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Integrating theoretical and experimental permeability estimations for provisional biopharmaceutical classification: Application to the WHO essential medicines

Miguel Ángel Cabrera-Pérez[1,2,3†] | Hai Pham-The[4†] | Mirna Fernández Cervera[5] | Rosario Hernández-Armengol[1] | Claudia Miranda-Pérez de Alejo[1] | Yudileidy Brito-Ferrer[6]

[1] Unit of Modeling and Experimental Biopharmaceutics, Centre of Chemical Bioactive, Central University of Las Villas, Santa Clara 54830, Villa Clara, Cuba

[2] Department of Pharmacy and Pharmaceutical Technology, University of Valencia, Burjassot 46100, Valencia, Spain

[3] Department of Engineering, Area of Pharmacy and Pharmaceutical Technology, Miguel Hernández University, 03550 Sant Joan d'Alacant, Alicante, Spain

[4] Hanoi University of Pharmacy, 13-15 Le Thanh Tong, Hoan Kiem, Hanoi, Vietnam

[5] Department of Pharmacy, Institute of Pharmacy and Foods, Havana 17100, Cuba

[6] University of Medical Sciences, Villa Clara, Cuba

Correspondence
Miguel Ángel Cabrera-Pérez, Unit of Modeling and Experimental Biopharmaceutics, Centre of Chemical Bioactive, Central University of Las Villas, Santa Clara 54830, Villa Clara, Cuba.
Email: macabreraster@gmail.com; macabrera@uclv.edu.cu

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Abstract

The accuracy of the provisional estimation of the Biopharmaceutics Classification System (BCS) is heavily influenced by the permeability measurement. In this study, several theoretical and experimental models currently employed for BCS permeability classification have been analysed. The experimental models included the *in situ* rat intestinal perfusion, the *ex vivo* rat intestinal tissue in an Ussing chamber, the MDCK and Caco-2 cell monolayers, and the parallel artificial membrane (PAMPA). The theoretical models included the octanol–water partition coefficient and the QSPeR (Quantitative Structure-Permeability Relationship) model recently developed. For model validation, a dataset of 43 compounds has been recompiled and analysed for the suitability for BCS permeability classification in comparison with the use of human intestinal absorption and oral bioavailability values. The application of the final model, based on a majority voting system showed a 95.3% accuracy for predicting human permeability. Finally, the present approach was applied to the 186 orally administered drugs in immediate-release dosage forms of the WHO Model List of Essential Medicines. The percentages of the drugs that were provisionally classified as BCS Class I and Class III was 62.4%, suggesting that *in vivo* bioequivalence (BE) may potentially be assured with a less expensive and more easily implemented *in vitro* dissolution test, ensuring the efficiency and quality of pharmaceutical products. The results of the current study improve the accuracy of provisional BCS classification by combining different permeability models.

KEYWORDS

Biopharmaceutics Classification System (BCS), Caco-2, human jejunal permeability, MDCK, PAMPA, permeability, rat permeability, WHO Essential Medicine List

1 | INTRODUCTION

Early in 1995, Amidon and colleagues introduced the theoretical frameworks of the Biopharmaceutics Classification System (BCS) for oral immediate-release (IR) solid drug products, with the core interests of reducing the regulatory burden and simplifying the drug development and approval processes (Amidon, Lennernäs, Shah, & Crison, 1995). This system is commonly represented as a four-quadrant plane on which the active pharmaceutical ingredients (APIs) or drug substances are positioned on the basis of their aqueous solubility and intestinal permeability properties. Accordingly, APIs are grouped into four classes expressed in Roman numerals as

[†] Co-first authors.

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follows: I (high solubility/high permeability), II (low solubility/high permeability), III (high solubility/low permeability), and IV (low solubility/low permeability). The validity and broad applicability of the BCS have been studied in depth for more than two decades (Bergstrom, Andersson, Fagerberg, Ragnarsson, & Lindahl, 2013). In particular, the BCS paradigm opened opportunities to avoid running numerous bioavailability and bioequivalence (BE) studies on human beings (also called biowaivers). As a result, many generic drugs could reach the market in less time and with lower cost-effectiveness compared with conventional clinical BE procedures, as long as the drug developer and/or generic companies can justify the BCS-based biowaivers (Cook, Davit, & Polli, 2010). This would be of great importance and benefit, especially for drugs used to treat diseases of high social impact but for which it is difficult to establish conventional BE tests, such as oncology drug products (Tampal et al., 2015).

To date, the BCS has been adopted by numerous organizations and individual regulatory authorities throughout the world, such as the Food and Drug Administration of United States (US-FDA), (CDER/FDA, 2015) the European Medicine Agency (EMA) (EMA, 2010) and the World Health Organization (WHO) (WHO, 2015) as a technical standard to grant biowaivers for immediate-release drug products by means of *in vitro* testing. The group of drugs eligible for BCS biowaivers was also extended from class I to several class III drugs, according to the latest FDA BCS draft guidance released in May 2015 (WHO, 2015).

Following the success of BCS, many experimental and theoretical studies have been carried out in order to simplify or at least to support the BCS classification in the early stages of drug development. One of these directions is the development of a provisional classification system that uses secondary references of aqueous solubility references and several permeability estimates to roundly predict the solubility and permeability class memberships. Various provisional classification schemes have been proposed and validated, using either theoretical and/or experimental tests. Some of the most relevant provisional systems included the modified BCS of Zaki, Artursson, & Bergstrom (2010), the quantitative biopharmaceutics classification system (QBCS) of Rinaki, Valsami, & Macheras (2003), the provisional BCS system of Kasim et al. (2004), the developability classification system of Butler and Dressman (2010), the biopharmaceutics drug disposition classification system (BDDCS) of Wu and Benet (2005), the provisional biopharmaceutical system (PBS) of Pham-The, Garrigues, Bermejo, Gonzalez-Alvarez, Monteagudo, and Cabrera-Perez (2013), the *in silico* BCS classification of Dahan et al. (2013) and the pH-dependent BCS classification of Varma et al. (2012).

In general, almost all these studies used the same criteria for the classification of BCS solubility since the aqueous solubility and the highest dosage strength had already been determined. However, the classification of intestinal permeability was carried out using different approaches. The heterogeneity of the permeability classification and the difficulty of demonstrating a high-permeability class may limit the broad application of the proposed provisional biopharmaceutical classification systems.

It is worth mentioning that several experimental methods are currently accepted to determine intestinal permeability. Among them, absolute bioavailability, the mass-balance determination or the human effective permeability (P_{eff}) across the jejunum membrane are the most reliable methods (Lennernäs, 1998). Other alternative methods such as *in vivo* or *in situ* intestinal perfusion using animal models (Kim et al., 2006) and *in vitro* permeation across cultured epithelial cells (e.g. Caco-2 (Artursson, 1990), MDCK (Irvine et al., 1999) etc.) are also commonly used. Similarly, the parallel artificial membrane permeation assay (PAMPA) (Avdeef, 2005) and the *in silico* models (Bergstrom & Bergström, 2005; Pham-The et al., 2011; Refsgaard et al., 2005) have been established as other possibilities to predict intestinal permeability.

In this work, we proposed a combined classification scheme, integrating a wide range of *in silico*, *ex vivo*, *in situ* and *in vitro* permeability models in order to improve the accuracy of provisional BCS classification. The correlation between different models as well as between experimental data and human absorption data were analysed in order to select the best combination. The final permeability model, based on a majority voting system, was validated with a dataset of 43 reference compounds and the provisional BCS classification obtained was compared with other provisional BCS approaches described in the scientific literature. The final model was rigorously applied to the WHO Essential Medicines List to achieve a provisional permeability classification based on BCS and to identify potential candidates for biowaiver.

