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Theoretical considerations on the in vivo intestinal permeability determination by means of the single pass and recirculating techniques

Mario Grassi [a,b,*], Giancarlo Cadelli [b]

[a] *Department of Chemical, Environmental and Raw Materials Engineering (DICAMP), University of Trieste, Piazzale Europa 1, Trieste I-34127, Italy*

[b] *Eurand Trieste, via del Follatoio 12, Trieste I-34148, Italy*

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Abstract

This paper deals with the development of proper mathematical models for the calculation of the in vivo rat intestinal drug permeability resorting to two different kinds of experimental methods: the single pass and the recirculating perfusion techniques. In particular, in the single pass case, attention is focused on the effect of water exchange between the flowing solution and the intestinal wall, as this can sensibly affect the permeability determination. In both the single pass and the recirculating perfusion method, a complete radial mixing of the flowing solution is supposed to hold, so that drug concentration and solution velocity are radius independent. Nevertheless, they depend on the intestinal axial position. Accordingly, two distinct models are built up by resorting to microscopic mass balances. The reasonably good data fitting performed by the recirculating perfusion model ensures that the most important factors affecting the passive drug (Antipyrine) diffusion through a rat intestinal wall are properly accounted for. Moreover, the reliability of the developed models and the experimental tests is proved by the fact that the drug (Antipyrine) permeability determined by means of the two methods is statistically equal.

Keywords: Modeling; Intestinal water uptake; Single pass technique; Recirculating perfusion technique

Nomenclature

$C(x)$	drug concentration at the abscissa x
C_I	inlet drug concentration
C_F	correcting factor (Eqs. (10) and (23))

* Corresponding author. Tel.: +39-040-67-63435; fax: +39-040-56-9823.
E-mail address: mariog@dicamp.univ.trieste.it (M. Grassi).

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C_{iANT}	inlet antipyrine concentration (single pass)
C_{iPR}	inlet Phenol Red concentration (single pass)
$C_M(x)$	marker concentration at the abscissa x
C_{Mi}	inlet marker concentration
C_{Mo}	outlet marker concentration
C_o	outlet drug concentration
C_{oANT}	outlet antipyrine concentration (single pass)
C_{oPR}	outlet Phenol Red concentration (single pass)
C_r	reservoir drug concentration at time t (recirculating perfusion)
C_{r0}	initial reservoir drug concentration (recirculating perfusion)
E_p	internal perimeter of the intestine
L	intestinal segment length
L_1	inlet connector length
L_2	outlet connector length
m_r	drug amount present in the reservoir at time t (recirculating perfusion)
P	drug intestinal permeability
P_M	marker intestinal permeability
$q(x)$	solution volumetric flow rate at the abscissa x
q_i	inlet volumetric flow rate
q_o	outlet volumetric rate
q_w	net water transfer rate between the flowing solution and the intestinal wall
R	internal intestine radius
R_1	internal radius of the inlet connector
R_2	internal radius of the outlet connector
S_1	internal section of the inlet connector
S_2	internal section of the outlet connector
t	time
t_1	time required to solution to go through the inlet connector
t_2	time required to solution to go through the outlet connector
t_{exp}	whole experimental time (recirculating perfusion)
t_{int}	time required to solution to go through the intestine
V_1	internal volume of the inlet connector
V_2	internal volume of the outlet connector
V_{int}	internal intestine volume
V_r	solution volume present in the reservoir at time t (recirculating perfusion)
V_{ri}	solution volume for $t = 0$ (recirculating perfusion)
V_{sf}	final solution volume (recirculating perfusion)
V_{si}	initial solution volume (recirculating perfusion)
$v_1 =$	solution speed in the inlet connector
$v_2 =$	solution speed in the outlet connector
$v_x =$	solution speed in the intestine
x	abscissa

