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Solubility, lipophilicity and membrane permeability of some fluoroquinolone antimicrobials

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ABSTRACT

Aqueous solubility and distribution of ciprofloxacin, enrofloxacin, norfloxacin and levofloxacin antimicrobials drugs in octanol/buffer system has been measured by the isothermal saturation method using buffer solutions pH 2.0 and 7.4 in the temperature range of 293.15–313.15 K. Thermophysical characteristics for the compounds have been determined by the DSC method. It has been established that the solubility of levofloxacin in these buffers is higher than that of the other fluoroquinolones. HYBOT descriptors for biologically active compounds have been calculated and the impact of the donor-acceptor capacity of the molecules on drugs solubility has been studied. According to the lipophilicity parameter fluoroquinolones are ranged in the following order: enrofloxacin > levofloxacin > ciprofloxacin > norfloxacin. The thermodynamic solubility and distribution functions of the studied compounds have been obtained. The permeability coefficients of the substances through an artificial phospholipid membrane were determined. The drugs with a lower aqueous solubility were estimated to have higher distribution coefficients and membrane permeability.

Keywords:
Fluoroquinolones
Solubility
Distribution octanol/buffer
Membrane permeability
Thermodynamics

1. Introduction

Fluoroquinolones are an important class of synthetic antibiotics that has proved to be efficient as broad-spectrum antimicrobial agents. They selectively inhibit one of the main enzymes of the microbial cell – DNA-gyrase - responsible for normal DNA synthesis, which results in bacteria death (Nix and Schentag, 1988). The most exciting achievements in synthesis of new fluoroquinolones are associated with obtaining drugs of high antimicrobial activity, optimal pharmacokinetics and low toxicity (Bolon, 2011). As medicinal fluoroquinolones are ampholytes, they are ionized in solutions to a varying extent depending on pH, influencing physical, chemical, thermal and biological properties important for their application (Florindo et al., 2014). So, the ionization processes affect the solubility and lipophilicity of the compounds – key parameters determining the availability of the substances to enter the biological membranes (Avdeef, 2003).

Many important biochemical interactions occur in both aqueous and lipophilic environment, influencing bioavailability, skin permeability, toxicity and environmental fate of drugs (Hansch, 2002; Sangster, 1997). Therefore, studying the drug-solvent interactions in both aqueous (hydrophilic) and organic (lipophilic) solvents is very important when designing new drug compounds with the enhanced characteristics. In our study we used buffers modeling pharmaceutically relevant aqueous media: buffer pH 2.0 models the medium of gastrointestinal tract, while pH 7.4 one – that of blood plasma. 1-Octanol (octanol) is a commonly used organic solvent for studying the biological systems. Amphiphilic character gives octanol the properties similar to those of lipids of the biological membranes and, consequently, the distribution coefficient in octanol-water system is applied for the assessment of the drugs membrane transport (Best et al., 1999). Despite the wide use of fluoroquinolones in medicine, rather few works (Florindo et al., 2014; Yu et al., 1994; Ross and Riley, 1990; Faller and Ertl, 2007; Zhang and Wang, 2008) deal with the effect of temperature and pH of the aqueous medium on solubility and lipophilicity parameters. However, the area of research in these works is limited by the pH range and temperature and the effect of temperature on these characteristics of the drugs are not taking into account. The thermodynamic properties of dissolution and distribution processes of the substances belonging to fluoroquinolone class are limited. At present, there is a great number of the approaches to determine the lipophilicity but the calculations for the compounds containing several functional groups as in the case of

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fluoroquinolones require many empiric parameters and their accuracy is insufficiently high as yet (Machatha and Yalkowsky, 2005).

Most fluoroquinolones are orally administered and, therefore, need to traverse several membrane barriers before receptor interactions can commence. Lipophilicity of the biologically active substances usually correlates with the ability to penetrate through the bacterial membranes that is of a great interest for the pharmaceutical industry. But an isotropic octanol nature poorly imitates the biomembranes. In this regard, the model membranes imitating the lipid layer of those in the living organism are used to investigate bioavailability of the compounds (Kerns and Li, 2008). According to Biopharmaceutics Classification System most of the drugs with good bioavailability (including fluoroquinolones) pass through the cellular membrane by passive transport. Investigation of the passive diffusion of fluoroquinolones ascertains the molecules of these substances to form bulk stacks and pass through the membranes in neutral form (Klosinrska-Szmurlo et al., 2014; Takacs-Novak et al., 1990). Barbato et al. (Barbato et al., 2007) investigated the interactions of quinolones and artificial immobilized membrane as compared to n-octanol lipophilicity. The obtained results suggest that quinolone/phospholipid data, encoding both lipophilic and electrostatic recognition forces, can be useful parameters to gain further information about the mechanism of interaction between highly ionized compounds and serum-proteins.

The aim of this work was to investigate the effect of the structure of a series of fluoroquinolone drugs (ciprofloxacin, enrofloxacin, norfloxacin and levofloxacin) on the solubility in the pharmaceutically relevant media, lipophilicity and membrane permeability. The obtained results may be helpful in predicting bioavailability of new medical fluoroquinolones and have been playing an important role in the rational drug design. The present study is a continuation of our investigation of physicochemical properties of antituberculous drugs in solid state and solution (Blokhina et al., 2015; Volkova et al., 2015; Blokhina et al., 2014).

