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Effect of Dielectric Constants of co-solvent DMF on Protonation Equilibria of 5-Sulfo Salicylic Acid and 5-Hydroxy Salicylic Acid.

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ABSTRACT

The solute-solvent interactions of 5-Sulfo salicylic acid and 5-Hydroxy salicylic acid have been studied in 0.0-60.0% v/v N, N'-Dimethyl formamide (DMF) - water media at an ionic strength of 0.16 mol L^{-1} and at 303.0 K. using the pH-metric method. The protonation constants have been calculated with the computer program MINIQUAD75. Selection of the best fit chemical model of the acid-base equilibria is based on standard deviation in protonation constants and residual analysis using crystallographic R-factor and sum of squares of residuals in all mass balance equations. Linear and Non linear variation of protonation constants with inverse of dielectric constant of the solvent mixture has been attributed to the dominance of the electrostatic forces. The trend is explained on the basis of solute-solute, solute-solvent interactions, solvation, proton transfer processes and dielectric constants of the media. Distributions of species, protonation equilibria and effect of influential parameters on the protonation constant have also been presented.

Keywords: Acid-base equilibria, 5-sulfo salicylic acid, 5-hydroxy salicylic acid, N, N'-Dimethyl formamide (DMF), protonation constant and dielectric constant.

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INTRODUCTION

5-Sulfosalicylic acid (5-SSA) is used in urine tests to determine urine protein content. The chemical causes the precipitation of dissolved proteins, which is measured from the degree of turbidity. It is chemically stable under normal temperatures and pressure and should be avoid from light, dust generation and excess heat. It has incompatibility with other materials such as, strong oxidizing agents and strong bases [1].

5-hydroxy salicylic acid (5-HSA) is also to be known as gentisic acid. It is a derivative of benzoic acid and a minor (1%) product of the metabolic break down of aspirin, excreted by the kidneys [2]. It is also found in the African tree *Alchorneas cord folia* and in wine [3].

The dielectric constant is a macroscopic property, which plays a significant role in the solution properties of the reaction medium. It is a useful technique in characterizing molecular ordering in solutions and the value of the dielectric constant is strongly related to the chemical structure of a molecule and to its intermolecular interactions [4].

The dielectric constant is one of the characteristics of liquid. The proton-ligand and metal-ligand stability constants are strongly affected by the dielectric constant of the medium because of the fact that at least one of the constituents is charged and other is either charged or has a dipole. Variations in the relative strengths of acids and bases with changing solvents should be a function of the charge, the radius of the ion and the dielectric constants of the medium. It has been reported that an acid in solvents of similar dielectric constants exhibits different strengths e.g. dissociation of substituted benzoic acids in aliphatic alcohols and aqueous dioxane with identical dielectric constants, is different [5].

The binary mixtures of dipolar aprotic and protic solvents have current interest in biological, chemical, pharmaceutical, technological and laboratory applications because mixed solvents manifest physicochemical properties as compared to those of pure constituents of the mixture amongst the physicochemical properties, dielectric constant of the mixed solvents, which is sensitive to molecular interactions specially H-bond formation, enhances or controls most of the applications, and hence precise the dielectric characterization is important. The dipolar solvent-solvent interactions are more complex in mixed solvents than in their pure form, and the characterization of these interactions are challenging in the liquid systems in which the molecules of the mixture constituents have a large number of possible atom-atom interactions.

Among the different solvents, the H-bonded structures of amides and their mixtures, especially with water and aliphatic alcohols have an important role in biological systems, because the hydrogen bonds are important as a force governing the structure and the dynamics of chemical and biological system. In the case of amides, the dipolar aprotic solvent, DMF is unable to engage in $N-H\cdots O=C$ hydrogen bonding with dialkyl substitution at nitrogen. However, with water and alcohols DMF forms the $C-H\cdots O$ and $C=O\cdots H$ type H-bond interactions. Which result in the complex H-bonded network structures of these systems. DMF is also able to form the H-bonded network structures with dipolar aprotic solvents the dielectric measurements have great potential for studying such H-bonded network structures with dipolar ordering, the strength of complexations and the stoichiometric ratio of stable adduct, and also their dynamics in the mixtures [6].

