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Review

In vitro biphasic dissolution tests and their suitability for establishing *in vitro-in vivo* correlations: A historical review

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ABSTRACT

For many decades, one of the most critical issues in the pharmaceutical industry has been the poor solubility of some drugs. Indeed, a prerequisite for drug absorption is the presence of dissolved drug at the absorption site and this can be challenging for compounds with low aqueous solubility such as BCS class II (low solubility, high permeability) and IV (low solubility, low permeability) compounds. If the development of oral delivery formulations of these compounds is frequently challenging to formulation scientists in the pharmaceutical industry, the *in vitro* evaluation of these new formulations is also a great challenge. One alternative approach to overcome the problems encountered with conventional dissolution methods is the use of biphasic dissolution systems. This review provides an overview of the origin and the evolution over time of the biphasic systems and the growing interest among scientists regarding their suitability for establishing *in vitro-in vivo* correlations. The evolution of these systems and their applications from the 1960s to the present day, such as in system variants and improvements, analysis of complex formulations, discriminatory power, bio-relevance, precipitation and supersaturation visualization, *etc.* will be discussed.

Contents

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1. Introduction

One of the most critical issues in the pharmaceutical industry for many decades has been the poor solubility of some drugs. Recent estimates suggest that approximately 70% of drugs within pharmaceutical pipelines possess a low aqueous solubility. Indeed, combinatorial chemistry and high-throughput screening used in drug discovery have resulted in an increase of poorly water-soluble drug candidates. These compounds mainly belong to the second or the fourth class of the Biopharmaceutics Classification System (BCS) described by Amidon et al. (1995). This BCS is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. BCS class II compounds are low solubility and high permeability drugs while BCS class IV are low solubility and low permeability drugs. If the development of oral delivery formulations of both BCS classes Active Pharmaceutical Ingredients (APIs) is frequently challenging to formulation scientists in the pharmaceutical industry, the *in vitro* evaluation of these new formulations is also a great challenge. The authorities recommend using a volume of dissolution medium larger than the amount of solvent needed to completely dissolve the drug. This would approach *in vivo* conditions where the drug dissolved in the gastrointestinal fluids is quickly absorbed. However, maintaining these "sink conditions" throughout the testing of poorly water-soluble drugs can be difficult in common single-phase dissolution systems. Possible techniques to address this concern include: (1) using relatively large volumes of dissolution medium or frequently replacing the medium, (2) altering the pH of the dissolution medium, (3) adding co-solvents to the medium (*e.g.* different alcohols, propylene glycol, glycerin, polyethylene glycol, sorbitol), and (4) adding nonionic, cationic or anionic surfactants to enhance API solubility. However, these approaches generally provide non-physiologic dissolution environments, and the rate-limiting dissolution of the suspended drug particles may be masked. Hence, *in vitro* results obtained from a single-phase dissolution system may not satisfactorily reflect the *in vivo* drug release and dissolution characteristics of formulations of poorly water-soluble drugs.

One attractive technique to overcome this problem is the use of biphasic dissolution systems. These systems consist of two immiscible phases: an aqueous phase and an upper organic phase. Following initial aqueous dissolution, the drug partitions into the organic layer, exploiting the lipophilicity of the compound. In this way, in theory, a complete dissolution of the poorly soluble drug can take place. As shown in Table 1, publications on biphasic tests have, for several years, demonstrated flexibility in accommodating different kinds of dosage forms, discriminative capability regarding formulations of poorly soluble drugs and good potential for establishing an *in vitro-in vivo* relationship. This review provides an overview of the origin and the evolution over time of the biphasic systems and their growing suitability for establishing *in vitro-in vivo* correlations (IVIVCs).

2. Historical review

2.1. The 1960s and 70s: origin, first use and kinetic studies

2.1.1. Origin of biphasic systems

Levy et al. (1965) reported the first development of a single *in vitro* dissolution rate test correlating quantitatively with gastrointestinal absorption in man. The study was limited to aspirin (three different types of dosage forms that differed in drug absorption rate), a drug which is relatively water soluble. However, drugs with the greatest dissolution problems are those with the lowest solubility and these drugs therefore cause the greatest difficulty with respect to maintenance of perfect sink conditions. There is thus a definite need for the development of methodologies to maintain sink conditions during the process of determining the dissolution rate of poorly soluble drugs. Following on from the study of **Levy et al.**, two possibilities were proposed: the addition of adsorbents to the aqueous medium or the use of an upper organic phase acting as a reservoir for the dissolved drug. The first proposal was based on the work of Wurster and Polli (1961), who studied the influence of an adsorbent (norite A) on the dissolution rate of a slightly soluble acidic solid (benzoic acid/pure API tablets). The authors demonstrated the ability of adsorbents to maintain "sink" conditions. The second proposal was inspired by the three-phase "rocking apparatus" (see Fig. 1) developed by Doluisio and Swintosky (1964). This apparatus consists of a tube forming a right angle gently rocked and containing two aqueous phases (one at pH 7.4 containing a known amount of dissolved drug and one free of drug with variable pH) separated by an immiscible phase (cyclohexane). Both approaches involved the same principle: the removal of dissolved drug from the dissolution medium and prevention of its accumulation. In fact, this phenomenon is analogous to the removal of a drug from gastrointestinal fluids by the absorption process in dissolution rate-limited absorption.

2.1.2. First use of a single system for simultaneously determining in vitro drug dissolution and partitioning rates

The three-phase "rocking apparatus" proved to be useful for drugs in solution but it could not be used to investigate the dissolution process (Doluisio and Swintosky, 1964). Thus the overall process of absorption was generally studied *in vitro* as two separate processes. However, it was known that certain factors had the capacity to increase the dissolution rate of a drug while simultaneously decreasing its partitioning rate. For example, surfactants can increase the *in vitro* dissolution rate of drugs but can also decrease their *in vivo* absorption. It was for this reason that **Niebergall et al.** decided to investigate the possibility of determining both the *in vitro* drug dissolution and the partitioning rates within a single system (Niebergall et al., 1967). The apparatus chosen for that study consisted of a 500 mL round bottom flask containing 250 mL of aqueous phase and 250 mL of organic phase (octanol). Hard non-disintegrating tablets of salicylic acid were tested in two different aqueous phases: a pH 2 buffer and a 0.1% w/v solution of polysorbate 80 at pH 2. The authors observed that in the early stage of the dissolution process, the API saturation concentration (C_S) was much higher than that of the drug present in the aqueous phase. Nevertheless, a steady state could be obtained quite quickly in the aqueous phase. In this case, the rate of change of the drug in octanol over time became constant and was equal to the dissolution rate. The authors concluded that these findings would be particularly useful for studies involving poorly water-soluble drugs, which would usually require a large volume of dissolution medium to obtain meaningful results. The presence of the lipid phase acting as a sink would obviate this difficulty. Regarding the effect of polysorbate 80, it was observed that the dissolution rate increased but that the partitioning rate decreased if the concentration is exceeding its CMC. For the authors, this was the necessary proof of the value of this system as a screening procedure, as opposed to the usual dissolution rate studies.

Table 1
Summary of biphasic systems described in the literature. This table shows the test configuration, the dissolution media used, the type of API and formulation as well as the observations made by the authors including *in vitro-in vivo* correlations (which are shown in bold).

Year of publication	Authors	Test configuration	Dissolution media	API/Type of formulation	Observations
1961	Wurster and Polli	–	+ norite A	Benzoic acid/pure API tablets	Ability of adsorbents to maintain sink conditions
1964	Doluisio and Swintosky	Three phase "rocking apparatus" (= tube forming a right angle is gently rocked)	Two aqueous phases (one at pH 7.4 and one with variable pH) separated by an immiscible phase (cyclohexane)	Salicylic acid, barbital, antipyrine, aminopyrine and tetracycline/pure API	System used to mimic partitioning of drug between GI fluid and lipoidal phase and between lipoidal phase and plasma
1965	Levy et al.	–	Use of an upper organic phase, which can act as a reservoir	Aspirin/3 different types of dosage forms that differed in drug absorption rate	Correlation of *in vivo* absorption and *in vitro* dissolution data
1967	Niebergall et al.	USP II	250 mL octanol + 250 mL pH 2 buffer or 0.1% (w/v) solution of polysorbate 80 at pH 2	Salicylic acid/hard non-disintegrating tablets	Simultaneous determination of the *in vitro* drug dissolution and partitioning rates in a single system
1967	Gibaldi and Feldman	500 mL three-neck round bottom flask immersed in a 37 °C bath	150 mL 0.1 M HCl + 150 mL mixture cyclohexane/octanol (1:1)	Benzoic acid and salicylic acid/non-disintegrating tablets of pure API	Correlation between dissolution rate and agitation speed
1971	Niebergall et al.	–	–	–	Establishment of an equation for the dissolution of a drug into an aqueous phase overlayered with a lipid phase in which back transfer from the lipid phase is assumed possible
1983	Stead et al.	USP I (200 rpm)	500 mL 0.1 M HCl + 400 mL hexane	Ibuprofen/5 different tablet formulations	Biphasic dissolution system was discriminant with a **satisfactory *in vitro-in vivo* correlation**
1985	Porges et al.	Flow-through cell of 4 variable designs with or without agitator (magnetic stirrer 50 rpm) combined to a spiral of extraction with organic phase	Aqueous dissolution medium (water, 0.1 N HCl, or aqueous solution with pH changes of 1.1–7.5 or 1.1–6.8) + chloroform	Nifedipine and nimodipine (pure API and 3 different tablet preparations)	First use of a flow-through cell with a biphasic system
1986	Fini et al.	Dissolution-partition apparatus (= a three-phase partition apparatus fitted with a dissolution cell)	3 phases: 25 mL pH 2 buffer + 25 mL octanol + 15 mL pH 7.4 buffer	Non-steroidal anti-inflammatory drugs (diclofenac, fenbufen, ibuprofen, naproxen, ketoprofen)	Partition improved the dissolution rate at high pH values (continuous extraction)
1993	Chaudhary et al.	USP II with additional paddle at the interface of the liquid (70 rpm)	500 mL simulated gastric fluid + 400 mL octanol	Nifedipine/prolonged release formulation	Consistent, reproducible results, which were correlated with the flow-through cell apparatus
1994	Takahashi et al.	Rotating dialysis cell	Variable pH buffers + octanol	Nifedipine/soft gelatin capsules containing API dissolved in a water soluble vehicle	Correlation between dissolution and absorption after oral administration
1995	Kinget and De Greef	USP II (60 rpm) with a paddle (in the organic phase) and a Petri dish (in the aqueous phase to maintain the dosage form)	250 mL phosphate buffer + 250 mL octanol	Methoxsalen/4 different semi-solid lipid matrices	Biphasic test more discriminant than the absorption simulator using a silicon membrane
1995	Takahashi et al.	Rotating dialysis cell	Variable pH buffers + octanol	Ibuprofen/soft gelatin capsules containing API in an oily semi-solid matrix (lipid-based formulation)	**Correlation between curve of release rate *in vitro* and the *in vivo* curve**
1996 and 1997	Ngo Thu Hoa et al.	USP II with dual paddle combined with a disintegration apparatus covered with thin filter paper	800 mL demineralized water + 200 mL organic solvent (chloroform, ethylacetate or mixture 1:1 octanol/cyclohexane)	Artemisinin/different tablet formulations containing surfactant or hydrophilic diluents	Biphasic system allowed sink conditions to be reached. **IVIVC level C established after rabbit PK study.**
1997	Grundy et al.	Basket-paddle hybrid system (= USP II with cylindrical basket attached to the base of the paddle)	750 mL simulated intestinal fluid USP without pancreatin + 250 mL octanol	Nifedipine/GITS tablet (Gastrointestinal therapeutic system)	**Suggestion of a better correlation with published *in vivo* studies than with classical dissolution techniques**
1997	Grundy et al.	Basket-paddle hybrid system (= USP II with cylindrical basket attached to the base of the paddle)	750 mL simulated intestinal fluid USP without pancreatin + 250 mL octanol	Nifedipine/GITS tablet (Gastrointestinal therapeutic system)	**Good correlation between the fraction of nifedipine absorbed-time profiles and the fraction of nifedipine transferred-time profiles after a human clinical trial (12 healthy subjects)**
1998	Pillay and Fassihi	USP II (with or without ring mesh assembly at the bottom)	750 mL phosphate pH 7.5 buffer + 250 mL octanol	Nifedipine/osmotic pump	The drug release profiles over the entire dissolution period were identical irrespective of the position of the delivery system.
1999	Pillay and Fassihi	USP I (75 rpm) or USP II with ring mesh assembly and 3 different positions of the paddle (75 or 100 rpm)	200 mL–400 mL phosphate buffer + 100 mL octanol	Nifedipine/lipid-filled capsules (Gelucire® 44/14 + Labrasol®)	Determination of which configuration gives the most reproducible results
2002	Grassi et al.	A flask with a dual paddle	150 mL pH 1.2 or 7.5 buffer	Piroxicam and nimesulide	Development of a mathematical model

