Vol. 5, Issue. 9, 2018, ISSN 2349-0780

Determination of Dissociation Constants of Drug-Like Molecule In Hydro-Organic Media By A Potentiometric Method

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Abstract :The distribution, transport behavior, bonding to receptors and contribution to metabolic behavior of the molecules depend on the dissociation constants. However its value depends on the structure of the molecules. The protonation behavior of hydroxamic acids was determined in binary mixture containing methanol: water in 60:40, 70:30, 80:20 and 90:10 ratio at 303.15K. Aqueous potassium hydroxide was used for the measurements done in binary mixture. The pKa values were obtained from the half–neutralization potentials which were obtained from an analysis of the potentiometric titrations. The result shows that hydroxamic acids act as weak acid in binary medium.

Keywords: Hydroxamic acid, pKa, Potentiometric titration,dissociation constants, binary medium.

1. Introduction

The acid base dissociation constant, pKa, is an important parameter in absorption, distribution, metabolism, excretion, and toxicity research because it helps to explain chemical phenomena such as absorption, distribution, and elimination of substances. These constants can have a profound effect on the physicochemical properties of a compound and are therefore essential for the optimization of absorption, distribution, metabolism, and excretion (ADME) characteristics. Notably, compounds in their unionized form are less soluble but can more easily penetrate lipophilic barriers encountered on the way to a biological target. Knowledge of the ionization state of a compound is also required for determining the correct binding-site interactions that occur and the development of reliable structure activity relationships (SAR). This important parameter has a lot of applications in research areas such as pharmaceutical drug development, solvent extraction, acid base titration, and ion transport. The toxicity, chromatographic retention behavior, and pharmaceutical properties of organic acids and bases are affected by acid baseproperties. In modern organic chemistry, a considerable amount of theoretical foundation is based on the observation of the effect on acid base equilibrium of changing molecular structure. [1] pKa equals the pH at which a drug is 50% ionized and 50% un-ionized. Experimental methods for deriving these constants involve exposing a compound to an environment of changing pH and monitoring changes that occur to a property dependent on the ionization state of the compound. In addition, the important role of the degree of ionization in the biological behavior of chemical substances, as well as in their ability to function in passive transcellular diffusion and/or in their

suitability as substrates for active transport, is well-established. [2]

The hydroxamic acid functionality, –C (=O).N.OH, is a key structural constituent of many biomolecules, some of which, are naturally occurring [3] and others, such as peroxidase, matrix metalloproteinase and urease inhibitors [4,5] are of synthetic origin. Hydroxamic acid derivatives have received increasing attention due to their biological activity especially as enzyme inhibitors [6] and metal chelators. [7] Hydroxamic acids are widely involved in pharmacological applications. These are used as antibiotics [8], anti-inflammatory [9], anti-virus [10], anti-tumor [11] and anti-cancer [12-17] agents. Some hydroxamic acids are reported as histone deacetylase [18-22], metalloprotinases [23-28], TNF-α converting enzyme [29-33] and peptide diformilase [34,35] inhibitors.

The dissociation constants of ionizableanalytes have been determined and discussed in terms of solvent characteristics by various authors in mixed solvents, such as methanol water, acetonitrile (MeCN) water, and tetrahydrofuran water mixtures. A knowledge of hydroxamic acids pKa values is useful to predict the extent of ionization of functional groups with respect to pH. This information is important in drug discovery and development. Present investigation deals with the determination of their acid dissociation constants (pKa) in non-aqueous solvents. pKa is useful physico-chemical parameter describing the extent of ionization state of a particular functional group so as to understand the pharmacokinetic and pharmacodynamic properties of new drug substances. At the same time the distribution, transport behavior and bonding to the receptor depends upon the ionization constant. [36–40] Furthermore, ionization constants allow the enumeration of likely chemical species (i.e., that are present at pH 7) prior to protein ligand docking studies. Ionization constants, typically represented as pKa, provide an insight into the degree of dissociation of hydrogen ions from a compound at a given pH. When a drug is ionized it will not be able to pass through the lipid membrane, it will only be able to do so when it is non-ionized and therefore has higher lipid solubility. The values of the dissociation constants can be related to macroscopic parameters [cosolvent percentage, the mole fraction of cosolvent (x), andthe dielectric constant (ε)] and to microscopic parameters (Kamlet and Taft's solvatochromic parameters, solventhydrogen-bond acidity; solvent hydrogen-bond basicity;π*, dipolarity/polarizability). [41-43]

2. Material and Method

2.1 Experimental Section

The knowledge of interactions of hydroxamic acids with aquo-organic solvent plays a very important role in understanding the nature of action of these bioactive molecules. Hydroxamic acids are very sparingly soluble in aqueous phase. In order to increase their solubility, a binary solvent system comprising of methanol and water was used. The potentiometric titrations of two hydroxamic acids have been measured at room temperature in 60%, 70%, 80%, and 90% (v/v) in Methanol/Water system.