EXPERIMENTAL

Solutions (0.05 mol L^{-1}) of 5-SSA (TCI, India) and 5-HSA (TCI, India) were prepared in triple-distilled water by maintaining 0.05 mol L^{-1} hydrochloric acid concentration to increase the solubility. DMF (Qualigens, India) was used. Carbonate free sodium hydroxide (Qualigens, India) pellets were used for the preparation of 0.4 mol L^{-1} solution. Hydrochloric acid (Qualigens, India) of 0.2 mol L^{-1} was prepared. Sodium chloride (Qualigens, India) of 2.0 mol L^{-1} was prepared to maintain 0.16 mol L^{-1} ionic strength in the titrand. Triple distilled water was used throughout the experiment. The strengths of acid and alkali were determined using Gran plot method [7,8]. To assess the errors that might have crept in to the determination of the concentrations, the data were subjected to analysis of variance of one way classification (ANOVA) [9].

Alkalimetric Titrations

All measurements were carried out at 303.0 ± 0.1 K. Equiptronics EQ 614 A pH meter with magnetic stirrer and combined glass and calomel electrode assembly used for pH measurements. The sensitivity of pH meter is 0.01 units. The instrument could read pH in the range 0.00 to 14.00 in the steps of 0.1. The pH meter was switched on half an hour before starting the titration for initial warm up of the instrument. It was calibrated before each titration with an aqueous standard buffer solution of pH 9.18 and 4.02 at 303.0 ± 0.1 K. The above said buffer solutions were prepared from a 'Qualigens' Potassium hydrogen phthalate (0.05 mol L^{-1}) and 'Qualigens' borax (0.01 mol L^{-1}) solutions. The glass electrode was equilibrated in a well stirred DMF-water mixture containing inert electrolyte for several days. At regular intervals strong acid was titrated against alkali to check the complete equilibration of the glass electrode. The calomel electrode was refilled with DMF-water mixture of equivalent composition of the titrand. The hydrogen ion concentration was measured with combined glass electrode.

Alkalimetric titrations were carried out with varying composition of DMF - water (0.0-60.0% v/v) maintaining an ionic strength of 0.16 mol L^{-1} with sodium chloride at 303.0 ± 0.1 K. The amounts of 5-Sulfo salicylic acid and 5- hydroxyl salicylic acid in the titrands ranged between 0.25 and 0.50 mmols. The details of the experimental procedure and titration assembly have been detailed elsewhere [10-12]. In each titration, the titrand contained approximately 1 mmol of hydrochloric acid. The initial concentrations of ingredients are given in (Table 1).

Table 1: Total initial concentrations of ingredients (in mmol) in proton-ligand titrations.

DMF% (v/v)	TLO	
	5-SSA	5-HSA
0	0.2493	0.2493
	0.3740	0.3740
	0.4987	0.4987
10	0.2499	0.2494
	0.3749	0.3741
	0.4999	0.4988
20	0.2497	0.2495
	0.3746	0.3743
	0.4995	0.4991
30	0.2495	0.2492
	0.3746	0.3738
	0.4990	0.4985
40	0.2496	0.2495
	0.3744	0.3742
	0.4992	0.4990
50	0.24196	0.2494
	0.3744	0.3741
	0.4993	0.4989
60	0.2495	0.2492
	0.3743	0.3738
	0.4991	0.4985

Modeling Strategy

The approximate protonation constants of 5-Sulfo salicylic acid and 5-Hydroxy salicylic acid were calculated with the computer program SCPHD [13-16] The best fit chemical model for each system investigated was arrived at using non-linear least-squares computer program, MINIQAD75 [17-19].

