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Application of chemometrics in determination of the acid dissociation constants (pK_a) of several benzodiazepine derivatives as poorly soluble drugs in the presence of ionic surfactants

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ABSTRACT

In this study, the acid dissociation constants (pK_a) of some benzodiazepine derivatives including chlordiazepoxide, clonazepam, lorazepam, and oxazepam in aqueous micellar solution were determined spectrophotometrically at an ionic strength of 0.1 M at 25 °C.

The effect of cetyl trimethylammonium bromide (CTAB) as a cationic and sodium n-dodecyl sulfate(SDS) as an anionic surfactant on the absorption spectra of benzodiazepine drugs at different pH values were studied. The acidity constants of all related species are estimated by considering the surfactant concept and the application of chemometric methods using the whole spectral fitting of the collected data to an established factor analysis model. DATAN® software (Ver. 5.0, Multid Analyses AB, and Goteborg, Sweden) was applied to determine the acidity constants. In this study, a simple and fast method to determine the ionization constant (pK_a) of poorly soluble drugs was developed using surfactants. The acidity constant (i.e. pK_a) for chlordiazepoxide, clonazepam, lorazepam, and oxazepam were reported as 4.62, pK_{a1} value of 1.52 and pK_{a2} value of 10.51, pK_{a1} value of 1.53 and pK_{a2} value of 10.92 and pK_{a1} value 1.63 and pK_{a2} value of 11.21 respectively. The results showed that the peak values in the spectrophotometric absorption spectra of drugs are influenced by the presence of anionic and cationic surfactants. According to the results, by changing the SDS concentration from 0 to 0.05 M, the pK_a of chlordiazepoxide was increased to 5.9, the pK_{a1} of lorazepam was decreased to 0.1 while the pK_{a2} was increased to 11.5. Increase in SDS concentration has not shown significant alteration in pK_a of clonazepam and oxazepam.

Results indicate that by Changing the CTAB concentration from 0 to 0.05 M, the pK_a of chlordiazepoxide was reduced to 4.4, the pK_{a1} of clonazepam was decreased to 0.1 and the pK_{a2} was decreased to 9.1, the pK_{a1} of lorazepam was decreased to 0.4 and the pK_{a2} was decreased to 9.4, the pK_{a1} of oxazepam was decreased to 0.3 and the pK_{a2} was decreased to 9.7.

Based on the results obtained from the study, charge of anionic and cationic surfactants leads to an electrostatic interaction between surfactant and the protonated form of the drug molecule. The electrostatic interactions can be attractive or repulsive forces and influence of separation of protons and consequently increase or decrease the acidity constants.

1. Introduction

pK_a determination is considered an interesting topic in a wide range of research fields such as reaction rates, biological activity, biological uptake, biological transfer, and environmental fate (Kara and Alkan, 2000). One of the most important physico-chemical characteristics of a substance used as a drug is considered its pK_a value. pK_a values can be used to determine the extent of drug absorption and applied in pharmacokinetic and bioavailability studies.

The pK_a of a molecule is defined as the pH at which the molecule is 50% protonated. The pK_a of a molecule predicts the degree of ionization of the molecule at a particular pH by using Henderson–Hasselbalch mathematical equation (Narasimham and Dnyandeo Barhate, 2011).

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Many drug molecules are either weak acid or weak base, and their related pK_a values represent their ionic strength. Determining the pK_a value of a drug allows the extent to which each drug is ionized at the pH of biological fluid (i.e., 7.4) to be predicted.

Poor solubility is considered an issue not only in the formulation of a drug, but it also imposes problems in evaluating the physico-chemical properties of the drug's molecules (Ravichandiran et al., 2011).

There are various techniques available to improve the solubility of poorly soluble drugs. Several approaches include: (I) physical modifications, including particle size reduction, crystal habit modification, drug dispersion in carriers, complexation, and solubilization by surfactants; (II) chemical modifications, including salt formation, co-crystallization, co-solvent, and hydrotropy (Patil et al., 2011).

One important property of micelles is their ability to solubilize a wide variety of compounds (i.e., drugs) that are insoluble or slightly soluble in water.

Benzodiazepines are considered an important class of poorly soluble drugs and categorized as psychoactive drugs whose core chemical structures are consisted of the fusion of a benzene ring and a diazepine ring.

Benzodiazepines enhance the effect of the neurotransmitter gamma-amino butyric acid (GABA) at the GABA receptors, resulting in sedative, hypnotic, anxiolytic, anticonvulsant, and muscle relaxant properties. It was also reported in applied pharmacology research that high doses of many shorter-acting benzodiazepines are amnesic-dissociative actions (Page et al., 2002).

