*Scientific paper*

# Physicochemical Properties of Lomefloxacin, Levofloxacin, and Moxifloxacin Relevant to the Biopharmaceutics Classification System

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## Abstract

The aim of this investigation was to identify the impact of physicochemical properties of three fluoroquinolones (second, third, and fourth generation) on bioavailability in relation to the Biopharmaceutics Classification System (BCS) by *in silico* and *in vitro* methods. These properties were estimated by analyzing the electrostatic potential pattern and values of the free energy of solvation as well as the distribution coefficients and true partition coefficients of the studied compounds. This study was based on theoretical quantum-chemical methods and the *in vitro* shake-flask technique with two immiscible phases (*n*-octanol and phosphate buffer) as well as the experimental potentiometric method to estimate protonation macro- and micro-constants. Properties identified in the *in vitro* and *in silico* studies were similar and indicated high lipophilic properties of the studied molecules as well as their good solubility in a polar medium. It appears that both the theoretical methods and simple *in vitro* studies are useful tools for predicting the bioavailability of medicinal substances based on the BCS principles.

**Keywords:** Fluoroquinolones; Bioavailability; Molecular modeling; BCS

## 1. Introduction

The bioavailability of drugs at a molecular level can be represented using a number of methods. One approach involves determining similarities in the *in vitro* dissolution using water and lipophilic solvents. The first imitates the environment of the release of the active pharmaceutical ingredient (API) from the drug dosage form and the second mimics biological membranes. The release of an API from its immediate-release drug dosage form in the gastrointestinal tract and its absorption by membrane permeation represent two factors that can be used to categorize drugs within the Biopharmaceutics Classification System (BCS). This system classifies the solid oral immediate-release drug dosage form into four classes based on their aqueous solubility and permeability through lipophilic barriers in biological membranes. In bioavailability studies, models of artificial membranes, cell culture lines, and human and animal tissues are widely used;[1] however, these are relatively expensive and represent ethically questionable methods.

The aim of this study was to use simple, measurable, and cost-effective *in vitro* and *in silico* models to estimate bioavailability determinants, that is, the distribution ($\log P_{n\text{-octanol/buffer}}$) and partition ($\log P_{n\text{-octanol/water}}$) coefficients, the free enthalpy of solvation, and the electrostatic potential surfaces for each studied molecule in order to verify a hypothesis stating that these properties are good criteria for the estimation of the bioavailability of fluoroquinolones. There is an important thermodynamic relationship between the free enthalpy of solvation and partition coefficient $\log P_{o/w}$. The negative enthalpy is a measure of the reaction spontaneity, and its change represents an interfacial transfer of solute molecules.[2] Additionally, the determinant that gives information about the extent of a interaction between the polar solvent and the molecule is the electrostatic potential range.

In this paper, the relationship between the physicochemical properties of the examined compounds and the efficacy of the models in predicting the bioavailability are described for lomefloxacin, levofloxacin, and moxifloxacin, which belong to second, third, and fourth generation fluoroquinolones, respectively.