RESULTS AND DISCUSSION

Normal biochemical processes occur in aqueous solutions at about neutral pH. Physiological pH is about 7.2 to 7.4. Drugs present in biomolecules can gain or lose protons depending on the availability of

hydrogen ions in the solution. This situation results in the simultaneous existence of a number of protonation-deprotonation equilibria in solution.

Secondary formation functions like average number of protons bound per mole of ligand \bar{n}_H and number of moles of alkali consumed per mole of ligand (a) are useful to detect the number of polymeric species and equilibria. Plots of \bar{n}_H versus pH (formation curves) for different concentrations of the ligand should overlap if there is no formation of polymeric species. Overlapping formation curves for 5-Sulfo salicylic acid and 5-Hydroxy salicylic acid (Figure 1) rule out the polymerization of the ligand molecules.

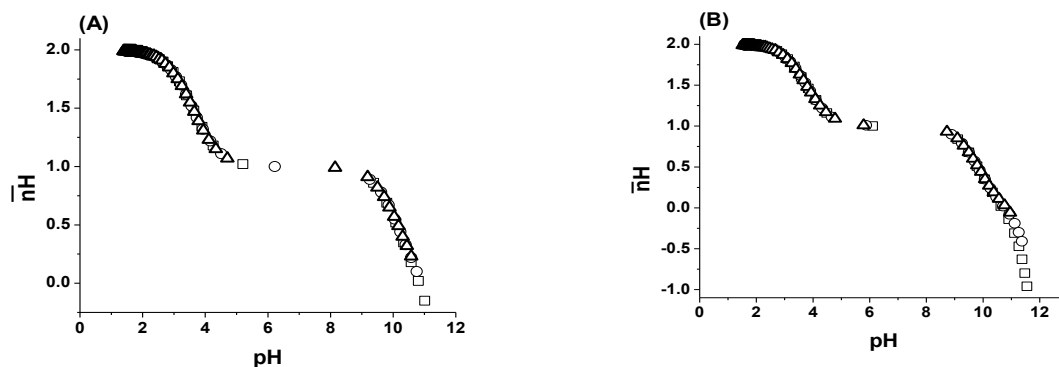


Figure 1: Plots of \bar{n}_H versus pH in 40.0 % v/v DMF-water mixture; (A) 5-SSA and (B) 5-HSA, (\square) 0.25 (\circ) 0.38 (Δ) 0.50 mmol.

The pH values at half integral values of \bar{n}_H correspond to the protonation constants of the ligands. Two half integrals (0.5 and 1.5) in the case of both the ligands (Figure 1) emphasize the presence of two protonation-deprotonation equilibria in the pH range of present study.

The plots of a versus pH for 5-SSA and 5-HSA were given in (Figure 2). The negative values of a corresponds to the number of moles of free acid present in the titrand and the number of associable protons. The positive values of a indicate the number of dissociable protons in the ligand molecules. The maximum value of a in (Figure 2) is +3, which indicates that both 5-SSA and 5-HSA have three dissociable protons.

