Investigation of the Lipophilicity of 2-Benzoylpyridine-Thiosemicarbazone Based on the Ion Transfer across the Liquid/Liquid Interface

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Abstract：   The ion transfer reaction of 2-benzoylpyridine-thiosemicarbazone (HL), which has antimicrobial and antifungal properties and anticancer activity, has been studied to determine its lipophilicity by cyclic voltammetry at the water/1,2-dichloroethane (1,2-DCE) interface. The physicochemical parameters such as standard partition coefficient ($\lg P_I$) and the standard Gibbs energy of transfer ($\Delta G_{tr,I}^{0, w \to o}$) of the protonated form of the ligand were measured as a function of pH in aqueous phase. The protonated form of the ligand exhibited reversible or quasi-reversible voltammograms at the 1,2-DCE in the range of pH 1−5. The protonation constants of the ligand, pK_{a1} and pK_{a2}, were determined spectrophotometrically and were found to be 12.14 and 3.24, respectively. The standard Gibbs energy of transfer ($\Delta G_{tr, N}^{0, w \to o}$) and the partition coefficient of neutral species ($\lg P_N$) were also determined by the shake-flask method. The standard Gibbs energy of transfer of this compound across the water/1,2-DCE interface was evaluated as the quantitative measure of its lipophilicity. The difference between $\lg P_I$ and $\lg P_N$ was related to the degree of charge delocalization and was used to evaluate qualitatively the lipophilicity of the ligand.

Key Words： Lipophilicity;   2-Benzoylpyridine thiosemicarbazone;   Ion transfer;   Protonation constant;   Liquid/liquid interface

Quantitative structure-activity relationships (QSAR) have been extensively used to correlate the biological activity of drugs with physicochemical parameters such as solubility, the partition coefficient in oil/water systems, electronic effect of substituents, electron density, and steric effects, in the last few decades [1]. Lipophilicity represents the affinity of a compound for a lipidic environment and it is the most widely used parameter to design drugs and to assess their performances. A quantitative measure of the lipophilicity of ions is related directly to the standard Gibbs energy of the ion transfer from water to organic phase[2].

The interface between an organic and a water phase has been frequently inferred as a simple model of biological membranes[3]. *n*-octanol is the most commonly used solvent in lipophilicity as the organic phase[4]. However, the *n*-octanol/water interface is not sufficient to model membrane permeation, owing to the major differences in the biophysical properties. For example, the presence of hydrogen bonds between water and *n*-octanol molecules at the interface introduces important changes in the interfacial structure. On the other hand, owing to very few base electrolytes that can dissolve in the *n*-octanol phase, the polarizability of this system is very difficult except the nano interface studies[5]. Owing

to the water/1,2-DCE interface being polarizable, this system is more suited for electrochemical studies[6]. Lipophilicities of some ionizable drugs have been determined by cyclic voltammetry at the interface between two immiscible electrolyte solutions (ITIES) across the water/1,2-DCE interface[1,7−10]. It has been demonstrated that cyclic voltammetry at ITIES is the method of choice to determine the lipophilicity of cations, in particular drugs that can be protonated[4]. The transfer of a base BH+ can be regarded either as a classical cation transfer reaction or as a proton transfer reaction assisted by the conjugated acid[11]. Therefore, these types of assisted ion transfer reactions concern with acid-base properties of molecules. The neutral form of acids and bases may reduce the Gibbs energy of transfer of the proton as a result of interfacial protonation and deprotonation reactions, respectively. The explanation of interfacial mechanism is of great importance in pharmacology, because the proton concentration is very tightly regulated in functional biological systems[12].

The partition coefficient of a given solute between two immiscible solvents is a measurement of its relative affinity for both phases. Since $\lg P$ is related to the free energy of transfer of the solute between the solvents, it encodes information on the differ-

ential solvation effects and has been correlated with the biological and pharmacological processes with regard to adsorption, transport through cell membranes, and hydrophobic binding[13].

Thiosemicarbazones (hydrazine carbothioamides) and their metal complexes exhibit a wide range of medicinal applications and represent some of the most potent known inhibitors of ri bonucleoside diphosphate reductase[14–20]. Metal complexes of 2-benzoylpyridine-derived thiosemicarbazone were characterized by spectroscopic techniques and their electrochemical and biological properties were also studied in detail[21–24]. Antifungal activities against candida albicans[22] and antibacterial activities in nutrient broth against *E. coli*-10536[25] of HL and its metal complexes have been studied. The cyctotoxic activity of palladium (II) complexes of 2-benzoylpyridine-thiosemicarbazone (HL), and its N(4)-methyl and -phenyl derivatives were also tested against the MCF-7, TK-10, and UHCC-62 human tumor cell lines[26].