Lomefloxacin hydrochloride (1-ethyl-6, 8-difluoro-7-(3-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic acid), levofloxacin hemihydrate ((*S*)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid), and moxifloxacin hydrochloride (7-[(4a*S*,7a*S*)-1,2,3,4,4a,5,7,7a-octahydropyrrolo[3,4-b]pyridin-6-yl]-1-cyclopropyl-6-fluoro-8-methoxy-4-oxoquinoline-3-carboxylic acid) are derived from nalidixic acid, which is a representative of the first generation of quinolones. This drug is becoming less commonly used because of its poor bioavailability and narrow spectrum of activity. The introduction of a fluorine atom to the quinolone core improved the pharmacokinetic drug profile in aqueous environments and, thus, improved both the bioavailability and the antibacterial activity.[3–5] Fluorine substitution at C6 (and, in the case of lomefloxacin, a second substitution at C8) increases the drugs penetration into the bacterial cell and, thus, improves the strength of activity. This substitution became the basis of the development of new generation of quinolone drugs. Activity against *Pseudomonas sp.* is ensured by the presence of a piperazine or *N*-methylpiperazine ring in the structure of the molecule. Replacement of the ethyl substituent on cyclopropyl at N1 extends the range of activity on some G-(+) aerobic bacteria. These compounds show activity against G-(+) as well as atypical bacteria, which lack a cell wall, and anaerobes as a result of the modification of the substituent at C1 and the introduction of a chlorine atom or a methoxy group at C8. Levofloxacin has bactericidal activity against G-(-) bacteria, such as *Salmonella sp.*, and G-(+) bacteria, such as *Enterobacteriaceae*. Moxifloxacin has increased activity against G-(+) and G-(-) bacteria and atypical microorganisms like *Mycoplasma* and *Chlamydia* as well as anaerobic bacteria. Thus, newer generations have a broader spectrum of activity, including most enteric Gram-(-) rods (*Escherichia coli, Klebsiella sp.*) and G-(+) bacteria, atypical strains such as *Chlamydia, Mycoplasma, Legionella*, and *Haemophilus influenza*, as well as methicillin-sensitive *Staphylococcus sp.* and *Streptococcus pneumoniae*.[6,7]

This group of drugs is distinguished by a specific mechanism of action, because quinoline–carboxylic acids and their fluorinated analogs are inhibitors of DNA gyrase subunit A (topoisomerase II) in bacterial cells and DNA topoisomerase IV. The mechanism of action of these enzymes is to terminate the bond between the chlorine and the phosphate in single-stranded DNA and DNA strand coiling. The effect of the topoisomerase inhibitors is to inhibit the metabolism of the bacterial cells by inhibiting DNA replication,[6] causing their death.[8]

The above-mentioned drugs are used either alone or in combination with other antibacterial drugs to treat certain bacterial infections including urinary tract infections, pneumonia, and abdominal infections.[3,4,7,8] Moxifloxacin is approved for acute bacterial exacerbations of chronic bronchitis and acute bacterial sinusitis when other antibacterial agents cannot be used or have failed.[9]

Each molecule of these chemotherapeutic agents, depending on the pH of the gastrointestinal tract, may take four forms (cation: $H_2X^+$, zwitterion: $HX^{+-}$, neutral: HX, and anion: $X^-$). Figure 1 shows their mutual relations, which particularly affects their bioavailability determination; although, in a physiological environment, the zwitterionic and cationic forms are prevelant.[10,11] Changes in the pH parameter shift the equilibrium between the different forms, which may obstruct release of the drug, absorption from the intestinal lumen, penetration through the pores of the cell bacterial wall, and bacterial topoisomerase binding.[12,13]

# 2. Experimental

## 2. 1. Materials

Lomefloxacin hydrochloride, levofloxacin hemihydrate, and moxifloxacin hydrochloride were obtained from Sigma–Aldrich, US. *n*-Octanol was purchased from Emplura Merck, Germany. Phosphate buffer solution, sodium hydroxide, and hydrochloric acid were also used.

The equipment included a UV/Vis spectrophotometer (JASCO 530), a mechanical shaker (Janke &Kunkel, IKA Labortechnik, HS 250), a pH meter with a glass combination electrode (Mettler–Toledo MP 225 model, InLab413, R program v 2.14.2 with R Studio graphic overlay v 0.95.262), an Intel® Core™ i5 CPU computer with an AMD Radeon HD 6800 series graphic card, Spartan software package 08, ACD/ChemSketch freeware v.12, and the Virtual Computational Chemistry Laboratory (VCCLAB) on-line software package (ALOGPS 2.1).

## 2. 2. Experimental Determination of $\log P_{n\text{-octanol/buffer}}$ ($\log P$)

A shake-flask technique was used to determine log*P* values.[14] Each substance was dissolved in 0.1 M phosphate buffer at pH 7.4, and then the pH of the solution was adjusted to pH 6.8 with 1 M HCl. Concentration versus UV absorbance calibration curves were drawn for five different concentrations. The absorbance was measured at a specific UV wavelength ($\lambda_{max}$) for each compound. The buffered samples were pre-saturated with 0.1 M *n*-octanol. The samples were shaken for 1 h in a flask before they were separated; after reaching an equilibrium state (after approximately 5 h), the absorbance of each sample was measured. The concentration of a compound in the aqueous phase was determined based on the calibration

curve. Then, the concentration of the compound in *n*-octanol and the apparent partition coefficient were calculated. Each of the values was an average of three parallel measurements. For each tested substance, the Pearson linear correlation coefficient was not less than 0.9993, whereas the *p*-value was in the range $1.35*10^{-6}$–$7.55*10^{-6}$. The separation media was controlled and maintained at a constant temperature.

