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Dissociation Constant Determination of Phenazopyridine Hydrocholoride: Using Three Different Extrapolation Techniques on Potentiometric Titration

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Introduction

Phenazopyridine hydrochloride (pzp HCl) (Figure 1) has been used for treatment of urinary tract infections for a long time. It has got analgesic effect in urinary tract and symptomatic relief effect in cystitis. It was adopted by USP in 1928[1]. Since it has no significant antibacterial effect, it has been used with other drugs such as sulfa drugs. It is absorbed from the gastrointestinal tract and excrete mainly from urine[2]. The pharmacokinetic properties of pzp have not been fully determined. It has mostly been studied in animal models, but they may not be very representative of humans. Rat models have shown that its half-life is 7.35 hours, and 40% of it is metabolized hepatically[3]

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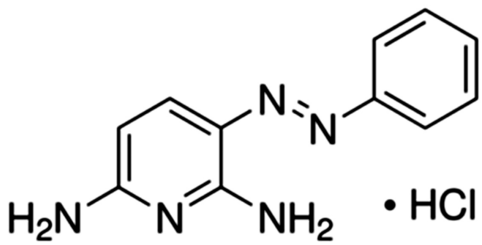

Figure 1
Chemical structure of pzp HCl

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Physicochemical profiling including determination of solubility, ionization constant, lipophilicity and permeability of drugs is one of the main stages in the early stage of drug discovery for drug candidate molecules. Absorption, distribution, metabolism and excretion of an active pharmaceutical ingredient in the system of the body depend on the physicochemical properties of it. From the dissociation constant(s), the major species (ionized or non-ionized form) of pharmaceuticals existing in a specific pH value could be estimated[4]. Dissociation constant(s) especially plays important role in absorption and distribution of molecules in body parts. Passive diffusion of a drug into the body parts at certain pH level depends on the ratio of its ionized and non ionized forms. Therefore, it is a great importance to know if a drug is in its ionized or non-ionized form at a certain pH value[5]. Hence, it is important to calculate properly the drug dissociation constant value of a pharmaceutical compound or a drug candidate. The Henderson–Hasselbalch equation (Equation 1) describes the derivation of pH as a measure of acidity (using pKa, the negative log of the acid dissociation constant) in biological and chemical systems.

Equation 1: $\mathrm{pH} = \mathrm{pKa} + \log\left(\frac{[A^-]}{[HA]}\right)$

Several methods including potentiometry[6-9], spectroscopy[10, 11] and chromatography[12, 13] have been reported to determine dissociation constant value(s) of active pharmaceutical ingredients. Potentiometric titration is a well known method used for a long time due to the fact that its simplicity and reliability. It has high accuracy and commercial advantages in comparison to other methods such like HPLC and spectrophotometry. Shortcomings of potentiometric titration include that compounds must be pure and must be at high amounts. The concentration of solution must be at least 10^{-4} M for significant change in titration curve[14].

The application of potentiometric titration to determine dissociation constant in aqueous solutions is often hindered by the low water solubility of samples. Since the new molecules in drug research are less water-soluble and more lipophilic, solubility is a frequently appeared problem in potentiometric titration[15]. Generally, potentiometry in aqueous medium is the method of choice for the pKa determination for molecules having solubility higher than 0.8 mM concentration in the whole pH interval of the titration. The mixed-cosolvent mixtures mainly methanol-water mixtures in potentiometric titration provide a good alternative for sparingly soluble compounds. However, experiences show that not all compounds dissolve in single component organic solvent-water mixtures. Thus, recently a new multicomponent

cosolvent mixture consisting equal amount of methanol (MeOH), dioxane and acetonitrile referred as MDM (from MeOH, dioxane and acetonitrile) was tried and found to be efficient for p*K*a measurements of various acids and bases[16]. This universal cosolvent system has a combination of polar and nonpolar solvents so that solubility is improved for hydrophobic compounds but is still good for polar molecules[17]. By using such a cosolvent system, the cosolvent ionization constants (ps*K*a) in different ratios of cosolvent mixtures are measured and the aqueous p*K*a is obtained by extrapolation.

