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Automated Potentiometric Titration Method for Determination of p*K* Values: An Application to Benzodiazepines

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ABSTRACT: The acid−base behavior of benzodiazepines was studied by using a new technique GL-pK_a in different ethanol−water [up to 50 % (v/v) ethanol] and methanol−water [up to 50 % (v/v) methanol] mixtures and at different temperatures. The ionic strength was maintained using potassium chloride. The primary goal of this work was to study the effect of different factors like dielectric constant, temperature, and ionic strength on the dissociation constant of benzodiazepines in detail as these are widely used in every day medical practice and forensic and clinical investigations. Potentiometric titrations were performed for determination of the p*K* values. This technique was found to be convenient, accurate, and easily applicable. The p*K* values obtained were in good agreement with literature values within experimental error.

INTRODUCTION

Benzodiazepines are heterocyclic compounds having a benzene nucleus fused with a seven-membered ring containing two nitrogen atoms. 1,4-Benzodiazepine and 1,5-benzodiazepine have been widely and thoroughly studied over several decades because of their relatively easy synthesis from common starting materials.[1] Benzodiazepines have recently become of great importance because of their wide range of therapeutic and pharmacological properties. Many members of the diazepine family are nowadays widely used as anticonvulsant, antianxiety, analgesic, sedative, antidepressive, and hypnotic agents.[2] The benzodiazepines are the most widely prescribed minor tranquillizers in current use, and they are known to act on the central nervous system.[3] Benzodiazepines are used as induction agents for general anesthesia in some forms of day surgery such as endoscopies and cardioversion.

Drugs used in this situation include midazolam and lorazepam. Lorazepam has also been used for the treatment of subjects with agitated, violent, or psychotic behavior. Recreationally, benzodiazepines are used to enhance the effects of heroin and cocaine and to reduce the impact of withdrawal symptoms between doses of harder drugs.[4,5] Benzodiazepines are the drugs of choice in the pharmacotherapy of anxiety and related emotional disorders, sleep disorders, status epilepticus, and other convulsive states.[6] Benzodiazepines are one of the largest classes of abused pharmaceuticals. While they do have potential for abuse, the recognized medical benefits are both physiological and psychological.[7] In general benzodiazepines act as hypnotics in high dosages, anxiolytics in moderate, and sedatives in low dosages. The activity of these 1,4-benzodiazepine compounds depends upon the substituent present on the main skeleton.[8]

A variety of methods exist in the literature for the detection and determination of 1,4-benzodiazepines like gas chromatography (GC),[9,10] high performance liquid chromatography (HPLC),[11−13] high-performance thin-layer chromatography (HPTLC),[14,15] high-performance liquid chromatography/electrospray ionization tandem mass spectrometry,[10] liquid chromatography/mass spectrometry,[16] spectrophotometry,[17,18] and voltammetry.[19] The acid−base behavior of the 1,4-benzodiazepines has also been investigated for determination of equilibrium constants in heterogeneous systems under different experimental conditions.[20−22]

In recent years, 1,5-benzodiazepines have been commonly used as substitutes to the regular 1,4-benzodiazepines. The simplest method for synthesis of these compounds involves the acid-catalyzed reaction of *o*-pheneylenediamine with ketones, *β*-haloketones, and α,*β*-unsaturated carbonyl compounds.[23] In addition to 1,4-benzodiazepines, 1,5-benzodiazepines also act as central nervous system (CNS) depressants,[24−26] antianxiety agents,[27] potential neuroleptics,[28] potential antidepressants,[29] and agonists[30] and have psychotropic[31] and pesticidal activity.[32] 1,5-Benzodiazepines are key intermediates for some fused heterocyclic rings such as triaxol and oxadiazole. Due to their wide range of pharmacological activity in synthetic and industrial applications, the syntheses of these compounds have recently received a great deal of attention for the discovery of improved protocols toward milder and high yielding approaches.[33]

Knowledge of the p*K* values of a substance plays an important role in the pharmaceutical industry for drug design and understanding the mechanism of action, in the new chemical manufacturing industry (environmental impact compliance), and in the environmental field (environmental fates of toxic substances). These values are constructive in determining the extent to which a drug is absorbed by the body's organs. The determination of the p*K* values, in aqueous media, is difficult and problematic for many new compounds which are very poorly soluble in water. Therefore it was decided to determine the p*K* values of benzodiazepines in different mixed solvent (aqua−organic)