## 2. 3. Experimental Determination of Protonation Macro- and Micro-constants

The following method of calculating the protonation constants has been validated and described by Takacs-Novak (1990),[11] Sun (2002),[10] and Langlois (2005).[12]

A pH meter (MP 225 model, Mettler-Toledo, USA) was used to measure protonation macro-constants $K_1$ and $K_2$ using a glass combination electrode (InLab413). The log*K* values were calculated by the attached software package. A 0.1 mM aqueous solution of sample (10 mL) was pre-acidified to pH 2 with 0.5 M HCl and then titrated with 0.5 M NaOH to pH 12. The titrations were carried out at constant ionic strength 0.15 M NaOH at 25°C. Each value is an average of three parallel measurements. The results were obtained by a complex computational process, according to the Yasuda-Shedlovsky standard extrapolation method.

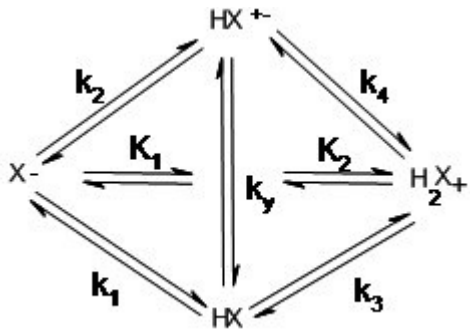


**Figure 1.** Ionization scheme of quinolones. $X^-$, HX, $HX^{+-}$, and $H_2X^+$ represent the anionic, neutral, zwitterionic, and cationic species, respectively.

Figure 1 presents the macro- ($K_1$, $K_2$) and micro-dissociation ($k_1$, $k_2$, $k_3$, $k_4$) constants.

$K_1$ and $K_2$ indicate the double-sided transition (proton accepting and proton donating) of the acidic and the basic moiety, respectively, to the zwitterionic and neutral forms:

$$K_1 = \frac{([HX^{+-}]+[HX])[H^+]}{[H_2X^+]} \quad (1)$$

$$K_2 = \frac{[X^-][H^+]}{[HX^{+-}]+[HX]} \quad (2)$$

Microscopic protonation constants specify the proton binding ability of the individual forms. They are characterized in Equations (3)–(6):

$$k_1 = \frac{[X^-][H^+]}{[HX]} \quad (3)$$

$$k_2 = \frac{[X^-][H^+]}{[HX^{+-}]} \quad (4)$$

$$k_3 = \frac{[HX][H^+]}{[H_2X^+]} \quad (5)$$

$$k_4 = \frac{[HX^{+-}][H^+]}{[H_2X^+]} \quad (6)$$

The $k_y$ constant is the tautomer equilibrium,[13] which expresses the concentration of the zwitterions and neutral species as a function of pH:

$$k_y = \frac{k_2}{k_1} = \frac{k_3}{k_4} \quad (7)$$

To determine four micro-protonation constants, the first step must be to experimentally determine the carboxyl group deprotonation fraction. The transition from the anionic to neutral forms expresses the $k_1$ micro-constant, and it was measured with the use of a UV/Vis spectrophotometer (JASCO 530). UV-metric methods provide p*K* results for the samples containing chromophores for which the UV absorbance changes as a function of pH. The protonation state of the acidic group of the quinolone is part of a conjugated region in the molecule with strong UV absorbance; the basic group is not part of the chromophore.[15] Each drug substance solution (50 μM) in 0.001 M HCl or 0.001 M NaOH was prepared, appropriate volumes of acid and base were mixed, and the spectrum of each solution was immediately recorded in the 200–400 nm wavelength range. To calculate the range of protonation and the acidic group at a given pH, the $\alpha_{coo-}$ value can be determined based on of absorbance measurements:

