

# Study on Dissociation Equilibria of Eberconazole Nitrate in Micellar Media by Spectrophotometry

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## To cite this article:

Marothu Vamsi Krishna, Salah Ali Mahgoub Idris, G. Madhavi, B. Jalachandra Reddy, M. Sowhardhra, D. Gowri Sankar. Study on Dissociation Equilibria of Eberconazole Nitrate in Micellar Media by Spectrophotometry. *International Journal of Pharmacy and Chemistry*. Vol. 5, No. 5, 2019, pp. 48-51. doi: 10.11648/j.ijpc.20190505.11

Received: August 15, 2019; Accepted: September 9, 2019; Published: October 11, 2019

**Abstract:** The equilibrium dissociation constant ( $K_D$ ) is the basic parameter to evaluate the binding property of the chemical structure of compounds. Thus, a variety of analytical methods have been established to determine the  $K_D$  values, including radioligand binding assay, surface plasmon resonance method, fluorescence energy resonance transfer method, affinity chromatography, and isothermal titration calorimetry. Here we present a detailed overview of the dissociation equilibria of Eberconazole nitrate (EBZ) in homogeneous and heterogeneous systems, focusing primarily on methods that are based on spectrophotometrically of the dissociation reaction. The Dissociation equilibria of Eberconazole nitrate (EBZ) in homogeneous and heterogeneous systems were studied spectrophotometrically in Britton-Robinson's (BR) buffer at 25°C. Acidity constant of EBZ in BR buffer was found to be 9.5. The effect of anionic, cationic and non-ionic surfactants applied in the concentration exceeding critical micellar concentration (cmc) on acid – base properties of EBZ were also examined. The results revealed a shift of pKa values in micellar media comparing to the values obtained in BR buffer. These shifts in pKa values are more in cationic and anionic micellar media compared with that of non-ionic. The observed differences in pKa values between micellar media and BR buffer solution ranged between -6.0 to -2.0 units. The micellar-mediated pKa shifts can be attributed to the differences between the mean intrinsic solvent properties of the interfacial and bulk phases, with an additional contribution from the electrostatic micellar surface potential in the case of the charged aqueous micellar solutions.

**Keywords:** Dissociation Equilibria, Eberconazole Nitrate, Micellar Media, Spectrophotometry

## 1. Introduction

Dissociation constants are important parameters to indicate the extent of ionization of molecules in solution at different pH values [1]. The acidity constants of organic reagents play a fundamental role in many analytical procedures such as acid-base titration, solvent extraction, complex formation, and ion transport. It has been shown that the acid-base properties affect the toxicity, chromatographic retention behavior, and pharmaceutical properties of organic acids and bases [2]. Various methods for the determination of dissociation constants, such as potentiometric titration,

spectrophotometric determination, conductometry, and spectroscopic methods, have been reported [3]. Among these, potentiometric titration [4] and spectrophotometric determination [5] are the most useful and widely used methods.

Drugs may be classified into three categories according to their physical behavior in aqueous solution: (1) they may exist entirely as ions, such as  $K^+$ ,  $Cl^-$ , or  $NH_4^+$  (strong electrolytes); (2) they may be undissociated, as with the steroids and the sugars (non electrolytes); or (3) they may be partially dissociated and exist in both an ionic and a molecular form, the relative concentrations of which will

depend on the pKa of the agent and the pH of the medium (weak electrolytes).

Most of the drugs are either weak acids or weak bases; their ionization state is controlled by both solution pH and acidic dissociation constants (i.e. Ka values). These different chemical species (cationic, neutral, or anionic) often have vastly different properties with respect to water solubility, volatility, UV absorption, and reactivity with chemical oxidants. The ionized form is usually more water soluble, while the neutral form is more lipophilic and has higher membrane permeability. The extent of ionization is one of several paramount properties used to estimate the absorption, distribution, metabolism and excretion of compounds in biological systems [6].