The electrochemical behavior of HL was first described on the basis of classical and differential pulse polarographic techniques[27]. The complexes of 2-benzoylpyridine thiosemicarbazone as well as of its N(4)-methyl and N(4)-phenyl analogues with metals such as iron (II), nickel (II), and zinc (II) were characterized and the electrochemical studies revealed that the iron (II) complexes undergo oxidation giving the iron (III) analogues, which can be reduced back by cellular thiols such as thioredoxine, suggesting that this process can occur in biological media[22]. The aim of this study was to examine the voltammetric behavior of the 2-benzoylpyridine-thiosemicarbazone (HL), which has antimicrobial and antifungal properties and anticancer activity that can have some advantages for biological systems, to characterize its ion transfer reaction across the water/1,2-DCE interface, and to determine the lipophilicity scale to obtain some conclusion to the origin of its pharmacological activity in biological systems.

1 Experimental

1.1 Chemicals

Deionized water (Milli-QSP reagent water system, Millipore) and 1,2-dichloroethane (1,2-DCE) of the highest available purity (Merck) were used as organic phase, without any treatment. The organic supporting electrolyte was prepared by metathesis of bis-(triphenylphosphoranylidene) ammonium chloride (BTPPA-Cl) (Fluka) and potassium tetrakis(4-chlorophenyl)borate (KTP-BCl) (Lancaster, UK) in 1:1 methanol/water mixture providing a BTPPATPBCl precipitate as described[12]. LiCl (Fluka) was the aqueous electrolyte and HCl or LiOH (Fluka) was added to the water phase of electrochemical cell to fix at the desired pH value.
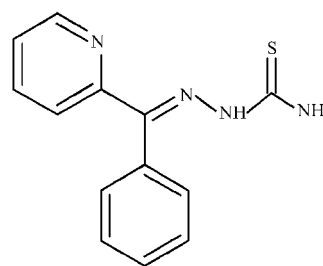

Fig.1 Chemical structure of 2-benzoylpyridine-thiosemicarbazone (HL)

2-benzoylpyridine-thiosemicarbazone (Fig.1) was prepared according to the method as described in the literature[25]. The structure of ligand was confirmed by elemental analysis, ^{1}H-NMR, and IR spectrometric techniques.

In the spectrophotometric experiments, Britton and Robinson buffer solution was used in pH interval 2−12.

1.2 Cell and apparatus

The ion-transfer reaction across the water/1,2-DCE interface was studied by means of cyclic voltammetry using a four-electrode cell system described elsewhere[10]. The cell was powered by a four-electrode potentiostat featuring *IR* compensation by positive feedback, PAR-263/A2 (PAR, USA). The area of the interface was 0.273 cm^2 and the experiments were carried out at room temperature (24±1) ℃. In all experiments, the cell was placed in a Faraday cage.

The electrochemical cell can be represented by Scheme 1.

The potential E applied between the two Ag/AgCl reference electrodes is related to the Galvani (or the inner) potential difference ($\Delta_o^w\phi$) across the interface by:

$$E=\Delta_o^w\phi+\Delta E_{ref} \quad (1)$$

Tetraethyl ammonium cation (TEA$^+$) was added to the aqueous phase after each experiment, to reference all the observed standard transfer potentials, $E_{tr,I}^0$, deduced from the peak potentials in the voltammograms (Fig.2).
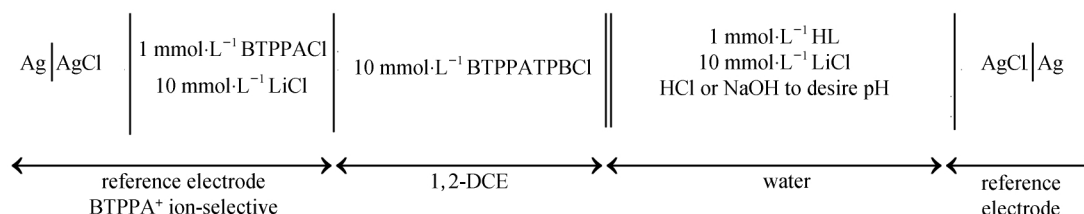
These values were then transposed to the absolute scale by applying the following relationship,