$$\alpha_{COO-(pH)} = \frac{A_{pH} - A_{COOH}}{A_{COO-} - A_{COOH}} \quad (8)$$

where $A_{pH}$ is the absorbance at a specified pH, at which the deprotonation fraction of the acid moiety ($\alpha_{COO^-(pH)}$) exists, and $A_{COO-}$ and $A_{COOH}$ are the experimental absorbance values at the deprotonated and protonated carboxyl group, respectively. $\alpha_{COO^-(pH)}$ shows the relationship of the individual fractions:

$$\alpha_{COO^-(pH)} = \frac{[HX^{+-}]+[X^-]}{[H_2X^+]+[HX^{+-}]+[HX]+[X^-]} \quad (9)$$

$$\alpha_{COO^-(pH)} = \frac{k_1[H^+]+K_1K_2}{[H^+]^2+K_1[H^+]+K_1K_2}$$

Subsequently, after the rearrangement of Equation (9), we obtained:

$$k_1 = \frac{\alpha_{COO\text{-}(pH)}(1 + K_1[H^+] + K_1K_2[H^+]^2) - K_1K_2[H^+]^2)}{[H^+]} \quad (10)$$

After the calculation of $k_1$, the other three coefficients can be determined. The relationship between the $k_2$ micro-constants (the conversion of the anionic form to neutral form $-k_1$) and the other factors follows:

$$k_2 = K_1 - k_1 \quad (11)$$

Micro-constants $k_3$ and $k_4$ describe the conversion of the neutral and zwitterionic forms to the cationic form, respectively. The equilibrium with the other forms is described by:

$$k_3 = K_1K_2/k_1 \quad \text{and} \quad k_4 = k_1k_3/k_2 \quad (12)$$

The intrinsic lipophilicity, $P_i$, is specified by only the concentration of the neutral species of the aqueous and lipophilic phases in equilibrium, but the experimentally obtained distribution of the octanol/buffer coefficient refers to a pH-dependent mixture of all electrical species present at a given pH in the polar phase and uncharged species in the octanol phase.[16] In which case, to define the intrinsic partition coefficient, one has to take into account the concentration of all species in the aqueous solution and the neutral species in the lipophilic solution.

$$P_{o/b} = \frac{[HX]_o}{[H_2X^+]_b + [HX^{+-}]_b + [HX]_b + [HX^-]_b} \quad (13)$$

From known macro- and micro-protonation constants in aqueous solvent, the concentration of the cation, anion, zwitterion, and neutral forms can be found, and the intrinsic partition coefficients can then be calculated for the partition of the neutral form in water to the neutral form in an organic solvent. In order to determine the intrinsic partition coefficient ($P_i$), the following equation can be used:[13,17,18]

$$LogP_i = LogP_{o/b} + Log(1 + \frac{1}{k_1[H^+]} + \frac{k_3}{k_4} + k_3[H^+]) \quad (14)$$

where the part in parentheses indicates the correction taking into account a given pH and the micro-constants.

## 2. 4. Molecular Modeling

The conformational space was found for each compound in order to determine the most stable and lowest-energy conformers. At first, the lowest energy conformers of each examined compound were found with the use of the Monte Carlo method with an implemented MMFF94 force field.[19] In the next stage, all of the conformers were used as starting-point structures in the calculations. Subsequently, the free energy of solvation in water and diethyl ether (dielectric constant of diethyl ether is close to *n*-octanol, which is not parameterized within Spartan 08) was determined for the most stable conformer following the SM8 continuum model and the density functional theory (DFT) method with the gradient-corrected B3LYP (Beck-Lee-Yang-Parr) functional and the 6-31*G basis set, as implemented in the Spartan 08 software package.[20] The electrostatic potential surfaces around the molecule were calculated using the same functional and basis set as the geometry optimization in order to evaluate the role of the electron donor and electron acceptor sites in the electrostatic pattern of the molecules.