Knowledge of pKa values as a function of solvent composition is also useful in applying liquid chromatography (LC) or capillary electrophoresis (CE) for the separation of ionizable compounds. The chromatographic retention and electrophoretic behavior of ionizable compounds strongly depend on the pKa of the compound and the mobile-phase pH [7, 8]. Satisfactory knowledge of the acid–base behavior of substances in hydro-organic media is therefore essential to optimize analytical procedures for the separation of ionizable compounds by LC [9, 10] and CE [11]. Moreover, the acid–base property of a drug molecule is the key parameter for drug development because it governs solubility, absorption, distribution, metabolism and elimination. Particularly for developing new active pharmaceutical ingredients (APIs), the pKa has become of great importance because the transport of drugs into cells and across other membranes is a function of physicochemical properties, and of the pKa of the drugs [12].

Micellar systems have important application in the field of pharmaceutical sciences (drug solubility and delivery) and analytical chemistry [13-16]. Amphiphilic molecules, containing both hydrophobic and hydrophilic moieties, associate in water above a certain concentration to form colloidal particles called micelles [17, 18]. Micellar systems can shift acid–base equilibria. This shift can be explained in terms of differences between the properties of the bulk solvent and of the interfacial region and perturbation of the acid–base equilibria by the electrostatic field effect of the charged interface. The dissociation equilibria of substituted benzoic acids in cationic and anionic micelles have been investigated potentiometrically [14]. It was shown that their pKa values shift to about less than 1.0 in cationic micelles and by about 0.5–3.0 in anionic micelles. The acid–base equilibria of a number of phenols, amines and carboxylic acids in aqueous micellar solutions have been examined [19]. The spectral and acid–base properties of some solvatochromic acid–base indicators were studied in self-assembled surfactant aggregates [20, 21]. Similar studies have been done for complexing agents amino acids [22, 23] and peptides [24] and medicinal compounds [25, 26]. Surfactant media also effect the complexation [27, 28] and other electrochemical phenomena [29, 30], which in turn have been exploited for electroanalysis of ascorbic acid and other vitamins [31, 32]. Eberconazole nitrate (EBZ) is an

imidazole derivative [33], used topically as a 1% cream in the treatment of superficial fungal infections [34]. EBZ (Figure 1), 1-(2, 4-dichloro-10, 11-dihydro-5H-dibenzo [a, d] cyclohepten-5-yl)-1H-Imidazole nitrate, acts by inhibition of fungal lanosterol 14 $\alpha$ -demethylase [35]. This leads to an alteration in its structure and function, thereby inhibiting the growth of the fungus. Eberconazole has antifungal as well as potent anti-inflammatory effects. It is a basic, white, amorphous powder which is readily soluble in methanol and dichloromethane. The present work was aimed at the study of acid–base equilibria of EBZ in anionic (sodium lauryl sulphate), cationic (cetyl trimethyl ammonium bromide) and non-ionic (Tween 80) surfactant micellar solutions by using Spectrophotometry.

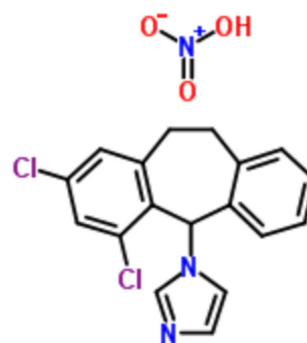


Figure 1. Chemical structure of Eberconazole nitrate (EBZ).

## 2. Materials and Methods

### 2.1. Reagents

Britton-Robinson's (BR) buffer solution (a mixture of acetic, boric and phosphoric acids each at 0.04 M) was prepared and the required pH (1.0-11.0) adjusted with 2 M, 5 M NaOH and 1:5 *o*-phosphoric acid: water. The surfactants, sodium lauryl sulphate (SLS), cetyl trimethyl ammonium bromide (CTAB), and Tween 80, were of analytical reagent grade and were used at the concentration of 0.01 M.

### 2.2. Apparatus

Analytical Technologies double beam UV-Visible spectrophotometer of UV-WIN 5 software with 1cm quartz cells for spectra and absorbance measurement. A Systronics digital pH meter was used for all pH measurements.