1. Introduction

One of the most important factors affecting the efficacy of an oral administrated drug acting at the systemic level is represented by its intestinal permeability. Therefore, drug permeation studies result of paramount importance for the development of those strategies aimed to improve the

drug absorption and the necessity of understanding the basic mechanisms ruling the drug transfer through the intestinal epithelium arises. Although some preliminary information about intestinal drug permeability can be obtained by resorting to theoretical predictive approaches (Breitkreutz, 1998; Palm et al., 1997) or to in vitro experiments (Grassi et al., 1999), in vivo tests represent the most reliable techniques. Recent works (Fagerholm and Lennernäs, 1995a) demonstrated that in vivo drug permeation through the intestinal mucosa mainly takes place according to a passive diffusive mechanism whose rate determining step is represented by the cellular membrane crossing. A little effect would be exerted by the aqueous stagnant layer arising at the intestinal wall (Fagerholm and Lennernäs, 1995a). Although it is usually affirmed that lipophilic drugs follow a transcellular pathway in their intestinal membrane crossing, while hydrophilic ones undertake a paracellular pathway (they would diffuse through the water filling the intercellular voids), today the transcellular way is though to be the main transport mechanism, both in the rat and in the human being, regardless the drug physicochemical properties (Fagerholm et al., 1996). Moreover, it is almost verified that the presence of nutrients in the intestinal lumen does not sensibly affect the drug absorption neither in the rat, nor in the human being (Lennernäs et al., 1994; Nilsson et al., 1994; Fagerholm et al., 1995b; Uhing and Kimura, 1995). In the light of these considerations, this paper is aimed to define mathematical models allowing the calculation of the in vivo intestinal drug permeability by resorting to experimental data collected by means of the well known single pass perfusion and recirculating perfusion techniques (Schurgers et al., 1986; Farraj et al., 1988; Savina et al., 1981). In the single pass modelling particular care is devoted to the effect of water exchange between the flowing solution and the intestinal wall.

Antipyrine is used as model drug, while male Wistar rats intestines are employed for the in vivo measurements.

2. Modelling

2.1. Single pass perfusion

This technique implies a solution, of known drug concentration C_i, to flow, at constant volumetric flow rate q_i, through an intestinal segment of length L at the end of which the drug concentration C_o is measured (Fig. 1). As it is well known (Yuasa et al., 1993; Lindahl et al., 1997) that the C_o value depends on both the drug intestinal absorption and on the water volumetric flow rate (q_w) exchanged between the flowing solution and the intestinal wall, the drug solution has to contain a second solute, the marker, that should not be absorbable by the intestinal wall. Thus, a difference between the marker inlet (C_{Mi}) and outlet (C_{Mo}) concentration indicates the presence of water exchange that is also responsible for a variation of the outlet volumetric flow rate (q_o).

In the past 15 years, the theoretical study of the intestinal drug absorption has been widely accounted for because of the key role played by this phenomenon in the reliability and the efficacy of oral administrated drugs (Amidon et al., 1980; Sinko et al., 1991; Yuasa et al., 1995). Thus, several aspects of drug absorption such as the

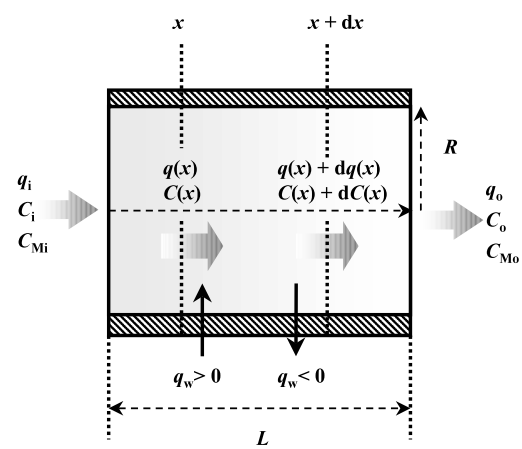

Fig. 1. Schematic view of the single pass perfusion method. Due to the intestine water (q_w) and drug absorption, the drug and marker concentrations (C_i, C_{Mi}) in the inlet solution differ from the drug and marker concentration (C_o, C_{Mo}) in the outlet solution.