2 | MATERIALS AND METHODS

2.1 | Data analysis

A dataset of 43 compounds, with human jejunal permeability values, was collected from the literature (see Table I in Supplementary document 1) (Lennernäs, 2007b, 2014b). These are the only compounds for which intestinal permeability values have been reported in humans, and many of them have been used to correlate with other *in vitro*, *in situ* and *ex vivo* methods of permeability. This dataset was complemented with permeability values obtained from other theoretical and experimental methods; and then used to analyse each experimental permeability model and to validate the suitability of a model based on the majority voting system for provisional BCS permeability classification.

The applicability of the previous permeability model based on the majority voting system was evaluated with the WHO Model List of Essential Medicines (2015). Only the immediate-release solid oral dosage forms were considered and the final list was confirmed by 186 oral drugs of different pharmacological classes (see Table I in Supplementary document 2). Sixteen drugs belonging to the WHO Essential Medicines List are included in the database of 43 references compounds.

2.2 | Permeability models

A variety of theoretical and experimental methods are currently accepted to determine drug permeability and most of them have been applied to classify drugs according to the biopharmaceutical classification system.

In this work, the permeability data were collected from the following approaches: *in situ* permeability in rats ('single pass' and 'closed loop' perfusion) (Doluisio, Billups, Dittert, Sugita, & Swintosky, 1969; Kim et al., 2006), *ex vivo* rat intestinal permeability in an Ussing chamber (Li, Jin, Shim, & Shim, 2013), *in vitro* permeability in Caco-2 and MDCK cell-cultures (Pham-The, Garrigues et al., 2013; Varma et al., 2012), *in vitro* permeability methods for the evaluation of passive transcellular permeability (PAMPA) (Kerns et al., 2004; Zhu, Jiang, Chen, & Hwang, 2002) and *in silico* permeability estimations based on physicochemical properties influencing permeability such as lipophilicity (logP, ClogP), (Kasim et al., 2004) or general QSPeR models involving Caco-2 cell permeability (Pham-The, Garrigues et al., 2013).

Considering the large number of protocols available to determine permeability in cell-cultures (Caco-2 and MDCK) as well as for PAMPA assays, all experimental data were revised taking into account the similarity of the experimental assay in those factors significantly contributing to data variability in each permeability method. Additionally, a classification scheme by categories (high and low permeability) was applied, reducing the variability associated with measurement from different laboratories, assays, cell types and experimental conditions. Consequently, compounds with high permeability were selected with the following threshold values: *in situ* intestinal perfusion in rat: $\geq 20 \times 10^{-6}$cm/s (Kim et al., 2006), *ex vivo* rat intestinal permeability in Ussing chamber: $\geq 5 \times 10^{-6}$cm/s (Ungell, Nylander, Bergstrand, Sjoberg, & Lennernäs, 1998), *in vitro* Caco-2 cells: $\geq 6 \times 10^{-6}$cm/s (Hou, Wang, Zhang, & Xu, 2007), *in vitro* MDCK cells: $\geq 5 \times 10^{-6}$cm/s (Varma et al., 2012) and *in vitro* artificial membrane PAMPA: $\geq 2 \times 10^{-6}$cm/s (Avdeef, 2005). Permeability values for the reference compound metoprolol were also considered to separate high/low permeability compounds (Regardh, Borg, Johansson, Johnsson, & Palmer, 1974). In the case of QSPeR model a high accurate voting consensus model for permeability (overall accuracy of 85.3%), based on Caco-2 permeability data, was employed. (Pham-The, Garrigues et al., 2013) In order to support the permeability classification, information about the absolute bioavailability in human (F_{oral}) and the fraction absorbed in human (F_{abs}) was also considered.

To analyse the performance of each permeability classification model, several statistical parameters were calculated. All these statistical measures are described in the confusion matrix (see Table 1).

2.3 | Validation of the permeability model

Once the final permeability model based on the majority voting system was proposed, the dataset of 43 references compounds was provisionally classified according to the BCS categories. For this classification, the definition established by the WHO guideline for compounds with high permeability and high solubility was taken into consideration (WHO, 2015). The effectiveness of the final provisional BCS classification was compared with other biopharmaceutical classifications previously published (Kasim et al., 2004; Lindenberg, Kopp, & Dressman, 2004; Pham-The, Garrigues et al., 2013; WHO, 2006; Wu & Benet, 2005). The final permeability model was applied to the provisional classification of orally administered drugs in immediate-release dosage forms of the WHO Model List of Essential Medicines.

The aqueous drug solubility values (mg/ml) were obtained from the Merck Index and USP. The dose number (D_0) was calculated using the following equation:

$$D_0 = \left(\frac{M_0}{V_0 * S}\right)$$

where, M_0 is the highest dose strength (mg, obtained from the WHO Essential Medicines Core List) (WHO Model List of Essential Medicines, 2015) S is the reference or calculated aqueous solubility (mg/ml) and the water volume V_0 is assumed to be 250 ml. Drugs with $D_0 \leq 1$ were classified as high-solubility compounds and drugs with $D_0 > 1$ were assigned as low solubility drugs (Amidon et al., 1995; Kasim et al., 2004).

3 | RESULTS

3.1 | Permeability model survey

The effective intestinal permeability in humans is a major determinant of the fraction of dose absorbed (Amidon et al., 1995). The human intestinal perfusion technique has been widely applied to investigate drug absorption, pre-systemic metabolism, *in vitro–in vivo* correlation and to establish an *in vivo* human permeability database (Lennernäs, Abrahamsson, Persson, & Knutson, 2007). This experimental method is also a good alternative to study different mechanisms of transport across the membrane (e.g. passive diffusion, carrier-mediated absorption, paracellular absorption, etc.). Although a limited number of compounds have been studied by human intestinal perfusion, more than 80% of the available data were obtained in one laboratory (Lennernäs, 2014b). This small and homogeneous dataset has been used to directly compare the consistency of permeability data obtained with several *in situ*, *ex vivo*, *in vitro* and *in silico* models and to assess their utility in predicting the fraction of dose absorbed in human studies. (Lennernäs et al., 2007) Nevertheless, the experimental measurements of human intestinal perfusion are not routinely

TABLE 1 Confusion matrix and the statistical parameters to evaluate the performance of permeability classification models

	High permeability predicted (+)	**Low permeability predicted (−)**
High permeability observed (+)	True-positive (TP)	False-negative (FP)
Low permeability observed (−)	False-positive (FP)	True-negative (TN)
Specificity (Sp) = TN/TN + FP		
Sensitivity (Se) = TP/TP + FN		
Precision (Pe) = TP/TP + FP		
Accuracy (Q) = (TP + TN)/(TP + TN + FP + FN)		

performed in early drug development phases and thus several experimental and theoretical methodologies, with different throughput screening capacity, have been used to evaluate the intestinal permeability of drugs (Amidon, Langguth, Lennernäs, Yu, & Amidon, 2011). A comparison between different experimental and theoretical models for the prediction of human intestinal permeability is depicted in Table 2.

3.2 | Experimental and theoretical model correlation

The experimental values of intestinal permeability in humans for 43 reference compounds, along with the permeability values determined by *in situ*, *ex vivo*, *in vitro* and *in silico* methodologies are described in detail in Supplementary document 1 online. In this dataset there are compounds absorbed by carrier-mediated processes (peptide transporter, amino acid transporters, P-gp substrate and an organic cation transporter substrate) as well as by passive diffusion. Sixteen of these compounds are listed in the FDA Waiver Guidance as recommended drugs for permeability classification (CDER/FDA, 2015).