These properties make benzodiazepines useful in treating anxiety, insomnia, agitation, seizures, muscle spasms, alcohol withdrawal, and as a pre-medication for medical or dental procedures (Olkkola and Ahonen, 2008). Interactions between benzodiazepines and surfactants as a way to increase the solubility of benzodiazepines have been investigated in several studies (Akbay et al., 2007; Nokhodchi et al., 2003; Shokri et al., 2001; Park and Cho, 1990). Although a few studies have been done estimating the pK_a values of poorly soluble drugs (Ravichandiran et al., 2011), no reports could be found determining the values using surfactants and the application of chemometric methods.

Since middle of the 1960s, computers have acquired increasing importance in the evaluation of equilibrium measurement data using multiple wavelengths or the full spectrum to determine the stability and acidity constants.

In this study, the data analysis software DATAN® (Ver. 5.0, Multid Analyses AB, Goteborg, Sweden) was used to analyze the spectroscopic data and to determine the dissociation constants and the effects of anionic and cationic surfactants on the pK_a values of studied benzodiazepine derivatives, including chlordiazepoxide, clonazepam, lorazepam, and oxazepam that are classified as poorly soluble drugs in pure water (Scarminio and Kubista, 1993). The chemical structures of studied benzodiazepines have been illustrated in Fig. 1(a–d).

In this study, the effects of sodium n-dodecyl sulfate (SDS) as an anionic surfactant and cetyl trimethylammonium bromide (CTAB) as a cationic surfactant were studied on the dissociation constants and the pure spectra of several benzodiazepine derivatives.

2. Materials and methods

2.1. Materials

Chlordiazepoxide, clonazepam, lorazepam, oxazepam, SDS, CTAB, hydrochloric acid, sodium hydroxide, and potassium nitrate were supplied as the analytical and pharmaceutical-grade chemicals from Merck (Darmstad, Germany). These reagents were used without further purification. The stock solutions of surfactants were prepared by dissolving weighted amounts of substances in appropriate amounts of distilled water.

All other chemicals were of pharmaceutical grade and were used as received.

2.2. Instrumentation and software

A lambda 25 Perkin Elmer® spectrophotometer (Waltham, Massachusetts, US) controlled by a computer and equipped with a 1-cm path length quartz cell was used for uv–vis spectra acquisition. Spectra were acquired between 200 and 500 nm. A PB-11 Sartorius® pH-meter (Gottingen, Germany) furnished with a combined glass-saturated calomel electrode was calibrated with at least two buffer solutions at a pH of 3.0 and 9.0.

All absorption spectra were acquired at five data points per nanometer in the wavelength range of 200–500 nm and transferred (in ASCII format) to an AMD 2000 XP (256 Mb RAM) computer for subsequent analysis using DATAN® package.

2.3. Spectrophotometric titrations

For the chlordiazepoxide, clonazepam, lorazepam, and oxazepam (3.5×10^{-6} M) in pure water, water-SDS, and water-CTAB mixtures, titrations were performed and the related absorption spectra were measured with a titration set-up consisting of a computer interfaced to a spectrophotometer.

After each pH adjustment, based on previous studies (Kara and Alkan, 2000; Narasimham and Dnyandeo Barhate, 2011), using hydrochloric acid and sodium hydroxide, solution was transferred into the cuvette and the absorption spectra were recorded. Ionic strength was maintained at 0.1 M by adding appropriate amounts of KNO_3. All measurements were carried out at a temperature of 25 ± 0.5 °C.

2.4. Statistical analysis

All experiments were performed in triplicate and the related values were reported as Mean ± SD. Statistical significance of

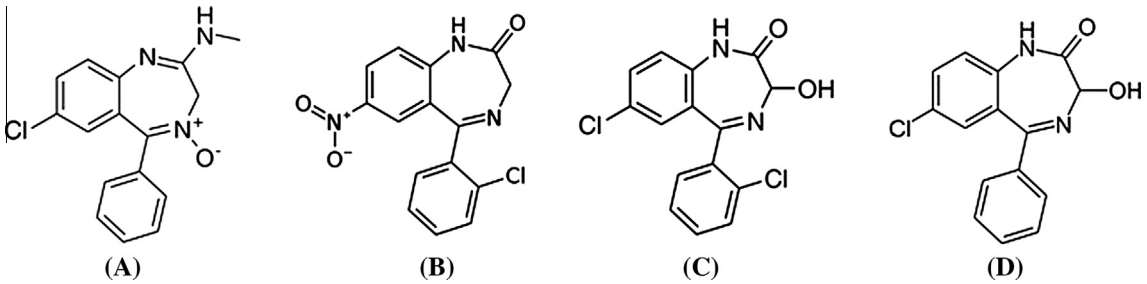

Fig. 1. Structure of some benzodiazepine derivatives; (A) chlordiazepoxide; (B) clonazepam; (C) lorazepam; (D) oxazepam.