The aim of this study is to calculate the dissociation constant of pzp HCl through using potentiometric titration with cosolvent system including different ratios of MDM-Water mixtures. Three different extrapolation methods were used to evaluate water dissociation constant and a brief discussion was performed to present the advantages and disadvantages of these methods for further studies.

Materials and Methods

Materials

MeOH, 1,4- dioxane, acetonitrile that are solvent of MDM system were HPLC grade and obtained from Merck chemical (Germany). Solutions and solvent mixtures were made up of distilled water. Potassium chloride and sodium hydroxide were supplied from Sigma (USA). Mettler Toledo A 235 pH/Ion analyzer was used for monitoring titration.

MDM-Water Solutions

Numerous examples may be cited of water ionization constant values determined by extrapolation in mixtures of methanol[18], ethanol[19], propanol[20], dimethyl sulfoxide[21], dimethylformamide[22], acetone[23] and dioxane[24]. Results of all these experiments showed that reliable results can be calculated in the range of 0-60 percent weight of organic solvent in solvent mixtures. Generally psKa values from titrations with $R > 60$ wt% are not suitable for extrapolation to zero organic solvent because ion-pairing starts to play a significant role[25]. According to this approach, MDM solution was prepared containing equal volume of methanol, 1,4-dioxane, and acetonitrile. Water-MDM mixtures were prepared by weight percentages of MDM at 39.9%, 32.5%, 24.4%, and 16.3% in identical ionic strengths by adding 0.15 M KCl.

Potentiometric Titrations

For pKa determination, solutions of pzp HCl equivalent to 3.2 x 10^{-4} M were titrated with 0.01 M aqueous sodium hydroxide in 0.15 M KCl solution. In neutralization titration, carbonate error could be occurred by dissolving of carbon dioxide in solution. Nitrogen gas was passed from the analyte to repress carbon dioxide and avoid carbonate error[26]. In each step of the titration in the present study, 0.25 ml titrant was added into the analyte solutions. The stirrer was turned on while the titrant was being added. Vigorous stirring continued for 10 s after the addition of titrant. Then the stirrer was turned off to measure the pH of the analyte solution. Three different extrapolation methods were used to evaluate water ionization constant of pzp HCl.

The first method depends on percent weight of organic solvent and ionization constant value. This method has been used since 1925. The linear ship between these parameters can be exhibited in equation 2 [25]. Rwt% describes the percent weight of organic solvent in the solvent mixtures. Terms a and b mean slope and intercept of the plot, respectively.

Equation 2: psKa= aRwt%+b

Second method (Equation 3) is to use solvent dielectric constant (Ɛ). Ionization constant changes linearly with Ɛ of the solvent system. Terms a and b symbolize the slope and the intercept of the plot, respectively. psKa is the cosolvent ionization constant.

Equation 3: psKa = a/Ɛ+ b

The third method is called Yasuda-Shedlovski. Yasuda[27] and Shedlovsky independently derived a correlation whereby a plot of psKa +log [H2O] versus a/Ɛ +b In this equation (Equation 4), [H2O] represents the molar water concentration. Terms a and b symbolize the slope and the intercept of the plot, respectively[17].

Equation 4: psKa + log[H_2O] =a/Ɛ + b

Result and Discussion

The first extrapolation technique was used identical as Mizutani reported in 1925 [25]. Figure 2 presents the titration curves of pzp HCl with NaOH at different water- MDM solvent mixtures. As seen from the graphic, equivalence point of titration shifts to higher values through increasing water percent in mixtures. Since MDM-Water mixtures have lower Ɛ values, it causes

changing of extension of ionization and acid-base strength of compound. Acids have lower psKa values and bases have higher psKa values than aqueous pKa. The equivalence point could be observed easily from the sigmoidal curve given in Figure 2. As a result of Henderson–Hasselbalch equation (Equation 1), the pH value of half equivalence point of titration curve is equal to the ionization constant. Measured ionization constant values with weight percent of MDM in solvent systems are listed in Table 1.