(continued on next page)

Table 1 (*continued*)

Year of publication	Authors	Test configuration	Dissolution media	API/Type of formulation	Observations
		immersed in a 37 °C bath	+ 50 mL octanol		able to describe partitioning kinetics
2004	Gabriëls and Plaizier-Vercammen	USP II (with paddle at the interface – 50 or 100 rpm)	150 mL aqueous phase (7 different tested) + 100 mL organic phase (different tested)	Artemether and dihydroartemisinin/tablets with different crushing strengths	Selection of the most appropriate organic solvent and evaluation of the ability to discriminate the tablets tested
2009	Vangani et al.	USP II combined with USP IV (after optimization of 12 test parameters)	300 mL pH 6.8 phosphate buffer + 200 mL mixture 1:1 nonanol/cyclohexane	AMG517, griseofluvin, lovastatin, carbamazepine/2 different tablet and capsule formulations (slow and intermediate release)	**Excellent rank order correlation between *in vitro* release and *in vivo* absorption (monkey)** + discrimination between bioequivalent and non-bioequivalent formulations
2010	Heigoldt et al.	USP II + pH controller to induce pH changes in the aqueous phase	500 mL phosphate buffer (from pH 2 to 6.8) + 100 mL octanol	Dipyridamole and BIMT17/modified release formulations (monolithic-coated tablets, matrix tablet, multi-unit pellet formulation)	Conversely to conventional dissolution testing, **rank order correlation found between *in vitro* release and *in vivo* absorption (human in fasted state)**
2010	Shi et al.	USP II (dual paddle – 75 rpm) combined with USP IV (30 mL/min)	250 mL pH 6.8 phosphate buffer + 200 mL octanol	Celecoxib/3 different formulations (Celebrex® capsules, solution, S-SEDDS)	**Level C correlation** (AUC organic phase up to 2 h *vs.* AUC or C_{max} *in vivo*)
2012	Phillips et al.	USP II (50 or 100 rpm) + μDiss Profiler to monitor the organic layer	600 mL water + 350 mL octanol	Nifedipine/3 different controlled release formulations (slow, medium, fast) with different HPMC loadings	Best discriminatory power compared to conventional dissolution testing.
2012	Mudie et al.	3 different types of apparatus with different agitation speeds (40-50-75-77 rpm)	150–250 mL buffer at different pHs + 150–250 mL octanol	Ibuprofen, nimesulide, piroxicam	Mechanistic drug-transport analysis of the partitioning process suitable for many drugs and experimental set-ups.
2014	Frank et al.	miBldi (= mini biphasic dissolution system with pH-shift for the aqueous phase)	50 mL aqueous phase +15 mL octanol	Dipyridamole/pure API BIXX (poorly soluble weak base) / 4 different formulations	miBldi predicted *in vivo* precipitation (dipyridamole) + superior ranking + **IVIVC level A (*in vivo* study in dogs)**
2015	Pestieau et al.	USP II (dual paddle – 50 rpm) combined with USP IV (8 mL/min)	300 mL 0.1 M HCl + 200 mL octanol	Fenofibrate/solid dispersions and PGSS formulations (containing Gelucire® 50/13)	API supersaturation in the aqueous phase allowed differentiation of formulations tested (maximum supersaturation ratio calculated)
2016	Thiry et al.	USP II combined with USP IV in a closed-loop and open-loop configuration	Closed-loop configuration: 400 mL 0.1 M HCl + 400 mL octanol Open-loop configuration: from 200 mL up to 800 mL 0.1 M HCl + 400 mL octanol	Itraconazole/3 different formulations (pure API, Sporanox® capsules, extrudates with Soluplus®)	Observation of an interaction between the small amount of octanol dissolved in the aqueous phase and Sporanox® excipients
2016	Al Durdunji et al.	USP II (120 rpm) combined with USP IV (30 mL/min)	300 mL pH 6.8 phosphate buffer + 500 mL octanol	Deferasirox/4 different dispersible tablets	Best discriminatory power between formulations + **IVIVC level A in accordance with the FDA acceptance criterion with dissolution profile in the organic phase (*in vivo* human study in fasted state)**
2016	Pestieau et al.	USP II (50 rpm) alone or combined with USP IV (8 mL/min)	300 mL 0.1 M HCl + 200 mL octanol	Fenofibrate/3 different formulations (pure API, Lipanthyl® 200, Fenogal® 200)	USP II apparatus alone not suitable as a biphasic system due to the migration of undissolved API particles in the organic phase. As a result, USP II was combined with USP IV apparatus.
2016	Locher et al.	miBldi-pH v2 (improved version)	50 mL aqueous phase (McIlvain buffer from pH 2.2 to pH 6.5) + 30 mL octanol	BCS class II APIs (telmisartan, dipyridamole, ibuprofen, griseofulvin, itraconazole, fenofibrate) with different excipients and different concentrations	Study of the influence of experimental model parameters such as rotation speed, pH-shift, influence of excipients, influence of API concentration, *etc.*
2016	Shi et al.	USP II (large or small model – 50 rpm) combined with USP IV (5 mL/min)	Small scale: 50 mL buffer (from pH 2 to 6.5) + 30 mL octanol Large scale: 200 mL pH 6.8 buffer phosphate + 200 mL octanol	ABT-072 (BCS class II weak acid)/different formulations (API capsule, suspension, amorphous SD and wet granulation formulations)	Observation of degree of supersaturation and precipitation inhibition + **IVIVC level C for some formulations** (with AUC *in vivo* and API concentration in the organic phase after 2 h for small scale *in vitro* test)
2017	Pestieau et al.	USP II (50 rpm) combined with USP IV (8 mL/min)	300 mL 0.1 M HCl + 200 mL octanol	Fenofibrate/4 different formulations (3 PGSS formulations and 1 solid dispersion)	**IVIVC level A in accordance with the FDA acceptance criterion (*in vivo* study in pigs) for all PGSS formulations** and with dissolution obtained with the sum of both phases (aqueous phase + organic phase)

2.1.3. Kinetic studies of dissolution and partition rates

Also in 1967, the use of an organic solvent reservoir for the determination of first-order dissolution rates was discussed by Gibaldi and Feldman (1967). The authors tested two different APIs (benzoic acid and salicylic acid/non disintegrating tablets of pure API) with two different dissolution procedures: one in 150 mL of 0.1 N HCl and one in 150 mL of 0.1 N HCl with an upper organic solvent phase (150 mL of a 1:1 mixture cyclohexane and octanol). The selection of this upper organic solvent phase (nature and volume) was dictated by the consideration of drug saturation solubility in the organic solvent. In order for

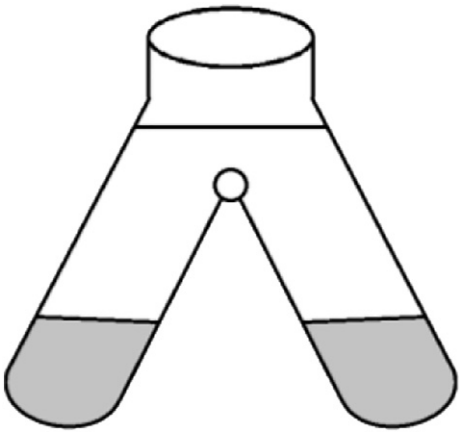

Fig. 1. The three-phase "rocking apparatus" developed by Doluisio and Swintosky (1964) (aqueous phases are shown in grey and the organic phase in white).

sink conditions to be achieved, the total dose of drug to be studied needed to represent less than 20% of its solubility in the selected volume of organic solvent. Regarding the aqueous phase, the dissolution occurred under sink conditions for benzoic acid because its concentration in the dissolution fluid never exceeded 12% solubility. In this case, the dissolution process would have followed zero-order kinetics. By contrast, the salicylic acid concentration in the aqueous phase at the end of the test approximated to 80% solubility (non-sink conditions). This condition was selected to illustrate the different dissolution kinetics that result from a lack of sink conditions and to demonstrate the resolution of these differences by means of an organic solvent reservoir. In practice salicylic acid in 0.1 N HCl was found to follow an apparent zero-order dissolution up to about 25% solubility, and when the concentration exceeded 30%, the dissolution appeared to follow first-order kinetics. Regarding the results obtained with the biphasic system, the authors found that the steady-state values of the concentrations of benzoic acid represented only 0.8 to 1.8% of the compound's solubility in 0.1 N HCl. This is an excellent indication of the efficiency of the organic solvent in maintaining near-perfect sink conditions in the aqueous phase. In the same way, the maximum accumulation of salicylic acid in the aqueous phase represented less than 5% solubility. Regarding the rate of drug appearance in the organic medium, this followed zero-order kinetics after an initial lag phase required to establish steady-state conditions. In other words, by using the organic reservoir, it was possible to demonstrate that the release rate from the model dosage form of a quantity of drug in excess of its total solubility in 0.1 N HCl adhered to apparent zero-order kinetics. Furthermore, the authors observed that the calculated apparent zero-order dissolution rate constant was in agreement with the apparent initial zero-order rate constant determined under non-sink conditions. Thus the authors concluded that an organic solvent reservoir that functions to maintain approximate sink conditions was also applicable to the determination of first-order dissolution rates. However, both previously reported methods were found to be only suitable for non-disintegrating type formulations and the investigation did not relate the relevance of methods to *in vivo* studies. Moreover, it was assumed that the lipid phase acted as a perfect sink, and that no transfer of the drug from the lipid phase back into the aqueous phase would occur.

Four years after the Gibaldi and Feldman (1967) study, **Niebergall et al.** hypothesized that a back transfer of the drug from the lipid phase into the aqueous phase might take place and that the lipid phase must therefore be chosen with great care to ensure that perfect sink conditions have, in fact, been achieved and maintained (Niebergall et al., 1971).

2.2. The 1980s: first comparison of a biphasic system with in vivo data and use of a new apparatus

2.2.1. First comparison with in vivo data

It was not until 1983 that the first comparison was made of a biphasic system with *in vivo* data by Stead et al. (1983). Firstly, the relative bioavailability of five different oral ibuprofen formulations was determined in 15 healthy volunteers. Secondly, a simple rotating-basket method, with sink conditions, was tested. The dissolution medium was 900 mL of phosphate buffer solution at pH 6.9. Given the acidic nature of ibuprofen, the total solute concentration remained at this pH below 10% saturation solubility, thus satisfying sink conditions. This method initially seemed to offer some useful correlations, but it failed to adequately separate the most highly bioavailable tablet from the rest of the formulations. Moreover, it separated two formulations that were bioequivalent, *i.e.* the dissolution procedure detected a manufacturing or formulation difference that had no influence on bioavailability. Finally, an acidic biphasic dissolution system was used. In this part of the study, the rotating-basket apparatus was again employed but the basket was immersed in a dissolution medium comprised of 500 mL of 0.1 N HCl (non-sink conditions), with an overlying layer of 400 mL hexane, in order to provide sink conditions (see Fig. 2). In comparison with the first test performed, this system provided the discrimination required for the most highly bioavailable tablet. In fact, this biphasic system resulted in some satisfactory *in vitro-in vivo* correlations, but these correlations would be improved in other futures studies with appropriate variation in dissolution methodology, *e.g.* pH of medium, presence of surfactant, stirrer speed, *etc.*

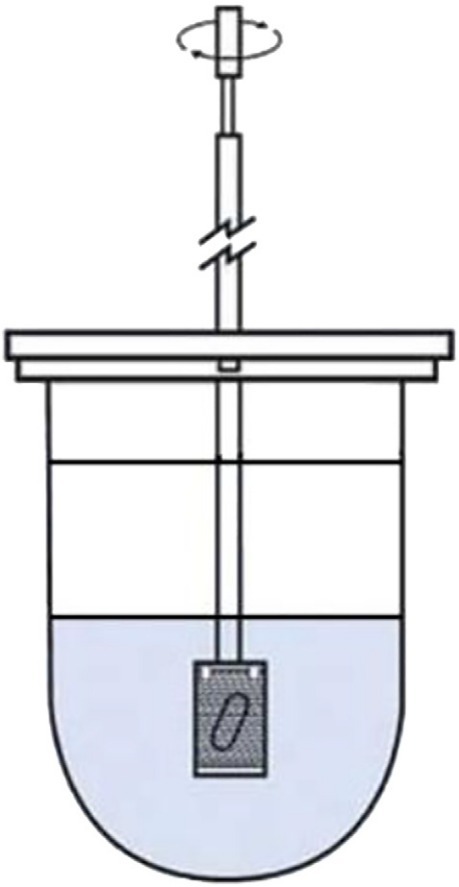

Fig. 2. Biphasic system using USP apparatus I. The basket is immersed in the aqueous phase (in blue) and the upper phase is an organic solvent (in white).