2.2 *Preparation of Solution*

0.001mol/litre of each hydroxamic acid was prepared in 60%, 70%, 80%, and 90% methanol/ water mixed solvent by direct weighing 0.001mol/litre potassium hydroxide solution was prepared in triple distilled water.

2.3 Procedure

Vol. 5, Issue. 9, 2018, ISSN 2349-0780

The pKa values of the studied hydroxamic acids were determined by means of the data obtained from potentiometric titrations in 60:40, 70:30,80:20, 90:10 and 100:0 volume fraction methanol: water mixtures at room temperature.0.001 mol/L potassium hydroxide solution(KOH) use as titrant for binary. pH titration was carried out as follows: in a first step, the electrodicsystem was calibrated by Gran's method as to obtain the standard electromotive force (emf) values, E0, of the potentiometric cell. The calibration parameters were checked from the Gran plots. The standardization of the electrode system was carried out each time the solvent medium was changed and the constancy of E0values ensured by continual surveillance by means of periodic calibrations. In a second step, a solution of hydroxamic acids (50.0 ml containing 0.001mol/litre) to be analyzed atthe required conditions of temperature, ionic strength and solvent composition was added to the pre titrated background solution. During titration, the titrant was added in 0.5ml after each addition, the potential was allowed to stabilize and the potential value was used to calculate the pH of the solution using the value of E^0 calculated in the calibration step. These amounts should be high enough to provoke a measurable change in the pH of the test solution, but also small enough to allow the increase of volume to be neglected.

3. Results and Discussion

The influence of an organic solvent added to a medium on the dissociation of ionizable analytes is extensive in many cases and must be accounted for the variation of the pKa values with the content of the organic modifier can be explained by consideration of the preferential salvation of electrolytes in organic solvents. The values of HNP and pKa of N-arylhydroxamic acids in 60, 70, 80 and 90 % methanol /water mixture at 25^0C are given in **Table 1.**

TABLE 1. Hnp, Pka Values And Amount Of Base Required For Equivalence Point/Ml of Hydroxamic Acids In Methanol : Water Mixtures.

S.No	N-phenylbenzohydroxamic acid			
	% methanol	HNP(mV)	Amount of base required for equivalencepoint/ml	pKa
1	60	-108	11.95	8.13
2	70	-107	6.51	8.22
3	80	-99	3.64	8.37
4	90	-63	1.99	8.61
	N-p-Tolylbenzohydroxamic acid			
1	60	29	4.01	7.53
2	70	47	3.99	7.61
3	80	48	1.49	7.76
4	90	52	1.39	7.93