## 2. 5. *In silico* Models

Values of the partition coefficient were also calculated using substructure-based methods.[21] The fragmental method developed by Rekker and Mannhold[22,23] splits the molecules into fragments and applies correction factors such as tautomerization effects, dipolar effects, ion effects, proximity effects, electronic interactions within one fragment, conjugated multi-hetero atomic effects, steric effects, H-bonding interactions, and so forth.[24] Algorithms like ACD/log*P* or AB/log*P* included in ACD/ChemSketch freeware v.12 I-Lab 2.0 (v 5.0.0.184) are based on the contributions of atoms, structural fragments, and intramolecular interactions between fragments.[21] MiLog*P* takes into account charge interactions.[25] The AC/logP algorithm from the VCCLAB on-line calculator relies on the construction of fragment sets derived from the measured log*P* data of simple molecules.[26] Below, the equation shows, schematically, the principle of the algorithm that calculates the value of the partition coefficient:

$$\log P = \sum k_1 f_1 + \sum l_2 F_2 \quad (15)$$

where $k$ is the number of repeats of fragment $f$ of type 1, and $l$ is the number of repeats of correction factor $F$ of type 2.

# 3. Results and Discussion

The physicochemical parameters of lomefloxacin, levofloxacin, and moxifloxacin, as potential bioavailability descriptors, have been determined by *in vitro* and *in silico* methods (Table 1).

The geometry of the lomefloxacin, levofloxacin, and moxifloxacin was optimized using the density functional theory (DFT) method with the gradient corrected B3LYP (Beck-Lee-Yang-Parr) functional and the 6-31*G basis set, as implemented in the Spartan 08 software package (Figure 2). Molecular determinants specified

**Table 1.**[1] values from ACD/Labs v 5.0.0.184; [2] values from experimental database VCCLAB (ALOGPS 2.1.); [3] values from ACD/ChemSketch v 2.0; [4] miLogP – www.molinspiration.com.

| | | Lomefloxacin hydrochloride | Levofloxacin hemihydrate | Moxifloxacin hydrochloride |
|---|---|---|---|---|
| **Free Energy of Solvation (kcal/mol)** | **in water** | –55.41 | –7.89 | –51.63 |
| | **in diethyl ether** | –66.06 | –18.27 | –61.57 |
| **Electrostatic Potential (kcal/mol)** | | –30.63 – +160.51 | –64.41 – +47.99 | –3.27 – +152.40 |
| **Experimenatal Partition Coefficient** | **$LogP_{n\text{-}oct/buf}$ pH = 6.8** | –0.47 | –0.25 | –0.28 |
| | **$LogP_i$** | 0,19 | 0,74 | 0,96 |
| **Protonation Macroconstants** | **$LogK_1$** | 8,81 | 8,20 | 9,28 |
| | **$LogK_2$** | 5,94 | 6,23 | 6,30 |
| **Protonation Microconstants** | **$Logk_1$** | 8,24 | 7,31 | 8,15 |
| | **$Logk_2$** | 8,71 | 8,22 | 9,19 |
| | **$Logk_3$** | 6,35 | 7,14 | 7,52 |
| | **$Logk_4$** | 5,82 | 6,29 | 6,23 |
| ***In Silico* Obtained Partition Coefficient Data** | **ACD/logP[1]** | 1.71±1.45 | 0.84±1.5 | 1.6±1.54 |
| | **AC/logP[2]** | 0.28(+0.6) | 0.26(+0.6) | 0.58 |
| | **AB/logP[3]** | –0.67 | 0.01 | 0.79 |
| | **miLogP[4]** | 0.08 | –0.262 | 0.39 |