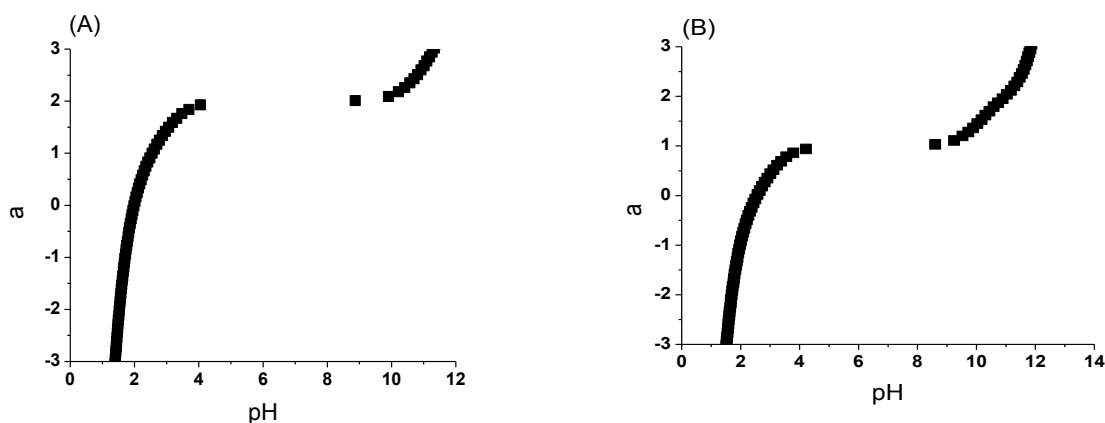


Figure 2: Variation of a with pH in 10.0 % v/v DMF-water mixture: (A) 5-SSA, (B) 5-HSA.

The best fit models containing the type of species and log values of overall formation constants ($\log \beta$) along with some of the important statistical parameters of the present study are given in (Table 2). A very low standard deviation (SD) in $\log \beta$ values and U_{corr} (sum of the squares of deviations in concentrations of ligand and hydrogen ion at all experimental points corrected for degrees of freedom) indicate that the experimental data can be represented by the model. Small values of mean, standard deviation and mean deviation for the systems corroborate that the residuals are around a zero mean with little dispersion.

Residual Analysis [20]

In data analysis with least squares methods, the residuals (the differences between the experimental data and the data simulated based on the model parameters) are assumed to follow Gaussian distribution. When the data are fit into the models, the residuals should be ideally equal to zero. Further, a model is considered adequate only if the residuals do not show any trend. Respecting the hypothesis of the least squares analysis, the residuals are tested for normal distribution. Such tests are χ^2 , skewness, kurtosis, and R-factor. These statistical parameters of the present data show that the best fit models portray the acido-basic equilibria of 5-SSA and 5-HSA in DMF-water mixtures, as discussed below.

χ^2 test

χ^2 is a special case of gamma distribution, whose probability density function is an asymmetrical function. This distribution measures the probability of residuals forming a part of standard normal distribution with zero mean and unit standard deviation. If χ^2 calculated is less than the table value, the model is accepted.

Crystallographic R-Test

Hamilton's R factor ratio test [21] is applied in complex equilibria to decide whether inclusion of more species in the model is necessary or not. In pH-metric method, the readability of pH meter is taken as the R_{limit} , which represents the upper boundary of R beyond which the model bears no significance. When different values are obtained for models containing different number of species, models whose values are greater than R-table are rejected. The low crystallographic R-values given in (Table 2) indicate the sufficiency of the model.

Skewness

It is a dimensionless quantity indicating the shape of the error distribution profile. A value of zero for skewness indicates that the underlying distribution is symmetrical. If the skewness is greater than zero, the peak of the error distribution curve is to the left of the mean and the peak is to the right of the mean if skewness is less than zero. The values of skewness recorded in (Table 2) are between -0.56 and 2.11 . These data evince that the residuals form a part of normal distribution; hence, least-squares method can be applied to the present data.

Table 2: Best-fit chemical models of acido-basic equilibria of 5-SSA and 5-HSA in DMF- water mixtures.