2.2.2. Use of new types of apparatus

2.2.2.1. Flow-through cells. In the years that followed the Stead et al. (1983) study, different types of dissolution/extraction apparatus using two phases gradually began to be used. For example, **Porges et al.** described an automated flow-through method combined to a spiral of extraction with chloroform to determine the dissolution rate of slightly soluble substances: nifedipine and nimodipine (Porges et al., 1985). In this case, the aqueous phase was pumped through flow-through cells of variable design (4 different with or without agitator) in an open system (system without return of the dissolving medium). This configuration was selected to avoid saturation effect since in the gastro-intestinal tract, dissolved API proportions are constantly removed by absorption. A separate extraction chamber was designed to extract the drug and determined the API content in the chloroform phase. The model was very complex and chloroform was selected as the organic component of the biphasic medium in order to achieve sink conditions. Regarding the aqueous phase, four different media were used during this work: water, 0.1 N HCl without pH change and aqueous solutions with pH changes of 1.1–7.5 or 1.1–6.8. The test was performed on pure API and on some tablet preparations. On pure API, the flow cells with agitator (magnetic stirrer 50 rpm) showed a 2–3 times higher release rates compared to the two other flow cells without agitator. The authors concluded that this effect was probably due to the better wettability of the API caused by the mechanical action of the stirring process. Regarding the tablet preparations, the most suitable flow cell configuration was variable depending on the formulation tested.

2.2.2.2. Three-phase dissolution-partition systems. In parallel, three-phase dissolution-partition systems appeared. One of these was developed by Fini et al. (1986), who studied the dissolution of non-steroidal anti-inflammatory drugs using the apparatus shown in Fig. 3, consisting of a buffer (25 mL aqueous phase 1) at various pH values (2–4–6.5), an octanol phase (25 mL organic phase) and another buffer at pH 7.4 (15 mL aqueous phase 2). In fact, considering its principle of operation, this system was very similar to the three-phase "rocking apparatus" developed by Doluisio and Swintosky (1964). From the point of view of **Fini et al.**, this three-phase system simulated satisfactorily the lipid layer of the cell membrane and the intra- and extra-cellular aqueous phase around it. In practice, the active compounds were suspended in a dissolution cell connected with aqueous phase 1 by means of a peristaltic pump. At the output of this cell, the dissolution medium and the solute passed through the organic phase. This step allowed the first solute partition from the aqueous phase 1 to the organic phase. Then, this organic phase containing the solute partitioned was pumped to the aqueous phase 2. In this aqueous phase 2, the solute was continuously extracted from the organic phase by a second partition phenomenon. The authors hypothesized that this second partition (from the organic phase into aqueous phase 2) was the main driving force of the whole process because it was irreversible due to the pH value. In practice, the tested molecules were found to be sparingly soluble undissociated forms of weak acids (HA) in aqueous phase 1 at pH 2. This uncharged form was easily able to partition into the organic phase and into aqueous phase 2. However, once in aqueous phase 2 at pH 7.4, the HA species changed into their charged conjugated base A^-, which has a structure unsuited to partition. It is for this reason that the reverse partition in the organic phase was found to be negligible. One important thing to note with this system is that the dissolved molecule must saturate the organic phase before it will be observed in aqueous phase 2. This can take a long time, but the volume of octanol needs to be reduced in order to simulate the absorption membrane correctly. However, **Fini et al.** observed that a powder that dissolved in a three-phase apparatus became available to a greater extent and in a relatively shorter time, than in the case of a simple dissolution in buffered solution or in a two-phase apparatus (buffered solution/octanol). In practice, in this two-phase system, it was found that the back partition could interrupt the dissolution process. This was found to be especially true for ionizable compounds or compounds with limited solubility in organic solvent. Bearing this consideration in mind, the second part of the study involved observing the influence of increasing pH values in aqueous phase 1. Since the tested compounds were acids, when the pH value of the dissolution medium was increased, the amount and rate of dissolution increased as well. However, solutes were also present in their ionized form and the easily partitionable form was consequently reduced. Therefore a conflicting influence of the two steps on the whole process could be expected and the pH value of aqueous phase 1 would need to be adapted in accordance with the pKa of the studied molecule.

2.3. The 1990s: system variants and analysis of more complex formulations

In the 1990s, the biphasic dissolution systems started to be used and adapted for the analysis of more complex formulations of poorly soluble drugs, such as prolonged release formulations, semi-solid lipid/oily matrices, formulations containing surfactant or hydrophilic diluents, *etc.*

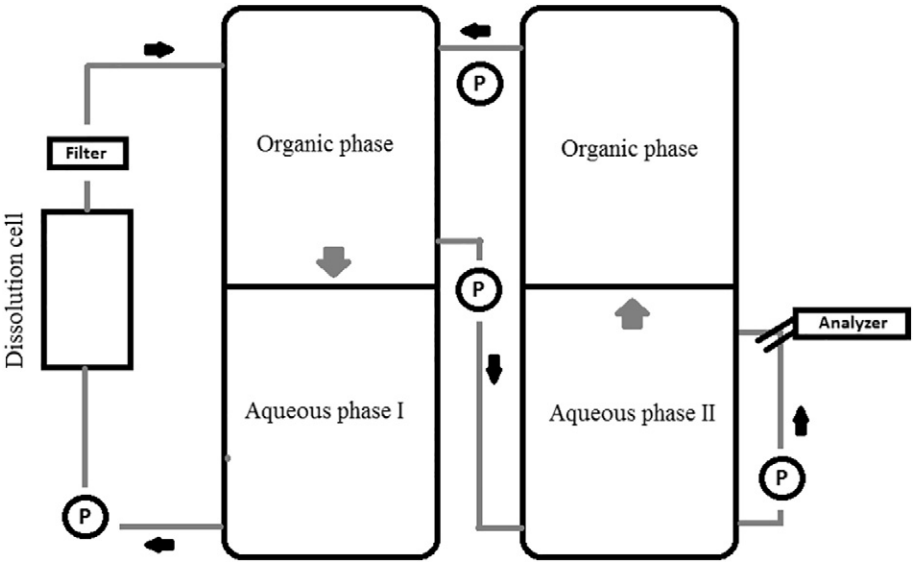

Fig. 3. The three-phase dissolution-partition system developed by Fini et al. (1986), which consists of a buffer (aqueous phase 1) at various pHs, an octanol phase (organic phase) and another buffer at pH 7.4 (aqueous phase 2). These three phases are connected together by peristaltic pumps symbolized by P.

2.3.1. Prolonged release formulations

Chaudhary et al. proposed an acidic biphasic dissolution system using simulated gastric fluid and octanol for insoluble drugs to simulate the flow-through cell method, usually used for prolonged release formulations (Chaudhary et al., 1993). The authors chose nifedipine as the model for their study because its absorption is dependent on the dissolution rate. A first dissolution test was performed in a USP apparatus type 2 with 900 mL of 0.54% sodium lauryl sulfate solution in distilled water. For the second dissolution test, the same apparatus was used but an additional paddle was introduced at the interface of the biphasic system (see Fig. 4). This biphasic system consisted of 500 mL simulated gastric fluid and 400 mL octanol. In the first dissolution system, the results were not reproducible and the variation observed was high. By contrast, the second dissolution system gave reproducible results and could be well correlated with the flow-through type of apparatus. The authors concluded that this biphasic system was for the *in vitro* evaluation of prolonged release formulations in the absence of a flow-through type of dissolution apparatus.

Other modified release (MR) formulations of BCS class II compounds were also studied in subsequent works as for example by Heigoldt et al., 2010. However, this study including a pH shift in the aqueous is detailed later in the text (see Section 2.5.3.).

2.3.2. Lipid-based formulations

Takahashi et al. were the first to describe the testing of a lipid-based formulation using a buffered solution coupled with octanol (Takahashi et al., 1994). The authors had previously carried out dissolution tests on soft gelatin capsules containing an oily, semi-solid matrix, using a paddle method from the Japanese Pharmacopeia and then the bead method reported by Machida et al. (1986). However, both these methods had proved unsatisfactory. By contrast, in their study, Takahashi et al., 1994 obtained a good dissolution pattern corresponding to *in vivo* observations using the rotating dialysis cell (RDC) method (see Fig. 5). Usually, in this method, the same aqueous solution is placed both inside and outside the dialysis cell (internal phase, external phase) (Takahashi et al., 1995). But in the 1995 study, buffers and octanol were used in the internal phase and external phase. A test was performed using octanol as the external phase and a buffer at pH 1.2 as the internal one. The RDC method was shown to simulate the transfer of drugs from the intestinal lumen into the tissues and thus the *in vivo* pharmacokinetics. Indeed, dissolution patterns similar to *in vivo* patterns were observed with this RDC method.

In a study reported by **Kinget and De Greef**, another biphasic dissolution system was used to determine the drug release characteristics of a poorly water soluble drug (methoxsalen) from different semi-solid lipid matrix formulations (Kinget and De Greef, 1995). The biphasic system consisted of 250 mL of 0.2 M phosphate buffer (pH 6) and 250 mL of octanol saturated with water placed in a 1 L round-bottom flask. A paddle was used to homogenize the octanol layer, and the dosage form was placed under a Petri dish (see Fig. 6). The challenge here was to develop a dissolution test for lipophilic compounds, because the accumulation of these compounds leads very quickly to non-sink conditions *in vitro*. However, in the gastrointestinal environment, which functions as a perfect sink, these compounds do not accumulate. The authors compared this biphasic dissolution model with a model based on the Absorption Simulator® by Stricker (Sartorious, Göttingen, Germany), which consists of a donor and an acceptor compartment separated by a membrane. Four different semi-solid lipid formulations were tested and an almost zero-order release and a lag time were obtained for all formulations with this membrane-based model. Both results can be explained

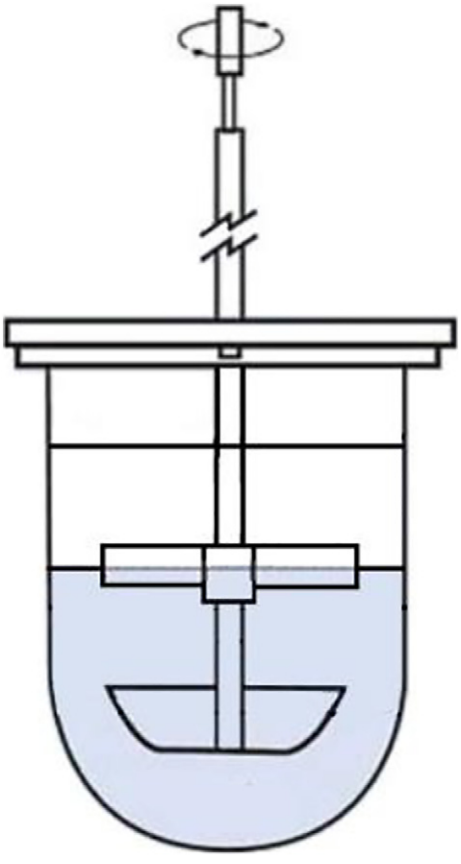

Fig. 4. Biphasic system using a modified USP apparatus II: an additional paddle is introduced at the interface of the two phases (lower aqueous phase in grey and upper organic phase in white).

