Biophysical Models as an Approach To Study Passive Absorption in Drug Development: 6-Fluoroquinolones

VIRGINIA MERINO, JOAN FREIXAS[x], Mª DEL VAL BERMEJO, TERESA Mª GARRIGUES, JOAQUIN MORENO[†], AND JOSE M. PLÁ-DELFINA

Received March 14, 1994, from the *Department of Pharmaceutics, Faculty of Pharmacy, University of Valencia, Av. Vicente Andrés Estellés sn, 46100 Burjassot, Valencia, Spain.* Accepted for publication February 14, 1995[⊗]. [x] Present address: I+D Department, CENAVISA S. A., Passeig Prim 32–6°, Reus, Spain. [†]Present address: Department of Pharmaceutics, Faculty of Pharmacy, University of Barcelona, Diagonal sn, 08028 Barcelona, Spain.

Abstract □ A preliminar study attempting to assess and explain the intestinal absorption of a series of antibacterial 7-piperazinyl-6-fluoroquinolones is presented. The synthesis, *n*-octanol partition coefficients, intrinsic rat gut *in situ* absorption rate constants, and *in vitro* antibacterial activity data found for these homologous compounds are described. A fluorimetric, reverse-phase HPLC method was performed for the quantification of the quinolones in absorption and partition samples. Equations based on two classic biophysical absorption models are given for predicting the intrinsic absorption features of the series according to the partition data or merely single structural parameters. *In situ* absorption rate constants were found to increase by a factor of 9.7–13.5 for moderately lipophilic derivatives relative to the simplest compound, while antibacterial activity decreased only by a factor of 4. *In vivo* absorption tests with two representative members of the series were carried out and the results showed a good accordance with those found *in situ*. This makes these compounds or related ones with similar partition features excellent candidates for further pharmacokinetic and pharmacological testing. The study can serve as an example of how to prevent potential absorption problems associated with the development of new drugs.

Intrinsic *in situ* absorption kinetics of drugs and other xenobiotics,[1,2] with the assessment of some of their physicochemical properties—such as lipophilicity and other structural features[3]—have resulted since the 1970's in the creation and use of a biophysical absorption model.[4–9] The model has helped explain the passive absorption mechanisms of the substances as well as predict the absorption potentialities of newly developed compounds, particularly when related molecules (i.e. belonging to a homologous series) are investigated. Notwithstanding, the use of such models at the early stages of drug development studies has not become as extensive as desirable. Provided that the suitable model was applied, it should be a powerful source of information in order to calibrate absorption potentialities and to prevent absorption failures of drug candidates, particularly when they are intended to be orally administered, as is often the case.

In this paper a tentative study is presented which, on the basis of a simple *in situ* rat gut absorption technique,[1,2] classic bulk-phase partition coefficient determinations,[3] or merely structural features and the use of selected biophysical model approaches, compartmental in nature,[4–9] can serve as a representative example of how to assess and explain the mechanisms of passive intestinal absorption of drugs and drug candidates and to predict their bioavailability potentialities. Although for purposes of example a perfect homologous series of compounds has been selected (6-fluoroquinolone derivatives, differing only in a CH_2 group, two of which are being used clinically), several aspects of the study can be extended to compounds similar to those tested here but with some different functional groups, leading, for example, to a better antibacterial activity, in order to select both pharmacologically active and bioavailable derivatives for further phase I studies.

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Experimental Section

Test Compounds—Eight compounds, derived from norfloxacin by progressive alkylation in *N'*-piperazinyl, were synthesized to perform the study. The procedure was previously described by Koga.[10] It involves the reaction of norfloxacin (3.3 g, 0.01 mol), triethylamine (1.5 g, 0.015 mol), and alkyl bromide (0.012–0.02 mol) in *N,N*-dimethylformamide (40 mL). The mixture was heated at 80–90 °C for 2 h, with continuous stirring, and evaporated to dryness. The residue was suspended in water, filtered, desiccated, and recrystallized in a suitable solvent.

Every compound was identified by its infrared spectrum. Its purity was checked by ion-paired reversed phase HPLC and shown to be above 99.9%. The names, structures and molecular weights of the compounds are shown in Table 1. They exhibited a pK_{a1} between 5.5 and 6.5 and a pK_{a2} between 7.5 and 8.5, so at the working pH they were in their zwitterionic form.

Absorption Studies—*Biological Technique*—The *in situ* rat gut technique,[1] adapted as previously described[2] and using the whole small intestine, was performed on male Wistar rats weighing 210–295 g (six animals per compound). In order to prevent enterohepatic recycling, the bile duct was cannulated before the perfusion.