The human permeability data obtained with the *in vivo* perfusion technique were correlated with the results achieved for the same dataset with different *in situ*, *ex vivo* and *in vitro* permeability models in order to identify the experimental techniques that better explain the human permeability values. The equations and the determination coefficient values (r^2) for each correlation are shown in Figure 1. In general sense, there was a good agreement between the permeability values obtained from different experimental methods and that in human. The best correlations with human permeability were ranked in the following way: *in situ* rat permeability > *in vitro* permeability in Caco-2 cells > *ex vivo* rat permeability in an Ussing chamber > *in vitro* permeability in MDCK cells > *in vitro* permeability in PAMPA membrane > *in silico* permeability. Although it has been widely documented, the poor *in vivo*/*in vitro* permeability correlation for drugs which are carrier-substrates, this behavior is different among the experimental methods.

3.3 | Model validation

A dataset of 43 reference compounds was used to analyse the suitability of a permeability model based on a majority voting system for provisional BCS classification (see Table 3). The number of compounds evaluated in each permeability method is different, being lower for experimental models. Figure 2 shows the percentage of drugs correctly classified compared with human intestinal permeability (P_{Hum}) and according to high/low permeability classes of BCS.

Classification using different permeability approaches produced dissimilar results. However, it is clear that the single-pass *in situ* intestinal perfusion in rats offers the best alternative to correctly classify compounds with high (I and II of BCS) and low intestinal permeability (III and IV of BCS), in accordance with human intestinal values (96% accuracy in the prediction). For the *ex vivo* Ussing-chamber system with rat intestine, the accuracy was slightly lower (90%) due to the lower fraction of compounds correctly assigned as class I-II out of the assigned class I-II compounds (83.3%). Both systems can be used to investigate carrier-mediated and passive transport across the intestinal membrane.

In vitro systems such as Caco-2, MDCK and PAMPA have good accuracy predictions (between 84% and 87%). Compounds with high intestinal permeability (Class I and II) had similar percentages of classification, suggesting that they permeated across PAMPA, Caco-2 and MDCK cells mainly by passive transcellular transport. In the case of compounds with low permeability (class III and IV of BCS) the prediction was 100%, but this result has around 25% of under-classification (false-negatives), since most of the compounds with carrier-mediated transport and high permeability were assigned to the low permeability group.

The predictions of permeability by logP and ClogP were quite lower with accuracy values of 52.4% and 58.1%, respectively. This approach only permits the evaluation of passive trans-membrane transport, and drugs with active transport or efflux mechanism could be wrongly classified. However, when we used the QSPeR approach, the accuracy value was 76.7%.

Although the best correlation with human intestinal permeability was obtained with the *in situ* single-pass rat intestinal perfusion technique, its low high-throughput capacity limits its application as a screening tool during the drug discovery and development processes. For this reason, the best option is the combined use of *in situ*, *ex vivo*, *in vitro* and *in silico* models for permeability classification (consensus model based on the majority voting system). This synergic strategy will provide mechanistic information on the human intestinal permeation process, increasing confidence in the permeability conclusions and allowing better prediction of the absorbed fraction at a reasonable cost.

In order to combine the different experimental approaches, we selected the *in silico* models as primary screening methods for permeability (see Figure 3).

Once an initial assessment of lipophilicity/permeability is made (by LogP or QSPeR), we can select the best *in vitro* and/or the *in vivo* experimental permeability methods to validate the *in silico* permeability predictions. This selection is based on the correlation of each permeability method with human permeability values (see Figure 1). This strategy will be a relevant tool during the hit and lead identification as well as for candidate selection.

3.4 | Permeability model based on majority voting system for provisional BCS estimation of WHO essential medicines

The WHO Model List of Essential Medicines contains 186 orally administered drugs in immediate-release dosage forms. These compounds were provisionally classified into the BCS classes following the final permeability model based on the majority voting system and considering all theoretical and experimental permeability information described in the literature. From 186 orally administered drugs, 16 compounds were included in the reference compounds (see Table 3) and the rest of the drugs are shown in Supplementary document 2 online. A clear comparison of different provisional biopharmaceutical classification is also described in Figure 4.

TABLE 2 Comparison between different experimental and theoretical models for the prediction of intestinal permeability

Type of model	Experimental technique	Dataset	High permeability limit for BCS	Disadvantages of the method	Examples
In vivo	Human intestinal perfusion (Loc-I-Gut) (Lennernäs, 1998)	42 compounds (Lennernäs, 2007b)	P_{eff} >1.5 × 10^{-4}cm/s (Lennernäs, 2007b) P_{eff} =1.34 × 10^{-4} cm/s (metoprolol)	Expensive to perform and difficult to handle; ethical issues; low throughput screening method; not used in development or routinely	(Dahlgren et al., 2014)
In situ	Single-pass intestinal perfusion (SPIP) in rat	20 compounds (Kim et al., 2006), 17 compounds (Cao et al., 2006), 14 compounds (Zakeri-Milani et al., 2007)	P_{eff} ≥ 20 × 10^{-6} cm/s (Kim et al., 2006)	Animal usage; not a screening tool; used in development but not routinely	(Cao et al., 2006), (Lennernäs, 2014b), (Zakeri-Milani et al., 2007), (Kim et al., 2006)
	Doluisio's (closed-loop) intestinal perfusion in rat	15 compounds (Lozoya-Agullo et al., 2015)	P_{eff} ≥ 36 × 10^{-6}cm/s (Kim et al., 2006)		(Lozoya-Agullo et al., 2015)
Ex-vivo	Ussing chamber	19 compounds (Ungell et al., 1998), 12 compounds (Li et al., 2013)	P_{app} ≥10 × 10^{-6}cm/s (Ungell et al., 1998)	Limited viability and integrity of membrane; suboptimal stirring conditions; drug must cross the whole intestinal wall; not blood flow; not used for screening	(Ungell et al., 1998), (Li et al., 2013), (Lennernäs, 2007a)
In vitro	Caco-2	20 compounds (Artursson & Karlsson, 1991) 648 compounds (Hou et al., 2007) 1324 compounds (Pham-The, Garrigues et al., 2013)	Papp >1 × 10^{-6}cm/s (Artursson & Karlsson, 1991) Papp ≥6 × 10^{-6}cm/s (Hou et al., 2007) Papp ≥16 × 10^{-6}cm/s (Pham-The, Garrigues et al., 2013)	Inter-laboratory variability; labor-intensive; variable transporter expression; long culture time; lack of mucus layer; limited extrapolation to *in vivo* data; static model; not information regarding regional intestinal absorption; tighter junctions compared with *in vivo* situation	(Pham-The, Garrigues et al., 2013), (Hou et al., 2007)
	Madin-Darby canine kidney (MDCK)	55 compounds (Irvine et al., 1999) 105 compounds (Varma et al., 2012)	P_{app} ≥ 5 × 10^{-6}cm/s (Varma et al., 2012)	Not an intestinal model; similar to Caco-2	(Irvine et al., 1999), (Varma et al., 2012)
	Parallel artificial membrane permeation assay (PAMPA)	93 compounds (Zhu et al., 2002), 90 compounds (Kerns et al., 2004), 138 compounds (Avdeef, 2005)	P_{eff} ≥ 2 × 10^{-6} cm/s (Avdeef, 2005)	Transport dependent upon lipid composition and pH; membrane retention of lipophilic compounds; no active transport	(Zhu et al., 2002), (Kerns et al., 2004), (Avdeef, 2005)
In silico	Octanol–water partition coefficients (logP, ClogP, AlogP, MlogP, KlogP)	29 + 123 compounds, (Kasim et al., 2004), 29 + 154 + 363 compounds (Wolk et al., 2014)	LogP >1.72 and ClogP > 1.35 (Kasim et al., 2004) (metoprolol); CLogP > 1.49, ALogP > 1.61 and KlogP > 1.87 (Wolk et al., 2014) (metoprolol)	Oversimplification of the physiological processes; limited accuracy prediction; limited experimental validation	(Kasim et al., 2004), (Wolk et al., 2014)
	Quantitative Structure-Permeability Relationship (QSPeR)	674 compounds (Pham-The et al., 2011), 1301 compounds (Pham-The, Garrigues et al., 2013), 1324 compounds (Pham-The, Garrigues et al., 2013), 648 compounds (Hou et al., 2007)	P_{app} > 8 × 10^{-6} cm/s (Pham-The et al., 2011); P_{app} >16 × 10^{-6}cm/s (Pham-The, Garrigues et al., 2013), (Pham-The, González-Álvarez et al., 2013); P_{app} ≥ 6 × 10^{-6}cm/s (Hou et al., 2007)		(Pham-The et al., 2011), (Pham-The, González-Álvarez et al., 2013), (Pham-The, Garrigues et al., 2013), (Hou et al., 2007)