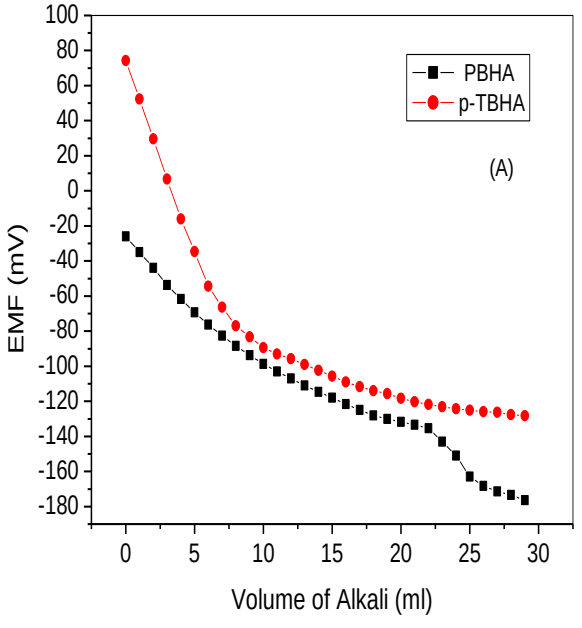
Vol. 5, Issue. 9, 2018, ISSN 2349-0780

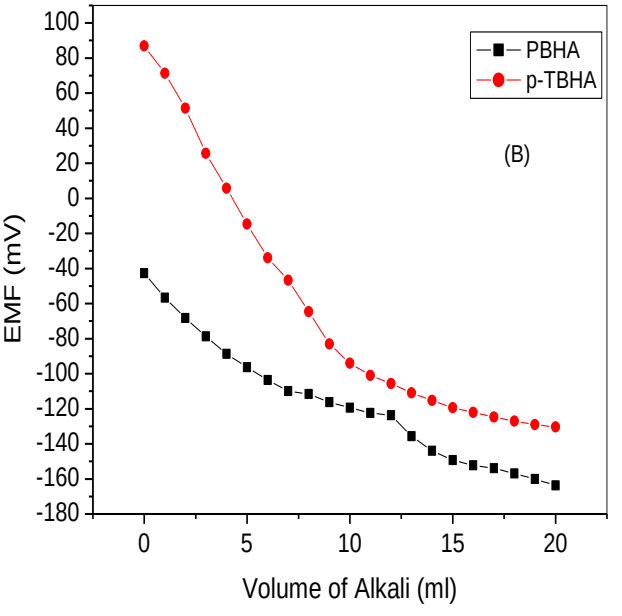
The values of HNP for N-phenylbenzohydroxamic acid in 60 to 90% methanol/water mixture are in the range from -63 to -108 mV and for N-p-tolybenzohydroxamic acid are in the range from 29 to 52 mV, respectively. The pKa values of N-phenylbenzohydroxamic acid in 60 to 90 % methanol/water media are in the range from 8.13 to 8.61 and that for N-p-tolybenzohydroxamic acid are in the range from 7.53 to 7.93, respectively.

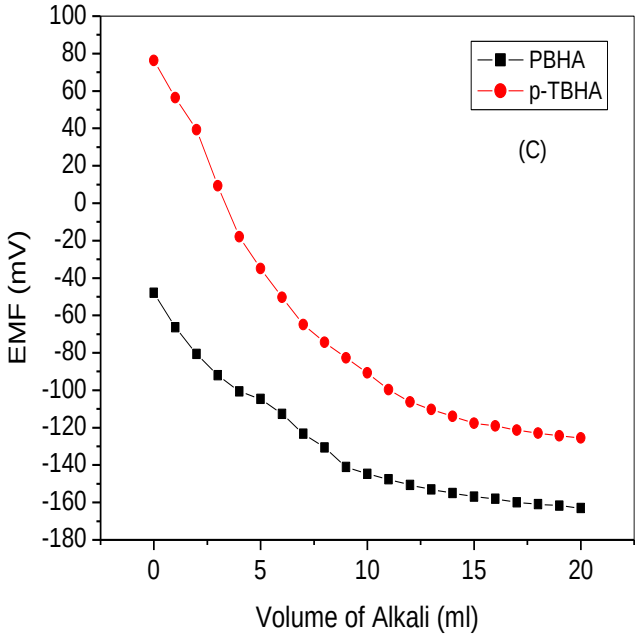
A typical titration curve of N-Phenylbenzohydroxamic acid and P-Tolylbenzohydroxamic acid using Methanol: Water system as binary solvent and aqueous potassium hydroxide as titrant is shown in **figure 1.**

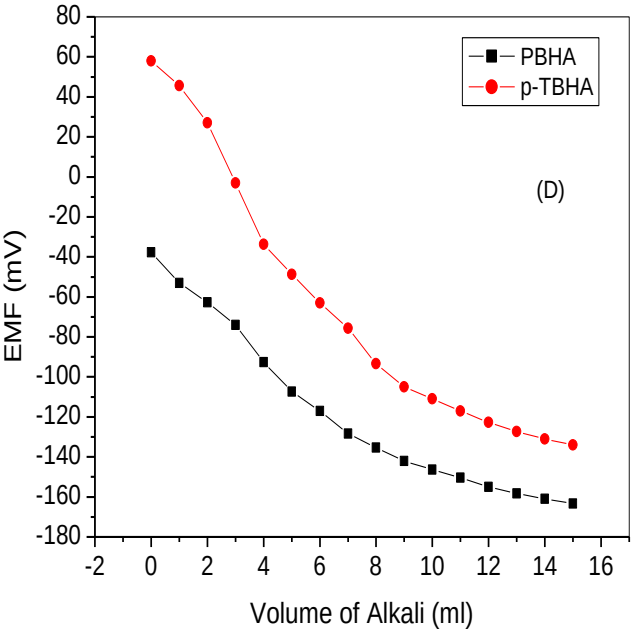
As seen from the figures all titration curves are S-shaped. From this curve pKa value of both hydroxamic acids is found to be very low. When these pKa values of hydroxamic acids are plotted against the mole fraction of methanol, a linear relationship is observed **(figure 2).**