presence of a boundary layer arising at the intestinal wall surface (Johnson and Amidon, 1988; Kou et al., 1991), the effect of perfusion rate (Komiya et al., 1980), the effect of intestinal lumen heterogeneity (Kalampokis et al., 1999; Macheras and Argyrakis, 1997; Dokoumetzidis and Macheras, 1997), the existence of a carrier mediated transport (Tsuji and Tamai, 1996), the drug dissolution phenomenon (Oh et al., 1993) and the gut metabolism (Ito et al., 1999), have been considered. Although the effect of water exchange has been previously considered (Lu et al., 1992; Yuasa et al., 1993), we believe that this topic needs further improvements. At this purpose, we suppose that the mass transfer inside the intestinal lumen can be properly described by means of a complete radial mixing model (Amidon et al., 1980). Accordingly, the stationary marker differential mass balance reads (see Fig. 1):

$$q(x)C_M(x) = (q(x) + dq(x))(C_M(x)) + (d(C_M(x))) + E_p P_M C_M(x) dx \quad (1)$$

where $q(x)$ and $C_M(x)$ are the volumetric flow rate and the marker concentration in the x position, respectively, while E_p ($= 2\pi R$) is the internal perimeter of the intestine and P_M is the marker permeability through the intestinal wall. Dividing Eq. (1) for dx and neglecting the second order differential $dq(x)\,dC_M(x)$, we get:

$$q(x)\frac{dC_M(x)}{dx} + C_M(x)\frac{dq(x)}{dx} + E_p P_M C_M(x) = 0 \quad (2)$$

if we further suppose that $q(x)$ has a linear trend through the intestinal length L (this is the simplest assumption to adopt):

$$q(x) = q_i + \frac{q_w}{L}x \qquad \frac{dq(x)}{dx} = \frac{q_w}{L} \quad (3)$$

we finally get:

$$\frac{dC_M(x)}{dx} = -\frac{((q_w/L) + E_p P_M)C_M(x)}{((q_w/L)x + q_i)} \quad (4)$$

The integration of Eq. (4), in the interval $x = 0$ to $x = L$, yields to:

$$C_{Mo} = C_{Mi}\left(\frac{q_i + q_w}{q_i}\right)^{-(1 + (E_P P_M L/q_w))} \quad (5)$$

Knowing C_{Mi}, C_{Mo}, q_i, L and E_p, it is possible to determine, by means of a numerical method, q_w. If P_M approaches to zero (that is what is required to a marker), Eq. (5) simplifies into:

$$q_w = q_i\left(\frac{C_{Mi}}{C_{Mo}} - 1\right) \quad (6)$$

Eq. (6) allows an easy determination of q_w by resorting to the experimental values of C_{Mi} and C_{Mo}. When water uptake occurs, q_w assumes a negative value, while it becomes positive on condition that the intestinal wall supplies water to the flowing solution. Notably, Eq. (6) could be deduced also from a marker macroscopic mass balance performed on the whole intestinal length.

Performing now an analogue differential mass balance on the drug, we get:

$$q(x)C(x) = (q(x) + dq(x))(C(x) + dC(x)) + E_p P C(x)\,dx \quad (7)$$

where $C(x)$ is the drug concentration in the x position and P is the drug permeability through the intestinal wall. Dividing Eq. (7) for dx, rearranging it and supposing a linear trend of $q(x)$ Eq. (3), we get:

$$\frac{dC(x)}{dx} = -\frac{((q_w/L) + E_p P)C(x)}{((q_w/L)x + q_i)} \quad (8)$$

The integration of Eq. (8), in the interval $x = 0$ to $x = L$, yields to:

$$C_o = C_i\left(\frac{q_i + q_w}{q_i}\right)^{-(1 + (E_P P L/q_w))} \quad (9)$$

Starting from this equation, we obtain the expression of the drug permeability P:

$$P = \frac{q_i}{L E_p}\ln\left(\frac{C_i C_{Mo}}{C_o C_{Mi}}\right)\left(\frac{(C_{Mi}/C_{Mo}) - 1}{\ln(C_{Mi}/C_{Mo})}\right) \quad (10)$$

Interestingly, this expression differs from the usual adopted equation for the P calculation (Fagerholm and Lennernäs, 1995a; Yuasa et al., 1993; Lindahl et al., 1997; Kou et al., 1991; Komiya et al., 1980) for the presence of the third right hand side term of Eq. (10). It will be shown