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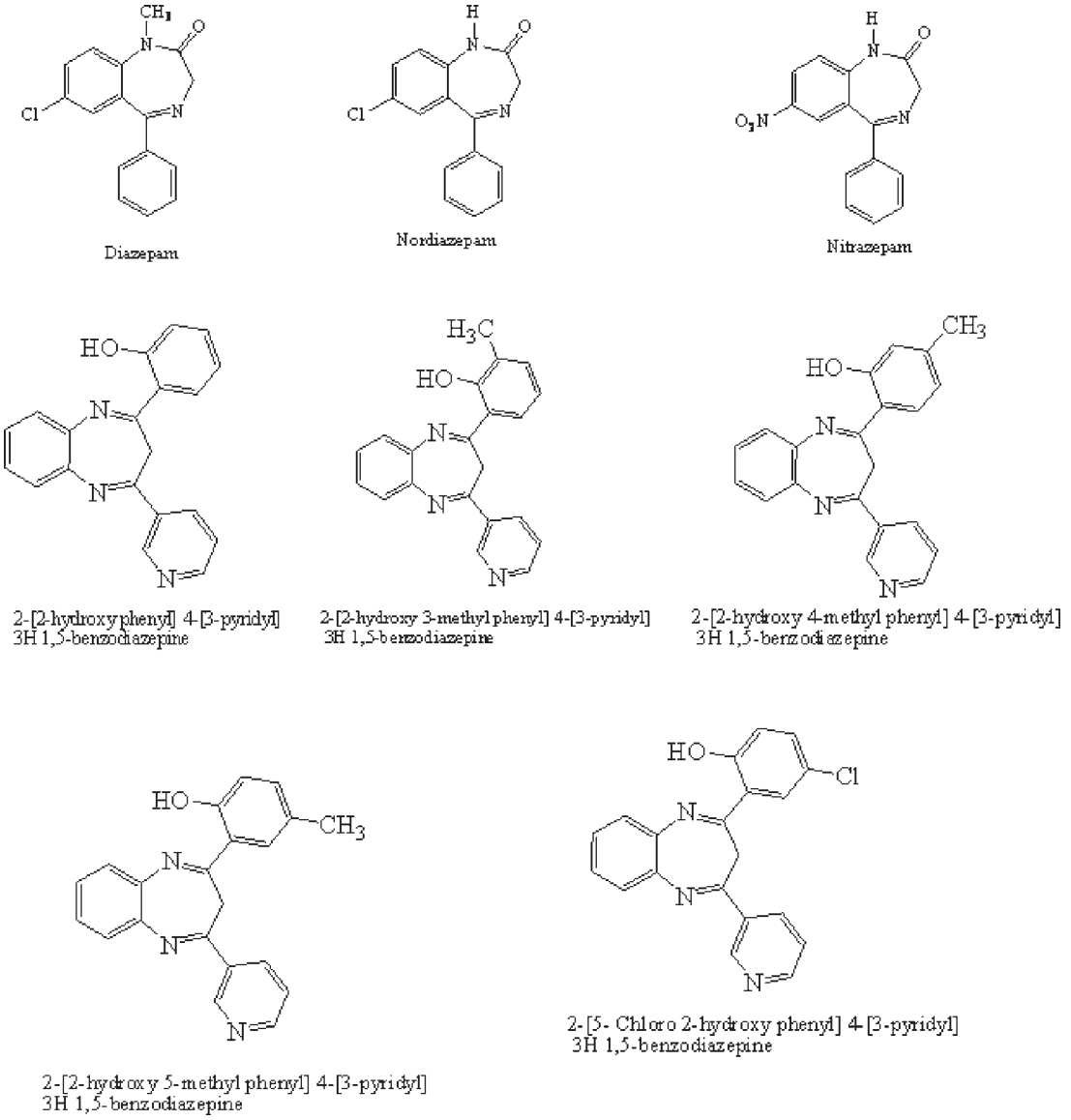

Figure 1. Structures of different benzodiazepines Diazepam (Dz), Nordiazepam (NDz), Nitrazepam (Nz), 2-(2-hydroxy phenyl) 4-(3-pyridyl)-3H-1,5-benzodiazepine (1−5 Ben 1), 2-(2-hydroxy 3-methyl phenyl) 4-(3-pyridyl)-3H-1,5-benzodiazepine (1−5 Ben 2), 2-(2-hydroxy 4-methyl phenyl) 4-(3-pyridyl)-3H-1,5-benzodiazepine (1−5 Ben 3), 2-(2-hydroxy 5-methyl phenyl) 4-(3-pyridyl)-3H-1,5-benzodiazepine (1−5 Ben 4), and 2-(5-chloro 2-hydroxy phenyl) 4-(3-pyridyl)-3H-1,5-benzodiazepine (1−5 Ben 5) used in the present investigation.