% v/v DMF	Log β_1 (SD)	Log β_2 (SD)	NP	Ucorr X 10^8	Skewness	Kurtosis	χ^2	R-factor
5-Sulfo salicylic acid (pH range: 1.5 -11.90)								
0	10.23(1)	13.05(2)	145	6.53	-0.56	2.90	6.88	0.0110
10	10.95(4)	13.84(5)	51	19.66	0.10	2.00	27.41	0.0154
20	9.89(3)	13.42(6)	53	20.33	1.54	7.07	8.57	0.0442
30	10.37(3)	13.95(5)	36	14.66	0.75	2.84	6.00	0.0239
40	10.18(2)	13.78(3)	72	26.66	0.37	3.57	5.22	0.0199
50	9.49(3)	13.27(6)	55	20.99	2.03	9.54	19.45	0.0506
60	10.09(4)	14.30(5)	20	9.333	1.42	5.78	10.80	0.0179
5-Hydroxyl salicylic acid (pH range: 1.5 -11.90)								
0	10.92(1)	13.23(2)	91	40.66	0.12	3.03	6.66	0.0166
10	10.15(1)	13.08(2)	114	23.66	1.34	5.11	11.72	0.0111
20	9.58(4)	13.17(8)	63	21.33	2.11	8.95	32.98	0.0635
30	9.91(2)	13.52(4)	56	23.33	0.63	2.90	4.14	0.0307
40	9.84(4)	13.61(8)	62	10.99	1.74	7.99	6.84	0.0558
50	9.59(3)	13.55(6)	25	12.66	1.13	4.43	3.76	0.0278
60	9.67(6)	13.88(9)	30	40.66	2.10	7.69	10.93	0.0553

$U_{\text{corr}} = U / (NP-m) \times 10^8$; NP = Number of points; m = number of protonation constants; SD = Standard deviation

Kurtosis

It is a measure of the peakedness of the error distribution near a model value. For an ideal normal distribution kurtosis value should be three (mesokurtic). If the calculated kurtosis is less than three, the peak of the error distribution curve is flat (platykurtic) and if the kurtosis is greater than three, the distribution shall have sharp peak (leptokurtic). The kurtosis values in the present study indicate that the residuals form leptokurtic pattern in the case of both 5-SSA and 5-HSA. Alkalimetric titration data are simulated using the model parameters given in (Table 2). These data are compared with the experimental alkalimetric titration data, to verify the sufficiency of the models. The overlap of the typical experimental and simulated titration data indicates that the proposed models represent the experimental data.

Effect of Systematic Errors in Concentrations on Best Fit Model

MINIQUAD75 does not have an inbuilt provision to study the effect of systematic errors in the concentration of ingredients like mineral acid, alkali and ligand. In order to rely upon the best fit chemical model for critical evaluation and application, a brief investigation was made by introducing pessimistic errors in the ingredients. This type of investigation is useful because the data acquisition was done under varied experimental conditions with different accuracies. The results of a typical system given in (Table 3) emphasize that the errors in the concentrations of alkali and mineral acid affect the protonation constants more than that of the ligand.

Table 3: Effect of errors in influential parameters on the protonation constants in 10.0 % v/v DMF-water mixture.

Ingredient	% Error	Log β_{mth} (SD)			
		5-SSA		5-HSA	
		LH	LH ₂	LH	LH ₂
	0	10.95(4)	13.84(5)	10.15(1)	13.08(2)
Alkali	+4	10.44(4)	13.93(5)	9.77(2)	12.43(3)
	-4	11.99(3)	15.40(3)	10.55(3)	13.84(5)
	+2	10.69(3)	13.37(4)	9.96(1)	12.74(2)
	-2	11.28(9)	14.41(10)	10.34(2)	13.44(3)
Acid	+4	11.37(12)	14.63(13)	10.42(4)	13.70(6)
	-4	10.64(4)	13.21(4)	9.88(1)	12.49(2)
	+2	11.13(6)	14.20(7)	10.28(2)	13.38(3)
	-2	10.28(5)	12.63(7)	10.02(1)	12.79(1)
Ligand	+4	11.19(5)	14.22(6)	10.26(1)	13.22(2)
	-4	10.73(3)	13.48(4)	10.03(1)	12.93(2)
	+2	11.06(5)	14.02(6)	10.20(1)	13.55(2)
	-2	10.84(3)	13.66(4)	10.09(1)	13.00(2)
Log F	+4	10.96(4)	13.87(5)	10.15(1)	13.07(2)
	-4	10.94(4)	13.81(5)	10.15(1)	13.09(2)
	+2	10.96(4)	13.86(5)	10.15(1)	13.07(2)
	-2	10.95(4)	13.83(5)	10.15(1)	13.09(2)
Volume	+4	10.95(4)	13.82(5)	10.15(1)	13.05(1)
	-4	10.95(4)	13.85(5)	10.15(2)	13.11(3)
	+2	10.95(4)	13.83(5)	10.15(1)	13.07(2)
	-2	10.95(4)	13.85(5)	10.15(2)	13.09(2)