### 2.3. pKa Determination

Different solutions of EBZ at a concentration of 20  $\mu$ g/ml were prepared in BR buffer, BR buffer with SLS and BR buffer with CTAB in the pH range of 1-11 and scan the solutions using corresponding blank solutions in the wavelength range of 200-400 nm. In the case of BR buffer with Tween 80, 300  $\mu$ g/ml solutions were prepared and scanned. pKa values of EBZ in the above media were determined using the absorbance (A) data at the respective absorption maximum by plotting first derivative curves between pH and  $\Delta A / \Delta pH$ .

### 3. Results and Discussion

pKa value of EBZ was determined spectrophotometrically in aqueous medium and in the presence of surfactants (SLS, CTAB and Tween 80; 0.01 M). The concentration of the surfactants of 0.01 M chosen in order to examine their effects on pKa value of EBZ was well above critical micelle concentration (cmc) and thus, the changes in cmc due to the solute could be neglected. Absorption spectra of EBZ in different media are shown in Figure 2.

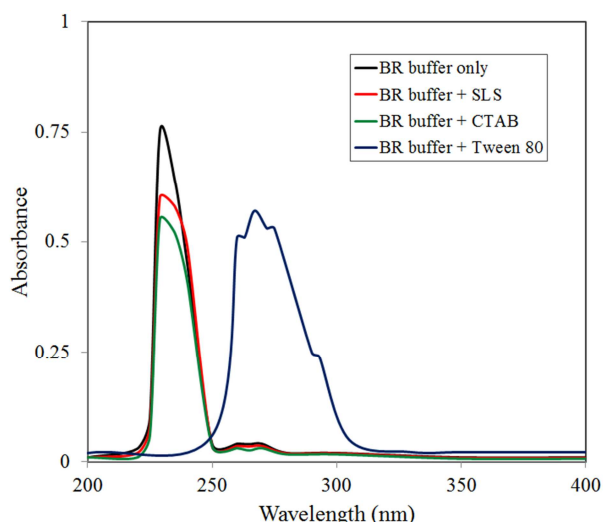


Figure 2. Absorption spectra of EBZ (20 µg/ml) in (BR buffer, BR buffer with SLS, BR buffer with CTAB and BR buffer with Tween 80) pH 1.0-11.0.

The absorption maximum of EBZ in BR buffer was 229 nm and in presence of ionic surfactants; SLS and CTAB, it

was 231 nm. In the presence of non-ionic surfactant (Tween 80) the  $\lambda_{max}$  was shifted to 266 nm. UV spectroscopic data of EBZ in BR buffer and in different surfactant media was presented in Table 1.

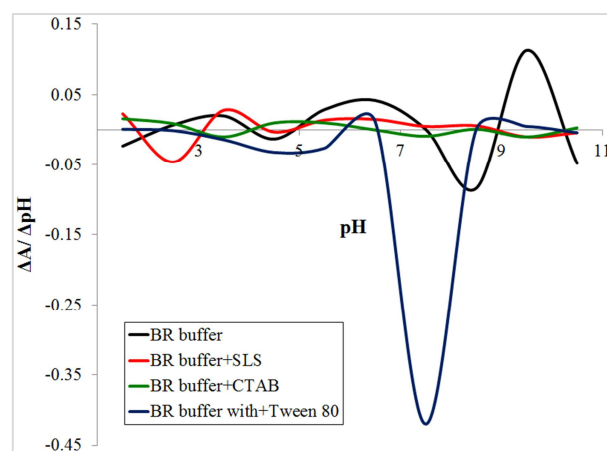


Figure 3. First derivative graph between pH and  $\Delta A/\Delta pH$  in BR buffer, BR buffer with SLS, BR buffer with CTAB and BR buffer with Tween 80.