systems. In these mixed solvent systems, some solvent properties like dielectric constant, solvent polarity, etc. can be changed and have certain advantages over aqueous as well as organic solvent systems. The effect of different factors like dielectric constant, ionic strength, and temperature was also studied.[34]

EXPERIMENTAL SECTION

Reagents and Solutions. All solvents were of HPLC-grade, while other chemicals were analytical-reagent (AR) grade. All chemicals were purchased from Merck (Mumbai, India). 1, 4-Benzodiazepines like Diazepam (Dz), Nordiazepam/Dismethyldiazepam (NDz), and Nitrazepam (Nz) were obtained from the Food and Drug Administration (FDA, Mumbai) and were used as received. The 1,5-benzodiazepines used were synthesized by the reported methods[35] and were used after purification. These compounds are 2-(2-hydroxy phenyl) 4-(3-pyridyl)-3H-1,5-benzodiazepine (1−5 Ben 1), 2-(2-hydroxy 3-methyl phenyl) 4-(3-pyridyl)-3H-1,5-benzodiazepine (1−5 Ben 2), 2-(2-hydroxy 4-methyl phenyl) 4-(3-pyridyl)-3H-1,5-benzodiazepine (1−5 Ben 3), 2-(2-hydroxy 5-methyl phenyl) 4-(3-pyridyl)-3H-1,5-benzodiazepine (1−5 Ben 4), and 2-(5-chloro 2-hydroxy phenyl) 4-(3-pyridyl)-3H-1,5-benzodiazepine (1−5 Ben 5). A 1 mg sample was used for three sets of titrations. The structures of the benzodiazepines are illustrated in Figure 1.

Instrumentation. The Sirius automated potentiometric method (GL-pK_a) was used for determination of the pK values of different compounds. The GL-pK_a instrument was supplied by Sirius Analytical Instruments (Forest Row, UK). It has five dispenser units that deliver all necessary reagents automatically. The correct mixing of regents was achieved by an overhead stirrer with variable speed. The assay temperature was controlled by using an external recirculating water bath which was monitored

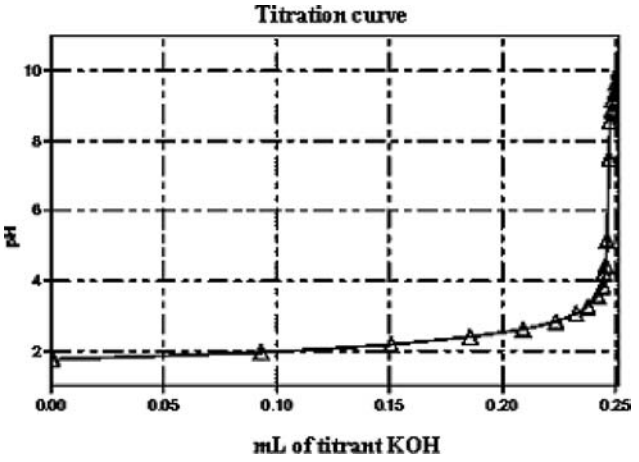

Figure 2. Titration curve of Diazepam in 30 % methanol−water system at μ = 0.15 M (KCl) and T = 25 °C.

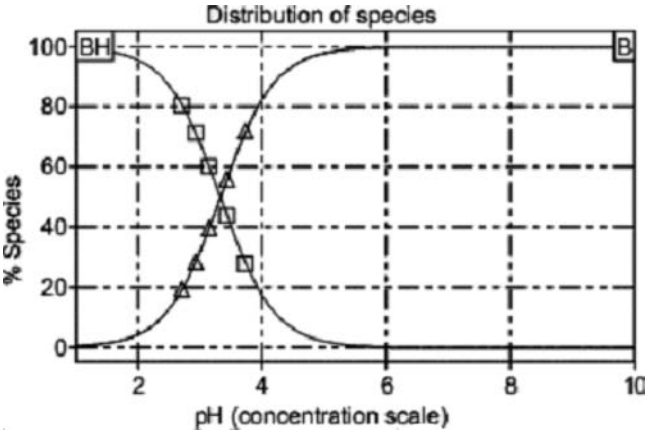

Figure 3. Distribution of species for Diazepam in 30 % methanol−water system at μ = 0.15 M (KCl) and T = 25 °C.

by a PT100 probe. The sophisticated software RefinementPro version 2.0.1.1 allows detailed iterative calculations to be made and values to be carefully refined.