$$E_{tr,I}^0-\Delta_o^w\phi_{tr,I}^0=E_{tr,ref}^0-\Delta_o^w\phi_{tr,ref}^0 \quad (2)$$

where, $\Delta_o^w\phi_{tr,I}^0$ is the standard Galvani transfer potential of protonated ligand, and $\Delta_o^w\phi_{tr,ref}^0$ is the standard Galvani transfer potential of TEA$^+$ ($\Delta_o^w\phi_{tr,TEA^+}^0$=44 mV according to Ref.[28]).

1.3 Determination of protonation constants

The protonation constants of the ligand, K_{a1} and K_{a2}, were determined spectrophotometrically at 25 ℃ and ionic strength of 0.1 mol·L^{-1} KNO$_3$. UV-Vis spectrophotometric measurements were carried out with Shimadzu UV-160 spectrophotometer.

| Ag | AgCl | 1 mmol·L^{-1} BTPPACl / 10 mmol·L^{-1} LiCl | 10 mmol·L^{-1} BTPPATPBCl | 1 mmol·L^{-1} HL / 10 mmol·L^{-1} LiCl / HCl or NaOH to desire pH | AgCl | Ag |

| reference electrode BTPPA$^+$ ion-selective | 1,2-DCE | water | reference electrode |

Scheme 1 Schematic diagram of the electrochemical cell
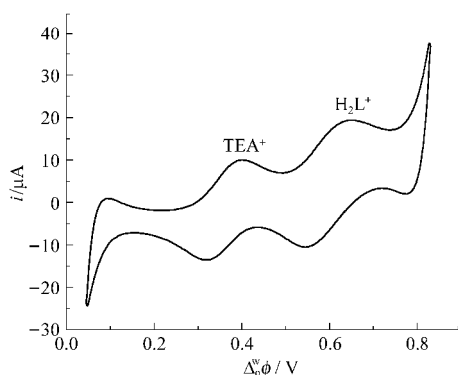
Fig.2 Obtained voltammograms for the transfer of TEA⁺ and the protonated form of ligand (H₂L⁺) at pH 3

Fig.4 Distribution of ionic species for the thiosemicarbazone

Crison micropH 2002 pH-meter with combined electrode (Metter Toledo-Inlab 412) was used for the pH measurements. Standardizations of HCl and NaOH solutions were adjusted potentiometrically.

The applied method depends upon the direct determination of the ratio of molecular species (neutral molecule) to ionized species in a series of non-absorbing buffer solutions, whose pH values are either known or measured. All spectra were recorded in the range of 250–450 nm, at slow scan speed, against corresponding blank (the number (n) of parallel measurements was 6).

1.4 Shake-flask method

The partition coefficient of neutral form of the ligand in the water/1,2-DCE system was measured by the shake-flask method[29]. Prior to each experiment, the organic and aqueous phases were mutually saturated for 6 h. The ligand was dissolved in the 1,2-DCE phase containing 0.01 mol·L⁻¹ BTPPATPBCl, while the aqueous phase composition was 0.01 mol·L⁻¹ LiCl (pH 7.4). Both phases were shaken (36 h) together before separation and analysis of the organic solution was done by UV spectroscopy (n=6).

2 Results and discussion

2.1 Protonation constants

Fig.3 shows the absorption spectra at different pH ranges 1–14. Clearly discernible isosbestic points in pH-dependent absorption spectra indicate that the acid-base equilibrium is not changed by side-reactions. The pK_{a1} and pK_{a2} values were
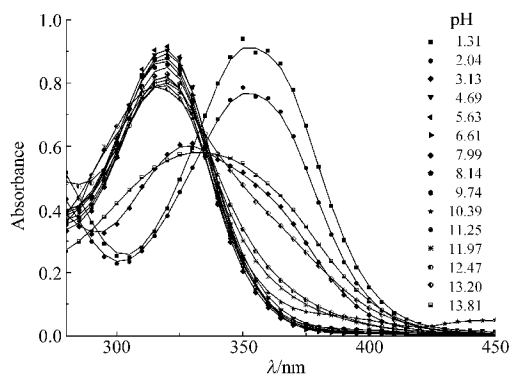