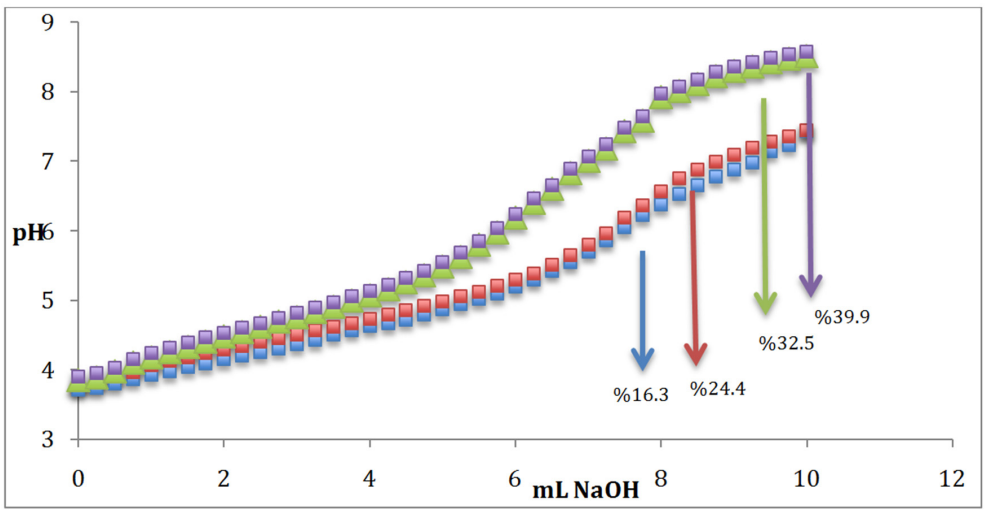

Figure 2
Titration curves of pzp solutions that contain %39.9, %32.5, %24,4 and %16.3 percentange weight of MDM with NaOH

TABLE I
Percent of weight of MDM in solvent systems and ionization constant values

%Wt of MDM in solvent	PsKa
16.3	5.13
24.4	5.02
32.5	4.72
39.9	4.57

As described before[17], in the range of % 0-39.9 weights of organic solvent, good linearity was observed between weight percentage of organic cosolvent

mixtures and ionization constant (Figure 3). By the help of the correlation between parameters, water dissociation constant could be assumed by trend line equation. Dissociation constant of pzp HCl was found 5.57 by using this approach.

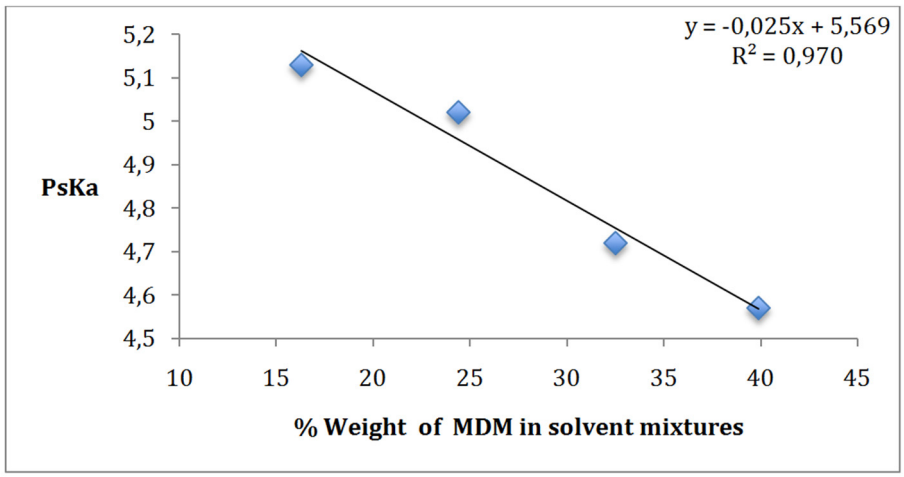

Figure 3
The first extrapolation method (correlation between percent weight of the organic mixtures (MDM) and dissociation constant

The second extrapolation technique is about the relationship between Ɛ of solvent mixture and cosolvent dissociation constant. Table 2 shows the percent volume of MDM in mixtures and dielectric constant values. The Ɛ values of all the selected MDM solvents in the present study are lower than that of water, which affects the ionization equilibria.