Sirius GL-pK_a Potentiometric Method. The Sirius GL-pK_a instrument is an excellent example of engineering science useful for chemical analysis. It is an easy, rapid, and convenient method for determination of a pK_a. It is equipped with a PCA101 automatic titrator which is useful for accurate pH determination. This method is universal and takes (30 to 60) min/titration (i.e., 10 to 30 titrations/24 h) which is far better than what we could perform by manual titration. It requires an extremely small concentration of substance (0.1 mM), whereas traditional methods require very large concentrations of substance.

This technique basically consists of two linked titrations: a normal titration followed by a two-phase titration in the presence of a partition solvent. Octanol was used as the partition solvent. Any partitioning by the compound will shift the equilibrium and cause a change in the apparent pK_a. From this shift, the log P may be calculated. Nitrogen gas was purged into sample solution by an internal flow meter to remove dissolved oxygen from the solution. The sample solution was titrated against 0.1 M hydrochloric acid and 0.1 M sodium hydroxide. The electrolyte potassium chloride was used to maintain the ionic strength. Different solvent−water systems were used during the course of titration, i.e., methanol−water system, ethanol−water system, etc.

RESULTS AND DISCUSSION

pK values are useful physicochemical parameters describing the extent of ionization of functional groups with respect to pH. These are a function of solvent composition and are also useful in the application of reversed-phase HPLC for the separation of ionizable compounds.[36] 1,4-Benzodiazepines possess weak basic properties due to the nitrogen atom at position 4 that can be protonated. The pK values of the protonation of N_4 of 1, 4-benzodiazepines are between 1.5 and 3.5.[37] All 1,4-benzodiazepines studied have the pK values in the range of 2.70 to 3.55. 1,5-Benzodiazepines can be deprotonated at higher pH due to the presence of a hydroxyl group (−OH). These have pK values in the range 9 to 10.5. pK values are affected by different factors like dielectric constant, temperature, ionic strength, etc.

Effect of Dielectric Constant on pK Values. A number of changes are observed by an addition of solvent to aqueous systems like a decrease in activity coefficient of water, change in dielectric constant, the gradual break down of the structure of water, and solvation of protons at a high concentration of solvent. Earlier studies show that the stability constant is linearly related to mole fraction of the solvent and varies with solvent. The addition of organic solvent to water leads to a gradual break down of the tetrahedral lattice and formation of a hydrogen bond. The stability of the hydroxonium ion increases with decreases in proton donating ability.[38]

Irving and Rossotti[39] observed that the stability constant of compounds containing a O−H bond increases, and a N−H bond decreases with an increase in the content of solvent. An increase in the content of organic solvent in an aqueous system leads to the interaction between protons and negatively charged oxygen atoms to a greater extent than the ion−dipole interaction between protons and the solvent. An increase in the solvent content leads to an increase in the ion−dipole forces between protons and nitrogen atoms in the ligand to a lesser extent than that of more electronegative donor oxygen atoms of the solvent.[39]

Determination of pK in Various Methanol−Water Systems. Methanol is widely accepted as a cosolvent, and its effect on pK has been investigated extensively.[40] The pK values of benzodiazepines were determined in different methanol−water proportions at various ionic strength and temperature up to 50 % of methanol. The data obtained for (10, 20, 40, and 50) % of methanol−water were not uniform. The pK values obtained at constant ionic strength and constant temperature are summarized in Table 1. With an increase in the percentage of methanol (decrease in dielectric constant), the pK values of 1,4-benzodiazepines were decreased as these are weak bases, while an increase in the pK values of 1, 5-benzodiazepines was observed since these are weak acids. Hence 30 % methanol−water was selected for further studies. A representative titration curve (Figure 2) and distribution of species (Figure 3) for Diazepam in a 30 % methanol−water system at 0.15 M (KCl) as ionic strength and T = 25 °C are given.

Determination of pK in Various Ethanol−Water Systems. All titrations were carried out at constant ionic strength (μ = 0.15 M KCl) and constant temperature (25 °C). The pH measurements were made in ethanol−water systems (up to 50 % of ethanol). The pK values obtained are summarized in Table 2. With an increase in the percentage of ethanol (decrease in dielectric constant), a decrease in the pK values of 1,4-benzodiazepines and an increase in the pK values of 1,5-benzodiazepines were observed.