Effect of Dielectric Constant of Medium

When the ionization of an acid gives a net increase of ions, a decrease in the dielectric constant of the solvent should be accompanied by an increase in the protonation constant of a weak acid dissolved in it. One

of the most important factors determining the equilibrium constants is the reaction medium, so, the solvent effect on protonation constants could be explained on the basis of dielectric constant of the medium, solvent structure, preferential solvation, and microscopic parameters (as Kamlet-Taft solvatochromic parameters) [22]. This variation of protonation constant or change in free energy with the organic solvent content depends up on two factors: an electrostatic one, which can be estimated by the Born equation and a non-electrostatic one, which includes specific solute-solvent interaction. When the electrostatic effects predominate, according to Born's equation, the energy of electrostatic interaction is related inversely to dielectric constant. Hence, the logarithm of step-wise protonation constant ($\log K$) should vary linearly as a function of the reciprocal of the dielectric constant ($1/D$) of the medium [23]. The linear variation of protonation constants of 5-SSA and 5-HSA (Figure 3) in DMF-water mixtures shows the dominance of electrostatic interactions.

In this study the linear variation of $\log K$ values of 5-SSA and 5-HSA as function of $1/D$ (Figure 3) in DMF - water mixtures shows the dominance of electrostatic interactions. Thus, the observed variation in $\log K$ values when the amount of the solvent DMF in the medium is increased can be mainly attributed to ions association reaction, solute-solvent interaction, proton-solvent interaction and solvent basicity (acidity) effects.

These Drugs can exist in anionic and cationic forms in different equilibria investigated. The cation stabilizing nature of co-solvents, specific solvent-water interactions, charge dispersion, and specific interactions of Co-solvent with solute, account for the deviation of classical linear relationship of $\log K$ with $1/D$. K_1 and K_2 are step-wise protonation constants for the reactions mentioned in (Figure 3).

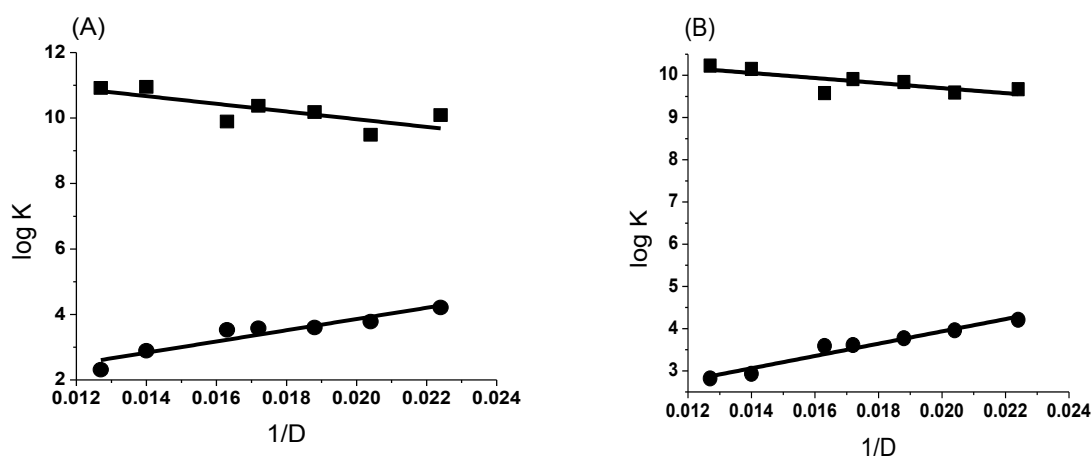


Figure 3: Variation of step-wise protonation constants ($\log K$) with reciprocal of dielectric constants in DMF-water mixture, (A) 5-SSA, and (B) 5-HSA (■) $\log K_1$ (●) $\log K_2$