An isotonic saline solution was prepared and buffered to pH 7.00 by addition of 1% (v/v) Sörensen phosphate solution (0.066 M). Such a concentration prevents the disturbing effects of phosphates on the intestinal membrane.[11] The test solutions were prepared immediately before use by dissolving a fixed amount of compound in the vehicle solution (w/v), according to its solubility. The concentrations are shown in Table 1 and are low enough to avoid precipitation in the lumen during the absorption tests. After dissolving the xenobiotic, the pH of the solution was checked and readjusted when necessary. Sampling of the perfusate was carried out at fixed times, after 5 min, at intervals of 5 min, into silanized glass tubes. All samples were immediately analyzed.

Water Reabsorption Studies—Reduction in the volume of the perfused solutions at the end of the experiments was significant (up to 20%) and a correction became necessary in order to accurately calculate absorption rate constants. Water reabsorption was characterized as an apparent zero-order process.[2,12] A method based on the direct measurement of the remaining volume of the test solution was employed.[2] The volume at the beginning of the experiment (V_0) for each compound was determined in groups of three animals, while the volume at the end (V_t) was measured in every animal used. In accordance with the results, the concentration in the samples (C_e) was corrected as follows:

$$A_t = C_e \frac{V_t}{V_0} \tag{1}$$

where A_t represents the concentration in the gut that would exist in the absence of the water reabsorption process at time t. The A_t values were used to calculate the actual absorption rate constant.

In Situ Absorption Rate Constants—The absorption rate constants of the compounds, k_a, were determined by nonlinear regression

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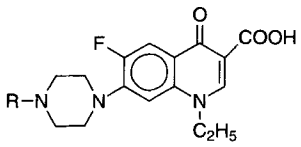
Table 1—Name, Structure, and Molecular Weight of the Test Compounds



R Alkyl Chain	Name	Molecular Weight	Perfusion Concentration (μg/mL)
H	Norfloxacin, 1-ethyl-1,4-dihydro-4-oxo-6-fluoro-7-(*N*-piperazinyl)-3-quinolinecarboxylic acid	319	100
CH_3	*N*-Methylnorfloxacin (Pefloxacin)	333	100
CH_2CH_3	*N*-Ethylnorfloxacin	347	100
$(CH_2)_2CH_3$	*N*-Propylnorfloxacin	361	100
$(CH_2)_3CH_3$	*N*-butylnorfloxacin	375	100
$(CH_2)_4CH_3$	*N*-pentylnorfloxacin	389	50
$(CH_2)_5CH_3$	*N*-hexylnorfloxacin	403	25
$(CH_2)_6CH_3$	*N*-heptylnorfloxacin	417	6

analysis of the remaining concentrations (A_t) versus time, using Sigma Plot 4.0, as it had been previously established that the process follows first-order kinetics. In order to prevent adsorption on the intestinal mucosae[13] and residual sample dilution[2] effects, only the calculated values after 5 min were used for regression, for it had been demonstrated that, in general, after that time, equilibrium had been reached.[2]

In Vivo Verification Tests—The *in situ* k_a values were determined on the basis of the disappearance rate of quinolones in free solution from the luminal perfusate, which usually reflects their rate of incorporation to the bloodstream. But, in order to assess this, an *in vivo* verification, based on the pharmacokinetic analysis of the plasma levels found after oral administration of some of these compounds to whole nonanaesthetized animals, was carried out. Two representative quinolones (norfloxacin, a marketed drug, and *N*'-propylnorfloxacin, a promising drug candidate) were used for this purpose.

Blood levels of these quinolones were measured after (1) an intravenous bolus injection (6.5 mg/kg), which was used as a reference for pharmacokinetic absorption rate constant calculations, and (2) an oral administration, by gastric sounding of a 26 mg/kg dose, both in propylene glycol–water vehicle to prevent compound precipitation. Four sets of five selected, permanently cannulated[14] animals were used. Samples were taken at 5, 10, 15, 30, 60, 90, 120, 180, and 240 min for intravenous tests and at 5, 10, 15, 30, 60, 90, 120, 180, 240, 300, and 360 min for orally administered rats. Plasma samples were prepared according to the following procedure: 200 μL of plasma was mixed with 600 μL of methanol and shook. After centrifugation, 400 μL of the supernatant was evaporated and restituted with the mobile phase, the sample was analyzed using the general procedure described below (recovery was 95%). The results were submitted to pharmacokinetic analysis which included (a) an estimation of the mean normalized AUC values, in order to determine the absolute extent of oral bioavailability; (b) an estimation of the intravenous disposition constants and intercepts, in order to interpret the oral plasma level data; and (c) a calculation of the *in vivo* absorption rate constants, k_v (or rates of bioavailability) for the orally administered animals, in order to be compared with those found *in situ* for the corresponding quinolones. Pharmacokinetic analysis was developed according to the usual recommended methods[15,16] for the four data sets, with the aid of the PCNONLIN 3.0 program.