Based on the absorbance values of the examined drug solutions at different pH values, pKa value was determined by plotting first derivative graph between pH and  $\Delta A/\Delta pH$ . The first derivative graphs obtained in BR buffer and in the presence of surfactants are shown in Figure 3. From the graphs, the pKa values obtained are 9.5, 3.5, 5.0 and 7.5 in BR buffer, BR buffer + SLS, BR buffer + CTAB and BR buffer + Tween 80 respectively. The values of molar absorbance for EBZ in the various media were also found and are given in Table 2 along with the pKa values.

Table 1. UV spectroscopic data for EBZ in the BR buffer and in the presence of surfactants.

pH	BR buffer only	BR buffer + SLS	BR buffer + CTAB	BR buffer + Tween 80
	Absorbance	Absorbance	Absorbance	Absorbance
	$\lambda_{max} = 229 \text{ nm}$	$\lambda_{max} = 231 \text{ nm}$	$\lambda_{max} = 231 \text{ nm}$	$\lambda_{max} = 266 \text{ nm}$
1.0	0.627	0.524	0.510	0.597
2.0	0.604	0.547	0.526	0.598
3.0	0.611	0.501	0.535	0.597
4.0	0.631	0.529	0.525	0.583
5.0	0.618	0.526	0.535	0.551
6.0	0.647	0.540	0.545	0.525
7.0	0.689	0.555	0.545	0.537
8.0	0.690	0.560	0.536	0.117
9.0	0.606	0.566	0.537	0.117
10.0	0.719	0.556	0.527	0.122
11	0.671	0.552	0.530	0.118

Table 2. pKa and Molar absorbance data of EBZ in different media.

Medium	pKa	Molar absorbance ( $L \cdot mol^{-1} \cdot cm^{-1}$ )
BR buffer	9.5	$1.18 \times 10^4$
BR buffer + SLS	3.5	$9.82 \times 10^3$
BR buffer + CTAB	5.0	$1.04 \times 10^4$
BR buffer + Tween 80	7.5	$7.02 \times 10^2$

Based on the obtained results it was observed that the shifts in pKa values are more in case of anionic and cationic surfactants (SLS and CTAB) compared with the non-ionic surfactant (Tween 80). EBZ behaves more strongly in case of SLS; its

interfacial layer is negatively charged. This is mainly because the cation is strongly attracted to the interfacial layer of negative surfactant due to attraction between opposite charges. This creates strain on the EBZ molecule causing it to dissociate strongly and so less pKa value was observed. In case of cationic surfactant (CTAB) interface is positively charged and the cation is repelled. In case of CTAB the hydrocarbon chain contains a large number of methyl substituent's that restrict the rotational degree of freedom around the C - C bond causing the chains to tilt, thus preventing them from packing in a close manner (Chattopadhyay and Mittal, 1996). This decreases the

interfacial charge on the CTAB micelles. So there is a less degree of repulsion between cation from EBZ and CTAB micellar interface. This leads to more strain on EBZ molecules followed by high dissociation and less pKa value was observed. In case of non-ionic surfactant, the EBZ dissociates lesser due to the absence of electrostatic effect and so higher pKa value was observed. These results obtained in this paper showed that by using simple method which can provide the guidance for researchers to utilize the most appropriate analytical tool to determine the  $K_D$  values compared with more complicated methods which summarised in ref [36].

## 4. Conclusions

The Dissociation equilibria of Eberconazole nitrate (EBZ) in homogeneous and heterogeneous systems were studied spectrophotometrically in Britton-Robinson's (BR) buffer at 25°C. The results showed that surfactant micellar media can affect the dissociation equilibria of EBZ. It was found that shifts in pKa values are more in BR buffer+SLS and BR buffer+CTAB compared with that of BR buffer+Tween80. The values of pKa for EBZ in various media was found to increase in the order of BR buffer+SLS < BR buffer+CTAB < BR buffer+Tween80 < BR buffer. This study of acid-base behavior in surfactant media is important to the understanding of mechanisms of reactions in both in vitro and in vivo environments; and such a study is also useful in analytical and pharmaceutical applications.

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