Distribution Diagrams

Typical distribution plots produced by DISPLOT [24-27] using protonation constants from the best fit models are shown in Figure 4. A single representative plot is shown for each system at a particular DMF-water concentration. As the alkali is added to the titrand containing the ligands, the protonated forms of the ligands lose their protons. In the pH range 1.5-11.9, 5-SSA and 5-HSA lose carboxylic and hydroxyl protons successively.

The distribution plot of 5-SSA (4(A)) shows the existence of LH_2^- , LH^{2-} and L^{3-} at pH ranges 1.5-8.5, 1.5-11.9 and 4.7-11.9 respectively. The LH^{2-} is present to an extent of 96% in the pH range 1.5-11.9.

In the case of 5-HSA, the distribution plot (4(B)) shows the existence of LH_3 , LH_2^- and LH^{2-} at pH ranges 1.5-8.5, 1.5-12.0 and 4.7-12.0 respectively. LH_2^- is present to an extent of 96% in the pH range 1.5-12.0. The corresponding protonation-deprotonation equilibria are shown in (Figure 5).

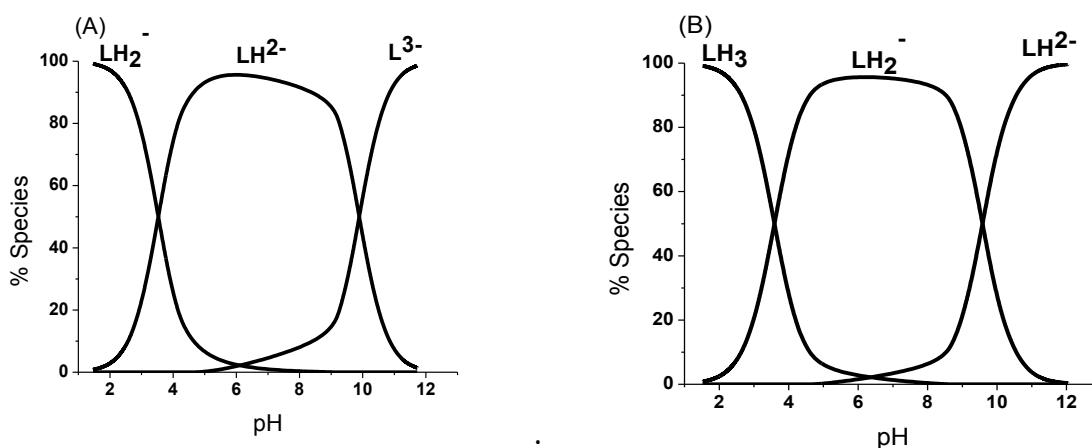


Figure 4: Species distribution diagrams of (A) 5-SSA and (B) 5-HSA in 20.0% v/v DMF- water mixture