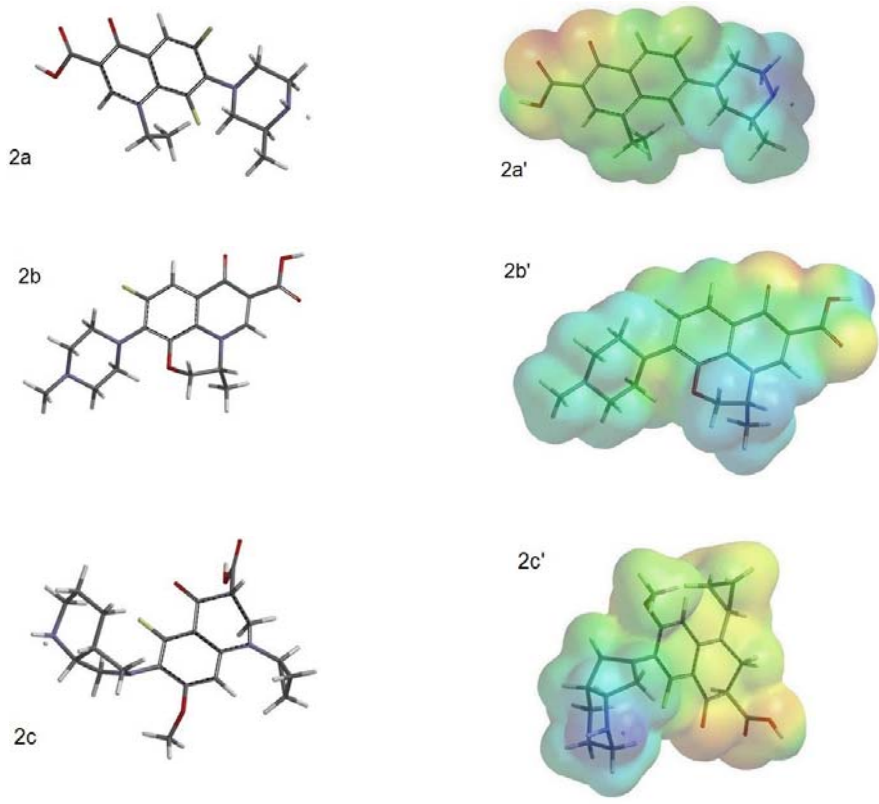


**Figure 2.** Optimized structure conformers of the lowest-energy and electrostatic potential on surfaces of the lowest-energy conformer. **2a, 2a' – Lomefloxacin** (1-ethyl-6, 8-difluoro-7-(3-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic acid); **2b, 2b' – Levofloxacin** ((*S*)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid); **2c, 2c' – Moxifloxacin** (7-[(4a*S*,7a*S*)-1,2,3,4,4a,5,7,7a-octahydropyrrolo[3,4-b]pyridin-6-yl]-1-cyclopropyl-6-fluoro-8-methoxy-4-oxoquinoline-3-carboxylic acid).

the most stable conformers in water and diethyl ether. The free energy of solvation and electrostatic potential values are presented in Table 1. The highly negative values of $\Delta G$ in water for lomefloxacin and moxifloxacin hydrochloride appear to correlate well with the electrostatic potential range of the examined molecules. The $\Delta G$

value is less negative for levofloxacin hemihydrate, but an electrostatic potential range may indicate that this compound is highly soluble in a polar medium. The free energy of solvation in an organic solvent, which is more negative than in water, demonstrates good lipophilic properties of the molecules.

The transfer of particles from the ideal gas phase, with a high concentration of 1 mol/L, to an ideal solution of the same concentration at a given temperature results in a change in the Gibbs function. This is a good example, describing the thermodynamics of a dissolution reaction, which occurs in the human body after oral administration of the drug substance in the form of an immediate release tablet, and the diffusion of the dissolved active substance through the lipophilic biological membranes of the small intestine, which have lipophilic character. Negative values of the free solvation energy indicate that the dissolution reaction occurs spontaneously.

The particles in diethyl ether have a large negative thermodynamic potential of solvation and, remarkably, for levofloxacin it is two times higher than in water (Table 1). Higher values of $\Delta G$ in an organic solvent than in water show the high intrinsic lipophilicity of the molecule. Therefore, these molecules, depending on the physiological pH of the small intestine, change protonation state and exist in the zwitterionic or cationic form with a significant dipole.[10,11] However, the high intrinsic lipophilicity indicates that the molecules can pass through the membrane by passive transport, which is not consistent with their zwitterionic character.[27] Researchers that studied the mechanism of transfer of ciprofloxacin using a simulated biological membrane noticed that the diffusion of ciprofloxacin occurs in its neutral form. Such neutralization may be caused by the intermolecular transfer of protons, which is favored by total desolvation of the neutral form or partial desolvation of the zwitterionic form. It was found that fluoroquinolones form dimer or trimer stacks, thus their electrostatic potential is reduced, and they pass through the membrane by passive diffusion.[28]