Lipophilicity Indexes—*Partition Coefficients*—Bulk phase partition coefficients, P, between *n*-octanol (Merck analytical grade) and Sörensen phosphate buffer (0.066 M, pH 7.00) were determined for every compound of the series. Phase/volume ratios were selected for each compound according to the classic approaches[3] in order to minimize deviations. Aliquots of the aqueous phases were removed and assayed for xenobiotic content. The partition coefficient was then calculated as the ratio between octanol concentration—calculated by difference—and the actual aqueous concentration. Six values per compound were used to establish the average value to be used in the correlations.

Number of Methylene Groups—The number of methylene groups was used as a lipophilicity index free of the influence of solvent interactions. Provided that a perfect homologous series is studied, it has been demonstrated to be equivalent to other indexes such as capacity factors, K', or partition coefficients, P.[8,9]

Analysis of the Samples—An original HPLC procedure was used to quantify the solute concentration in both biological and partition samples. The method was carried out on a Novapak C18 column (3.9 × 150 mm), using as mobile phase a mixture of methanol and 15 mM phosphate buffer, adjusted to pH 2.4 with orthophosphoric acid. The percentage of each component of the mobile phase was selected for every compound of the series in order to obtain the best chromatographic resolution with as short as possible retention times.

The equipment consisted of a Series 10 Perkin-Elmer pump, a Rheodyne injector, a Model 420 AC Waters fluorescence detector, and an LCI 100 Perkin-Elmer integrator.

Quantification was done by fluorometry, with an excitation wavelength of 338 nm and an emission wavelength of 425 nm. This technique offers a high degree of selectivity and specificity.

The procedure was validated for inter- and intraday runs before use. Accuracy was calculated by means of the percentage of error associated to the measure of 5–8 standards, analyzed at least three times. It was demonstrated to be less than 15%, regardless of the concentration of the analyte. Precision was calculated as the coefficient of variation of five determinations over the same standards. It was shown to be less than 5%. Linearity was established over the range of concentrations present in the samples for every compound (correlation coefficients were always over 0.999).

Fitting of Models to Data—*Comparison of the Absorption Rate Constants*—In order to validate the influence of lipophilicity on the absorption rate constant of the test compounds, they were compared after proving the homogenity of variances with the Cochran test. The multiple comparison test of Scheffé with a probability of 0.05 was performed.

Fitting of Models—Absorption–partition data were fitted to the previously established hyperbolic[6] or collapsed bihyperbolic[8] equation:

$$k_a = \frac{k_m P^a}{B + P^a} \tag{2}$$

where k_m represents the limiting asymptotic value of the intestinal absorption rate constant and the terms a and B are readily calculable constants arising from the technique used.

The Higuchi–Ho equation was also checked as a compartmental model, able to describe in more detail the two steps involved in absorption. The original equation[4,7] was readapted upon taking into account the fact that the permeability in the aqueous layer depends inversely on the square root of the molecular weight and that the permeability in the lipoidal phase of the membrane can be included as a constant for all the compounds of the series. According to that, the equation is as follows:

$$k_a = \frac{CP^d}{1 + E\sqrt{M}P^d} \tag{3}$$

where C, d, and E are fitted parameters.

In agreement with the postulates of the diffusion model, the total resistance can be expressed as the sum of the partial resistances on both layers[5] as follows:

$$\frac{1}{k_a} = \frac{1}{k_{aql}} + \frac{1}{k_{mem}} \tag{4}$$

where k_{aql} equals the diffusion through the aqueous boundary layer and k_{mem} equals the diffusion through the lipophilic membrane.

Since k_{aql} is proportional to $\sqrt{M}$ and k_{mem} has a potential relationship with lipophilicity, the above equation can be expressed as

$$k_a = \frac{\frac{J}{\sqrt{M}} HP^f}{\frac{J}{\sqrt{M}} + HP^f} \quad (5)$$

where J, H, and f are parameters. Equation 5 is equivalent to eq 3.

All fitting operations were developed by a computer using the PCNONLIN 3.0 program. To appreciate the goodness of fits, correlation coefficients between experimental and model-predicted k_a values were calculated, as well as AIC values. Precision in the estimation of the parameters and residual sum of squares (SS) were also used as a criterion to evaluate the results.[17]

In Vitro Antibacterial Activity—The minimum inhibitory concentration, MIC_{90}, was considered the lowest concentration of the drug that inhibited the growth on agar of 90% of the strains. It was determined on 100 strains of *Escherichia coli*, using the dilution in the agar plate method. The procedure was validated by means of the following typified strains: *Pseudomonas aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *Streptococcus faecalis* ATCC 29212, and *Staphilococcus aureus* ATCC 29213. The assay was carried out following the specifications of the National Committee for Clinical Laboratory Standards.[18] Müeller–Hinton agar plates were prepared with known concentrations of the quinolone tested, covering a suitable range. The bacterial suspension was then inoculated into the plates with a Steers replicator. The plates were cultured at 37 °C for 20 h and evaluated immediately thereafter.