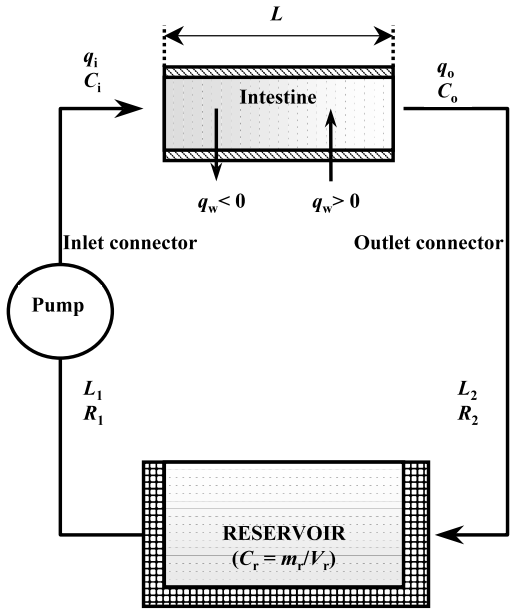

Fig. 2. Schematic view of the recirculating perfusion method. A peristaltic pump makes possible the solution circulation inside the loop made up by the reservoir, the inlet connector, the intestine and the outlet connector.

in the results and discussion section that this term can be not negligible.

2.2. Recirculating perfusion

This technique (Schurgers et al., 1986; Farraj et al., 1988) consists in connecting an intestinal tract of length L to a close loop containing a reservoir characterised by an initial drug concentration C_{r0} (Fig. 2). A pump guarantees the solution circulation while the drug concentration is measured, at fixed time, by sampling the reservoir fluid. Wherever the single pass perfusion has been widely studied, the recirculating perfusion method, at our knowledge, has never been modelled, except for an empirical approach (Nook et al., 1988). To match this target, some considerations are required on both the determination of the water amount exchanged between the solution and the intestine and the modelling of the drug absorption inside the intestine. While in the single pass perfusion the assumption of negligible marker absorption can reasonably hold (the solution does not spend a very long time inside the intestine), in the recirculating case it can not be still true. Indeed, re-circulation obliges the solution to go through the intestine tract many times, so that the marker absorption probability is considerably increased. In this light, according to Nook (Nook et al., 1988), the simplest way to determine the exchanged volumetric water flow q_w is to measure the initial (V_{si}) and final (V_{sf}) solution volume and supposing it time independent:

$$q_w = \frac{V_{sf} - V_{si}}{t_{exp}} \tag{11}$$

where t_{exp} is the whole experimental time.

Supposing a complete radial mixing condition (Amidon et al., 1980), the drug differential mass balance in transitory conditions reads:

$$\frac{\partial C}{\partial t}\pi R^2\,\mathrm{d}x = qC - (q+\mathrm{d}q)(C+\mathrm{d}C) - E_p\,\mathrm{d}x PC \tag{12}$$

where R is the internal intestinal radius and, of course, C and q depend on both x and t. Dividing Eq. (12) for $\pi R^2\,\mathrm{d}x$, neglecting the second order differential $\mathrm{d}q\,\mathrm{d}C$ and assuming a q linear trend inside the intestinal tract (Eq. (3)), we get:

$$\frac{\partial C}{\partial x}\left(\frac{q_i}{\pi R^2} + \frac{q_w}{\pi R^2 L}x\right) + \frac{\partial C}{\partial t} = -C\left(\frac{q_w}{\pi R^2 L} + \frac{2P}{R}\right) \tag{13}$$

The solution of this equation, performed by the characteristic method (Lapidus and Pinder, 1981), leads to:

$$C_o = C_i\left(\frac{q_i + q_w}{q_i}\right)^{-(1+(E_P PL/q_w))} \tag{14}$$

where C_i is now time dependent and it represents the drug concentration in the reservoir fluid at time $t-t_1$, where t_1 is the time required to solution to pass from the reservoir to the intestinal beginning (Fig. 2) and it can be defined by:

$$t_1 = \frac{L_1}{v_1} = \frac{S_1 L_1}{q_i} \tag{15}$$

where L_1 and S_1 are, respectively, the inlet connector length and internal section.