A solvent will be more likely to promote ionization of a dissolved acidic molecule in the following circumstances:

a) A protic solvent can form hydrogen bonds and will promote ionization.

b) A solvent with a high donor number is a strong Lewis base.

c) A solvent with a high dielectric constant will promote ionization

For a given acid, p*K*a values will vary depending on the solvent. The degree of dissociation of an acid increases with increase of solvent basicity. On the other hand, dissociation is relatively less for solvents having low Ɛ values. Also we observed this results in our study (see table 1). As increasing MDM percent in solvent system, pKa values shift to lower values.

TABLE II

%Wt of MDM in solvent system and dielectric constant values

% Weight percent MDM	**39.9 %**	**32.5 %**	**24.4 %**	**16.3 %**
ε	53.5	59	64.6	70.2

Observed ionization constant values are satisfactory and have a good correlation with 1/ε (Figure 4). By using tredline equation, water ionization constant value was calculated as 5.44.

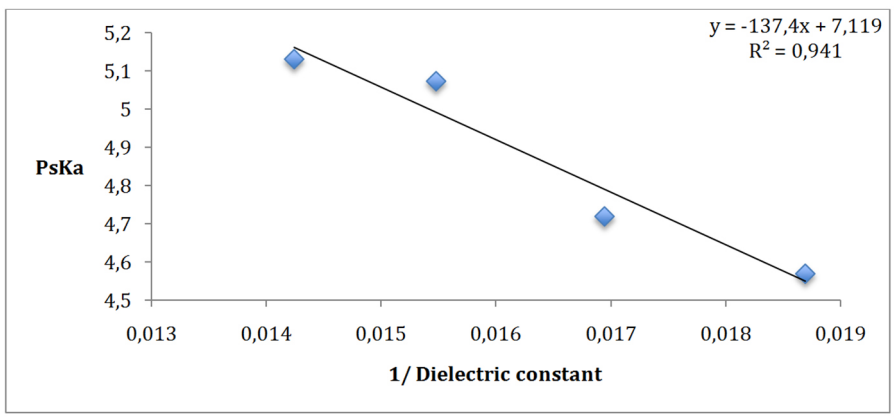

Figure 4
Second extrapolation method (correlation between dielectric constant and dissociation constant)

In literature, the last method called Yashuda-Shedlovski is described as the most suitable method for cosolvent mixture systems. It was observed in some extrapolation experiments that basic functional groups have negative slopes and produce straight lines in the total interval. The acids have positive slopes. Ionization constant calculated as 5.25 at zero percent of weight MDM mixtures by using Yashuda-Shedlovski technique. The Figure 5 shows the linear relationship between psKa + (H_2O) and 1/ dielectric consta. PsKa +(H_2O) value of Solution that contains %16.3 weight percent of MDM showed a deviation form linearity so three points in this graph were used.