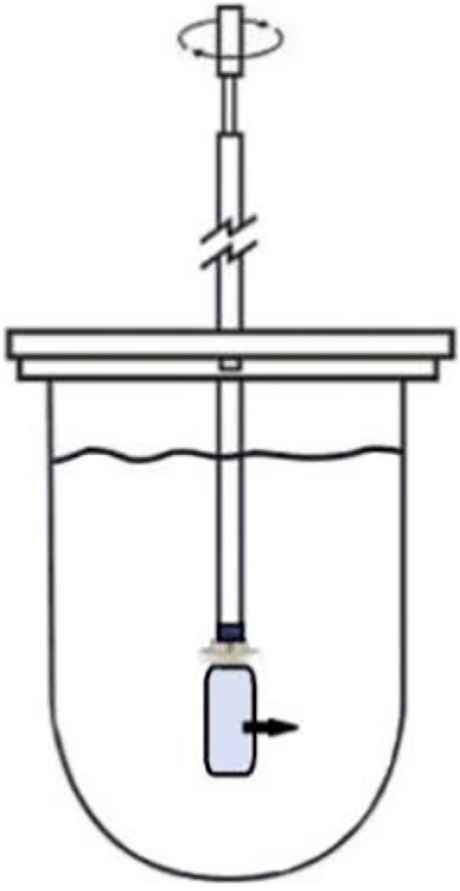

Fig. 5. Biphasic system using a rotating dialysis cell. The organic phase is used as the external phase (in white) and the aqueous buffer (in blue) as the internal phase.

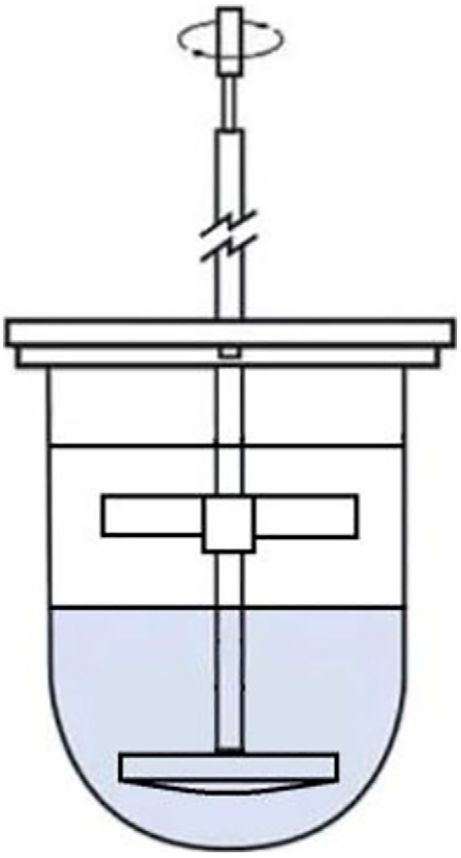

Fig. 6. Biphasic system using a modified USP apparatus II: an additional paddle is introduced to homogenize the organic layer (in white) and the dosage form is placed in the aqueous phase (in blue) under a Petri dish.

by the existence of a rate-limiting barrier due to the presence of a membrane. Only one formulation differed statistically significantly from the other products tested. Thus this test does not seem to be ideal for differentiating between formulation variables, and it is possible that the introduction of an additional process such as membrane transport would have the effect of masking the actual release of the drug delivery form. For the biphasic test, two commercial products were included as reference products. These products had shown different plasma concentrations *in vivo*, with one of the formulations showing a concentration six times greater. This same difference in concentration could also be observed with the *in vitro* biphasic test. More generally, the biphasic test makes it possible to distinguish between several semi-solid lipid formulations and other formulations containing the same active ingredient. Following these results, the authors concluded that it should be possible to establish an *in vitro-in vivo* correlation.

2.3.3. Tablet formulations containing surfactants or hydrophilic diluents

Ngo et al. used the same partition-dissolution method for solid dosage forms with a high content of a very water insoluble drug (artemisinin) (Thu Hoa and Kinget, 1996). The authors showed that this method guaranteed sink conditions in the aqueous dissolution medium for the total length of the experiment. Generally, the types of system employed to maintain sink conditions are large fixed fluid volume, multiple phase (partition method) and continuous flow (flow-through cell). The study by **Ngo et al.** demonstrated that when the official dissolution methods were used, sink conditions did not prevail for the duration of the test for some of the artemisinin formulations tested. Thus the objective of the study was the development of a suitable dissolution method for artemisinin solid oral dosage forms. The volume of the organic phase was 200 mL and the aqueous phase consisted of 800 mL of demineralized water. Water was used as the dissolution fluid given that artemisinin is a neutral compound unaffected by pH. Octanol/cyclohexane (1:1), ethyl acetate and chloroform were tested as potential organic phases. First, the solubility of artemisinin in these different organic solvents was determined in order to evaluate the dissolving ability of each of the solvents for artemisinin. Chloroform was found to be a very good solvent for this API. However, the high density of the chloroform made it impossible to immerse the tablet in the aqueous phase by placing it on the bottom of the round-bottom dissolution vessel. Moreover, the authors did not mention the undesirable physical-chemical properties of this solvent: a relatively high water solubility (0.742 g/100 g·H_2O), and high volatility and toxicity. In practice, both phases were placed in a USP II apparatus and the solid dosage form was encased in a paper filter bag. This bag was fastened to the shaft of the rotating paddle and suspended in the aqueous phase. The dissolution proceeded very slowly with this technique. Following these results, the authors decided to modify the apparatus. The filter bag was replaced by a glass cylinder with dimensions identical to those prescribed for the glass cylinders of the USP disintegration apparatus. The bottom of this glass cylinder was covered with filter paper, and the dosage form was laid inside. This cylindrical device was then attached to the shaft of the disintegration apparatus and was raised and lowered through the water phase (see Fig. 7). During the first hour of the experiment, the artemisinin concentration in the aqueous phase was always below 10% of the solubility with the three tested formulations. These experimental conditions were designed to maintain sink conditions in the aqueous phase. On the other hand, when the same formulations were tested with the classical paddle method or the flow-through cell, sink conditions were not present in the dissolution medium.

A second article by the same primary author (Ngo et al., 1997b) was published a few months later regarding the influence of some formulation variables on the time required to achieve a fast and complete dissolution of artemisinin. The effects of five parameters of the formula were investigated using this *in vitro* dissolution method. The last part of the study was the evaluation of bioavailability (in rabbit plasma) of three different formulations in order to correlate the results with *in vitro* data (Ngo et al., 1997a). Results showed a linear correlation between the mean AUC values and the time required for dissolution of 50% of the tablet content (T_{50}). These *in vivo* data confirmed the validity of the developed partition-dissolution method.

2.3.4. Suspended particles

By the mid-1990s, the *in vitro* release rate of suspended nifedipine particles from the gastrointestinal therapeutic system (GITS), a controlled-release drug formulation, had already been characterized by classical, differential and flow-through type dissolution methods. However, the results had been found not to correlate satisfactorily with *in vivo* drug absorption. In practice, these dissolution methods measured the total amount of nifedipine released from the tablet but did not distinguish between the amount of nifedipine in suspension and in solution. It was for this reason that, as part of their study, **Grundy et al.** developed an *in vitro* two-phase dissolution system to test GITS (Grundy et al., 1997a). This system consisted of 750 mL simulated intestinal fluid without pancreatin (SIF) and a 250 mL octanol phase. Both phases were placed in a modified USP type II apparatus. A single stainless steel cylindrical basket was attached near the base of the steel paddle to form a basket-paddle hybrid stirrer (see Fig. 8). GITS tablets were placed in the basket and lowered into the aqueous SIF. This hybrid system was developed because the authors observed that a paddle stirrer did not displace the GITS tablet adequately and that the basket method did not provide sufficient mixing at the interface between the organic and aqueous phases. The hybrid method demonstrated an improvement, as it enabled a streamlined hydrodynamic flow of dissolution medium adjacent to all sides of the tablet, and adequate stirring of the two phases. Furthermore, the degree of mixing obtained did not allow nifedipine particles to settle at the bottom of the dissolution vessel. With this

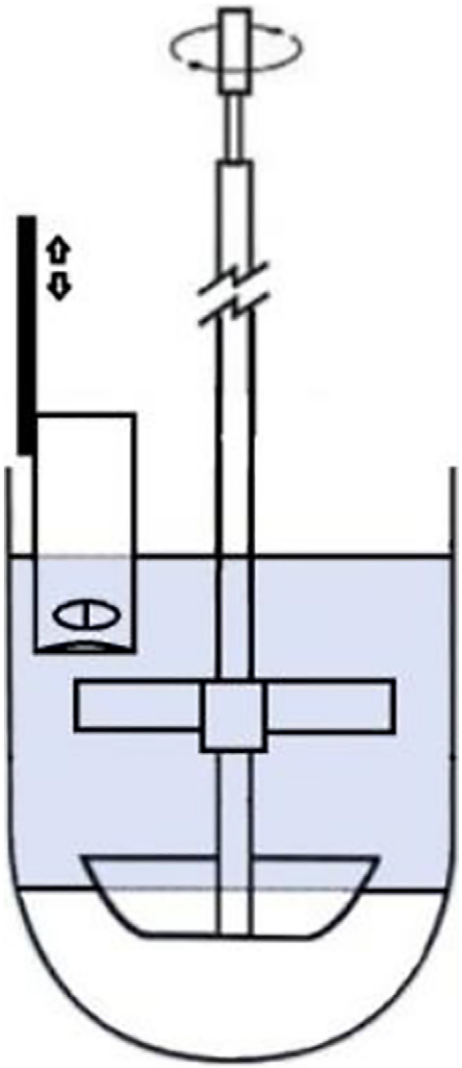

Fig. 7. Biphasic system using a modified USP apparatus II. The paddle is at the interface of the two phases (lower organic phase in white and upper aqueous phase in blue). An additional paddle is introduced to homogenize the upper aqueous phase. A glass cylinder from the USP disintegration apparatus, and containing the dosage form, is placed in the aqueous phase.

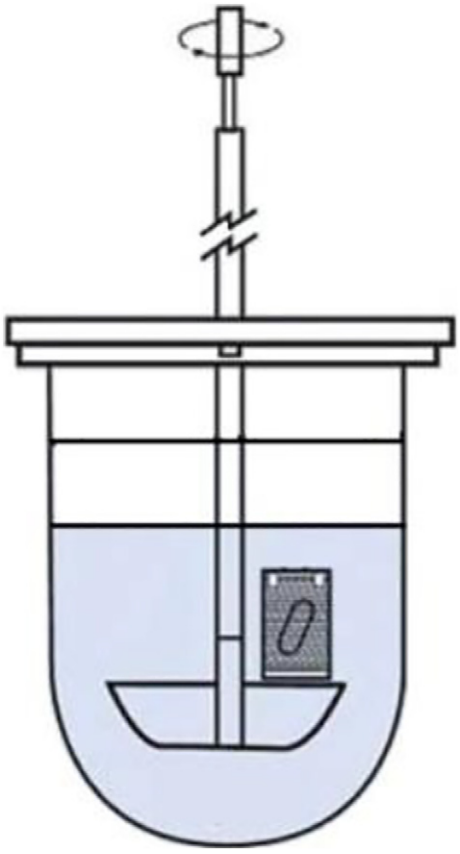

Fig. 8. Biphasic system using modified USP apparatus II: the paddle is immersed in the lower aqueous phase (in blue) and a single stainless steel cylindrical basket is attached near the base of the steel paddle to form a basket-paddle hybrid stirrer.

system, authors determined nifedipine "transfer" rate which was considerably less than the zero-order delivery rate values reported by single-phase methods. This could be explain by the fact that these dissolution methods measure the total amount of nifedipine released from the tablet but do not distinguish between the amount of nifedipine in suspension and in solution in contrast to two-phase method. At the end of this work, the authors concluded that comparison of nifedipine transfer rate-time profiles obtained in this study with plasma nifedipine concentration-time profiles obtained in several clinical trials appeared to suggest an improved IVIVC.