Results and Discussion

Lipophilicity Indexes—The partition coefficient in *n*-octanol was selected as a classic system of reference. Partition coefficients found for each compound are shown in Table 2 (first column of data). In order to prove that genuine partitioning processes are responsible for the different values obtained, the correlation between log P (π) and the number of straight chain CH_2 groups (N) (Figure 1) was established, except for the two first elements ($n = 6$). The correlation was highly significant ($r > 0.999, p < 0.00001$), thus implying the constancy of $\Delta\pi$. That means that the interactions between solute and solvent can be considered constant and that the system quantifies only the relative lipophilicity.

When correlations between log P and the number of methylene groups are calculated, the intercept that should correspond to the log P of norfloxacin differs from the experimental value obtained. This effect seems to be due to specific interactions between the solvent and this compound, which is different from the others because norfloxacin is the basic nucleus, devoid of any alkyl chain. This determines physicochemical parameters (ionization degree and steric and electronic characteristics) that lead to less affinity for *n*-octanol. In fact, an entirely similar effect has been observed with other homologous series of compounds.[19] On the other hand, the compound *N'*-methylnorfloxacin has a higher lipophilicity value than predicted. Such a phenomenon is called a *first element effect* and seldom occurs in homologous series with low lipophilicity components.[20]

However, the lipophilicity experimentally determined for those compounds matches their behavior *in vivo*, as can be seen from the results obtained in the correlations with biological parameters. In fact, the number of methylene groups in the linear chain is a theoretical lipophilicity index. This parameter can be used only in perfect homologous series, in which it can be assumed that the interactions between the compounds and the lipophilic membrane will be similar. But it should always be borne in mind that sometimes it cannot reproduce the partition characteristics of the absorbing membranes. In the present results, the correlation between absorption rate constants and N was established without norfloxacin and *N'*-methylnorfloxacin, because those compounds have demonstrated a peculiar partitioning behavior. Nevertheless, as this type of correlation has the advantage of having a nonaleatory independent variable, they were performed.

Table 2—*n*-Octanol Partition Coefficients (P) and First-Order Intestinal Absorption Rate Constants (k_a) Obtained for the Compounds of the Series

Compound	$P \pm$ SD	$k_a \pm$ SD (h^{-1})
Norfloxacin	0.028 ± 0.002	0.42 ± 0.07
N-methylnorfloxacin	1.883 ± 0.050	1.92 ± 0.18
N-ethylnorfloxacin	2.363 ± 0.018	2.75 ± 0.21
N-propylnorfloxacin	11.27 ± 0.27	4.07 ± 0.40
N-butylnorfloxacin	30.34 ± 1.62	5.59 ± 0.26
N-pentylnorfloxacin	127.7 ± 7.3	5.69 ± 0.55
N-hexylnorfloxacin	515.9 ± 16.2	5.98 ± 0.69
N-heptylnorfloxacin	1664.7 ± 92.9	6.00 ± 0.55

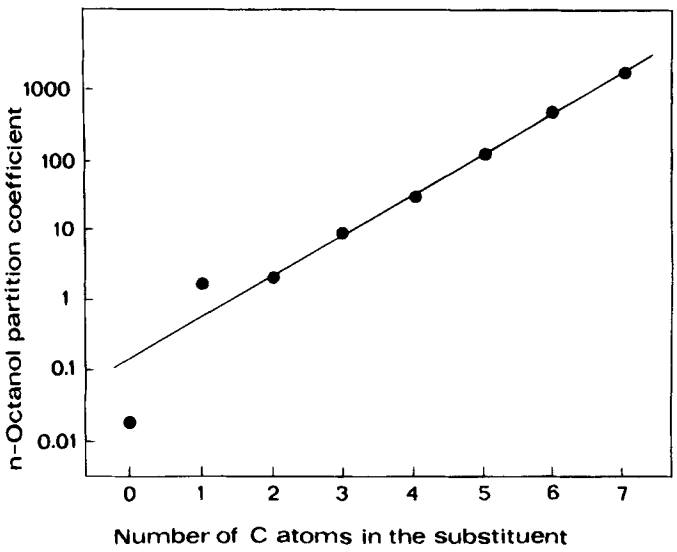


Figure 1—Log–linear regression between *n*-octanol coefficient parameters and number of methylene groups for the test compounds.

Absorption-Partition Correlation—Compartmental theories (eqs 2 and 3) are a logical approach to describing the intrinsic mechanism of absorption. They have an advantage, over the probabilistic trends in that they can explain the nature of the process while also being predictives. Equation parameters and statistical figures associated with the fits of each model equation are shown in Table 3. The correlations found using *n*-octanol partition coefficients are graphically outlined in Figure 2.