The electrostatic potential of the hydrochloride molecules has concentrated and diffuse negative charges at the carboxyl group and nitrogen of the piperazine ring, respectively. This phenomenon is depicted by the blue and red clouds of potential areas. In the case of levofloxacin, these areas are less pronounced as a result of the charge diffusion on the benzoxazine and piperazine ring as well as the negative charge on the oxygen of oxochinoline group.

The electrostatic potential value gives additional information that can explain solute–solvent interactions and the polarity of the examined compound.[29] The wide range of lomefloxacin hydrochloride electrostatic potentials (–30.63 to 160.51 kcal/mol) proves its exceptional hydrophilic properties (Figure 2a'). The electron cloud of moxifloxacin hydrochloride may be scattered or concentrated, depending on the interactions between the pyrrole-bi-pyridine ring and the methoxy group, thus the hydrophilicity is slightly lower (Figure 2c'). On the surface of the levofloxacin molecule (Figure 2b'), the diffusion of electrons occurs in the vicinity of 3-methyl-7-oxo-7H-pirido substituent and the hydroxy group, and makes the range of dipole electrostatic interactions narrower (–64.41 to 47.99 kcal/mol), which modifies the interaction of the molecule with a polar solvent.

The experimental and theoretical log*P* values reveal the lipophilicity of the active substances. The logarithm of the partition coefficient of *n*-octanol/water was also calculated using *in silico* fragmentary methods, such as ACD/log*P*, AB/log*P*, AC/log*P*, miLog*P*. Fragmentary methods show a large discrepancy in the results (Table 1). *In silico*-obtained partition coefficients indicate different lipophilic properties of the compounds.

The log*P* value obtained by *in silico* calculations with use the fragmental model, does not give conclusive evidence of the hydrophilic or lipophilic properties of the tested compounds (Table 1). It follows that the theoretical models, even if taking into account various corrective factors such as atom hydrophobicity, rotatable bond count, hydrogen bonds, steric hindrance, intramolecular interactions, electronic effect and so on,[30] cannot yield sufficiently reliable and precise results.

The experimental distribution of the *n*-octanol/buffer coefficients obtained at pH = 6.8 by the shake-flask method indicates a high affinity of the particles to the aqueous phase as well as their good solubility in this medium.

The micro-constant values allowed us to calculate the intrinsic partition coefficients [Eq. (14)]. The calculated values are listed in the Table 1. The protonation micro-constant values are within the pH limits of 5.82–9.19 in the small intestine. Tautomer equilibrium ($k_y$) values indicative of the wide range of zwitterionic species compare well to the neutral species. The intrinsic partition coefficient was 0.19 for lomefloxacin, 0.74 for levofloxacin, whereas it was 0.96 for moxifloxacin. The resulting values of the partition coefficient indicate a relatively high affinity for the lipophilic phase, wherein the second generation of fluoroquinolones shows lower lipophilic membrane permeability in comparison to the third and fourth generations.

The results of the *in vitro* experiments indicate high intrinsic lipophilicity of the moxifloxacin hydrochloride and levofloxacin hemihydrate. However, for lomefloxacin hydrochloride, despite its high solubility in a polar environments, the lipophilic barrier is more difficult to overcome (Table 1). According to the BCS guidelines, insufficient solubility of the compound in aqueous medium limits the drug bioavailability of the solid oral dosage form.[1] The studied fluoroquinolones possess high solubility in aqueous media, but the question arises as to how they overcome the lipophilic barrier, as all available data indicate that these compounds have good

bioavailability and yield an high overall efficiency for the therapy.[31]