This observation was followed by a second article by the same authors (Grundy et al., 1997b) describing in detail a comparison of the data obtained with the developed two-phase dissolution with pharmacokinetic data obtained from a human clinical trial (12 healthy subjects) with nifedipine GITS tablets. The fraction of nifedipine absorbed-time profiles and the fraction of nifedipine transferred-time profiles, determined using the two-phase dissolution method, appeared to be similar. Thus, this work confirmed the results suggested in the first article by the same authors.

This nifedipine GITS was also studied with a biphasic system (750 mL phosphate buffer at pH 7.5 and 250 mL octanol) in another study published by Pillay and Fassihi (1998). The objective of that study was to investigate the effect of the positioning of the delivery system during a dissolution test on drug release from different controlled release systems including the GITS. Given the low aqueous solubility of nifedipine, the authors chose to use two phase solvent system for this formulation while the two other tested categories of delivery system were tested by a single-phase method. This two phase solvent system was tested in two separate dissolution designs: one where the tablet was dropped into the dissolution medium and another where the tablet was placed above a ring/mesh assembly (see Fig. 9). Results showed that the drug release profiles over the entire dissolution period were identical irrespective of the position of the delivery system. In contrast, the two other categories of delivery system tested by a single-phase method behaved differently: their release capacities depended on the system design and their position in the dissolution vessel. Perhaps these observations could be explained by a greater robustness of the biphasic test compared to other tests and not by a difference in the type of controlled release systems used.

2.3.5. Self-emulsifying drug delivery systems

Self-emulsifying drug delivery systems (SEDDS) offer great potential for the oral delivery of insoluble hydrophobic drugs. However, no official dissolution method for lipid-based formulations is provided by the United States Pharmacopeia, the European Pharmacopeia or other official compendia. There is only the recognition that the liquid nature of capsule contents presents different technological problems due to their ability to form fine oil-in-water emulsions. **Pillay and Fassihi** proposed the development of a modified two-phase dissolution media system for lipid-filled capsules (Pillay and Fassihi, 1999). Once again,

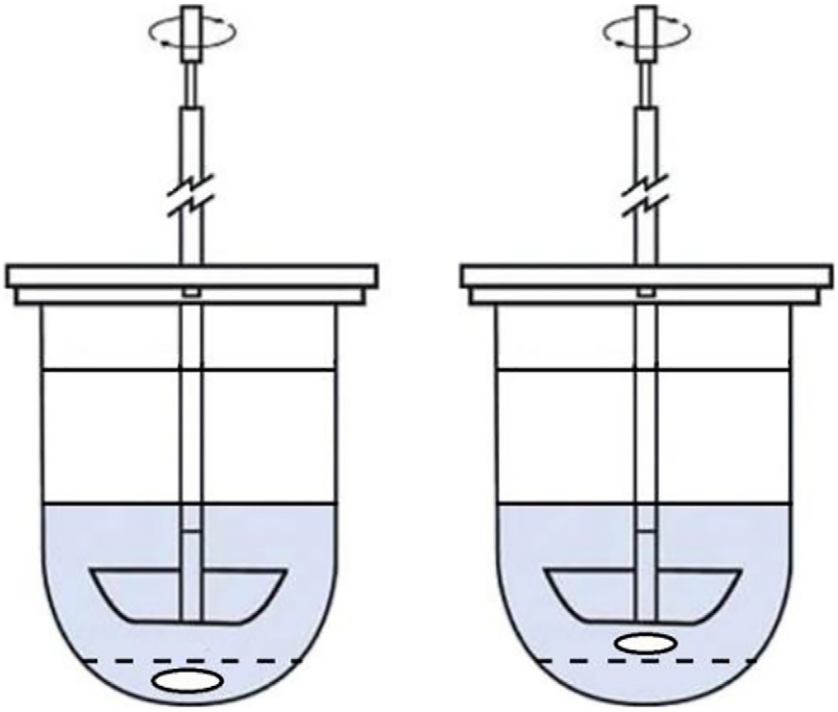

Fig. 9. Biphasic system using USP apparatus II where the tablet is placed below (see left picture) or above (see right picture) a ring/mesh assembly.

nifedipine was chosen as the model compound due to its water insoluble nature and high octanol-water partition coefficient. In order to form the SEDDS and solubilize nifedipine, this API was dissolved in a Gelucire® 44/14 – Labrasol® mixture, two emulsifying agents. Dissolution studies were conducted with a rotating basket (Fig. 2) or a paddle or a modified paddle method with a designed ring/mesh stainless steel device under the paddle (Fig. 9), in order to prevent flotation of the capsule (Pillay and Fassihi, 1998). For all tests, the dissolution medium consisted of a lower phase of phosphate buffer and an upper phase of octanol (100 mL). The volume of aqueous phase was 200 mL, 300 mL or 400 mL according to the design used. The same formulation was tested with four different dissolution designs (see Fig. 10) in order to determine the optimum hydrodynamic and drug transfer conditions. Different positions for the paddle were selected in order to determine the most favorable hydrodynamic condition to facilitate complete drug transport from the lower into the upper phase. The four tested designs were: (a) a rotating basket centrally positioned in the aqueous phase between the boundaries of the organic phase and the bottom of the vessel, (b) a paddle positioned halfway at the air/organic phase interface, (c) a paddle positioned halfway at the organic/aqueous phase interface, (d) a paddle centrally positioned in the aqueous phase between the boundaries of the organic phase and the ring/mesh assembly. A stirring rate of 75 rpm was used in all the designs with the exception of design (d), for which a 100 rpm-stirring was also tested. Results showed that configuration (a), the rotating basket apparatus, did not allow complete drug transfer into the aqueous phase. In fact, after 6 h of dissolution, most of the viscous oily vehicle still remained entrapped within the basket. This could be explained by the fact that the pores of the dissolution basket and the hydrodynamic conditions within the basket were not adapted for oleaginous formulations. With configuration (b), the paddle at the air/organic surface, drug transfer was negligible (5% in 6 h). By contrast, configuration (c), the paddle at the organic/aqueous interface, was effective in encouraging rapid dissolution of the capsule shell (complete drug transfer in 6 h) and subsequent self-emulsification of the formulation. This self-emulsification could be observed by the appearance of opaqueness in the aqueous phase. This

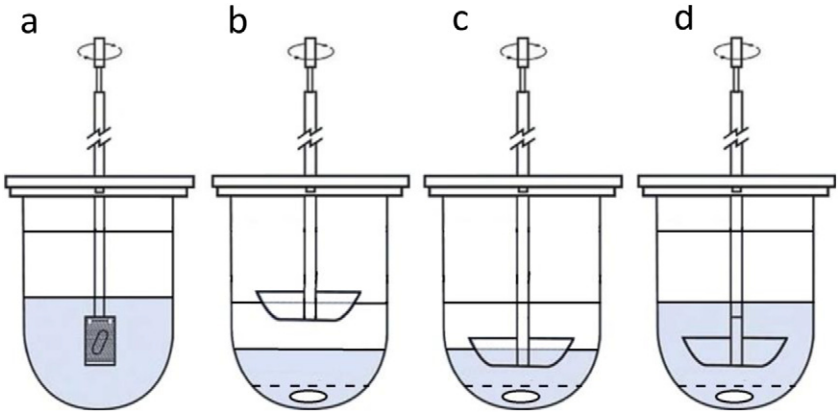

Fig. 10. The four biphasic dissolution systems tested by Pillay and Fassihi (1999): (a) a rotating basket (USP I) centrally positioned in the lower aqueous phase (in blue), (b) a paddle (USP II) positioned halfway at the air/organic phase interface, (c) a paddle (USP II) positioned halfway at the organic/aqueous phase interface, (d) a paddle (USP II) centrally positioned in the aqueous phase between the boundaries of the organic phase and the ring/mesh assembly.

micro-emulsion caused by both lipid excipients with the aqueous phase did not exhibit partitioning into the organic phase even though Gelucire® and Labrasol® when used alone are soluble in octanol. This observation was confirmed by an ultraviolet spectrophotometric scan of pure octanol and octanol samples obtained from the described two-phase dissolution set-up containing blank formulations without the drug. However, the authors did not specify with which dissolution design this experiment was performed. Due to the emulsification properties of Gelucire® and Labrasol®, configuration (c) could have promoted an undesirable emulsion of both phases. In this case, the solubility of the API in this aqueous phase would be not relevant due to the presence of octanol and the drug transfer into the organic phase would be also modified and irrelevant. To ensure that this was not the case here, it would have been interesting to determine the API concentration *versus* time in the aqueous phase too. The risk of emulsion was less present with configurations (d) and (e), where the paddle was centrally positioned in the aqueous phase. For both these configurations, the agitation speed of 100 rpm seemed to be more appropriate for lipid-filled products because the reproducibility in drug transfer rates was found to be better than at 75 rpm.

2.4. The 2000s: modeling, system improvements

2.4.1. Modeling for sparingly soluble drugs

Grassi et al., 2002, proposed the development of a mathematical model able to describe the partitioning kinetics of a drug between a polar (water buffer) and an apolar (octanol) liquid phase, particularly for use with sparingly soluble drugs (Grassi et al., 2002). A detailed study of drug partitioning between a water buffer and octanol was greatly needed at that time, given the increasing number of applications of biphasic tests or partition tests. Drug transfer between two phases had already been experimentally and mathematically analyzed. However, the existing models were being used for describing the partitioning of sufficiently soluble drugs. It was unlikely that these old models could be applied effectively in the case of sparingly soluble drugs in one or both phases. It was for this reason that the authors proposed a model that would properly take into account this solubility problem. In this model, it was supposed that drug fluxes occurring between the polar and apolar phase depend also on drug solubility, and not only on both the kinetics constants and the instantaneous drug concentration in the two phases. Piroxicam (low water soluble) and nimesulide (very low water soluble) were chosen as the model drugs due to their low water solubility and to the fact that their water solubility is pH dependent. Two different kinds of experimental conditions were considered: a first case with a water buffer at pH 1.2 and a second case with a water buffer at pH 7.5. Initially, the octanol phase (50 mL) was drug free, while the aqueous phase (150 mL) contained a known amount of drug, leading to a concentration gradient between the two phases. The decrease in drug concentration in the aqueous phase was monitored with an on-line UV spectrophotometer. The obtained results were compared to predictions made with both the old model and the new model. With the old model, the meaningless prediction clearly revealed its unsuitability in describing the oil-water partition of sparingly water-soluble drugs. By contrast, predictions made by the new model showed very good agreement with experimental data for both APIs and both tested pH values.

2.4.2. Selection of the best organic solvent

In a study presented by **Gabriëls and Plaizier-Vercammen**, the best organic solvent was determined for the evaluation of artemether and dihydroartemisinin tablets (Gabriëls and Plaizier-Vercammen, 2004). The study was based on the observations made by **Ngo et al.** in 1996 (Thu Hoa and Kinget, 1996), but this time, the system used the usual position of the paddle, and the dissolution of the tablet was performed on the bottom of the dissolution vessel. In order to achieve this, the selected upper organic phase needed to have a density lower than 1 (conversely chloroform). Furthermore, its volume needed to be kept as low as possible to allow direct measurement by HPLC (sufficient concentrations), but at least 100 mL volume was required in order to allow space for sampling. In order to select this organic solvent, API solubility tests were performed in seven different solvents: cyclohexane, chlorobutane, isooctane, n-Butanol, n-Hexane, petroleumether and methyl-tert-butylether. Isooctane (100 mL) was chosen as the most suitable extraction solvent for artemether tablets. For dihydroartemisinin tablets, it was found that sink conditions could be maintained in the organic solvent only with chlorobutane (150 mL). Finally, the discrimination ability of the method was confirmed with self-made tablets of different crushing strength. The authors concluded that in contrast with the approach of Ngo et al. (Thu Hoa and Kinget, 1996), these methods allowed samples to be taken easily from the upper phase. However, the authors did not mention volatility, toxicity or the potential miscibility between the aqueous phase and the various organic solvents tested, when in fact these factors could have strongly influenced the dissolution profiles obtained.

