



RESEARCH ARTICLE

Spectrophotometric determination of pKa of schiff base ligand

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Manuscript No: IJPRS/V6/I1/00026, Received On: 28/03/2017, Accepted On: 08/04/2017

ABSTRACT

Schiff bases are a significant group of organic compounds that have biological activities and miscellaneous applications because of their antibacterial, antiviral activities, metal complexation and other Pharmacological activities. The simple and meticulous method used for the determination of the stability constants of the complexes by means of UV-visible spectrophotometry depending on the theoretical interpretation of the stoichiometry, Jobs and Yoe-Jones, methods was proposed. In present work, proton ligand stability constant (pKa) of substituted hydroxy Schiff base ligand 2,2'-((ethane-1,2-diylbis(azanylylidene))bis(ethan-1-yl-1-ylidene))bis(4-methylphenol) (H₂L) have been investigated by spectrophotometric method. Determination of pKa values has studied at different concentrations of ligand solution. pKa values are calculated at pH 4.82, 5.12, 5.40, 5.70 & 5.90 by using standard literature method. The ruggedness of the determined result was also validated in this study for producing exact pKa value. The results obtained from this work were compared with those obtained using the potentiometric method. The difference between the results found with these two methods was approximately 0.04 units.

KEYWORDS

Schiff base, Spectrophotometric method, pKa determination.

INTRODUCTION

The Schiff bases and their metal complexes have got more importance recently¹⁻⁶ because of their application as biological, biochemical, analytical, antimicrobial, anticancer, antibacterial, antifungal and anti tumor activity. They have been studied as a class of ligands^{7,8}. Recently there has been a considerable interest in the chemistry of Schiff base compounds because of their potential pharmacological applications⁹. Schiff base complexes showed catalytic activity in carbonylation of alcohols and alkenes at low pressure to produce arylpropionic acids and their esters^{10, 11}, which are used as non-steroidal anti-inflammatory

In addition to monometallic, the bimetallic Schiff base complexes also showed catalytic activity in carbonylation reactions. The Heck reaction¹², an industrially useful process to synthesize fine chemicals and pharmaceutical was successfully catalyzed using Schiff base complexes.

The study of proton ligand stability constant¹³(pKa) has gained much importance as it affects solubility, absorption across biological membranes, distribution to the site of action, renal elimination, metabolisms and binding of the drug with protein and receptor. The pKa plays very important role in drug discovery. The determination of pKa of drug is of interest from the stand point of its influence on pharmaceutical compound synthesis. The FDA of all countries has made it mandatory to estimate pKa value of new drug molecules¹⁴. The pKa of the drug is cited in literature^{15, 16} by

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several authors. The first real report ¹⁷ on the determination of pK_a value of drug appeared in which the dissociation constant was determined by a potentiometric method employing mixed solvent techniques using methanol–water mixtures. But pK_a of Schiff base ligand at different concentration by spectroscopic method have not been studied yet. So in this report we determined the pK_a of Schiff base ligand spectrophotometrically. Spectrophotometry is one of the most powerful methods for the investigation of solution equilibria, although potentiometric pH titrations are more convenient and more commonly used due to the simplicity of the equipment and minimum time requirement. Spectrophotometric titrations are useful for determining the ionization constants of acids and bases even below pH 2 and above pH 11, where potentiometry becomes inconvenient. Spectrophotometric titrations also yield additional spectral information about the species formed during the titration and requires very less amount of the compound ¹⁸, therefore we prefer this method in our experimental work.