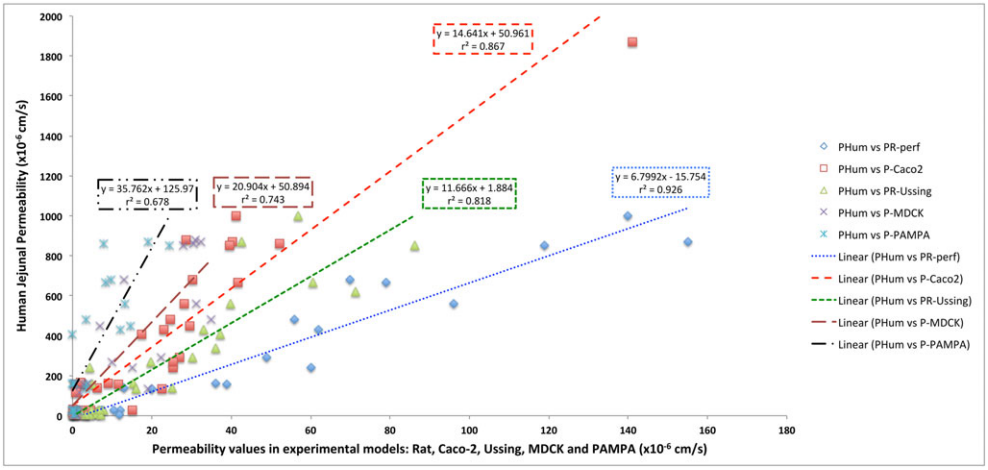

FIGURE 1 Correlations between apparent permeability coefficients (P_{app}) of *in situ* and *in vitro* models and the P_{app} from human small intestinal obtained with *in situ* perfusion technique

4 | DISCUSSION

4.1 | Permeability model survey

As can be seen in Table 2, there is a wide variety of experimental and theoretical methods for predicting permeability in humans; however, we cannot consider a general model, as each of them has advantages, limitations and a specific domain of application.

The single-pass and recirculating perfusion of the rat intestine are *in situ* models with a high predictive value of human intestinal permeability for compounds with passive diffusion and a carrier-mediated absorption mechanism (Cao et al., 2006; Zakeri-Milani et al., 2007). This evidence is also supported by the high correlation reported (r^2 = 0.88) between the fraction of the oral dose absorbed in humans and rats (Zhao et al., 2003). The intestinal perfusion in rat provides very similar conditions to the normal physiological state in humans, which has made this technique the best preclinical model for the prediction of human intestinal permeability, and provides permeability data that may be applied to the provisional classification of drugs according to the biopharmaceutical classification system (Kim et al., 2006; Zakeri-Milani, Valizadeh, Tajerzadeh, & Islambulchilar, 2009).

The *ex vivo* Ussing chamber with a rat jejunal segment is a valuable technique to study drug permeability, mainly for passive transported drugs (Lennernäs, 2007a) Nevertheless, differences between human and rat permeability, for drugs with passive diffusion or a carrier-mediated transport mechanism and high or low permeability, could be explained by the blood supply for membrane maintenance, the sink condition, the unstirred water layer in the Ussing Chamber at various sites along the trans-mucosal pathway and a lower supply of cofactor *in vitro*, which is important for the optimal function of transport proteins (Lennernäs, Nylander, & Ungell, 1997).

On the contrary, the *in vitro* permeability models such as Caco-2 and MDCK (Madin-Darby canine kidney) cells as well as the parallel artificial membrane permeability assay (PAMPA) are the most frequent and successful preclinical experimental procedures to predict intestinal permeability in humans (Artursson & Karlsson, 1991; Cheng, Li, & Uss, 2008; Irvine et al., 1999; Volpe, 2011). The PAMPA assay is a good alternative as primary high-throughput screening for passive permeability ranking; while the Caco-2 is the best choice to study the transport mechanism and absorption routes, and MDCK to evaluate the role of individual transporters of drug absorption (Avdeef, 2005; Teksin, Seo, & Polli, 2010). At present, these permeability models are very useful diagnostic tools to characterize and determine drug permeation according to the BCS (Teksin et al., 2010; Thiel-Demby et al., 2009).

Finally, among the most relevant high-throughput methods for studying intestinal permeability and the oral absorption of drugs are the *in silico* approaches. Several quantitative structure–property relationships (QSPR) have been proposed for the prediction of human intestinal absorption, membrane permeability as well as for BCS classification (Kasim et al., 2004; Newby, Freitas, & Ghafourian, 2015a; Pham-The, González-Álvarez et al., 2013; Pham-The et al., 2011; Sun et al., 2013; Wolk, Agbaria, & Dahan, 2014). *In silico* models are inexpensive tools with a high-throughput capacity, but their accuracy is limited by the quality of the raw *in vivo* data upon which they are based, the statistical approach and the kind of molecular descriptors used in the models. However, despite the drawbacks of the computer models they have been used widely during the lead optimization and candidate selection stages.

As previously mentioned, each *in situ*, *ex vivo*, *in vitro* and *in silico* model has its own limitations for the classification of permeability, and there is no universal permeability model that allows the provisional estimation of the BCS with similar accuracy to the BCS based on human permeability data. In this sense, the proposal of a general permeability model based on the majority voting system is probably the best way to improve the accuracy of prediction for this property in order to obtain a better provisional BCS classification. A detailed quantitative analysis of the relationship among different permeability models will be discussed in the next section.