In studies that came after the Gabriëls and Plaizier-Vercammen (2004) study, the organic phase most often used in biphasic systems was octanol. Octanol is also the solvent used to calculate the partition coefficients (log P) of drugs. Indeed, this solvent has desirable physical-chemical properties, including: (1) being practically insoluble in water (0.05 g/100 g H_20), (2) being less dense than water, thereby allowing ease of sampling, (3) presenting low volatility, meaning that octanol will not readily evaporate at 37 °C and that a relatively constant upper phase volume can thus be maintained, and (4) the fact that, generally, BCS class II compounds possess an acceptable solubility in octanol to reach sink conditions with acceptable volumes (Grundy et al., 1997a; Heigoldt et al., 2010).

2.4.3. Paddle apparatus combined with flow-through cell apparatus

Vangani et al., 2009 published for the first time a study where a flow-through cell apparatus (USP IV) was coupled with a paddle dissolution apparatus (USP II) and a biphasic dissolution medium (Vangani et al., 2009). The flow-through cell apparatus was used because this apparatus generates within the cell similar hydrodynamics to that found in the gastrointestinal tract (GIT). The limitation of this technique for poorly water-soluble drugs is that a large volume of dissolution medium is required to maintain sink conditions. This results in drug concentrations that are below the limit of detection. In fact, in the Vangani et al. (2009) experiment, using the USP IV apparatus alone did not provide any meaningful data, due to the low solubility of the chosen model compounds. Dissolution rate determination experiments were also performed on the same model compounds using the USP II apparatus and the same biphasic dissolution medium. This time, the release rate was much higher than expected and the resulting profiles showed no discrimination between the different formulations. With this new configuration, the cells in the USP IV system were used to hold the formulations, while dissolution vessels were used to retain the dissolution medium and maintain it at 37 °C. In the USP II apparatus, an additional small paddle (adjustable) was mounted perpendicularly on the classical paddle, in order to obtain sufficient hydrodynamics in both phases. Once the formulations were placed in the USP IV cell, the aqueous phase of the biphasic dissolution medium was pumped through the flow-through cells and then returned into the USP II apparatus (see Fig. 11). The experimental parameters optimized during the development of the method were the choice of the aqueous phase (pH 6.8 phosphate buffer) and the organic phase (nonanol or a 1:1 mixture of nonanol and cyclohexane), the volume of the two phases (100 mL to 500 mL for each), the type of paddle, the distance between the dual paddles, the position of the fiber optic probe, the position of the filter, the paddle speed (0 rpm to 100 rpm), the flow rate (0 mL to 35 mL/min) and the dimensions of the USP IV cell. A phosphate buffer at pH 6.8 was used as the aqueous phase because of its physiological relevance: most of the absorption of a drug is known to occur in the small intestine (Pang, 2003). Octanol is recognized to mimic the action of fatty tissues inside

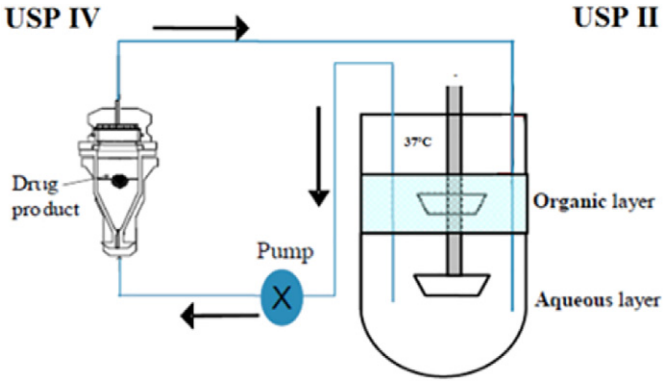

Fig. 11. Biphasic system using USP apparatus II combined with USP apparatus IV in a closed loop configuration.

the body, but due to its nauseating smell, it was decided to replace this in the organic phase with a similar solvent, nonanol. However, using nonanol as the organic component of the biphasic medium resulted in the formation of an emulsion at the interface of the biphasic medium. By contrast, using a mixture of nonanol and cyclohexane resulted in a well-defined interface and no error in optical measurements. Finally, the volume of the dissolution medium was limited to 500 mL (300 mL of aqueous phase and 200 mL of organic phase) because of its physiological relevance (Mudie et al., 2010). The authors observed that the use of a single paddle (stirring in the aqueous phase only) caused a negligible partitioning of the drugs into the organic phase. Regarding the paddle speed, a lower paddle speed (less than 60 rpm) was found to lead to lower drug partitioning, while a higher speed (100 rpm) resulted in the formation of an emulsion at the interface of the biphasic medium. So, a paddle speed of 75 rpm was preferred. Two different tablet and capsule formulations (slow releasing and intermediate releasing), with known *in vivo* exposure from monkey studies, were tested with this optimized system. The model drug (AMG 517) chosen for the development was a BCS class II compound with no pKa in the physiological range. The *in vitro* release profiles obtained from this optimized dissolution model enabled the formulation changes to be distinguished. In fact, excellent rank order correlation was achieved between the *in vitro* release and the *in vivo* absorption of the chosen model drug. The *in vitro* dissolution model was then further evaluated using three commercial formulations. Here, the model successfully discriminated between the bioequivalent and non-bioequivalent formulations. In conclusion, the Vangani et al. (2009) study demonstrated IVIVCs for several poorly soluble compounds, and for different kinds of dosage forms and formulations.

2.5. The 2010s: discriminatory power, bio-relevance, pH-adjusted systems, precipitation/supersaturation visualization, IVIVCs

2.5.1. Discriminatory power and bio-relevance of biphasic systems compared to common dissolution media

In one of their studies, **Phillips et al.** emphasized the need for a biphasic dissolution system that would be sensitive enough to detect changes in the release rates of controlled-release formulations containing different HPMC loadings in a manner not possible with traditional aqueous media (Phillips et al., 2012). In order to assess the relative utility of the biphasic technique, the results were compared with those generated in dissolution media incorporating inorganic salts, surfactants and co-solvents in order to reach sink conditions. In practice, three nifedipine formulations were tested in monophasic (600 mL water) and biphasic (600 mL water, 350 mL octanol) dissolution media and in conventional aqueous medium (900 mL) using a paddle apparatus (USP type II). These formulations differed in their polymer loading (HPMC 10, 20 or 40% w/w). An increase in the polymer loading amplifies the tortuosity of the matrix, and therefore decreases the release rate of a poorly soluble drug. The three tested formulations were characterized as slow-, medium- and fast-release tablets. Results showed that dissolution in monophasic media (600 mL water) failed to provide sink conditions. All formulation dissolution profiles were found to reach a plateau at approximately 28% and all profiles were statistically similar. The authors found that it was impossible to discriminate between the formulations in water. Dissolution was then performed in the biphasic medium and a clear discrimination between all formulations was found to be possible. Moreover, complete drug dissolution was observed. The last part of the study involved evaluating the ability of aqueous media with different ionic strengths, surfactant concentrations or a hydro-alcoholic medium to provide the same degree of discrimination. Given their widespread use in dissolution media, dibasic sodium phosphate (10 mM or 150 mM) and sodium dodecyl sulfate (SDS) (5%) were chosen as the salt and surfactant, respectively. The final pH of the dissolution media (900 mL) was fixed at pH 6.8, due to its physiological relevance. Dissolution in phosphate buffers failed to provide sink conditions and it was not possible to differentiate the formulations. The addition of SDS afforded complete dissolution because of an increase in drug solubility. Dissolution in buffer with 5% SDS enabled discrimination of the slow-release formulation, while the other two remained statistically similar to each other. Even when the addition of surfactant enabled all the drug to dissolve, dissolution was no longer fully predictive. Finally, the ability of a dissolution medium of 60/40 water/ethanol (sink conditions) to discriminate formulations was evaluated. This hydro-alcoholic medium enabled all formulations to be discriminated in a manner only obtained previously using the biphasic medium. However, the discrimination was not as powerful as that produced with the biphasic model. Moreover, the addition of such solvents had no physiological relevance.

In another study, **Pestieau et al.** investigated various *in vitro* dissolution tests in order to select one that would be able to discriminate different fenofibrate formulations and that would be as biorelevant as possible (Pestieau et al., 2016). In practice, three fenofibrate formulations, for which *in vivo* data are available in the literature, were tested using different dissolution tests: a test under sink conditions (3 L 0.1 M HCl + 1% polysorbate 80), different tests under non-sink conditions in non-biorelevant (300 mL 0.1 M HCl) and biorelevant media (300 mL FaSSGF, FaSSIF and FeSSIF) and two biphasic dissolution systems (300 mL 0.1 M HCl + 200 mL octanol in apparatus type II alone or apparatus type II combined with type IV). The results of the study showed that the single phase dissolution tests (sink, non-sink and biorelevant) were highly dependent on the type of drug formulation and that they consequently provided poor biorelevance. Moreover, in the different tests under non-sink conditions, the dissolution medium became rapidly saturated, thereby limiting the dissolution process and the comparison of the formulations. The biphasic systems were used to bypass this limitation. However, the utilization of apparatus type II alone was found to be inadequate in this case. Indeed, the undissolved hydrophobic fenofibrate particles rose to the interface of the two phases and dissolved in the organic solvent without a previous dissolution in the aqueous phase. In order to solve this problem, the authors coupled apparatus type II with apparatus type IV (as shown in Fig. 11). This meant that the undissolved API particles remained trapped inside the dissolution cell and that only fenofibrate dissolved in the aqueous phase was available to move into the organic phase. In this configuration, the biphasic system was able to discriminate the formulations. Moreover, after comparison with the published *in vivo* data, the biphasic system appeared to be the most biorelevant of all the tested dissolution systems.

2.5.2. Biphasic system and physiological relevance studies

Mudie et al. performed a mass transport analysis regarding the partitioning kinetics of drug substance solutions from the aqueous phase into the organic phase of a two-phase dissolution apparatus (Mudie et al., 2012). Furthermore, the authors demonstrated the

effectiveness of this theory in predicting the *in vitro* partitioning profiles of three BCS II weak acids (ibuprofen, nimesulide and piroxicam) in three different types of *in vitro* two-phase dissolution apparatus. This proved that their model was suitable for many drugs and experimental set-ups. More importantly, the authors discussed how a two-phase apparatus could be scaled to reflect *in vivo* absorption kinetics, and for which drug substances the two-phase dissolution systems might be appropriate tools for measuring oral bio-performance. During the study, various volumes of buffer (150, 250 mL), volumes of octanol (150, 200, 250 mL), impeller rotational speeds (40, 50, 75, 77 rpm), pH values and doses (2.5, 3.75, 4, 5, 6.25, 12.5, 15.0 mg) were used for the experiments. Three different types of two-phase dissolution apparatus were tested. At the start of the experiment, the drug in solution was injected into the aqueous buffer. The concentration in each phase was measured with UV fiber optic probes as a function of time. The purpose of these case studies was to demonstrate how a two-phase system could be set up (the vessel size, aqueous volume, organic volume and dose that would be required) to be physiologically relevant when conducting an experiment using a solid dosage form. Using this approach, the saturation conditions in the aqueous medium of the two-phase system would be expected to be similar to the saturation conditions *in vivo*, and the *in vitro* partitioning rate would be expected to be similar to the *in vivo* absorption rate, facilitating potential IVIVCs for some drug candidates. However, in order for a drug substance to be suitable for the two-phase system, it would need to have a relatively high absorbed fraction *in vivo*, and to be relatively hydrophobic. In addition, the absorbed fraction of the drug would need to be similar to its bioavailable fraction (*i.e.* offering low first-pass metabolism and gut metabolism/degradation). Moreover, the authors also gave some tips for gaining optimal results from such a two-phase apparatus. They recommended that the solubility and the drug dissolution rate in the chosen aqueous media (*e.g.* surfactant level, buffer species, constant or variable pH) should both be compared in the chosen buffers saturated with organic medium. This was because the presence of organic medium in the buffer containing surfactant could have effects on the solubility and dissolution rates as well as on the rate and extent of partitioning into the organic medium.