Table 1. Variation in p*K* Values of 1,4-Benzodiazepine and 1,5-Benzodiazepine at Various Methanol−Water Systems (v/v) at Constant Ionic Strength (μ) = 0.15 M (KCl) and Constant Temperature (T) = 25 °C

	p*K* values[a]							
methanol	1,4-benzodiazepine			1,5-benzodiazepine				
(%, v/v)	Dz	Ndz	Nz	1−5 Ben 1	1−5 Ben 2	1−5 Ben 3	Ben 4	Ben 5
10	3.424	3.597	3.104	9.119	9.341	9.345	9.420	9.913
20	3.399	3.527	3.061	9.196	9.345	9.398	9.345	9.387
30	3.325	3.475	2.947	9.271	9.504	9.484	9.423	9.467
40	3.260	3.406	2.909	9.341	9.579	9.561	9.506	9.554
50	3.208	3.339	2.866	9.406	9.663	9.629	9.594	9.619

[a] n = 5.

Table 2. Variation in p*K* Values of 1,4-Benzodiazepine and 1,5-Benzodiazepine at Various Ethanol−Water Systems (v/v) at Constant Ionic Strength (μ) = 0.15 M (KCl) and Constant Temperature (T) = 25 °C

	p*K* values[a]							
ethanol	1,4-benzodiazepine			1,5-benzodiazepine				
(%, v/v)	Dz	NDz	Nz	1−5 Ben 1	1−5 Ben 2	1−5 Ben 3	1−5 Ben 4	1−5 Ben 5
10	3.314	3.522	3.053	10.113	10.120	10.049	9.860	10.080
20	3.230	3.459	3.002	10.192	10.193	10.126	9.939	10.165
30	3.149	3.378	2.928	10.267	10.268	10.212	10.016	10.224
40	3.083	3.302	2.862	10.345	10.337	10.295	10.095	10.321
50	2.992	3.233	2.774	10.428	10.415	10.374	10.181	10.405

[a] n = 5.

Table 3. Variation in p*K* Values of 1,4-Benzodiazepine and 1,5-Benzodiazepine in a 30 % Methanol−Water System (v/v) at Constant Ionic Strength (μ) = 0.15 M (KCl) and Various Temperatures

	p*K* values[a]							
temperature	1,4-benzodiazepine			1,5-benzodiazepine				
(°C)	Dz	NDz	Nz	1−5 Ben 1	1−5 Ben 2	1−5 Ben 3	1−5 Ben 4	1−5 Ben 5
25	3.325	3.475	2.947	9.271	9.504	9.484	9.423	9.467
35	3.275	3.426	2.922	9.209	9.451	9.429	9.370	9.423
45	3.222	3.356	2.876	9.153	9.396	9.364	9.309	9.358

[a] n = 5.

Table 4. Variation in p*K* Values of 1,4-Benzodiazepine and 1,5-Benzodiazepine in a 30 % Methanol−Water System (v/v) at Various Ionic Strengths (μ) (KCl) and Constant Temperature (T) = 25 °C

	p*K* values[a]							
ionic strength	1,4-benzodiazepine			1,5-benzodiazepine				
(M)	Dz	NDz	Nz	1−5 Ben 1	1−5 Ben 2	1−5 Ben 3	1−5 Ben 4	1−5 Ben 5
0.15	3.325	3.475	2.947	9.271	9.504	9.484	9.423	9.467
0.30	3.295	3.433	2.899	9.226	9.473	9.449	9.390	9.429
0.40	3.268	3.386	2.854	9.189	9.435	9.408	9.349	9.385

[a] n = 5.

Effect of Temperature on p*K* Values. The effect of temperature was examined, and the p*K* values showed a decrease with an increase in temperature. The pH measurements were made in 30 % methanol−water systems. Titrations were carried out at constant

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ionic strength (μ = 0.15 M KCl) and various temperatures (Table 3). The pK values of 1,4-benzodiazepines as well as 1,5-benzodiazepines decreased with increasing temperature.

Effect of Ionic Strength on pK Values. In the present work, pK values were determined in a 30 % methanol−water system (v/v) at various ionic strengths and at constant temperature (25 °C). The stability constant of all ligands decreases with an increase in ionic strength. The decrease in stability is in the range of (0.030 to 0.060) units. The variation in stability constant is shown in Table 4. The pK values of 1,4-benzodiazepine as well as 1,5-benzodiazepine were decreased with an increase in ionic strength.

CONCLUSIONS

In the field of pharmacy, pK values are the most important characteristic property of biologically active compounds and are useful in drug design studies and for the evaluation of biopharmaceutical properties. These are also used in the determination of the degree at which these drugs are absorbed by body organs. A decrease in dielectric constant, decrease in pK values of 1, 4-benzodiazepines, and increase in pK values of 1,5-benzodiazepines were observed. With increasing temperature and ionic strength, a decrease in pK values of 1,4-benzodiazepine as well as 1,5-benzodiazepine was observed. The data obtained will be utilized in the development of different chromatographic methods by means of an appropriate selection of buffer and solvent system.

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