Passive absorption, globally considered, is the result of two simultaneous processes: diffusion through the aqueous channels occurring in the small intestine and diffusion through the lipophilic membrane. The bihyperbolic model can be applied in these cases[8] whenever the two pathways are involved, that is, when the test compounds have a low molecular weight (<300) and are assayed in the small intestine.[2,8] When diffusion through the aqueous pathway is not possible, as occurs in our case, the absorption process is reduced to lipidic and aqueous layer diffusion. Such a process can be quantified by either the model proposed by Wagner-Sedman,[6] i.e. the collapsed Plá-Moreno model,[8] or the one proposed by Higuchi–Ho.[4,7]

In the present study, a thorough assessment of their implications and usefulness was carried out by comparing the process through the lipophilic absorbing membrane in the two models.

Table 3—Equation Parameters and Statistical Figures Associated with the Correlations between Absorption Rate Constants and Lipophilicity Indexes

Model	Lipophilicity Index: *n*-Octanol Partition Coefficient	Lipophilicity Index: Number of Methylene Groups
eq 2	$k_m = 6.081 \pm 0.237$ (3.90%)	$k_m = 6.056 \pm 0.161$ (2.65%)
	$a = 0.845 \pm 0.153$ (18.09%)	$a = 0.484 \pm 0.079$ (16.22%)
	$B = 3.024 \pm 0.643$ (21.26%)	$B = 11.780 \pm 5.046$ (42.82%)
	$r > 0.993$	$r > 0.988$
	SS = 0.4953	SS = 0.1292
	AIC = 0.379	AIC = −6.276
eq 3	$C = 2.007 \pm 0.399$ (19.90%)	$C = 0.606 \pm 0.223$ (36.78%)
	$d = 0.775 \pm 0.129$ (16.59%)	$d = 0.436 \pm 0.063$ (14.54%)
	$E = 1.65 \cdot 10^{-2} \pm 3.15 \cdot 10^{-3}$ (19.05%)	$E = 4.93 \cdot 10^{-3} \pm 1.74 \cdot 10^{-3}$ (35.22%)
	$r > 0.993$	$r > 0.992$
	SS = 0.4857	SS = 0.1194
	AIC = 0.223	AIC = −6.752

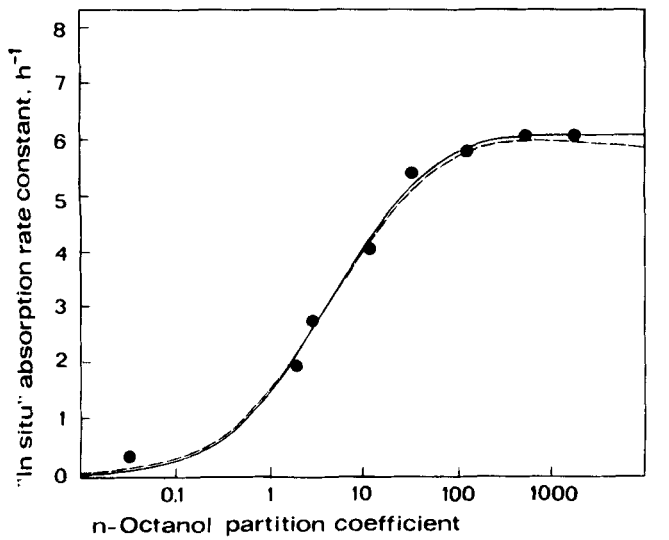


Figure 2—Plot of the correlations between the absorption rate constants and *n*-octanol partition coefficients. The continuous line represents the fit for eq 2 and the dotted line represents the fit for eq 3.

The biophysical model applying, following Wagner–Sedman, is a three-compartmental one (two aqueous compartments limiting a central lipidic one). Absorption is described as a diffusion through the bulk lumen (the first compartment), then a second step, which is the partitioning into the lipidic phase of the membrane and diffusion through it (the second compartment), and, finally, extraction by the plasma, which functions as a sink, after diffusing through an aqueous limiting layer, called *serosa* (the third compartment). A hyperbolic equation relating absorption rate constants, k_a, and lipophilicity, P, can be derived from that model (eq 2), with its asymptote, k_m, being a constant value for every homologous series and representative of the diffusion rate constant through the serosa for a large lipophilic structure belonging to the series.

The model proposed by Higuchi and Ho introduces some modifications with respect to the other one. These authors also consider a three compartmental model, but the aqueous limiting step is located at the lumen interphase. Besides that, they implicitly describe the diffusion through the aqueous boundary layer as a function of the molecular weight, so that the equation describes a reduction in the absorption rate constants as *in vivo* lipophilicity increases over values higher than 10^3 (eq 3).