According to the BCS, the most bioavailable drugs penetrate through the cellular membrane in passive transport mode.[1] Fluoroquinolones, owing to changes in the gastrointestinal tract pH, can exist in various protonation forms, but the protonation micro-constants ($k_1$–$k_4$, $k_y$) indicate that the compounds usually have a zwitterion form at physiological pH in the absorption site. Many sources indicate that, despite the wide electrostatic potential range on the surface of the compound, these compounds pass through cell membranes by the diffusion pathway.[27,32] This is attributed to the intermolecular transfer of protons in the zwitterion molecule by dehydration and the formation of a so-called stack, as shown elsewhere.[28] Such agglomerates are formed in an aqueous medium and possess a tendency to separate during movement in the lipophilic environment of a biological membrane. Agglomerates formed by zwitterions eliminate the high total charge of the electric field, thus molecules can naturally pass through biological membranes.

In addition to drug penetration through the cell membrane (also in bacterial strains) through the formation of stacks,[28] some researchers have found that higher partitioning occurs at a lower pH (where dominate cationic species) than at physiological pH (where dominate zwitterionic form). Electrostatic forces between the positive charge of fluoroquinolone and the negative charge of acidic phospholipids increased the binding.[17,33] Others have noted that fluoroquinolones can pass in a »self-promoted« manner, similarly to the route used by cationic compounds.[34] A further study concerned the evaluation of the possible electrostatic contributions to the interaction of moxifloxacin with liposomes (dimyristoyl-L-α-phosphatidylglycerol). Their observation suggests a strong affinity of the N-protonated diazabicyclonyl ring of moxifloxacin towards the negatively charged phosphate liposome groups at physiological pH, and subsequently enhanced electrostatic interactions.[35] In summary, the behavior of the cationic species is important for the understanding of the mechanisms of fluoroquinolone penetration through biological membranes.

The results of our study indicate high intrinsic lipophilicity of moxifloxacin and levofloxacin, moderate intrinsic lipophilicity of lomefloxacin, as well as good solubility of the drugs in the environment at physiological pH.

From the BCS viewpoint, these physicochemical properties are relevant to the description of the rate of release of the drugs from their pharmaceutical form in order to achieve a sufficiently high concentration at the absorption site and in the bloodstream.[1]

Determination of the lipophilicity of the compounds is a key element for *in vitro* studies on bioavailability, which justifies the choice of methods used here as useful tools in the classification of drugs within the system.

# 4. Conclusion

Defined physicochemical parameters could be used to evaluate the activity of drug substances in the gastrointestinal tract. As is evident from the Experimental Section, newer generations of fluoroquinolones have better physicochemical parameters and, hence, could have better bioavailability. So far, the WHO has classified three therapeutic substances from the group of fluoroquinolones within the BCS: ciprofloxacin into the third class as well as levofloxacin and ofloxacin into the first.[29] Classification of levofloxacin into the first class of this system has been confirmed by studies carried out in the framework of our study. From the experiments, it appears that lomefloxacin could be included in the I/III BCS class, because of its high hydrophilicity and the possibility for its active passage through the membrane, which results from the effect of a P glicoprotein that belongs to a group of so-called efflux proteins.[36] Moxifloxacin can be classified into the I BCS class. The methods used in our study are measurable, cost effective, and provide a fast assessment of a drugs biological activity based on the BCS concept.

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## Povzetek

Z *in silico* in *in vitro* metodama smo proučevali vpliv fizikalno-kemijskih lastnosti treh fluorokinolonov (druge, tretje in četrte generacije) na biorazpoložljivost v povezavi z biofarmacevtskim klasifikacijskim sistemom (BCS). Analizirali smo modele elektrostatskega potenciala in vrednosti proste energije solvatacije kot tudi porazdelitvene koeficiente preiskovanih snovi. Raziskava je temeljila tako na teoretičnih kvantno-mehanskih metodah kot tudi na *in vitro* stresalni tehniki z dvema fazama, ki se med seboj ne mešata (*n*-oktanol in fosfatni pufer). S potenciometrično metodo smo določili konstante protonacije. Izkazalo se je, da so tako teoretične metode kot tudi enostavna *in vitro* študija primerne za napovedovanje biorazpoložljivosti medicinskih substanc na osnovi BCS načel.