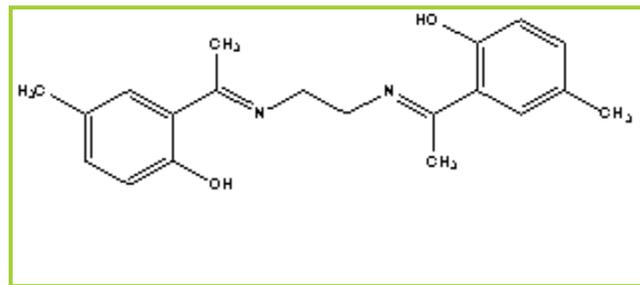
An extra aspect of our study is the validation of pK_a value. This study was done by S. Singh et al for pK_a determination of nimesulide¹⁹. For this, the result was validated from determination of pK_a by using different concentration of H_2L ligand on same spectrophotometer.

EXPERIMENTAL AND MATERIALS

All chemicals were used as obtained from Rankem and Sd fine chemicals. Anal grade solvent from Sd Fine chemicals was used without further purification. Freshly prepared triple distilled water was used for making solutions.

The Schiff base ligand was synthesized by condensation of the acetophenone with ethylene diamine in suitable stoichiometric proportion as per the literature method ²⁰. The pH of the solutions were measured on a pH meter (Systronics Ltd. India) equipped with a combined glass electrode. It was standardised at 27°C using standard buffers. The UV-Visible

absorption spectra were recorded on Systronics UV-Visible spectrophotometer-119.



2, 2'-((ethane-1, 2-diylbis (azanylylidene)) bis (ethan-1-yl-1-ylidene)) bis (4-methylphenol)

H_2L ligand

Method

The determination of pK_a by spectrophotometric method is based on the principle that ionisation of the acidic or basic compound is pH dependent. Hence with change in pH of the solution is the ratio of ionised form to the unionised form changes. At definite wavelength the ionised and unionised forms have different absorptions. The UV-Visible absorption spectra were recorded on Model Systronics UV-Visible Spectrophotometer -119 operated at a wavelength range of 200-400 nm. The dissociation constant (pK_a) of H_2L ligand were determined at room temp, according to the experimental method reported by Albert and Serjeant ²¹. The pH values of all solutions were measured on a Systronics digital pH meter 335 equipped with combined glass electrode which was calibrated at 27⁰ using standard buffers at pH 4.0 and 7.0.

The synthesized H_2L ligand was used after recrystallisation, DMF was purified by distillation process which was given in Vogel's TextBook of Practical Organic Chemistry ²². Buffer chemicals and all other reagents were of analytical grade. Freshly prepared double distilled deionised water were used throughout. All of the experiments were carried out at room temperature; 27±1⁰C. The procedure reported by Albert & Serjeant was used for the determination and calculation of pK_a . For the experiments at different pH, the acetate buffers

were used. Aliquots of five different sets were prepared by varying volume of buffer solⁿ. 0.01M solution of H₂L ligand in DMF was prepared. To each set 10 μ L of this stock solutions was added to the buffer solutions of varying pH to get the final conc. of the ligand as 10 μ ML⁻¹. The buffer solutions of varying pH were prepared by mixing suitable proportions of 0.04M sodium acetate, 0.02M Acetic acid, 0.1M HCl and 0.1M NaOH. Relevant quantity of sodium chloride was added in order to maintain the ionic strength of all solutions to 0.1. The solutions were mixed and the all five sets were placed in a High precision water bath set at 27^oc. The absorbance variation was illustrating this result for each solution at 294 nm.

The absorbances of neutral and ionised species of the ligand at 0.1M ionic strength of NaCl were measured. For validation of the determination of pKa value the total study of measurement of absorbance was repeated. This ruggedness study was done by absorbance measurements in three studies were done by using different conc. of ligand solution on same spectrophotometer.

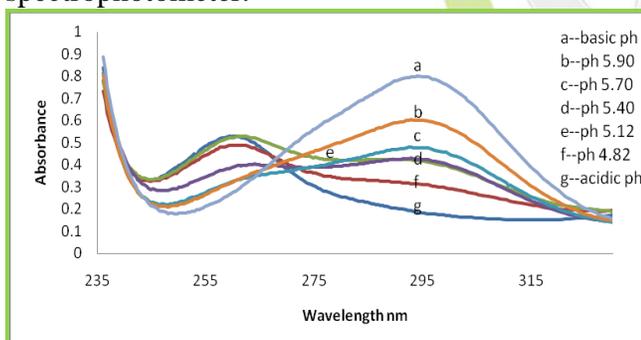


Fig 1: UV Spectrum of H₂L at Various pH.