Table 4—Result after Decomposition of the Absorption Rate Constants Obtained Using Equation 5 after Decomposition of the Absorption Rate Constants Obtained Using Equation 5

N	k_a (h^{-1}): exp	k_a (h^{-1}): theor	k_{aql} (h^{-1})	k_{mem} (h^{-1})
0	0.420	0.123	6.649	0.126
1	1.922	2.180	6.795	3.210
2	2.747	2.461	6.514	3.908
3	4.074	4.353	6.386	13.125
4	5.593	5.208	6.266	28.256
5	5.689	5.840	6.152	86.069
6	5.978	6.006	6.044	253.972
7	6.000	5.986	5.942	629.714

[a] k_{aql} represents the diffusion through the aqueous boundary layer constant and k_{mem} represents the lipoidal membrane diffusion constant.

Our results show that both treatments are reliable and functional. As can be observed in Figure 2, the plots are practically coincident in the range of lipohilicity assayed; the residual distributions are thus similar in both cases. The multiple range test shows that there are no statistical differences among the absorption rate constants of the four most lipophilic compounds (see Table 2, second column). Our data suggest that the absorption rate constants rise to an asymptotic value. In fact, the mean value of these constants represent more than 92% of the limiting value k_m predicted by the Wagner–Sedman fit.

Nevertheless, as can be seen in Table 3, eq 3 fits the data mathematically better. It provides lower values of residual sum of squares than eq 2, and with eq 3 the correlation coefficients are similar or higher than with eq 2. The Higuchi–Ho equation probably describes the absorption–lipophilicity correlation better than the Wagner–Sedman equation does because it takes into account the molecular weight of the compounds. The importance of considering the molecular weight in diffusion through aqueous layers of membranes was also pointed out recently.[21] In addition, the Higuchi–Ho biophysical model seems to give a more exact picture of the real situation, for there is clear evidence that the limiting barrier for the absorption of lipophilic compounds is located adjacent to the membrane at its luminal side.[22] Moreover, it has been demonstrated that it can be removed by perfusion of synthetic surfactants at their critical micelle concentration.[9]

Further analysis of the selected equation shows that more information about absorption can be derived, as shown in the treatment specified in eq 4. According to this, the diffusion through the aqueous boundary layer decreases more than 1 h^{-1} from the most hydrophillic to the most lipophilic compound, as can be seen in Table 4, and this indicates the importance of the molecular weight in this step of the process. On the other hand, diffusion through membrane can be accurately quantified by means of a potential relationship with lipophilicity. This was perfectly demonstrated in recent investigations where surfactants were added to the perfusing solution, resulting in a severe disturbance of the aqueous boundary layer.[9] Thus, the aqueous boundary layer effectively limits the absorption rate constants, as shown in Figure 3.

Our data suggest that when the *n*-octanol partition coefficient is above 100, the diffusion through the aqueous boundary layer is critical and limits the actual absorption rate constant. As can be observed in Table 4, only the norfloxacin rate constant is entirely determined by diffusion through the membrane. When the lipophilicity increases, the importance of the diffusion constant through the membrane is reduced

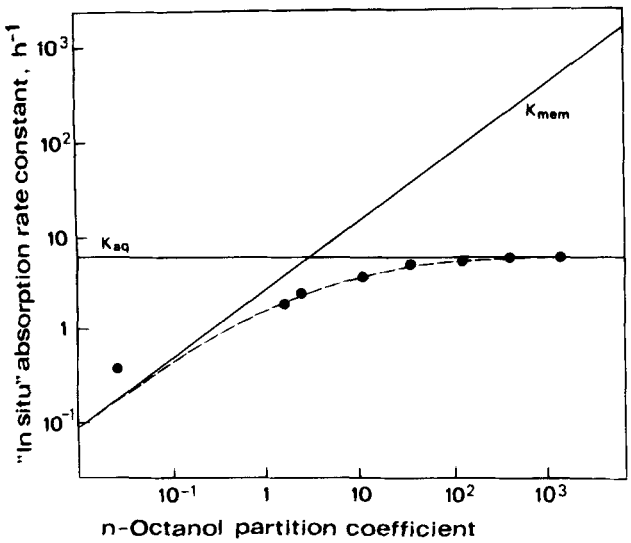


Figure 3—Decomposition of the Figure 4 curve into the diffusion constant through the aqueous boundary layer (k_{aql}) and diffusion through lipoidal membrane (k_{mem}).