Conventionally, the initial experimental time ($t=0$) corresponds to the completion of the first loop by solution (Fig. 2). At this time, the reservoir solution volume V_{ri} will be ($q_w<0$ implies intestinal water absorption):

$$V_{ri} = V_{si} - V_1 - V_{int} + q_w(t_{int} + t_2) - V_2 \quad (16)$$

where V_1 and V_2 represent the internal volume of the inlet and outlet connectors, respectively, V_{int} is the internal intestine volume, while t_{int} and t_2 are, respectively, the time required to solution to go through the intestine and the outlet connectors. t_2 can be defined by:

$$t_2 = \frac{L_2}{v_2} = \frac{S_2 L_2}{q_0} \qquad q_0 = q_i + q_w \quad (17)$$

where L_2 and S_2 are, respectively, the outlet connector length and internal section while q_2 is the flow rate entering the outlet connector. To determine t_{int}, we remind that the solution flow rate $q(x)$ in the x intestinal position will be:

$$q(x) = \pi R^2 v_x = \pi R^2 \frac{dx}{dt} = q_i + \frac{q_w}{L} x \quad (18)$$

Integrating Eq. (18) in the interval $0<x<L$ and $0<t<t_{int}$, we get the t_{int} analytical expression:

$$t_{int} = \frac{\pi R^2 L}{q_w} \ln\left(\frac{q_i + q_w}{q_i}\right) \quad (19)$$

It is to verify (Demìdovič, 1975) that in the limit $q_w = 0$, t_{int} correctly approaches ($\pi R^2 L/q_i$).

As time goes on, according to Eq. (11), the reservoir fluid volume V_r modifies in a linear manner:

$$V_r = V_{ri} + q_w t \quad (20)$$

The mathematical model assumes a different formal expression for $t < t_1 + t_2 + t_{int}$ and for $t > t_1 + t_2 + t_{int}$. In the first case, the differential equation governing the absorption is given by:

$$\frac{dm_r}{dt} = -q_i \frac{m_r}{V_r} + (q_i + q_w) C_{r0} \times \left(\frac{q_i + q_w}{q_i}\right)^{-((E_p PL/q_w)+1)} \quad (21)$$

where m_r is the drug amount present in the reservoir at time t. This equation imposes that the m_r reservoir variation depends on the exiting flow (first right hand side of Eq. (21)) and on the incoming fluid (second right hand side of Eq. (21)). Notably, the concentration of the incoming fluid is constant with time and it is strictly related to the initial solution concentration. Indeed, for $t < t_1 + t_2 + t_{int}$ a single pass perfusion takes place. For larger times this is not still true and the following equation holds:

$$\frac{dm_r(t)}{dt} = -q_i \frac{m_r(t)}{V_r(t)} + (q_i + q_w) \frac{m_r(t - t_{int} - t_1 - t_2)}{V_r(t - t_{int} - t_1 - t_2)} \times \left(\frac{q_i + q_w}{q_i}\right)^{-((E_p PL/q_w)+1)} \quad (22)$$

This equation, physically equal to Eq. (21), accounts for the fact that the drug concentration of the fluid feeding the reservoir is time dependent since the inlet intestinal fluid concentration is now time dependent (see Eq. (14)).

Both Eqs. (22) and (23) has to be numerically solved (fourth order Runge–Kutta method (Press et al., 1992).

3. Materials and methods

Antipyrine (Sigma Chemical, Steinheim, Germany) and Phenol Red (Sigma Chemical, Steinheim, Germany) were chosen as model drug and marker, respectively, for their wide use in this field (Fagerholm et al., 1996; Farraj et al., 1988; Lindahl et al., 1997). Male Wistar rats (Centro servizi di Ateneo, Settore Stabulario e Sperimentazione animale, Trieste University, Trieste, Italy) weighing approximately 300–350 g were fasted for 16 h (water ad limitum) prior to each experiment. Anaesthesia was induced with i.m. injection of Ethyl Urethane (Sigma Chemical, Steinheim, Germany) (Yuasa et al., 1993) dissolved in physiological solution (1.5 g/kg). To maintain normal body temperature rats were placed on a heated slide (37 °C). The abdomen was opened with a midline longitudinal incision and a jejunal segment of approximately 30 cm was measured and cannulated with a plastic tubing (outlet and inlet tubes diameter equal to 0.4 cm) inserting the outlet tube at 30 cm far from the cecal intestine.

