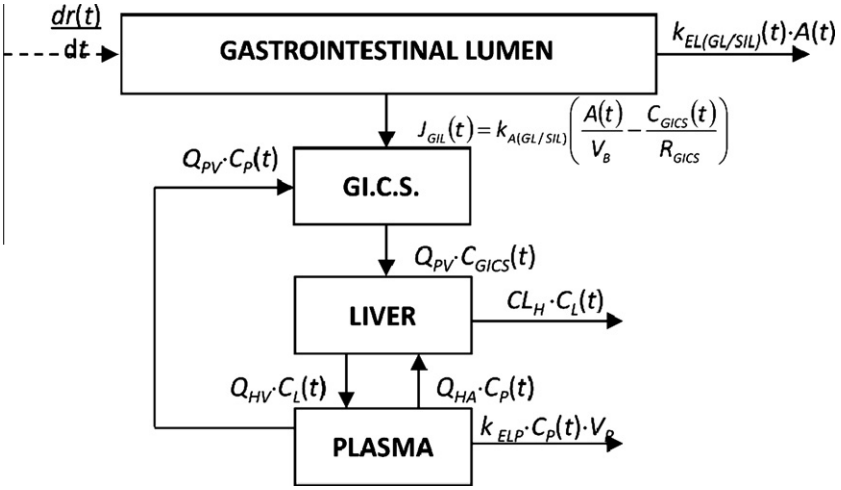

Fig. 3. Schematic of the pharmacokinetic model, which is a simplified version of the model proposed by Di Muria et al. [22]. The boxes represent the single compartments, the full lines are the mass fluxes, and the dashed lines are the administration routes.

into account are $K_{ELGIL}(t)$ and K_{ELP} (kinetic constants of elimination), Q_{PV}, Q_{HA}, and Q_{HV} (blood volumetric flow rates of the portal veins, of the hepatic artery, and of the hepatic vein), CL_H (hepatic clearance), and V_P (distribution volume of the plasma compartment). Moreover, J_{GIL} is the time evolution of the mass flow rate of the drug exchanged between the gastric lumen and the gastrointestinal circulatory system. It takes into account the volume of the bolus crossing the gastrointestinal tract, the drug partition coefficient in the gastrointestinal circulatory system, and the kinetic constants of absorption. $A(t)$ (time evolution of the drug contents in the gastrointestinal tract mass), $C_P(t)$, $C_L(t)$, and $C_{GICS}(t)$ (time evolution of the drug concentration in the plasma, in the liver, and in the gastrointestinal circulatory system, respectively) are outputs of the model. The model needs an input function to describe the drug administration kinetics, $r(t)$ is the time evolution of the *in vitro*-measured drug release. The derivative of the *in vitro* drug release has to be used as forcing function in the model. Then, the experimental data obtained using the *in vitro* model have to be fitted to obtain continuous function which can be differentiate. Both the release kinetics obtained by the conventional method and by the novel technique have been fitted by the following equations:

$$r(t) = \begin{cases} a \cdot [1 - exp[-b \cdot (t - t'_o)]] & t \geqslant t^* \\ c \cdot [1 - exp[-d \cdot (t - t''_o)]] & t > t^* \end{cases} \quad (1)$$

in which t^* represents the time in which the pharmaceutical form remains in the gastric environment (minutes), a, b, c, d, t'_o, and t''_o are parameters that have to be fitted. The function parameters for both the conventional and the novel dissolution technique are reported in Table 1. The function is plotted in Fig. 2 as dashed line for the conventional method and as continuous line for the novel technique.