Table 5—Pharmacokinetic Parameters Found after Intravenous Bolus Injection and Oral Administration (Gastric Tube) of the Tested Quinolones

Pharmacokinetic Parameter	Norfloxacin[a]	*N*-Propylnorfloxacin[a]
Fast disposition rate constant, α (h^{-1})	1.92(±0.22)	2.01(±0.19)
Slow disposition rate constant, β (h^{-1})	0.134(±0.059)	0.194(±0.012)
Peak level, C_0 (mg/L)	5.08(±0.53)	7.81(±0.70)
Area under the iv curve, AUC_0^∞ (mg·h/L)	7.44	7.46
In vivo absorption rate constant, k_v (h^{-1})	1.12(±0.18)	5.12(±0.47)
Peak level, C_{max} (mg/L)	2.132	8.730
Peak time, t_{max} (h)	0.78	0.34
Area under the po curve, AUC_0^∞ (mg·h/L)	3.53	28.90
Absolute bioavailability, F(%)	11.86	96.79

[a] Numbers in parentheses represent the standard deviation.

as a limiting step. In fact, while for the methyl derivative it represents 75% of the k_a value, for the ethyl derivative it has the same importance as aqueous diffusion. The relationship progresses up to the pentyl derivative, in which the membrane diffusion constant represents only 6% of the global constant.

Absorption Rate Constants—The mean *in situ* absorption rate constants, k_a, found for each compound are shown in the second column of Table 2. As can be observed, the k_a values increase several fold as lipophilicity increases until they are almost stabilized at high lipophilicity values. In Table 5, pharmacokinetic data analysis of plasma levels after intravenous and oral administration of two of the assayed quinolones are shown, in an attempt to verify whether or not such increments in absorption rate are reproduced in whole rats. In Figures 4 and 5 the plasma curves are displayed. As can be observed, the absolute bioavailability of norfloxacin is clearly incomplete (F = 11.86%); apart from the effect of presystemic losses, this should be mainly attributed to its *in vivo* absorption rate constant (1.122 h^{-1}), which is too low for providing a complete absorption of the dose during the normal rat intestinal transit time. In contrast, the absolute bioavailability of the *N'*-propyl derivative is much higher (F = 96.79%), thus indicating a rapid (5.123 h^{-1}) and lucrative absorption; presystemic losses may have prevented a 100% bioavailability. The propylnorfloxacin/norfloxacin bioavailability ratio is, therefore, about 8.2. On the other hand, the *in vivo* k_v values compare fairly well with *in situ* k_a ones since an outstanding difference exists between the two compounds both *in situ* and *in vivo*. Under this latter condition the existence of enterohepatic cycles probably tends to reduce the greater differences

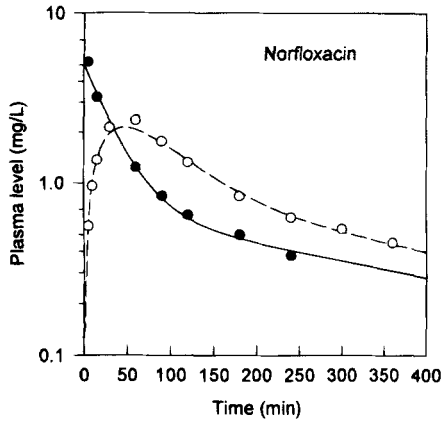


Figure 4—Mean plasma level after intravenous injection (●) and oral administration (○) of norfloxacin.

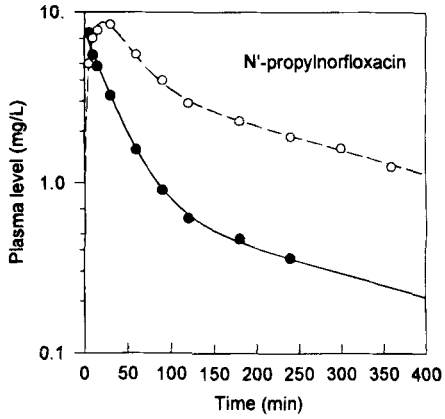


Figure 5—Mean plasma level after intravenous injection (●) and oral administration (○) of propylnorfloxacin.

Table 6—Minimal Inhibitory Concentration (MIC_{90}) of the Compounds against *E. coli* ATCC 25922

Compound	MIC_{90} (μg/mL)
Norfloxacin	0.1
N-Methylnorfloxacin	0.1
N-Ethylnorfloxacin	0.2
N-Propylnorfloxacin	0.4
N-Butylnorfloxacin	0.4
N-Pentylnorfloxacin	0.4
N-Hexylnorfloxacin	1.6
N-Heptylnorfloxacin	3.2

found *in situ*, but they are still highly significant ($p < 0.001$). The opinion of the authors is that there is sufficient proofs to take the *in situ* k_a values as realistic bioavailability criteria.

Biopharmaceutical Implications—The lipophilicity increment of the tested molecules, from norfloxacin to *N'*-heptylnorfloxacin, implies a significant improvement in the *in situ* absorption rate, which increases more than 14-fold. Such a modification could overcome the bioavailability problems that norfloxacin has in man,[23] as it does in rat (Table 5).