RESULTS AND DISCUSSION

Fig. 1 shows a schematic representation of UV spectrum of H₂L ligand with pH. Table 1 shows variation in the UV spectrum of one of the determination in buffer solution of various pH.

Table 1: Analysis Data of Calculations of pKa from Absorbance at Aarious pH

pH	Absorbance (d)	$pK_a = pH + \log \left(\frac{d_i - d}{d - d_m} \right)$
4.82	0.30469	5.3573
5.12	0.4182	5.4224
5.40	0.4255	5.6384
5.70	0.4752	5.7586
5.90	0.6020	5.6055

Average: pKa = 5.5564

$d_m = 0.1875$ (in 0.1M HCl) ; $d_i = 0.7996$ (in 0.1M NaOH).

Table 2: Validation of Ruggedness of the pKa Value

Instrument 1			
Person 1,day 1,ligand Strength= 5 μ ML ⁻¹	Person 1,day 1,ligand Strength= 10 μ ML ⁻¹	Person 1,day 2,ligand Strength= 15 μ ML ⁻¹	Person 1,day 2,ligand strength = 20 μ ML ⁻¹
5.5533	5.5564	5.5578	5.5585

In fig.1 we observed that the overlapping spectra of all the sample solution which were taken and absorbance at 294 nm was recorded. In this spectrum clearly two isobestic points were observed at 241 and 269 nm. The sharp isobestic points were observed which only used for calculation of pKa. The absorbance data was used to estimate pKa values by three methods of calculation. Table 1 shows the pH dependance of absorption spectra of H₂L.

(i) The distinct pKa value was calculated by using following equation:

$$pKa = pH + \log (d_i - d / d - d_m)$$

Where d_i is the absorbance of ionized species, d is the absorbance of respective buffers tested solution and d_m is absorbance of unionized species. The average pKa was calculated by taking antilogarithm of each individual calculated pKa value, by taking average of all the antilogarithmic values, and taking logarithm of the averaged value. This calculation method gives more precise pKa value.

(ii) The plot of pH Vs $\log (d_i - d / d - d_m)$ gave a straight line with intercept as pKa equal to 5.5545. It is a good agreement with these determinations by equation.

(iii) The values of degree of ionization (α) are determined from the absorption data. The plot of α Vs pH, gives the value of pKa equal to 5.7502 at $\alpha = 0.5$.

Table 3: pKa Calculation by Various Methods of Calculation.

pKa Calculation from Equation	pKa by Intercept	pKa from Calculation of α
5.5564	5.5545	5.7502

To establish validation of ruggedness of the result the same process was repeated for all other working solutions of different concentration of ligand. The validation result of pKa was listed in Table 2. This result of the validation study were obtained very close, that

means it indicate the accuracy of result and technique. The final determined pKa value calculated from our experiment is 5.55. the lower value of pKa as compared to standard value of 10.0 for phenolic -OH group, might be due to presence of unsaturated -N=C- linkage at ortho position.

In this work, the pKa value for H₂L ligand determined spectrophotometrically was found to be 5.55 which is about 0.04 unit different from 5.51 obtained by Calvin- Bjerrum method using potentiometric technique. The observed differences between the pKa values obtained by both method might be due to different experimental methods.

Finally we report that the close agreement of pKa value among spectrophotometric method and with potentiometric method shows robustness of the spectrophotometric method.

ACKNOWLEDGEMENTS

The authors are grateful to the Principal, Vidyabharati Mahavidyalaya, Amravati, and Maharashtra, India for providing excellent infrastructure facility to carry out this research work.

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