2. Experimental section

2.1. Materials

Ciprofloxacin, enrofloxacin, norfloxacin and levofloxacin were obtained from commercial sources. The origin, CAS numbers and purity of all samples are presented in Table 1.

Bidistilled water (with electrical conductivity 2.1 μS cm^{-1}) was used for preparation of buffer solutions. Phosphate buffer pH 7.4 (I = 0.15 mol/l) was prepared by combining the $KHPO_4$ (9.1 g in 1 L) and $NaH_2PO_4 \cdot 12H_2O$ (23.6 g in 1 L) salts. For the preparation of the buffer solution pH 2.0 ($I = 0.10$ mol/l) 6.57 g of KCl was dissolved in water, 119.0 mL of 0.1 M hydrochloric acid was added and the volume of the solution was adjusted to 1 L with water. The pH values were measured by using pH meter FG2-Kit (Mettler Toledo, Switzerland) standardized with pH 1.68, 6.86 and 9.22 solutions.

Egg phosphatidyl choline, Lipoid E-80 was obtained from Lipoid (Germany). Culture inserts (Transwell-Clear, $d = 6.5$ mm) and plates were purchased from Corning Inc. Corning, USA, filters in existence were removed, and mixed cellulose ester filters (0.65 μm pore size) from Millipore, Billerica, USA were fused on.

2.2. Differential scanning calorimetry

Temperatures and enthalpies of melting of the compounds under investigation have been determined using a Perkin-Elmer Pyris 1 DSC differential scanning calorimeter (Perkin-Elmer Analytical Instruments, Norwalk, Connecticut, USA) with Pyris software for Windows NT. DSC runs were performed in an atmosphere of flowing 20 $cm^3 \cdot min^{-1}$ dry helium gas of high purity 0.99996 (mass fraction) using standard aluminum sample pans and a heating rate of 2 $K \cdot min^{-1}$. The accuracy of weight measurements was 0.005 mg. The DSC was calibrated with an indium sample from Perkin-Elmer (P/N 0319-0033). The value determined for the enthalpy of fusion corresponded to 28.48 $J \cdot g^{-1}$ (reference value 28.45 $J \cdot g^{-1}$). The fusion temperature was 429.5 ± 0.1 K (determined from at least ten measurements).

2.3. Solubility

All the experiments were carried out by the isothermal saturation method at five temperature points: 293, 298, 303, 310, 315 ± 0.1 K. The essence of the above method includes determination of the compound concentration in the saturated solution. Glass ampoules containing the tested substance and the solvent were placed into the air thermostat supplied by the stirring device. The time required for establishing a constant value of the solution concentration was determined from the solubility kinetic dependences and averaged 24 h. The time of sedimentation of solid phase after stirring was 2 h. The solution aliquot was taken and centrifugated in centrifuge under the temperature control Biofuge stratos (Germany) during 5 min under the fixed temperature. The solid phase was removed by isothermal filtration with filter MILLEX®HA 0.45 μm (Ireland). The saturated solution was diluted with the correspondent solvent to the required concentration. The molar solubilities of drugs were measured by means of spectrophotometer Cary-50 (Varian, USA) with an accuracy of 2–4%. The experimental results are reported as an average value of at least three replicated experiments. It should be noted, that sediment DSC analysis showed the absence of crystallosolvates for all the tested compounds.

The standard Gibbs energies of dissolution processes ΔG_{sol}^0 were calculated using the following equation:

$$\Delta G_{sol}^0 = RT \ln a_2 \quad (1)$$

where $a2 = \gamma 2 \cdot x$ is the activity of the solute molecule; x is the drug molar fraction in the saturated solution; γ2 is the activity coefficient of the solute molecule. The standard solution enthalpies ΔH_{tr}^0 were calculated using the van't Hoff equation:

$$\partial(\ln a_2)/\partial T = \Delta H_{sol}^0/RT^2 \quad (2)$$

Due to very low solubilities of the compounds under investigation in a buffer solutions it was assumed that $\gamma 2 = 1$. The temperature dependencies of drug solubilities within the chosen temperature interval can be described by the linear function:

$$\ln x = A\text{-}B/T \quad (3)$$

This indicates that the change in heat capacity of the solutions with the temperature is negligibly small.

The standard solution entropies ΔS_{sol}^0 were obtained from the well-known equation:

$$\Delta G_{sol}^0 = \Delta H_{sol}^0 - T\Delta S_{sol}^0 \quad (4)$$

2.4. Distribution experiment

The experiments for determination of distribution coefficients octanol/buffer were carried out by the isothermal saturation method at five temperatures: 293.15, 298.15, 303.15, 308.15 and 313.15 K. The procedure was as follows: to a defined volume of a buffer saturated octanol solution an identical volume of octanol saturated buffer of defined compound concentration was added in an ampoule placed in a thermostat. The resulting solution was equilibrated for two days with continuous shaking. The drug concentrations in the both phases were determined by means spectrophotometer Cary-50 (USA) in UV spectral region (λ = 190–400 nm) with an accuracy of 2–4%. The reported experimental values represent the average of at least three replicated experiments.