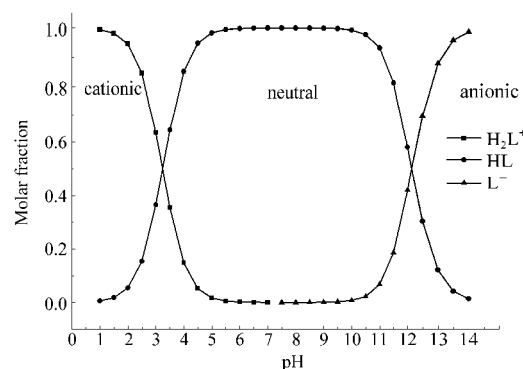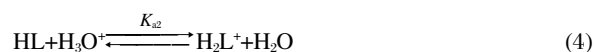**Fig.3 Plots of absorbance data *versus* wavelength for HL**

calculated from the classical spectrophotometric equation[30] and were found to be equal to 12.14 and 3.24, respectively.

The ligand possesses one acidic NH group and one basic pyridyl group, thus, it behaves as a weak base and a weak acid, and therefore, it has three independent species in aqueous solutions. The distribution of ionic species for the pH range from 1 to 14 is shown in Fig.4. The equilibrium between the species is given by the equilibrium equations as follows:

$$L^-+H_3O^+ \underset{K_{a1}}{\overset{}{\rightleftharpoons}} HL+H_2O \tag{3}$$

$$HL+H_3O^+ \underset{K_{a2}}{\overset{}{\rightleftharpoons}} H_2L^++H_2O \tag{4}$$

The pK_{a2} value is lower than that of pyridine (5.25). Compared to pyridine, it can be attributed to the decrease in electron density on the pyridine nitrogen caused by the electron-withdrawing effect of the thiureide group[20].

It has been found from the data of ¹H and ¹³C NMR spectroscopies that in the electronic spectra of the HL, the signals are doubled as a consequence of the existence of the Z and E configurational isomers in solution[22]. It can be pointed out that the isomer and resonance forms directly affect the acidity and basic properties of HL, i.e., pyridine nitrogen of HL in the E form with regard to the Z form can be easily protonated owing to the hydrogen bond in the molecules.

2.2 Thermodynamic parameters

Lipophilicity of a solute is commonly measured by its partition coefficient (P) in a biphasic system. Thermodynamically, this parameter is defined as the ratio of the activity of a species dissolved at equilibrium between two immiscible solvent phases and it is often expressed on a logarithmic scale as lg$P$.

The partition coefficient of protonated form (lgP_I) is calculated according to

$$\lg P_I = -\left(\frac{zF}{2.303RT}\right)\Delta_o^w\phi_{tr,I}^0 \tag{5}$$

where, lgP_I represents the proportion of ions present in each phase if the interface is not polarized. In dilute solutions, the standard ion-transfer Galvani potential is related to the standard Gibbs energy of transfer by the following definition

$$\Delta_o^w\phi_{tr,I}^0 = \frac{\Delta G_{tr,I}^{0,w\to o}}{z_IF} \tag{6}$$

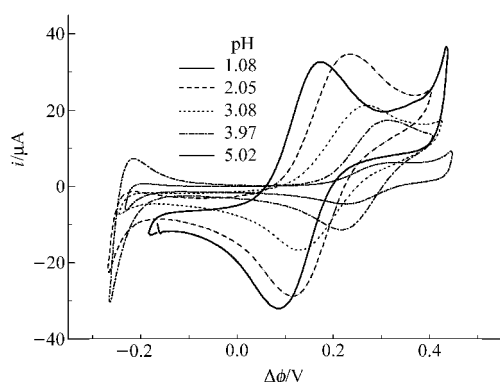The Gibbs energy of transfer from an organic solvent to water

Fig.5 Typical cyclic voltammograms obtained for the transfer of protonated form of HL across the water/1,2-DCE interface at different pH values

has been used as a quantitative measure of the lipophilicity of organic compounds. In the case of charged components, the standard Gibbs energy of ion transfer is a direct measurement of the lipophilicity[1].