Put the intestinal tract in its anatomical position so that the inlet and outlet tubes are out of the abdomen, the surgical area was covered with a cotton sheet constantly wetted by means of a 37 °C physiological solution. Approval of this study was given by the Italian Ministry of Health (D. LVO 116/92) in accordance with the 'Principle of Laboratory Animal Care'.

3.1. Single pass perfusion

The jejunal segment was rinsed with a saline solution (Schanker formula (Schanker et al., 1958): NaCl 145 mM/l; KCl 4.56 mM/l; $CaCl_2$ 1.25 mM/l, Na_2HPO_4 1.33 mM/l; NaH_2PO_4 0.33 mM/l; pH 7.2; flow rate 2.5 cm^3/min) until a clear perfusate was obtained (about 10 min). Then, the peristaltic pump feeding (see Table 1 for the flow rates considered) was connected to the reservoir containing a solution characterised by known Antypirine (C_{iANT}) and Phenol Red (C_{iPR}) concentrations. Due to the not high intestinal residence time of the marker (Phenol Red) and to literature evidences (Farraj et al., 1988), we can reasonably assume that the variations of the marker outlet concentration are only due to the water exchanged between the intestine and the flowing solution. Setting $t = 0$ when the saline solution has been completely pushed out (approximately 3 min), the solution flows for 105 min and the outlet solution is sampled (0.1 cm^3 for each sample) every 15 min starting from the 45th perfusion min. Each sample is then diluted 1:10 in Schanker solution (Schanker et al., 1958) and the absorbance was measured at the maximum wavelength for Antipyrine (254 nm). At this wavelength and dilution, intestinal secretions did not interfere.

3.2. Recirculating perfusion

The intestinal cleaning step is identical to the single pass one with the only difference that the saline solution is pushed out by means of air sent by the peristaltic pump (Nook et al., 1988). Then, the pump (flow rate $q_i = 0.62$ cm^3/min) and the outlet intestinal tube are connected to a thermostatic (37 °C) reservoir containing the Antipyrine solution (30 cm^3) at known concentration (C_{0ANT}). We set $t = 0$ when the circulating solution reaches the reservoir for the first time. Each test lasts 150 min and, at fixed times, a 0.1 cm^3 solution volume is sampled for the Antipyrine concentration determination. Each sample is then diluted 1:10 in Schanker solution (Schanker et al., 1958) and the absorbance was measured at the maximum wavelength for Antipyrine (254 nm). At this wavelength and dilution, intestinal secretions did not interfere.

Table 1
Single pass experimental set-up

Test number	C_{iANT} (μg/cm^3)	C_{oANT} (μg/cm^3)	C_{iPR} (μg/cm^3)	C_{oPR} (μg/cm^3)	q_i (cm^3/min)	L (cm)
1	98.35	93.9 ± 0.8	14.38	14.5 ± 0.28	0.41	15
2	121.13	109.6 ± 1.6	22.24	22.5 ± 0.31	0.41	16
3	121.13	111.5 ± 3.6	22.24	22.5 ± 0.32	0.41	21
4	105.32	81.8 ± 2.8	19.55	19.2 ± 0.30	0.23	27
5	105.32	103.7 ± 3.3	19.55	19.6 ± 0.23	0.23	9
6	105.32	90.7 ± 1.8	19.55	19.8 ± 0.13	0.62	27
7	105.32	103.2 ± 0.4	19.55	19.6 ± 0.05	0.62	9
8	104.00	97.1 ± 4.9	19.20	21.8 ± 0.37	0.52	31
9	104.00	98.8 ± 4.7	19.20	22.3 ± 1.20	0.52	34
10	104.00	89.6 ± 2.4	19.20	22.7 ± 1.20	0.52	32

C_{iANT} and C_{oANT} are, respectively, the antipyrine concentrations in the incoming and exiting solution, C_{iPR} and C_{oPR} are, respectively, the phenol red concentrations in the incoming and exiting solution, q_i is the inlet flow rate, L is the intestinal length ($R = 0.173$ cm internal intestine radius).

The small solution volume required for each concentration determination makes negligible the Antipyrine losses due to sampling. On the contrary, the whole sampled volume is considered for an exact determination of the remaining solution volume at the end of each experimental test. The complete solution elimination from the intestinal tract is led by air blowing using the peristaltic pump.