Furthermore, the assayed compounds keep their bactericidal activity, as demonstrated in preliminary trials of determination of minimal inhibitory concentration (MIC_{90}), which are shown in Table 6. The MIC_{90} increases when lipophilicity does, but for the intermediate compounds, this increment is lower than the absorption increase. For instance, for the ethyl derivative, the activity is reduced 2-fold while the absorption constant increases about 7-fold. The selection of a structure

belonging to the series, where some modifications to enhance bactericidal activity (i.e. 8-fluorinated derivatives, ring between C_8 and N_1) were introduced while the intrinsic lipophilicity was maintained, would be a useful way to carry out new drug development.

Our study serves to point out a different approach to be used in the early stages of development of new drugs. Biophysical compartmental models can help to explain the mechanism of the absorption process and they can serve as new and perhaps only tools for preventing bioavailability problems in the early phases of drug development studies.

References and Notes

1. Doluisio, J. T.; Billups, N. F.; Dittert, L. W.; Sugita, E. T.; Swintosky, J. V. *J. Pharm. Sci.* **1969**, *58*, 1196–1199.
2. Martín-Villodre, A.; Plá-Delfina, J. M.; Moreno, J.; Pérez-Buendía, M. D.; Miralles-Mir, J.; Collado, E. F.; Sánchez-Moyano, E.; Del Pozo, A. *J. Pharmacokin. Biopharm.* **1986**, *14*, 615–633.
3. Curry, S. H.; Whelpton, R. In *Manual of laboratory pharmacokinetics*; John Wiley and Sons Ed.: New York, 1983; pp 39–54.
4. Suzuki, A.; Higuchi, W. I.; Ho, N. F. H. *J. Pharm. Sci.* **1970**, *59*, 644–651.
5. Flynn, G. L.; Yalkowsky, S. H. *J. Pharm. Sci.* **1972**, *61*, 838–852.
6. Wagner, J. G.; Sedman, A. J. *J. Pharmacokin. Biopharm.* **1973**, *1*, 23–50.
7. Ho, N. F. H.; Park, J. Y.; Morozowich, W.; Higuchi, W. I. In *Design of biopharmaceutical properties through prodrugs and analogs*; APhA: Washington, 1977; pp 136–227.
8. Plá-Delfina, J. M.; Moreno, J. *J. Pharmacokin. Biopharm.* **1981**, *9*, 191–215.
9. Plá-Delfina, J. M.; Pérez Buendía, J. M.; Casabó, V. G.; Peris Ribera, J. E.; Martín Villodre, A. *Int. J. Pharm.* **1987**, *37*, 49–64.
10. Koga, H. *J. Med. Chem.* **1980**, *23*, 1358–1363.
11. Morishita, T.; Yamazaki, M.; Yata, N.; Kamada, A. *Chem. Pharm. Bull.* **1973**, *21*, 2309–2322.
12. Gabus-Sannié, C.; Buri, P. *S. T. P. Pharma.* **1987**, *3*, *11*, 856–860.
13. Doluisio, J. T.; Crouthamel, W. G.; Tan, G. H.; Swintosky, J. V.; Dittert, L. W. *J. Pharm. Sci.* **1970**, *59*, 72–76.
14. Torres-Molina, F.; Aristorena, J. C.; García-Carbonell, M. C.; Granero, L.; Chesa-Jiménez, J.; Plá-Delfina, J. M; Peris-Ribera, J. E. *Pharm. Res* **1992**, *9*, 1587–1591.
15. Wagner, J. G. *Fundamentals of Clinical Pharmacokinetics*; Drug Intelligence Publ.: Hamilton, 1983.
16. Gibaldi, M.; Perrier, D. *Pharmacokinetics*; Marcell Dekker Inc. New York, 1982.
17. Imbimbo, B. P.; Martinelli, P.; Rocchetti, M.; Ferrari, G.; Bassotti, G.; Imbimbo, E. *Bipharm. Drug Dispos.* **1991**, *12*, 139–147.
18. NCCLS Document M 100-S, Vol 6 N° 21. National Committee for Clinical Laboratory Standards (NCCLS). Villanova, Philadelphia, 1986.
19. Sánchez-Moyano, E.; Seco, C.; Santolaria, A.; Fabra-Campos, S.; Herráez, M.; Martín-Villodre, A. *J. Pharm. Sci.* **1992**, *81*, 720–725.
20. Bermejo, M. V.; Pérez-Varona, A. T.; Segura-Bono, M. J.; Martín-Villodre, A.; Plá-Delfina, J. M.; Garrigues, T. M. *Int. J. Pharm.* **1991**, *69*, 221–231.
21. Pérez-Buendía, M. D.; Gómez-Pérez, B.; Plá-Delfina, J. M. *Arzneim. Forsch* **1993**, *43(II)*, 789–794.
22. Hayton, W. L. *J. Pharmacokin. Biopharm.* **1980**, *8*, 321–324.
23. Bergeron, M. G. *Clin. Invest. Med.* **1989**, *12 (1)*, 20–27.

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