The neutral form (N) of an ionizable compound is distributed between the two phases, but its partition coefficient is independent of $\Delta_o^w \phi$ and is related simply to its standard Gibbs energy of transfer $\Delta G_{tr,N}^{0,w \to o}$ as defined by Eq.(5).

$$\lg P_N = \lg\left(\frac{a_N^o}{a_N^w}\right) = -\frac{\Delta G_{tr,N}^{0,w \to o}}{2.303RT} \tag{7}$$

The voltammograms corresponding to the transfer of the protonated form of the ligand are shown in Fig.5 between pH 1 and 5. The voltammograms obtained at different scan rates were analyzed at pH 1 (Fig.6). The dependence of the background-subtracted anodic peak-current (i_{pa}) on the square root of the scan rate (v) is shown in Fig.7, indicating that the current is proportional to $v^{1/2}$. The peak separation was 65 mV and the mid-point potential was determined as 134 mV from Eq.(2). The linear dependence of the peak current on the square root of the v in the range of 20 to 150 mV·s^{-1}, and the negligible shift of the peak potential with v indicates that the transfer of the protonated ligand at the water/1,2-DCE interface is diffusion-controlled and reversible at pH 1.
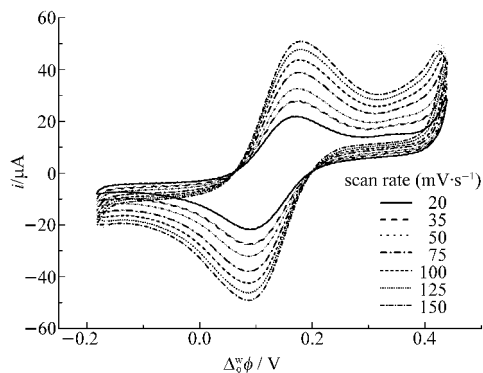

Fig.6 Typical cyclic voltammograms obtained at different scan rates for the transfer of HL across the water/1,2-DCE interface at pH 1
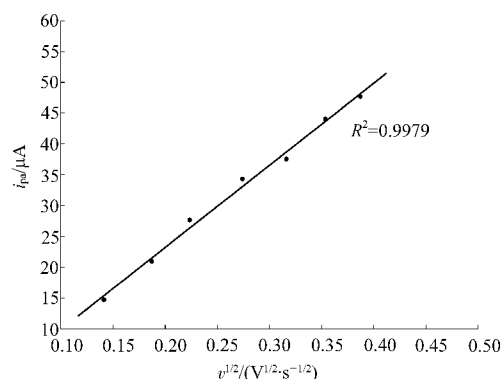

Fig.7 Dependence of the anodic peak current (i_{pa}) of protonated ion transfer of HL on the square root of the potential scan rate ($v^{1/2}$) at pH 1

The peak separation was approximately obtained as $\Delta E_p = 0.091$ V between pH 2 and 5, indicating that the voltammograms are of quasi-reversible nature, which can be partly attributed to the limited rate of protonation preceding ion transfer across the interface[31]. The formal Galvani transfer potential of the protonated ligand across the interface shifted to more positive values with the increase of pH (Fig.5).

The diffusion coefficient of the protonated form of the ligand was calculated from the Randles-Sevcik equation[32] using the voltammetric data at pH 1:

$$i_{pa} = 0.4463z_I F A c_I^{w*}\left(\frac{z_I F}{RT}\right)^{1/2}(v D_I^w)^{1/2} \tag{8}$$

where, i_{pa} is the value of the current peak, A is the interfacial area, z_I is the charge of the ion I, c_I^{w*} is the concentration of ion in the aqueous phase, D_I^w is the diffusion coefficient of ion in the aqueous phase, and v is the potential scan rate. The other symbols have their usual meanings. The diffusion coefficient of the protonated form of the ligand in the water phase was found to be $D_w = (2.09 \pm 0.37) \times 10^{-6}$ cm^2·s^{-1}. The diffusion coefficient of the species in organic phase was calculated as, $D_o = (2.39 \pm 0.37) \times 10^{-6}$ cm^2·s^{-1} using the D_w and the viscosity ratio of the contacting phases (Walden′s rule). The obtained values are close to the reported values for similar structure[3].

No current was observed between pH 5 and 12, indicating that assisted ion transfer reaction does not take place under these conditions. Above pH 12, a weak wave appeared (Fig.8), which could be attributed to the transfer of the anionic form (L$^-$). However, the nature of this transfer could not be satisfactorily evaluated by analyzing the cyclic voltammetric data.