4. Results and discussion

In order to check the reliability and the correctness of both the theoretical and experimental approaches, two different kinds of experimental tests were performed. The first one consists in measuring of the intestinal Antipyrine permeability using the single pass perfusion method, while the second adopted the recirculating perfusion technique for the intestinal Antipyrine permeability determination. Table 1, referring to single pass perfusion, reports the inlet Antipyrine (C_{iANT}) and Phenol Red (C_{iPR}) solution concentrations, the intestinal inlet volumetric flow rate q_i and the intestinal length L (intestine internal radius $R =$ 0.173 cm). The permeability determination is led applying Eq. (10) on the experimental outlet Antipyrine (C_{oANT}) and Phenol Red (C_{oPR}) concentrations (see Table 1) measured from $t = 45$ min on, in order to be sure that the water and solute transport reached the steady state (Lu et al., 1992). Table 2 shows the calculated Antipyrine permeability jointly with the average value characterising the ten experimental tests. Table 3, referring to the recirculating perfusion, reports the initial Antipyrine solution concentration C_{0ANT}, the inlet volumetric flow rate q_i, the intestinal length L (internal intestinal radius $R = 0.173$ cm) and the water exchanged volumetric flow rate q_w. The length and the internal radius of the inlet and out let connectors are, respectively, $L_1 = 30$, $R_1 =$ 0.03, $L_2 = 60$, $R_2 = 0.03$ cm, while the initial solution volume V_{si} is equal to 30 cm^3. The permeability value is calculated by fitting the model Eqs. (21) and (22) on the decreasing Antipyrine reservoir concentration. As an example, we report, in Fig. 3, the comparison between the model best fitting (solid line) and the experimental values (open circles), referring to the test 2 conditions (Table 3). It can be noted that a reasonably good agreement exists, this supporting the correctness of both the theoretical and experimental approaches performed. Table 4 shows the perme-

Table 2
Antipyrine permeability values (P) calculated applying Eq. (10) on the single pass experimental data

Test number	P (cm/min) $\times 10^3$
1	1.4
2	2.7
3	1.7
4	1.9
5	0.6
6	3.4
7	1.7
8	2.8
9	2.7
10	4.4
$P_{Average}$	2.31 ± 1.09

Table 3
Recirculating perfusion experimental set-up

Tests number	C_{0ANTP} (μg/cm^3)	q_i (cm^3/min)	q_w (cm^3/min)	L (cm)
1	112.62	0.62	−0.033	27
2	112.00	0.62	−0.033	31
3	84.47	0.62	−0.033	32
4	85.40	0.62	−0.033	31
5	110.43	0.62	−0.020	32
6	113.25	0.62	−0.026	29

C_{0ANT} is the initial antipyrine concentration, q_i is the inlet flow rate, q_w is the exchanged water flow rate while L is the intestinal length ($R = 0.173$ cm internal intestine radius).

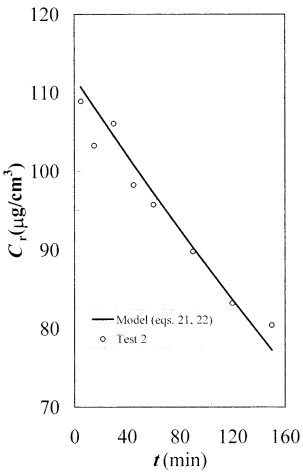

Fig. 3. Comparison between the model best fitting (solid line, Eqs. (21) and (22)) and the experimental data (open circles, test 2) in the case of the recirculating perfusion method.

ability values deriving from data fitting for each of the six experimental tests, jointly with the average value and its standard error. Notably, both the two adopted techniques (single pass and recirculating perfusion) lead to a statistically equal Antipyrine permeability value as the t-test clearly reveals ($t_{\text{calculated}} = 1.1 < t_{\text{tabulated}} = 2.145$; $N = 14$, $\alpha = 0.05$). This evidence is of great relevance since it ensures the reliability and the correctness of both the theoretical and experimental approaches performed. The Antipyrine permeability value determined in this study closely resembles that measured by Lindahl (Lindahl et al., 1997) while it is, more or less, 1/3 of that determined by Fagerholm (Fagerholm et al., 1996).