4.2 | Experimental and theoretical model correlation

In the present study, the *in situ* rat permeability ($P_{R\text{-}perf}$) values ranged between 0.1 × 10^{-6} cm/s and 155 × 10^{-6} cm/s and showed a good correlation (r^2 = 0.79, $p < 0.0001$) with human permeability (P_{Hum}). This correlation was improved when two substrates for amino acid

TABLE 3 A provisional BCS classification for 43 reference compounds, based on different *in vivo*, *in situ*, *in vitro* and *in silico* permeability models, the fraction of dose absorbed and the bioavailability values in humans

Drug	Do[1]	BCS_{Hum}[2]	BCS_{Rat}	BCS_{Ussing}	$BCS_{Caco\text{-}2}$	BCS_{MDCK}	BCS_{PAMPA}	BCS_{logP}	BCS_{ClogP}	PBC_{QSPeR}[3]	F_{abs}[4]	F_{oral}[5]	BCS_F	Ref. study
Acetaminophen[a]	0.09[b]	I	I	III	I	I	I	III	III	I	85; 89 ± 9	88 ± 15; 62–89	I	(Lennernäs, 2014b); (Kalantzi et al., 2006)
Amiloride hydrochloride	0.004[b]	I		I	III	III	III	III	III	I	80–90	50	I	
Amoxicillin trihydrate[a]	0.6[b]	III	III	III	III	III	III	III	III	III	> 90	89–97	III	(Thambavita et al., 2017)
Antipyrine	0.2	I	I	I	I	I	I	III	III	I	100	99 ± 1	I	
Atenolol	0.02	III	III	III	III	III	III	III	III	III	55	50–60	III	(Lennernäs, 2014b)
Benserazide	0.02	I						III	III	I	90	70	I	
Carbamazepine	1.8[b]	II	II	II	II	II	II	II	II	II	100	70–90	II	
Cephalexin	0.08[b]	I[c]	I	III	I	III	III	III	III	III	96 ± 4	90 ± 9	I	
Cimetidine	0.53	III	III		III	III	III	III	III	III	64	60 ± 7	III	(Amidon et al., 1995); (Lennernäs, 2014b)
Creatinine		III		III	III			III	III	I	80	80	III	(Karlsson, Ungell, Grasjo, & Artursson, 1999)
Cyclosporine	25[b]	II	II		II	IV	IV		II	IV	33; 40 ± 5	27 ± 9	II	(Varma, Sateesh, & Panchagnula, 2005)
D-Glucose		I	I	I	I			III	III	III	100	100	I	
Desipramine	< 0.03	I			I	I	I	I	I	I	100	70 ± 8	I	
Enalapril maleate[a] **(PD)**	0.03[b]	I			III	III	I	I	III	III	63; 60–70	41 ± 15; 29–50	III	(Zhao et al., 2001); (Verbeeck et al., 2017)
Enalaprilat	0.003	III	III	III	III			III	III	III	8	8	III	
Fexofenadine	0.32	III	III	III	III	III		I	I	III	5–10	30 ± 3	III	
Fluvastatin	< 0.9	I	I	III	I	I		I	I	I	95	95	I	
Furosemide[a]	8[b]	IV	IV	IV	IV	IV	IV	IV	II	IV	61	61 ± 17; 60–70	IV	(Granero et al., 2010)
Griseofulvin	71.4[b]	II			II	II	II	IV	II	II	> 90	95 ± 5	II	(Lennernäs, 2014b)
Hydro-chlorothiazide	0.16[b]	III	III	III	III	III	III	III	III	III	68; 65–72	71 ± 15	III	(Zhao et al., 2001)
Hydrocortisone	0.2[b]	I[c]			I	I	I	III	I	I	91; 84–95	75–100	I	(Zhao et al., 2001)
Isotretinoin	> 20	II						II	II	II	90	40	II	(Dahan, Lennernäs, Amidon, Lennernäs, & Amidon, 2012)
Inogatran	< 0.001	III		III	III			III	III	III	5–10	5–10	III	
Ketoprofen	1.54	II[c]	II	II	II	II	II	II	II	II	100	100	II	
L-Leucine		I		I	III			III	III	III	100	100	I	
L-Dopa	0.32[b]	I[d]	I	I	III			III	III	III	86; 90 ± 10	41 ± 16	I	(Zhao et al., 2001)
Lisinopril	0.002	III			III	III		III	III	III	35	27 ± 3	III	
Losartan	0.004	III			III	III		I	I	III	100	36	III	(Sica, Gehr, & Ghosh, 2005); (Lennernäs, 2014a)
α-Methyldopa	0.1[b]	III	III	III	III	III		III	III	III	50	42 ± 16	III	

(Continues)

TABLE 3 (Continued)

Drug	Do[1]	BCS_{Hum}[2]	BCS_{Rat}	BCS_{Ussing}	BCS_{Caco-2}	BCS_{MDCK}	BCS_{PAMPA}	BCS_{logP}	BCS_{ClogP}	PBC_{QSPeR}[3]	F_{abs}[4]	F_{oral}[5]	BCS_F	Ref. study
Metoprolol tartrate	0.0004	I	I	I	I	I	I	I	I	I	95–100	38 ± 14	I	(Zhao et al., 2001), (Regardh et al., 1974)
Naproxen	400	II[c]	II	II	II	II	II	II	II	II	100	100	II	
Phenylalanine	0.07	I	I	I	I		III	III	III	III	100	100	I	
Piroxicam	2.5	II	II	II	II	II	II	IV	II	II	100	100	II	
Propranolol[a]	0.004[b]	I	I	I	I	I	I	I	I	I	> 90	26 ± 10; 5–50	I	(Vogelpoel et al., 2004)
Ranitidine[a]	0.001[b]	III	III	III	III	III	III	III	III	III	57	50–60	III	(Yliperttula et al., 2005)
Salicylic acid	0.5	I		I	I	I	I	III	I	I	100%	100	I	
Sulforaphane		II			II			IV	IV	II	74	74 ± 29	II	(Petri et al., 2003)
Talinolol	0.09	III		III	III	III		I	I	I	40		III	(Lennernäs, 2014b)
Terbutaline	0.01	III		III	III	III	III	III	III	III	40–50	14 ± 5	III	
Triamcinolone acetonide		II						IV	IV	II	95	23	II	(Lennernäs, 2014b); (Dorwald, 2012)
Urea		I	III	I	I			III	III	I			I	
Valacyclovir (PD)	0.02	I[d]			III			III	III	III	> 80	54	III	
Verapamil hydrochloride[a]	0.03[b]	I	I	I	I	I	I	I	I	I	90	22 ± 8; 10–20	I	(Vogelpoel et al., 2004)

Drugs in bold are included in the WHO model list of essential medicines.

[a]Drugs whose biowaiver monographs have already been published for over 40 APIs and are available online through links to J. Pharm. Sci. and www.fip.org/bcs.

[b]The dose number values considered the maximum dose strength in the WHO model list of essential medicines (acetaminophen 500 mg, amiloride 5 mg, amoxicillin 500 mg, carbamazepine 200 mg, cephalexin 250 mg, cyclosporine 50 mg, enalapril 40 mg, furosemide 40 mg, griseofulvin 250 mg, hydrochlorothiazide 25 mg, hydrocortisone 20 mg, levodopa 250 mg (with carbidopa), methyldopa 250 mg, propranolol 40 mg, ranitidine 150 mg, verapamil 80 mg).

[c]These compounds were differently classified in the original papers (cephalexin BCS II, Do = 2 (Lennernäs, 2007b); hydrocortisone BCS II (Lennernäs, 2014b); ketoprofen BCS I, Do = 0.2 (Lennernäs, 2007b) and naproxen BCS I, Do = 0.06 (Lennernäs, 2007b)).

[d]High permeability due to carrier-mediated absorption, currently not included in BCS I.

PD, prodrug; BCS_{Hum}, biopharmaceutical classification using *in vivo* intestinal perfusion in human; BCS_{Rat}, biopharmaceutical classification using *in situ* intestinal perfusion in rat; BCS_{Ussing}, biopharmaceutical classification using *in vitro* permeation in Ussing chamber; BCS_{Caco-2}, biopharmaceutical classification using *in vitro* permeation in Caco-2 cells; BCS_{MDCK}, biopharmaceutical classification using *in vitro* permeation in MDCK cells; BCS_{PAMPA}, biopharmaceutical classification using *in vitro* permeation in artificial membrane (PAMPA); BCS_{logP} and BCS_{ClogP}, biopharmaceutical classification using different computational calculation of partition coefficient; BCS_{QSPeR}, biopharmaceutical classification using *in silico* permeability models of Caco-2; Fabs, fraction of dose absorbed in human; Foral, oral bioavailability in human; BCS_F, biopharmaceutical classification using the final permeability model based on majority voting system.