Born′s equation provides good estimates of ionic solvation energies[12]. The difference between a charged and neutral species, $\Delta \lg P(=\lg P_I - \lg P_N)$, can be expressed as:

$$\Delta \lg P = \frac{-(\Delta G_{IS}^o - \Delta G_{IS}^w)}{2.303RT} = \frac{(z^2 e^2 N_A)}{8\pi\varepsilon_0 r}\left(\frac{\varepsilon_r^o - \varepsilon_r^w}{\varepsilon_r^o \varepsilon_r^w}\right) \tag{9}$$

where, e is the charge of the proton, N_A is the Avogadro number, r is the molecular radius, ε_0 is the vacuum permittivity, ε_r^o and ε_r^w are the dielectric constants of the organic and aqueous phases, ΔG_{IS}^o and ΔG_{IS}^w are the differences in the chemical potential between 1 mol of ions and 1 mol of neutral molecules for equal
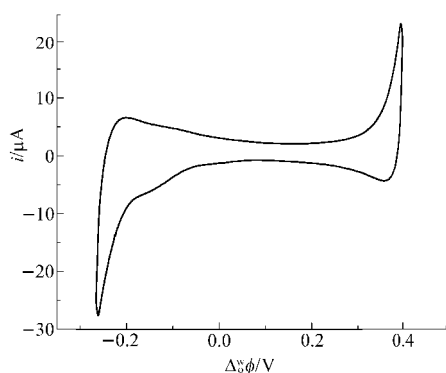
Fig.8　Voltammogram for the transfer of anionic form at pH 12.71

number of molecules of equal size in organic and aqueous phases, respectively. As a general characteristic, it can be concluded that the smaller $\Delta \lg P$ can be attributed to the higher molecular radius of the compounds. It indicates that this type of molecule has higher lipophilicity. Born's solvation model shows that ions with a delocalized or masked charge behave as larger ions than those that possess a more localized charge[12].

In the water/1,2-DCE system, the difference between the neutral and cationic forms of HL (-3.42) is smaller than the difference observed for simpler protonable compounds. This result indicates that the positive charge in the cationic form is less localized than regular cations. The physicochemical parameters for the neutral and protonated form of the ligand are found as follows: $\Delta_o^w \phi_I^0 = 134$ mV, $\lg P_I = -2.27$, $\Delta G_{tr,I}^{0,w \to o} = 12.93$ kJ·mol^{-1}, $\lg P_N = 1.15$, $\Delta G_{tr,N}^{0,w \to o} = -6.56$ kJ·mol^{-1}, and $\Delta \lg P = -3.42$. It is seen that the neutral form of ligand is more lipophilic than its protonated form.

The ligand exhibits considerable antibacterial activity[25]. It was reported that the thiosemicarbazone series exhibited a specific and consistent structure-activity relationship[33]. Lipinski *et al.*[34] described the desired ranges for certain properties thought to be important for pharmacokinetics and drug development. They are: $\lg P_{OCT} < 5$ (the logarithm of partition coefficient between water and octanol), number of hydrogen bond donors $\leqslant 5$, number of hydrogen bond acceptors $\leqslant 10$, and molecular weight < 500. The molecular weight of HL is 256.33 g·mol^{-1}. H-bond donating solutes have $\lg P_{DCE}$ lower than the $\lg P_{OCT}$ values. The partition coefficient was calculated using internet software products ($A\lg P_S$, $IA\lg P$, $C\lg P$, $\lg P_{Kowin}$, and $x\lg P$)[35]. The average $\lg P_{OCT}$ value for HL was obtained as 2.40±0.41.

3　Conclusions

Despite the wide research of thiosemicarbazones and the increasing interest in chemistry and biology, we are not aware of any previous investigation of the facilitated ion transfer by thiosemicarbazones at the ITIES about their lipophilicity except our group. In this study, the characterization of the ion transfer reaction and the lipophilicity scale of 2-benzoylpyridine-thiosemicarbazone at the water/1,2-DCE interface was characterized.

The studied ligand has high antibacterial activity; therefore, electrochemically, the determination of lipophilicity at the interfaces between water and 1,2-DCE as membrane model can be expected to contribute the pharmaceutical properties of the studied ligand.

The ligand possesses three independent species: the cationic (H_2L^+), the anionic (L^-), and the neutral (HL) forms. Transfer of H_2L^+ at macro ITIES was obtained between pH 1 and 5, and was analyzed by cyclic voltammetry. On the other hand, the obtained transfer of L^- above pH 12 could not be analyzed owing to its nature.

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