Once identified the function describing the time evolution of the *in vitro* drug release, the input function of the model, which consists of the release profile derivative, is known. To use the *in silico* model, it is necessary to know model parameters used to describe the diclofenac pharmacokinetics. With the purpose to evaluate these parameters, the model is used to fit an experimental plasma evolution of diclofenac after oral administration of a 50 mg dose [30]. The experimental data and the model prediction after parameters optimization are plotted in Fig. 4. The model parameters used to describe the diclofenac pharmacokinetics obtained are reported in Table 2. Once the parameters are obtained, the model is able to simulate the plasma concentration starting from the *in vitro* release profiles. The plasma profiles are obtained both starting from the *in vitro* release kinetics observed using the conventional USP method A (the continuous line in Fig. 5) and starting from the *in vitro* release kinetic observed using the novel apparatus (the dotted line in Fig. 5). It should be noticed that both the profiles are contained in the therapeutic window, which is represented by horizontal dashed lines in Fig. 5, even if the profile shapes are very different. Following the conventional method, an almost constant plasma profile is obtained, and the drug concentration remains in the therapeutic window for a long time. Analyzing this profile, it should be concluded that the pharmaceutical dosage form is very effective for the controlled oral release, and the rate of drug entering in blood is constant. In spite of these considerations, if the *in vitro* release kinetics used as input for the model consists of the dissolution profile obtained following the real pH evolution experienced by the pharmaceutical form, the simulated plasma profile is completely different. It could be noticed that the maximum concentration of the drug in the blood is six times higher and the drug remains over the minimum effective concentration

Table 1
Function parameters for the equation fitting the release kinetic both for the conventional dissolution method ($r_{cov}(t)$) and for the novel dissolution method ($r_{mod}(t)$).

Function	Parameter	Value	Parameter	Value
$r_{conv}(t)$	a	12.03057 mg	d	0.002464 min^{-1}
	b	0.016555 min^{-1}	t'_o	0 min
	c	116.4842 mg	t''_o	51.28305 min
$r_{mod}(t)$	a	18.61314 mg	d	0.095421 min^{-1}
	b	0.092511 min^{-1}	t'_o	−0.65673 min
	c	74.45371 mg	t''_o	124.5987 min

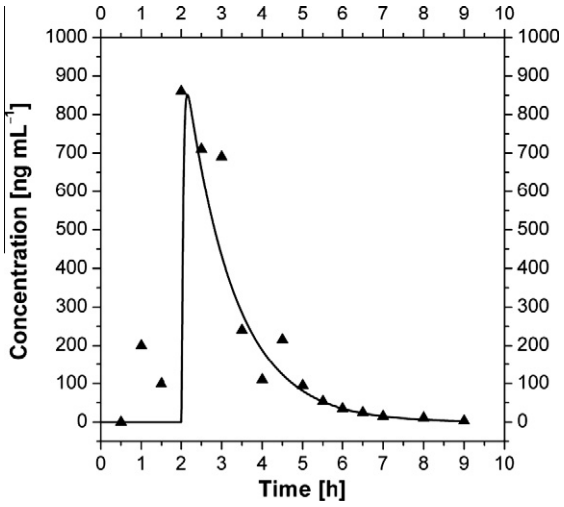

Fig. 4. Plasma profile of diclofenac after oral administration of a 50 mg dose [30]. Symbols are experimental data, and line is the model (Fig. 3) prediction after parameter optimization.

Table 2
The model parameters used to describe the diclofenac pharmacokinetics obtained.

Parameter	Value	Parameter	Value
t_{GL}	2 h	k_{AGL}	0 s^{-1}
t_{SIL}	9 h	k_{ASIL}	4.787×10^{-4} s^{-1}
V_L	1.5×10^{-3} m^3	k_{ELSIL}	1.167×10^{-4} s^{-1}
Q_{PV}	1.625×10^{-5} m^3 s^{-1}	k_{ELGL}	0 s^{-1}
Q_{HA}	5.417×10^{-6} m^3 s^{-1}	V_T	152×10^{-3} m^3
Q_{HV}	2.167×10^{-5} m^3 s^{-1}	V_P	0.098 m^3
C_{LH}	1.647×10^{-5} m^3 s^{-1}	V_{GICS}	9.956×10^{-6} m^3
R_{GICS}	6.667	V_B	0.1×10^{-3} m^3