Table 4
Antipyrine permeability values (P) calculated by fitting Eq. (22) on the recirculating experimental data

Test number	P (cm/min) $\times 10^3$
1	2.60
2	3.40
3	2.20
4	2.75
5	2.70
6	3.30
P_{Average}	2.83 ± 0.45

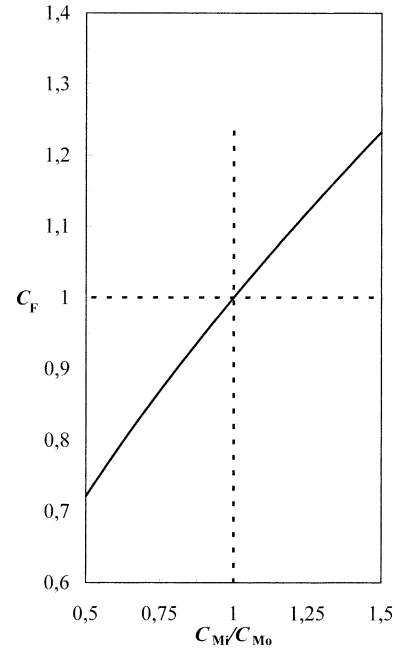

Fig. 4. Dependence of the correcting factor C_F (solid line) on the ratio C_{Mi}/C_{Mo} (Eq. (10)). The figure shows that for $C_{Mi}/C_{Mo} = 1$, $C_F = 1$ (dotted lines).

This discrepancy could be due to the more precise evaluation of the effect of the water exchange performed in this paper. The differences arising in the antipyrine permeability value calculated according to our model Eq. (10) or to the usual adopted one (Fagerholm and Lennernäs, 1995a; Yuasa et al., 1993; Lindahl et al., 1997; Kou et al., 1991; Komiya et al., 1980), represented by Eq. (10) in which the third right hand side term, indicated as correcting factor C_F:

$$C_F = \frac{(C_{Mi}/C_{Mo}) - 1}{\ln(C_{Mi}/C_{Mo})} \tag{23}$$

is set equal to 1, are $\leq 1\%$ of the P value reported in Table 2 for tests 1–7 while they are 6/8% for tests 8–10. Although these can be not big differences, from the work of Lu (Lu et al., 1992), it emerges that for an intestinal tract of 30 cm the percentage difference can be up to 20% (it means $C_F = 1 \pm 0.2$). Of course, this difference is directly proportional to the intestinal tract length consid-

ered and it becomes more and more important as q_w increases. Fig. 4, reporting the correcting factor C_F trend versus C_{Mi}/C_{Mo}, quantifies the difference arising between the P evaluation according the usual (Fagerholm and Lennernäs, 1995a; Yuasa et al., 1993; Lindahl et al., 1997; Kou et al., 1991; Komiya et al., 1980) and the new proposed (Eq. (10)) equation. When C_{Mi}/C_{Mo} is close to 1, this implying a vanishing q_w (Eq. (6)), the correction is negligible (by means of the L'Hôpital–Bernoulli theorem (Demìdovič, 1975), it is easy to verify that in the limit $C_{Mi}/C_{Mo} = 1$, it follows $C_F = 1$). On the contrary, however, when this ratio increases or decreases, C_F becomes more and more important. In particular, because of the Eq. (23) mathematical nature, the effect of the correction factor is more pronounced for $C_{Mi}/C_{Mo} < 1$, this corresponding to an intestinal water absorption. It is clear that C_F becomes especially important in the case of drugs inducing water exchanges between the flowing solution and the intestinal wall.

5. Conclusions

The main result of the present work consists in the development of proper mathematical tools suitable for the calculation of the in vivo intestinal permeability resorting to experimental data collected by means of the single pass and the recirculating perfusion techniques. In the single pass technique, in particular, the importance of the correcting factor connected with the water exchanged between the flowing solution and the intestinal wall, has been pointed out. Indeed, it can be up to the 20% of the permeability value. Conversely, for the recirculating perfusion technique, a not empirical mathematical model is for the first time proposed.

The reliability and the correctness of the developed models is proved by the fact that their application on the single pass and recirculating experimental data leads to a statistically equal permeability value for the considered drug. This, of course, proves also the good quality of the experimental tests performed.

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