Vol. 5, Issue. 9, 2018, ISSN 2349-0780



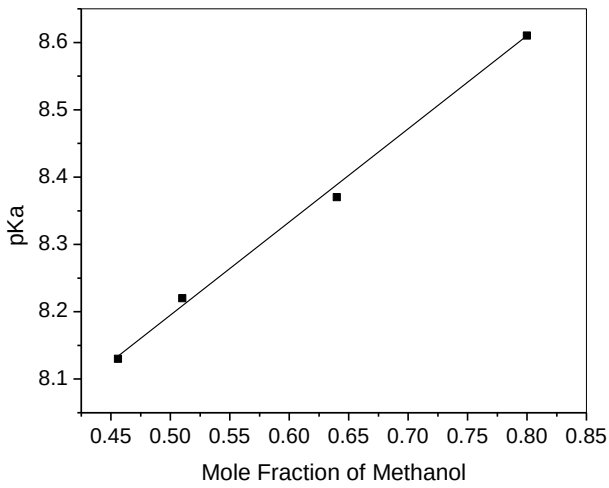






Vol. 5, Issue. 9, 2018, ISSN 2349-0780

FIG. 1 Potentiometric Titration Curve of N-Phenylbenzo- And N-P-Tolylbenzo- Hydroxamic Acid in 60% to 90 % Methanol/Water System.



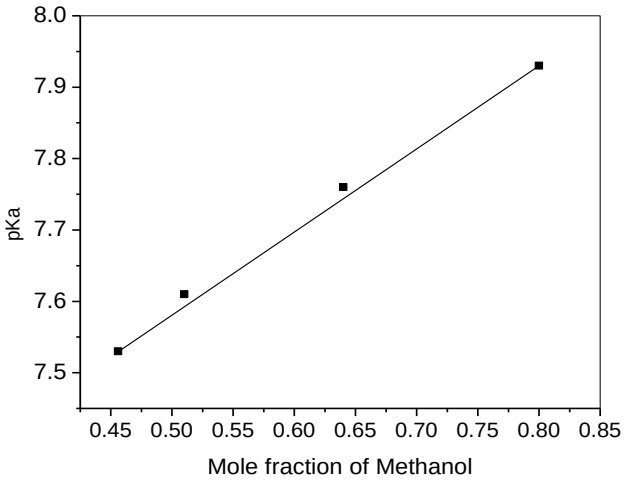


Figure. 2 Potentiometric Titration Curve of N-Phenylbenzo And N-P-Tolylbenzo- Hydroxamic Acid in 60% to 90 % Methanol/Water System.

Table 2. Emperical Relation Between Pka and $N_2$of Methanol At 25^0C

Hydroxamic Acids	pKa = mn_2 + c		
	m	C	r
PBHA	1.36	7.53	0.998
p-TBHA	1.14	7.01	0.998

The experimental values of pKa for both the hydroxamic acids from table 2 indicate a maximum deviation from linearity by the order of 0.05. The acidity of hydroxamic acids may be attributed essentially to the –OH group. The suppression of acidic character may be attributed to intramolecular hydrogen bonding present in the molecule.

4. Conclusion

It is of interest to examine this accuracy of the extrapolated values of pKa at 0 methanol concentration (n_2=0). For N-phenylbenzohydroxamic acid, pKa values obtained by extrapolated and experimental methods are 8.57 and 8.55, respectively and pKa by extrapolated method for N-p-tolylbenzohydroxamic acid is 6.01. Both the hydroxamic acids studied are very weak acids and their acidity may be attributed essentially due to their –OH group. This suppression of acidic character is due to presence of intramolecular hydrogen bonding in the molecule.

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