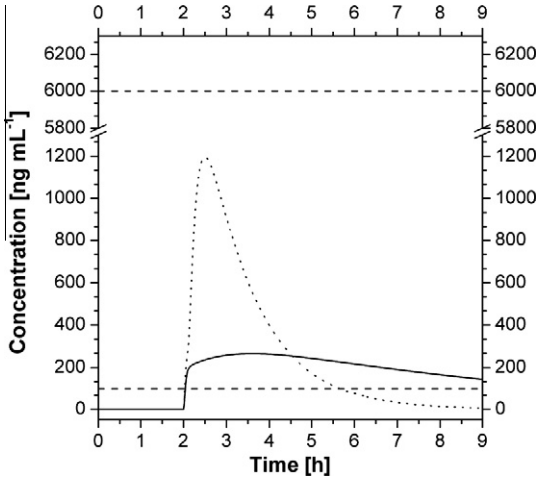

Fig. 5. The plasma profiles obtainable: (a) if the real *in vitro* release kinetics would be the one observed using the conventional USP method A (the continuous line), (b) if the real *in vitro* release kinetics would be the one observed using the novel apparatus (the dotted line). The horizontal dashed lines represent the minimum effective concentration (the lower one, [31]) and the minimum toxic concentration (the higher one, [32]); therefore, they identify the therapeutic window.

for a shorter period. Thus, the differences between the dissolution profiles are reflected and amplified in the plasma simulations.

Once more, the availability of the modified dissolution technique, together with the pharmacokinetic model, suggests that a pharmaceutical form, designed to fulfill the requirements imposed by USP testing methods, in a real system would show a completely different behavior. The dissolution under a variable pH (similarly to what happens in a real stomach) causes a large release in the gastric environment and a fast release in the intestine, and this dissolution kinetics should cause an anticipated and higher maximum plasma concentration (with respect to that expected if the *in vitro* release kinetics would be the one observed during an USP test), and a faster exit from the therapeutic window.

7. Conclusions

A large set of dissolution apparatuses, both approved by USP and developed by single research groups, were critically analyzed in the first section of the present work. The general outcome is that the full reproduction of what happens to a pharmaceutical once swallowed is not reproduced by the USP-approved techniques. The particular apparatuses developed to the purpose are much better in the *in vitro* simulation, but they are not able to a full simulation yet.

Similarly, a review of the most interesting *in silico* models was performed, analyzing the pharmacokinetic models proposed in literature, focusing on the ones based on physiology (PBPK models). The most efficient balance between model complexity (and number of parameters) and details in simulation is still to be found, even if a recently proposed model [22] seems to be heading in the right direction.

In this work, a preliminary experiment was carried out testing the release kinetics from a commercial enteric tablet for the controlled release of diclofenac. Once tested in the presence of a pH evolution mimicking the real one, the release was found higher than expected in the gastric environment and faster than expected in the upper intestine. The comparative test, carried out following the USP recommendation, gives a very different release pattern (low amount of drug released in the stomach, very controlled drug release in the intestine). The pharmacokinetic model from [22], slightly simplified, was tuned against *in vivo* pharmacokinetic data for diclofenac, and then it was used to predict the plasma levels on the basis of the two different *in vitro* release kinetics. Of course, the predicted plasma levels are very different each other. In particular, the release kinetics obtained in the variable pH methods causes higher plasma peak concentrations (C_{max}) and a faster exit of the plasma concentration from therapeutic window.

In conclusion, the need for a very realistic dissolution device and of a detailed and simple pharmacokinetic model was emphasized, and a preliminary test – carried out mimicking accurately only the real pH evolution – demonstrated that a pharmaceuticals designed to obtain a given plasma profile (and tested successfully following the traditional methods) can give completely different results, once administered to living beings.

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