References:

[1](Pham-The, Garrigues et al., 2013), (Lennernäs, 2007b);

[2](Lennernäs, 2007b);

[3](Pham-The, Garrigues et al., 2013);

[4](Lennernäs, 2007b), (Newby, Freitas, & Ghafourian, 2015b);

[5](Avdeef & Tam, 2010), (Dorwald, 2012).

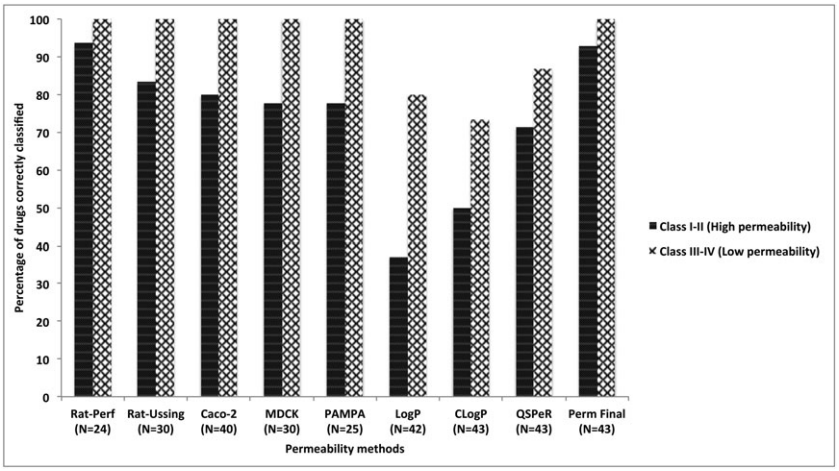

FIGURE 2 Classification of 43 oral drugs in immediate-release dosage forms, based on human permeability values, with different *in silico*, *in situ* and *in vitro* permeability models

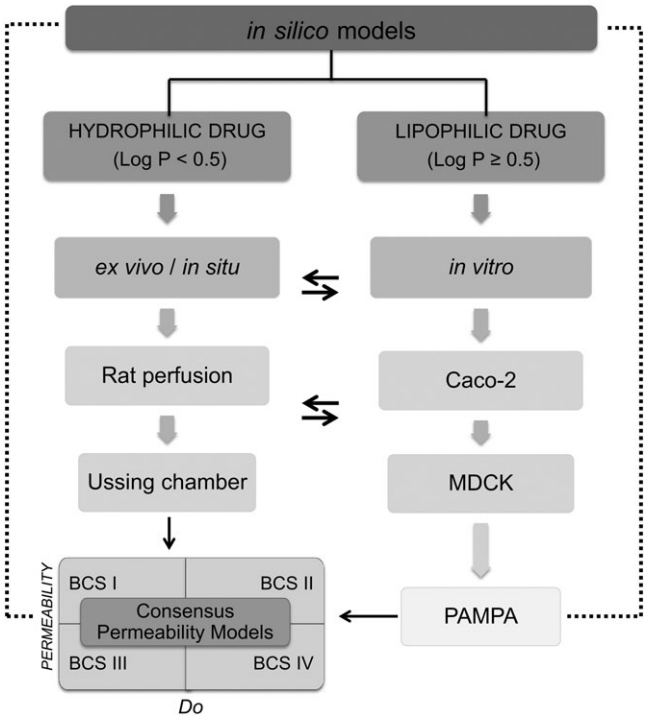

FIGURE 3 Permeability flowchart to propose a general model based on majority voting system for BCS permeability classification

transporters (L-Dopa and phenylalanine) were removed (r^2 = 0.93, p < 0.0001). These results are to be expected because the *in situ* rat permeability technique provides an intact blood supply and a functional intestinal barrier, conditions very similar to the normal physiological state in human (Zakeri-Milani et al., 2007). The results of this study are in agreement with other scientific reports, that have been confirmed the good correlation between intestinal absorption in rat and intestinal absorption in human for hundreds of compounds (Chiou, 1995; Chiou & Barve, 1998; Zhao et al., 2003). The reasonable correlation in transporter expression levels between rat and human small intestine also confirms the similarity of drug permeability correlations (Cao et al., 2006). According to the present results, the human permeability could be predicted adequately using the *in situ* rat perfusion model, where the final results are not biased with respect to carrier-mediated or paracellular transport. However, despite the limited high-throughput capacity of the rat *in situ* perfusion technique to determine intestinal permeability, it is a good alternative for the provisional classification of drug permeability based on BCS.

In the case of *ex vivo* rat intestinal permeability in the Ussing chamber ($P_{R\text{-}Ussing}$), the values ranged between 2.3 × 10^{-6} and 86.3 × 10^{-6} cm/s and the correlation with human permeability (P_{Hum}) was moderate (r^2 = 0.69, p < 0.0001). This correlation was improved when two highly lipophilic and Pgp substrates (acetaminophen and verapamil) were removed (r^2 = 0.82, p < 0.0001). The absent or limited

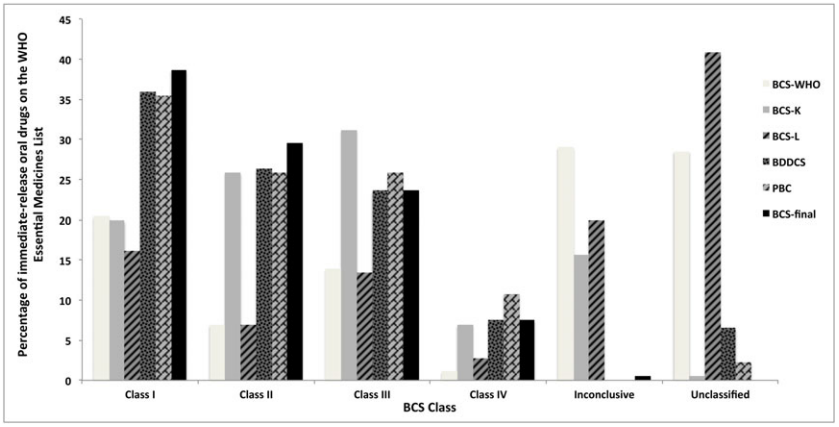

FIGURE 4 Permeability classification of 170 oral drugs in immediate-release dosage forms on the WHO Essential Medicines List, and comparison with other provisional BCS methods described in literature. BCS_{WHO}: biopharmaceutical classification by the WHO guideline; BCS_K: biopharmaceutical classification by Kasim et al.; BCS_L: biopharmaceutical classification by Lindenberg et al.; BDDCS: biopharmaceutics Drug Disposition Classification System; PBC: provisional biopharmaceutical classification using *in silico* permeability models of Caco-2; BCS-final: biopharmaceutical classification using consensus permeability model

sink conditions for the *ex vivo* model as well as the unstirred water layer could account for the low permeability values for these lipophilic compounds. Similar results have been described in the literature (Li et al., 2013), showing that the isolated rat jejunum model is a robust and reliable method for the mechanistic investigation of drug substance permeability and to predict the extent of absorption in humans (Peternel, Kristan, Petruševska, Rizner, & Legen, 2012).

From the *in vitro* cell monolayers, better results were obtained with Caco-2 cells ($P_{Caco\text{-}2}$). A general good correlation with human intestinal permeability was achieved (r^2 = 0.81, $p < 0.0001$) and the result became better when the amino acid transporters substrates L-Dopa and L-leucine were withdrawn (r^2 = 0.87 $p < 0.0001$) (Sun et al., 2002). In the case of MDCK permeability (P_{MDCK}), the correlation with the human value was quite a bit lower (r^2 = 0.61 $p < 0.0001$). Nevertheless, this correlation was improved when piroxicam and carbamazepine were removed (r^2 = 0.74, $p < 0.0001$).