This recommendation was followed in a publication by Thiry et al. (2016). In that study, three formulations containing itraconazole (a BCS class II weak base) were assayed in seven different conditions (using different USP apparatuses and media) in order to select a suitable *in vitro* dissolution test for itraconazole-based solid dispersions. In these various conditions, a biphasic dissolution, which combined USP apparatus types II and IV in a closed-loop configuration (Fig. 11), was tested. Regarding the total quantity of API within both aqueous and organic phases, only 40% of the itraconazole was released from the commercialized product Sporanox® with this test. The authors speculated that the presence of less than 1% octanol dissolved in the aqueous medium could be responsible for this phenomenon. In order to confirm this hypothesis, the C_s of itraconazole from Sporanox® was measured in 0.1 M HCl and in 0.1 M HCl previously saturated with octanol, and this C_s was found to decrease by half. Moreover, an open-loop biphasic dissolution test (see Fig. 12) was performed in order to verify that the octanol dissolved in 0.1 M HCl was the reason why only 40% had been released from Sporanox®. This was the first time that this open system had been described in the literature. The system had actually been chosen in order that only fresh medium (octanol free 0.1 M HCl) would come into contact with the formulation. At the beginning of the test, the dissolution media consisted of 200 mL 0.1 M HCl and 400 mL octanol. Fresh 0.1 M HCl was pumped at a flow rate of 1.5 mL/min into the aqueous phase so that it reached approximately 800 mL at the end of the test (after 6 h). Using this open-loop system, up to 90% of the itraconazole was able to be released from Sporanox®. These experiments confirmed that the presence of surfactants, but also of organic solvents (even in very small amounts) could dramatically influence the *in vitro* release profiles of a drug. Furthermore, increasing the

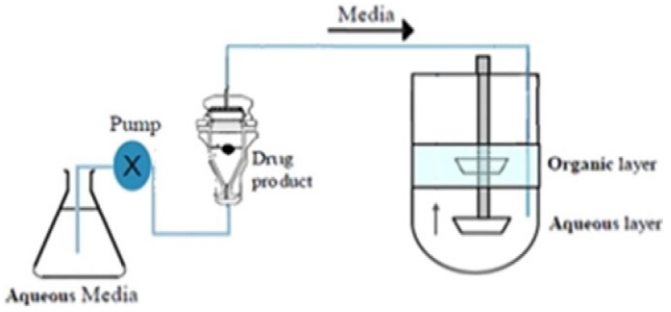

Fig. 12. Biphasic system using USP apparatus II combined with USP apparatus IV in an open loop configuration.

aqueous phase constantly could also influence these release profiles. In the open-loop configuration, conversely to the closed-loop configuration, the aqueous phase which came into with the product was free of dissolved API. This could increase the dissolution rate and reduce the risk of dissolution medium saturation. The authors concluded that choosing an *in vitro* dissolution test to evaluate the performance of one formulation against another could be very difficult and that, in order to be meaningful, the test would have to be very specific to the API and the excipients contained within the formulations.

In addition to the possible impact of surfactants on dosage form performance in the two-phase apparatus, the integrity of the aqueous-organic interface also needs to be considered. Research has shown that long-chain alcohols, such as octanol, can form mixed micelles with ionic surfactants (Moya and Schulz, 1999). However, Shi et al. (2010) successfully performed two-phase experiments at polysorbate 80 concentrations as high as 0.23 mM (*i.e.* 20 times the CMC). **Mudie et al.** demonstrated the formation of a clear, distinct aqueous-organic interface using FaSSIF and FeSSIF and 0.7 mM SDS in a USP II apparatus at 25, 50 and 75 rpm (Mudie et al., 2012). The interface was found to be somewhat obscured at 100 rpm. However, the authors recommended running USP II two-phase experiments at speeds lower than 75 rpm in order to minimize the formation of a vortex. Taking into account the results of these studies of biphasic systems, we could conclude that future studies would need to assess the potential applicability of two-phase systems based on key drugs (acid-base characteristics, particle size, pH-solubility profile, human jejunal effective permeation rate, dose, etc.) and the physicochemical properties of the excipients.

2.5.3. Biphasic dissolution combined with pH-gradient

In order to overcome the limitations of dissolution testing at a constant pH for MR formulations, **Heigoldt et al.** developed a modified USP apparatus II combining biphasic dissolution with a pH-gradient in the aqueous dissolution medium (Heigoldt et al., 2010). Using this approach, the dissolution measurements of pH-dependent poorly soluble drugs enabled an improved forecast of *in vivo* behavior and bioavailability compared to conventional dissolution testing at pH 1, pH 5.5 or pH 6.8. Nevertheless, there are enormous challenges in MR drug development regarding the establishment of proper dissolution test conditions for a predictive *in vitro* test because of the variability in physiological conditions of the GIT, such as pH, intestinal fluids and transit time. Particularly for drugs with pH-dependent solubility, dissolution needs to be performed with a series of different pH values of various media in one experiment. In order to set up consecutive pH changes, more advanced methods, such as USP apparatus III (reciprocating cylinder) and USP apparatus IV (flow-through cell) have been proposed. In the **Heigoldt et al.** study, the developed model combined consecutive pH changes in an aqueous dissolution medium with a biphasic approach, in order to maintain sink conditions. Several MR formulations of two weakly basic BCS II compounds with pH-dependent solubility were investigated *in vitro* and *in vivo*. The pH-adjusted biphasic dissolution system consisted of a conventional USP apparatus II

coupled with an automated pH titration and controlling device. Tests were performed in vessels with 500 mL of sodium dihydrate phosphate-buffered aqueous phase and 100 mL of octanol. In order to ensure biorelevant pH conditions throughout the test, a sequential pH-gradient was applied in the aqueous phase to simulate transit through the GIT in the fasted state with residence times of 1 h in the stomach and 4 h in the small intestine. At the beginning of the dissolution test, the aqueous medium was set at pH 2 for 1 h. Subsequently, the pH was adjusted to pH 5.5 within 5 min. After a total dissolution time of 3 h, the medium was readjusted to pH 5.5. Finally, after a total dissolution time of 5 h, the pH was set to pH 6.8 for the remaining time of the experiment, simulating further intestinal and colonic transit. The method was then compared to a conventional *in vitro* dissolution study. That study was carried out using USP apparatus I (basket apparatus) using 900 mL of 0.1 M HCl media or of USP 0.05 M sodium phosphate-buffered media at pH 1, pH 5.5 or pH 6.8. Cremophor® RH 40 (0.5–2%) was added to phosphate buffered dissolution media of higher pH in order to guarantee sink conditions. For the *in vivo* study, data regarding individual plasma concentration *versus* time were collected from healthy volunteers in the fasted state. It was shown that the ranking of release profiles obtained from dissolution media at a constant pH was inconsistent with their *in vivo* performance. However, the dissolution results obtained from pH-adjusted biphasic dissolution turned out to be qualitatively predictive for the *in vivo* performance of several formulations of two drugs. The authors conclude that this model could be a useful tool during the early MR development of BCS II drugs, especially if their solubility is pH-dependent.

Several years later, **Frank et al.** improved this system by developing a miniaturized biphasic system (miBIdi-pH) with pH shift (Frank et al., 2014). This equipment, which contained an aqueous phase (50 mL), whose pH was shifted during the experiment, covered by a lipophilic phase (15 mL of octanol), was used to study the kinetics of supersaturation and precipitation of dipyridamole as well as the kinetics of absorption. The pH shift from acid to neutral imitates the pH values in the stomach and in the small intestine. Indeed, in the acidic environment of the stomach, weak bases are fully ionized and thus well soluble. However, during their passage into the small intestine, after a short period of kinetically unstable supersaturation, the weak bases precipitate until they reach their thermodynamic equilibrium solubility. However, it is well known that *in vitro* dissolution studies with a pH shift may overestimate the precipitation tendency with respect to *in vivo* relevance. Firstly, in the Frank et al. (2014) study, dipyridamole was used as a model of a weak base for a comparative study of the mini-scale single phase dissolution model with pH shift and the miBIdi-pH (biphasic model). The kinetics of supersaturation and precipitation of crystalline API were evaluated with both models. With the miBIdi-pH, the supersaturation in the aqueous phase was more pronounced and the timeframe until the amount in solution decreased to the solubility equilibrium was prolonged. The single phase dissolution model with pH change clearly overestimated the influence of precipitation on *in vivo* absorption after a pH shift when compared with published *in vivo* results. In contrast, the results from the miBIdi-pH revealed a less marked precipitation, which was in better correlation with these *in vivo* data. In a second case study, the performance of four formulations of the weak base (BIXX) was also appraised with both dissolution approaches. The *in vitro* results were correlated with data from a pharmacokinetic study in dogs ($n = 5$). Correlation of relative fraction absorbed with relative fraction dissolved in octanol phase revealed a level A correlation with an overall $r^2 = 0.95$. However, the statistical evaluation of the IVIVC was not carried out in accordance with the acceptance criterion of the FDA (evaluation of the internal or external predictability of the model). The miBIdi-pH studies revealed a formulation-dependent extent and stability of supersaturation in the aqueous phase as well as a formulation-dependent concentration of BIXX in solution in the aqueous phase at the end of the experiment. The *in vivo* studies of BIXX showed that the absorption must be driven by two different absorption processes (two peaks in the plasma profile). The first absorption seemed to be determined by the supersaturation generated through the pH shift from acidic to neutral (gastric transit), and this was in good agreement with the results from the miBIdi-pH studies. The second absorption process, leading to the second maximum, was hypothesized to be caused by dissolution of the precipitated BIXX. This kinetics of precipitation and re-dissolution was reflected in the miBIdi-pH studies. In contrast, the single phase dissolution profiles were not predictive of the *in vivo* performance. Neither the ranking of formulations nor the dissolution rate was in accordance with the *in vivo* results. In previous studies investigating the correlation of a biphasic dissolution method with *in vivo* performance, no pH shift was performed and thus biphasic methods had not been specially adapted for basic compounds.

The most recent improvement of this system (miBIdi-pH-II) was made by Locher et al. (2016). The aim of this improvement was to mimic *in vivo* situations more realistically and to increase the robustness of the experimental model. In order to achieve this, six dissolved BCS class II APIs (telmisartan, dipyridamole, ibuprofen, griseofulvin, itraconazole and fenofibrate) were tested and the influence of experimental model parameters including various excipients, API concentrations, and the dual paddle and its rotation speed was investigated. Even though this study gave encouraging results, the model would need to be reevaluated on a larger dataset and ultimately correlated to *in vivo* data.

2.5.4. Biphasic system to analyze supersaturable self-emulsifying drug delivery systems

Shi et al. published an application of a biphasic test for the characterization of immediate release formulations of celecoxib (Shi et al., 2010). The biphasic *in vitro* test method used both the USP II and IV apparatus (Fig. 11). Three celecoxib formulations were investigated: (1) a commercial Celebrex® capsule, (2) a solution formulation containing a cosolvent and a surfactant and (3) a supersaturable self-emulsifying drug delivery system (S-SEDDS). These formulations were chosen because of the availability of their human pharmacokinetic data. For comparison, these formulations were also evaluated using a single aqueous medium under sink conditions (USP II with 900 mL of pH 6.8 phosphate buffer containing 2% (w/v) SDS) and another under non-sink conditions (USP IV with 250 mL of pH 6.8 phosphate buffer). The biphasic dissolution system consisted of 250 mL of pH 6.8 phosphate buffer and 200 mL of octanol in a USP II vessel. A volume of 250 mL for the aqueous phase was selected to represent the volume of GI fluid in human subjects. The choice of octanol volume was based on two considerations: that it would ensure a sink condition for the model drug, and that it would be suitable for use in a the USP II type vessel. Results showed that the API concentration profiles from the three formulations under sink conditions were very similar and that all the formulations displayed a rapid dissolution. During the test under non-sink conditions, the Celebrex® capsule displayed a consistent low concentration during the test. The authors concluded that this clearly indicated that the dissolution of this capsule was limited by its solubility. The concentration of API from the solution formulation and the S-SEDDS formulation was found to increase quickly up to a maximum and then to gradually decrease. In fact, both formulations produced a supersaturated state of API and they then precipitated. In the biphasic dissolution test, the Celebrex® capsules yielded a continuously low API concentration in the aqueous medium, similar to that obtained in the single phase dissolution test under non-sink conditions. This concentration-time profile appeared unaffected by the presence of octanol. A low API concentration in the octanol was consistently observed from Celebrex® capsules. The authors presumed that this was indicative of a steady state occurring between the dissolution and partition processes. The solution formulation generated high API concentrations in the aqueous phase (similar to those obtained under non-sink conditions). The API concentration in the octanol phase was slightly higher than that of the Celebrex® capsules. In contrast, the S-SEDDS formulation showed

noticeable higher API concentrations in the aqueous medium compared to those in the single phase dissolution under non-sink conditions. The concentration in octanol was also significantly greater than that observed with the solution formulation and with the Celebrex® capsules. In the *in vivo* study, the relative AUC and C_{max} showed the following trend: S-SEDDS ≫ solution ~ capsule. As distinctly different pharmacokinetic results were obtained in human subjects between the three formulations, the single phase dissolution test under sink conditions appeared to be nondiscriminatory. It is known that the sink condition disallows the establishment of supersaturation. This problem may be overcome by assessing levels of drug release in the test medium under non-sink conditions. However, this test condition has a detrimental influence on drug precipitation. In the Shi et al. (2010) study, the S-SEDDS formulation showed the metastable supersaturated state of API in the aqueous phase. This was stabilized by the organic phase, allowing better characterization of the formulation as compared to the single aqueous phase dissolution test. Finally, a rank order correlation among the three formulations was obtained between the *in vitro* AUC values from the octanol phase and the *in vivo* mean AUC (or C_{max}) values.