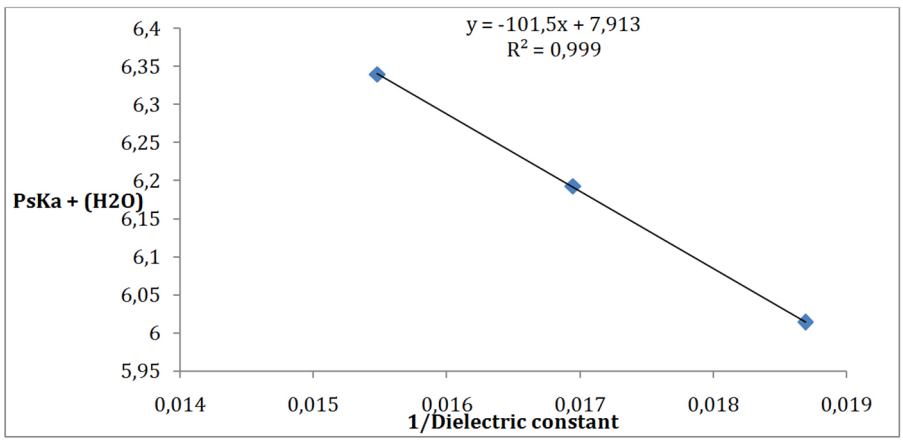

Figure 5.
Yashuda-shedlovsky extrapolation method

We compared the pKa of pzp HCl found by the extrapolation techniques in this study with the reported one in the literature[29]. Our results are in a harmony within each other and identical with the reported value. Yashuda shedlovski extrapolation method has the lowest shift and gave the closest result to the reported one. For the first method and the second method, the calculated pKa values are 5.57 and 5.44, respectively. For the Yashuda-shedlosky method, the closest result, 5.25, was found to the reference value 5.10 [29].

The dielectric constants of solvents effect the ionization of compounds. MDM-Water mixtures have lower ε than water, so the psKa values of acids are higher, where as the psKa values of bases are lower than their aqueous pKa values.

Experimental results exhibited that small changes of the amount of organic solvent have a great effect on the colsolvent dissociation constant values.

Conclusion

There are lots of publications reported about the biological activities of various synthesized compounds but it is rarely discussed the physicochemical properties of these substances as an additional/supporting data on biological activity. Acid dissociation constant is highly related with the ability or inability of the compounds to cross the cell membranes, and therefore it is a great importance to know the pKa of any potential active compound in or-

der to classify and understand the behavior related with the permeability in the system of the body. Potentiometric titration is a well-known and easy to apply technique for determination of the dissociation constant of any ionized compound. The disadvantage of potentiometric titrations is that the concentration of the pharmaceutical solution must be high enough to indicate the equation point on analysis. It is not always possible to work with high concentrations if the compounds are slightly soluble in aqueous solutions. The application of MDM–water mixtures improves the solubility of poorly water soluble compounds, thus, their ps*K*a values can be calculated easily in lower percentage of organic solvents. The aqueous p*K*a values could be easily obtained by using extrapolation techniques. The MDM–water mixtures do not cause large shifts on ps*K*a values. In this study, the application of three different extrapolation techniques was shown by using pzp HCl as a model molecule and it was concluded that extrapolation results were very close to the reported pKa value of pzp HCl. Thus, it was shown that the extrapolation techniques could be easily applied for determination of the pKa values of any drug candidate molecule.

Keywords: ionization constant, extrapolation, potentiometry, Yasuda-shedlovski, phenazopyridine.

Özet

Fenazopiridin Hidroklorür'ün İyonlaşma Sabitinin Analizi: Potansiyometrik Titrasyonda Üç Farklı Ekstrapolasyon Tekniğinin Kullanılması

Bu çalışmanın amacı potantisyometrik titrayondan elde edilen sonuçların değişik ekstrapolasyon yöntemleri ile değerlendirilerek iyonlaşma sabitlerinin bulunması ve bu ekstrapolsayon metodlarının karşılaştırılmasıdır. Suda az miktarda çözünebilen Fenazopiridin hidroklorür model molekül olarak seçilmiş ve çalışmalarda MDM (metanol, asetonitril ve 1,4-dioksan)– Su karışımları kullanılarak kosolvent iyonlaşma sabitleri hesaplanmıştır. Değişik oranlardaki MDM-Su karışımlarına 0.15M KCl eklenerek eşit iyonik kuvvet sağlanmıştır. Çalışmalarda 0.01M Sodyum hidroksit çözeltisi titrant olarak kullanılmış ve kosolvent iyonlaşma sabitleri belirlenmiştir. Sulu fazdaki iyonlaşma sabiti elde edilen deneysel veriler üç farklı ekstrapolasyon yöntemi ile hasplanmış ve karşılaştırılmıştır. Karşılaştırılan sonuçlar birbirinden çok farklı olamamakla birlikte litaratürde verilen sonuçlarla uyum içerisindendir.

Anahtar kelimeler: İyonlaşma sabiti, ekstrapolayon, Potansiyometri, Yasuda-Shedlovski, Fenazopiridin.

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