The permeability obtained with PAMPA assay demonstrated a relative moderate correlation with the human jejunal permeability (Avdeef, 2001). In this study, the correlation with the human value was the lowest (r^2 = 0.68, $p < 0.0001$) compared with the rest of the *in vivo* and *in vitro* experimental methodologies. The compounds removed, hydrocortisone, propranolol and salicylic acid, are highly lipophilic drugs.

Attempts to compare the performance of different *in vitro* models (Caco-2, MDCK and PAMPA) in terms of absorption screening as well as the provisional prediction for BCS permeability classes have been extensively performed in the literature (Avdeef & Tam, 2010; Cabrera-Pérez et al., 2012; De Souza, Benet, Huang, & Storpirtis, 2009; Jin et al., 2014; Larregieu & Benet, 2014; Teksin et al., 2010). However, there is not solid evidence demonstrating the overwhelming ability of the Caco-2 model to characterize permeability according to BCS. Despite some synergies of BCS predictions annotated in Table 3, the relative low-to-moderate pairwise correlation between permeability data calculated for our sample of 43 drugs ($r^2_{Caco\text{-}2/MDCK}$ = 0.67, $r^2_{MDCK/PAMPA}$ = 0.22, and $r^2_{PAMPA/Caco\text{-}2}$ = 0.47) suggested the diversity of the three models. Besides, PAMPA and MDCK have been reported to offer several advantages over Caco-2 cells for early permeability profiling, given their efficient, economical and higher-throughput screening characteristics. With the growing implementation of *in vitro* models, numerous studies have proposed the combination of Caco-2, MDCK and PAMPA data to enhance the confidence of permeability classification, as well as to rapidly assess mechanisms of permeation during drug discovery (Avdeef & Tam, 2010; Sevin et al., 2013). Therefore, in this study it is of primary interest to use complementarily the three *in vitro* models for provisional BCS permeability estimations.

At last, *in silico* models based on the logP and ClogP indices were computed using ChemDraw Ultra (CambridgeSoft version 12.0.3.1216). The correlation of experimental human jejunal permeabilities with the logP values revealed that the classification of permeability, based on metoprolol as the reference compound, was correct for 22 of the 42 test drugs (52.4%). Only for cyclosporine was it not possible to calculate the logP value due to missing fragments. For ClogP the correlation was correct for 25 of the 43 test drugs (58.1%). In both cases, molecules with high permeability and transported by carrier-mediated mechanisms (L-leucine, L-Dopa, L-phenylalanine, cephalexin and valacyclovir) were classified as low permeability drugs.

Another *in silico* approach, based on the prediction of Caco-2 permeability (QSPeR), was considered (Pham-The, Garrigues et al., 2013). The model had a 76.7% accuracy of prediction and seven compounds with carrier-mediated transport were wrongly classified (L-leucine, L-Dopa, L-phenylalanine, cephalexin, valacyclovir, D-glucose and enalapril).

At this point it has been clearly shown that theoretical and experimental permeability models have predictive limitations in terms of the physicochemical properties of compounds, mechanism of transport across membrane, type of membrane, etc., which means that each model can generate reliable permeability predictions within a defined applicability domain. This fact justified the use of several models with a different applicability domain, in a first stage a combination of high-throughput but less predictive models (*in silico* + *in vitro*) and later the application of low-throughput but more predictive models (*in situ* + *in vivo*). This strategy could be a valuable approach for better prediction of permeability during the drug discovery and development stages.

4.3 | Model validation

In order to select a primary screening method for intestinal permeability, it should be considered that this property varies greatly for lipophilic compounds and much more for hydrophilic drugs. Lipophilic compounds ($\log P \geq 0$) are mainly absorbed by passive transcellular processes across the intestinal mucosa, while the absorption of hydrophilic compounds ($\log P < 0$) depends on passive paracellular diffusion and/or carrier-mediated transport mechanisms. In this sense, it is not recommended to assess the BCS permeability classification for hydrophilic compounds using only *in silico* or *in vitro* methods where significant expression of transporters as well as the paracellular pathway do not exist or are limited (Larregieu & Benet, 2013). For this class of compounds, permeability methods that involve human or animal intestinal tissues are the most successful for predicting human intestinal permeability. For lipophilic compounds almost all *in silico*, *in vitro* and *in situ* models can accurately predict human permeability.

Taking into consideration all the permeability information of the reference compounds, their correlation with human intestinal permeability values (Figure 1), the fraction of dose absorbed and the bioavailability values in humans, as well as the limitations of each experimental method (see Table 2), a final model based on a majority voting system for BCS permeability classification was proposed (BCS_F).

With this provisional classification 95.3% of data (41/43) were correctly classified compared with the human values, and only two compounds (enalapril and valacyclovir) were misclassified. Valacyclovir, a prodrug of the potent antiviral agent acyclovir, is absorbed through a peptide transporter (PepT1) (Larregieu & Benet, 2013). For this compound its potential high intestinal permeability or the high extent of absorption will be dependent on the expression degree of carrier-mediated transport and it could be different between *in vivo* and *in vitro* models. Two BCS classifications have been described in the literature for valacyclovir (Class I and III). Around

90% of uptake for valacyclovir in small intestine is by PepT1, and its permeability by *in situ* jejunal perfusion studies in wild-type mice (1.68×10^{-4} cm/s) was almost the same as the *in vivo* permeability of valacyclovir in humans (Yang & Smith, 2013). Although these results suggest a BCS class I compound, the final model and the oral absorption in humans make the proposed classification for permeability low (BCS III).

In the case of enalapril, a prodrug of enalaprilat, the final permeability model suggests a BCS III. Although the effective intestinal permeability in human for enalapril is high (1.57×10^{-4} cm/s), similar to the reference compound of high permeability (metoprolol, 1.34×10^{-4} cm/s), enalapril is basically converted to enalaprilat after intestinal membrane permeation (Verbeeck et al., 2017). Almost 34% of the oral dosage is recovered in faeces (enalapril 7% and enalaprilat 27%), leading to an oral absorption fraction of lower than 85% which is the recommended value for high permeable compounds by regulatory authorities (Verbeeck et al., 2017). For this reason, to apply for BCS based biowaivers it must assumed that enalapril is not highly permeable.

According to *in situ*, *ex vivo*, *in vitro* and *in silico* permeability values, amoxicillin was considered a BCS class III, nevertheless the fraction absorbed is high ($F_{abs} > 90$). Taking into consideration the saturable nature of amoxicillin uptake (PepT1), it is logical that permeability values are not consistent with the high fraction absorbed and high absolute bioavailability reported for low doses (Thambavita et al., 2017). Considering the different solubility and permeability results, at different therapeutic doses, amoxicillin belong to BCS class I for dose strengths of 875 mg or less. (Thambavita et al., 2017).

A detailed analysis of the reference dataset for lipophilic compounds (LogP $\geq$ 0.5) reveals a 92% concordance among the *in vitro* permeability measurements (Caco-2, MDCK and PAMPA), the fraction of dose absorbed in humans (F_{abs}), and the final BCS permeability proposed (BCS_F). This result suggests that these compounds permeate across Caco-2 and MDCK cells mainly by a passive transcellular pathway (Sugano et al., 2010).