A similar biphasic dissolution system was used by **Pestieau et al.** for an optimization study of a fenofibrate lipid-based solid dispersion (Pestieau et al., 2015). In that study, a PGSS process (Particles from Gas Saturated Solutions) was optimized to produce a solid dispersion containing fenofibrate and Gelucire® 50/13. The selected response for this optimization was the *in vitro* drug dissolution profiles obtained using a biphasic dissolution system. As in the Shi et al. (2010) study, the biphasic system consisted of a slightly modified USP II apparatus combined with the USP IV apparatus (Fig. 11). The aqueous phase (300 mL 0.1 M HCl) and the organic phase (200 mL octanol) were placed in the USP II dissolution apparatus and agitated at 50 rpm. In the USP IV apparatus, the flow rate was 8 mL/min. The dissolution profile obtained in the organic phase was selected as the response for the design of experiments because organic concentrations had been most often used in previous *in vitro-in vivo* correlation studies. However, the dissolution profiles obtained in the aqueous phase also gave very important information, such as the establishment of API supersaturation due to the formulation. Drawing on these observations, the authors concluded that the physical mixture of fenofibrate and Gelucire® did not allow the establishment of supersaturation, while the classical micronized solid dispersion or the PGSS formulation did allow it. The maximum supersaturation ratio (SR^M) was then calculated for this type of supersaturated formulation. This SR^M has been proposed as a measure of the likelihood of drug precipitation and it can thus serve as a potential indicator for the *in vivo* performance of a drug (Thomas et al., 2014). Regarding this supersaturation (its duration and the SR^M values), the improvement of fenofibrate oral bioavailability is likely to be more pronounced with the PGSS formulation than with the classical solid dispersion formulation. The same authors later confirmed this hypothesis with an *in vivo* pharmacokinetic study in pigs (Pestieau et al., 2017).

The most recent adaptation of this biphasic system combining USP II and IV apparatus was performed by Shi et al., 2016 to analyze a range of different formulations of a weak acid compound with extremely low intrinsic aqueous solubility (ABT-072). This test used two aqueous dissolution media: one at pH 2 (dosage form soaked for 30 min in approximately 12 mL of 0.01 N HCl) and one at pH 6.5 (starting the circulation of phosphate buffer at pH 6.8 with a flow rate of 5 mL/min) in a sequential manner to simulate the transition of drug within the gastrointestinal tract (Shi et al., 2016). In this way, the concentration profiles observed in octanol represented the amount of drug absorbable as a result of the compromise between the dissolution, precipitation and partition processes. During the study, the authors also made many observations regarding precipitation of the drug in the aqueous medium and the degree and duration of supersaturation based on the nature of the excipients. At the end of the study, this allowed for the establishment of a quantitative relationship (level C IVIVC) between *in vitro* partition profiles in octanol and *in vivo* pharmacokinetic profiles in dogs and human subjects.

2.5.5. Biphasic system and level A IVIVC in accordance with the FDA acceptance criterion

It was only in 2015 that a study of the use of the first level A IVIVC in accordance with the acceptance criterion of the FDA with a biphasic dissolution system was published. **Al Durdunji et al.** studied the dissolution of dispersible tablets of deferasirox, a new BCS type II compound, in a biphasic dissolution medium using a flow-through dissolution apparatus coupled to a paddle apparatus (Fig. 11) (Al Durdunji et al., 2016). The experimental parameters associated with dissolution were optimized to discriminate between different formulations. A volume of 500 mL for the organic phase was chosen to allow the establishment of sink conditions during dissolution testing of 500 mg API tablets. An aqueous phase volume of 300 mL was selected as a compromise because the volume needed to be as low as possible in order to encourage partitioning of the API into the organic phase, while still allowing practical sampling and stirring in a USP apparatus II. An agitation speed of 100 rpm did not discriminate between formulations that showed a different *in vivo* performance. On the other hand, rotation speeds higher than 120 rpm resulted in vigorous stirring and emulsification at the interface. Following the observed poor solubility of the API in the aqueous phase, a high rate of flow was used with USP apparatus IV (30 mL/min) to enable a complete dissolution within a reasonable time frame. The effect of the presence or absence of glass beads inside the flow-through cell was also investigated, and the results prompted the decision to use them. Additionally, dissolution testing was performed in a single-phase dissolution medium (900 mL of pH 6.8 phosphate buffer + 0.5% polysorbate 20 in USP apparatus II at a paddle speed of 50 rpm) in order to compare the discriminatory power of the two types of media. As the *in vitro* results showed the ability of the dissolution profiles in the organic phase of a biphasic dissolution medium to rank order formulations based on their *in vivo* performance, the dissolution profiles obtained from the organic phase of the biphasic system were subsequently used to construct a level A *in vitro-in vivo* correlation. The internal validation results from this IVIVC model were in accordance with the acceptance criterion of the FDA. Thus, a level A IVIVC was successfully established. The biphasic system was shown to be superior to the one-phase system in terms of discriminatory power between different formulations and ability to contribute to a successful IVIVC.

Recently, **Pestieau et al.** carried out a study of the second level A IVIVC with a biphasic dissolution system (Pestieau et al., 2017). Once again, the apparatus used a combined flow-through dissolution apparatus and a paddle apparatus (Fig. 11). Initially, the aim of the study was to investigate the suitability of different *in vitro* dissolution media for the evaluation of fenofibrate self-emulsifying lipid-based formulations. The tested dissolution media were 0.1 M HCl + 1% polysorbate 80, 0.1 M HCl, FaSSGF, FaSSIF, FeSSIF and a biphasic medium combining 0.1 M HCl and octanol. The suitability of these media was evaluated by the establishment of a level A IVIVC after an *in vivo* study in pigs. At the end of the study, only the percentage of fenofibrate dissolved in the biphasic dissolution medium (sum of two phases) was able to correctly predict *in vivo* profiles in accordance with the FDA recommendations. However, the authors showed that the established IVIVC was technology-dependent. Indeed, the model was only able to predict adequately the *in vivo* profiles of the formulations produced by a PGSS process and not by a common melt mixing process. Another interesting observation made during this study was that the best IVIVCs were achieved with the sum of the concentrations found simultaneously in the aqueous and the organic phase. We have already discussed how, in the previous IVIVC studies, the best correlations were usually obtained with the concentrations found in the organic phase. The authors mentioned that this was probably due to the nature of the formulations. Indeed, the self-emulsifying lipid-based systems tested led to *in vitro* and probably also *in vivo* supersaturation and this supersaturation

seemed to be important to take into account for the biorelevance of the test. This is the likely explanation as to why the biphasic dissolution medium, the only one able to highlight this phenomenon, was found to be the most suitable to test these formulations.

3. Conclusions

Although several studies have produced some interesting findings, the biphasic dissolution method cannot be seen as a panacea. As evidenced from the reported methods, most of the biphasic systems were tested on specific formulations and customized equipment. The applicability of these systems for all poorly soluble compounds remains to be demonstrated. When developing such dissolution method, the simplest system that could be tested is an USP apparatus type II (paddle) or type I (basket). However, as already discussed throughout this work, both configurations are only suitable for tablets and capsules and have some limitations such as an inadequate mixing, a move of undissolved hydrophobic particles or the entire formulation to the interface of the two phases without a previous dissolution in the aqueous phase. In the literature, these problems have been solved by customized systems such as USP II apparatus with Petri dish to maintain the lipid-based dosage form (Kinget and De Greef, 1995) or USP II apparatus combined with a disintegration apparatus covered with thin filter paper (Thu Hoa and Kinget, 1996). However, these customized systems are complicated to implement and are quite different from dissolution equipment recommended by Eur.Ph. or USP. The customized system that seems most promising and not too atypical is the system that combined the USP type II apparatus with the USP type IV. The use of a flow through cell as a sample holder allows the analysis of multiple dosage forms including powders and allows easily altering the medium composition to run pH-gradient for example. However, even though two level A IVIVCs have already been established with of this type of biphasic system, this occurred in very specific circumstances in each case. Moreover, this success was greatly dependent on the type of formulation tested, as shown by Pestieau et al. (2017). Moreover, there are some important practical issues that still need to be considered. For example, the most popular organic solvent is octanol despite its nauseating smell. The selection of another organic solvent would be dependent on the API solubility, but also on solvent toxicity, volatility and miscibility with the aqueous phase. This may limit the number of applicable organic media. Regarding the selection of the aqueous phase, many possibilities were tested depending on the nature of the tested active ingredient and formulation. However, two important considerations to keep in mind were that the propensity for emulsification with heavily surfactant-enriched aqueous media, such as FaSSIF or FeSSIF, may create problems and that pH-shift would be more appropriate for pH-dependent poorly soluble drugs especially for MR formulations. In comparison with one-phase dissolution test with pH-shift, this type of two-phase dissolution test did not seem to overestimate the API precipitation tendency (Frank et al., 2014; Heigoldt et al., 2010). Although valuable as a bio-relevant dissolution tool to help predict the outcome of bioequivalence studies and assist in the formulation selection, the biphasic model at its current state of investigation could not be used as a regulatory test without discussion with the relevant authorities. However, this model seems promising considering that it is one of the simplest models to implement for mimicking the *in vivo* absorptive sink condition. Indeed, other approaches are used to mimic the permeability in the dissolution (Lu et al., 2017) as for example dissolution/permeation systems including Caco-2 monolayer or multiple compartments systems such as the gastrointestinal simulator (GIS) developed by Amidon group based on artificial stomach-duodenum model or the TNO Simulated Gastro-intestinal Tract Model 1 (TIM-1) developed at TNO Nutrition and Food Research (Zeist, The Netherlands). In practice, these models are much more complicated to implement and have also their own disadvantages. For example, Caco-2 cell models have some compatibility issues between dissolution media or excipients and monolayer integrity. However, the greatest disadvantage of all these above mentioned models is that due to their complexity, they do not provide an opportunity for the scientist to try multiple variables quickly, which is often critical in reaching the final conclusion of the most bio-relevant method for a given compound and formulation.

Abbreviations

API	Active Pharmaceutical Ingredient
AUC	Area Under Curve
BCS	Biopharmaceutics Classification System
C_{max}	Maximal Concentration
CMC	Critical Micelle Concentration
C_S	Saturation Concentration
FaSSGF	Fasted State Simulated Gastric Fluid
FaSSIF	Fasted State Simulated Intestinal Fluid
FeSSIF	Fed State Simulated Intestinal Fluid
FDA	Food and Drug Administration
GIT	Gastro Intestinal Tract
HPLC	High Performance Liquid Chromatography
IVIVC	*In Vitro-In Vivo* Correlation
MR	Modified Release
PGSS	Particles from Gas Saturated Solutions
PK	Pharmacokinetic
RDC	Rotating Dialysis Cell
RPM	Revolutions Per Minute
SDS	Sodium Dodecyl Sulfate
SEDDS	Self-Emulsifying Drug Delivery System
	S-SEDDS Supersaturable Self-Emulsifying Drug Delivery System
SIF	Simulated Intestinal Fluid
SR^M	Maximum Supersaturation Ratio
USP	United States Pharmacopeia

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