In the case of cyclosporine, a Pgp substrate, there was a difference between the permeability value obtained in Caco-2 cells and with those of the rest of the *in vitro* methods. For this drug the molecular size (MW > 1200) made the paracellular transport negligible, and the differences in the permeability observed between Caco-2 and MDCK could be a result of the difference in the relative expression levels of the total P-gp in each of them. The selection of the BCS_F permeability value for cyclosporine took into consideration the relevance of the Caco-2 permeability assay to predict the human absorption fraction. For isotretinoin, no *in vitro* permeability values in Caco-2, MDCK and PAMPA methods have been reported, and the final permeability classification took into consideration the *in silico* prediction of permeability.

On the contrary, for hydrophilic compounds (Log*P* < 0.5) the *in vitro* cell methods (Caco-2, MDCK and PAMPA) underpredict the intestinal permeability for drugs absorbed by the paracellular pathway or by a substrate of carrier-mediated transport. For this reason, the most suitable methods to characterize the intestinal permeability of this kind of compound could be the use of *in situ* intestinal perfusion in rat or in the *ex vivo* Ussing chamber system with rat intestine. In our dataset from the 18 hydrophilic compounds, 12 had good correlations (66.7%) between both rat intestinal tissue techniques for permeability and the fraction of dose absorbed in humans. In the cases of benserazide, sulforaphane, triamcinolone and valacyclovir there are no permeability data reported, but the results with the *in silico* QSPeR model and its correlation with the fraction of dose absorbed in humans were considered for the final BCS permeability values.

4.4 | Permeability model based on majority voting system for provisional BCS estimation of WHO essential medicines

According to the WHO BCS classification only 39.4% (67 compounds) were classified in the different classes of BCS, 30.6% of the data were unclassified and 30% had inconclusive permeability values to assign it to a BCS class. These values are quite low if we pretend to apply the bases of the BCS to demonstrate the therapeutic interchangeability of the immediate-release dosage forms of the WHO Model List of Essential Medicines. This result suggests the use of other biopharmaceutical classification alternatives in order to bring about a more comprehensive provisional classification.

Similar results were obtained when orally administered drugs on the WHO Model List of Essential Medicines were assigned BCS classifications, following the data available in the public domain as was published by Lindenberg et al. (2004). From the 170 orally administered drugs, 62 were classified in one of the BCS classes (36.5%), 72 compounds were not classified (42.3%) and 36 compounds had inconclusive data to assign to a specific BCS class (21.2%).

The BCS classification of the WHO Model List of Essential Medicines proposed by Kasim et al. (2004), based on the aqueous solubility of drugs reported in the available reference literature and their calculated partition coefficients (logP and ClogP), (Kasim et al., 2004) allowed a more complete classification of the data. From the 170 WHO oral drugs, 153 (90%) were classified in different classes of BCS (22.3% BCS Class I, 28.8% Class II, 30.6% Class III and 8.2% Class IV), 16 compounds had inconclusive data (9.4%) and only one compound was not classified. Figure 4 shows a very similar trend to the reference compounds. Nevertheless, this *in silico* permeability approach underestimates Class I and overestimates Class III drugs, failing to classify the permeability of drugs with active transport processes in their absorption (influx or efflux) (Wolk et al., 2014).

The application of BDDCS (Benet, Broccatelli, & Oprea, 2011) to the WHO Model List of Essential Medicines showed a good classification of results. Of the 170 oral drugs, 159 (93.5%) were classified in a specific BCS class. Unlike previous classifications, the BDDCS approach classified 35.9% of the data (61 compounds) as BCS Class I. This high value compared with the *in silico* BCS suggests a more flexible benchmark for metabolism (70%), in comparison with the strict permeability benchmark (metoprolol). The rest of the classifications were 27.6% BCS Class II, 22.3% BCS Class III and 7.6% as Class IV. Only nine compounds were not classified. Once the *in silico* BCS and BDDCS classification for the 170 oral drugs were compared, an excellent agreement was obtained for Class II and IV drugs, but not for Class I and III.

The PBC developed by Pham-The, Garrigues et al. (2013) provided comparable results to BDDCS. From the 170 WHO oral drugs, 166 (97.6%) were classified in the different classes of BCS (35.9% BCS Class I, 26.5% Class II, 24.1% Class III and 11.2% Class IV) and only four compounds were not classified. Although the results are quite good, it should be kept in mind that a possible underestimation can be achieved for class III of BCS. Some discordant predictions of human intestinal permeability have been described using the Caco-2 cell system (Larregieu & Benet, 2013), mainly for those hydrophilic compounds that are substrates of highly expressed intestinal transporters (amino acid, nucleoside, and peptide transporter families).

Taking into consideration the variable biopharmaceutical classifications, a final BCS classification considering the permeability model based on the majority voting system was proposed. The percentages of the drugs in immediate-release dosage forms that were provisionally classified as BCS Class I, Class II, Class III, and Class IV were as follows: 38.7% in Class I, 29% in Class II, 23.7% in Class III, and 7.5% in Class IV. Only 0.5% of compounds were classified as Class II/IV, due to the unavailability of permeability data. Although the results are quite similar to the BDDCS and PBC proposals, there is a tendency toward a better prediction of Class I and a reduction of false-negatives in the prediction of Class III compounds.

On the other hand, the accuracy of the general model in predicting high/low permeability for compounds with F_{abs} values close to metoprolol in BCS was analysed. Of the 84 compounds with $F_{abs} \geq 90$, 92.8% (78 drugs) were classified as high permeability (BCS class I or II). This result reinforces the model's good ability to classify compounds with high oral absorption.

Considering the final provisional biopharmaceutical classification of the 186 oral drugs in immediate-release dosage forms in the WHO Essential Medicines List, the percentage of drugs that was classified as BCS Class I and Class III was 62.4%. This result represents the potential number of immediate-release oral dosage forms of the WHO Model List of Essential Medicines that could be eligible for biowaivers of human bioequivalence by a rapid and affordable *in vitro* dissolution test. Of course, to be considered for biowaivers other drug product characteristics, such as the therapeutic index of the drug and the potential influence of the excipients on the rate or extent of absorption, have to be regarded as well.

For this reason, the application of BCS becomes an important tool to ensure the efficiency and quality of pharmaceutical products, especially in developing countries, where the resources for *in vivo* bioequivalence studies are limited (Benet et al., 2008). At the same time, biowaivers based on BCS reduces the unnecessary exposure of healthy humans to drugs, reduces the regulatory problems, and provides economic relief, ensuring the maintenance of the clinical performance of marketed products worldwide (Cook et al., 2010).

The current approach, combining theoretical and experimental methods, could be applied as a screening tool in the prediction of intestinal permeability of the top oral drugs in developed countries or for new molecular entities; improving success rates, reducing costs, and accelerating the development of oral drug products. In the future, all this information will be included in a comprehensive database that will offer, free of charge, the BCS provisional classification service for immediate-release solid oral dosage forms.

5 | CONCLUSIONS

Analysis of different *in silico*, *ex vivo*, *in situ* and *in vitro* models to predict human intestinal permeability, using a reference data set of 43 compounds, showed that *in situ* intestinal perfusion in rats is the best option for correctly classifying the permeability of compounds according to human data. However, considering the throughput limitations of *in situ* methods in screening a large number of compounds, a combined application of permeability models based on a majority voting system was proposed to improve the accuracy of the provisional BCS permeability classification (overall accuracy up to 95.3%). The potential of this approach was assessed for the data of 186 orally administered drugs in immediate-release dosage forms from the WHO Model List of Essential Drugs, for which 62.4% of these data were classified as BCS Class I-III compounds. These results suggested the relevance of the present approach to identifying drug candidates that could be eligible for biowaivers of human bioequivalence by a rapid and affordable *in vitro* dissolution test.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

ORCID

Miguel Ángel Cabrera-Pérez http://orcid.org/0000-0001-5897-2230

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