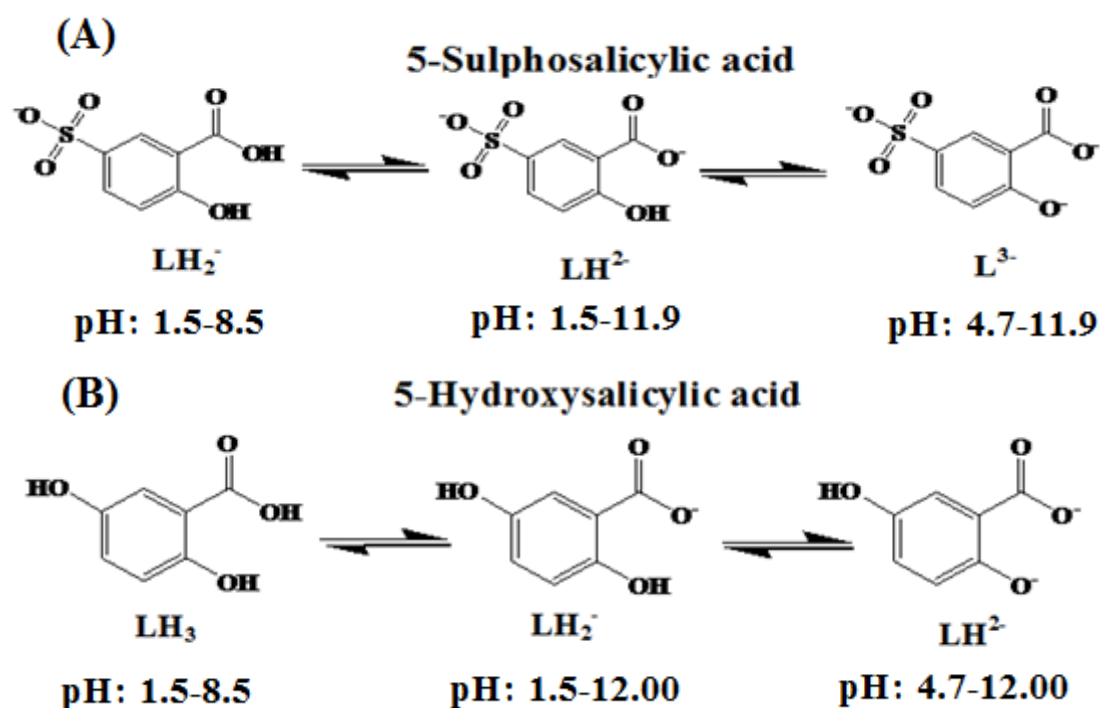


Figure 5: Protonation-deprotonation equilibria of (A) 5-SSA and (B) 5-HSA

CONCLUSIONS

- 5-SSA has three dissociable protons and can exist as LH₃ at very low pH. In the present investigation, it can exist as LH₂⁻ and gets deprotonated with the formation of LH₂⁻, and L³⁻ successively with increase in pH.
- 5-HSA has three protons and can form LH₃ at low pH and gets deprotonated with the formation of LH₂⁻ and LH₂⁻ with increase in pH.
- Secondary formation functions confirm the existence of two protonation equilibria for 5-SSA and 5-HSA.
- 5-SSA and 5-HSA exhibit a linear trend. The linear variation of log values of protonation constants of 5-SSA and 5-HSA with increasing dielectric constant of DMF-water mixtures indicates the dominance of electrostatic forces in the protonation-deprotonation equilibria.

- The effect of systematic errors in the influential parameters shows that the errors in the concentrations of alkali and mineral acids will affect the protonation constants more than that of the ligand.

REFERENCES

- [1] Wikipedia: the free encyclopedia, https://en.wikipedia.org/wiki/5-Sulfosalicylic_acid
- [2] Levy G and Tsuchiya T. *J Medicine*, 1972; 287: 430-432.
- [3] Rong-Rong T, Qiu-Hong P, Zn Ji-Cheng, Jing-Ming L, Si-Bao W, Qing-Hua Z and Wei-Dong H. *Molecules* 2009; 14: 827-838.
- [4] Partha pratim S, Pradip K and Adhikar B. *Bull Mater Sci* 2011; 34: 261-270.
- [5] Thakur S D, Munot K P, Mahajan D T, Deshmukh R D and Tihile M S. *J Chem Phar Res* 2012; 14: 450-455.
- [6] Sengwa R J, Vinita K and Sonu S. *Indian J Chem* 2009; 48: 512.
- [7] Gran G. *Anal Chim Acta* 1988; 206: 111-123.
- [8] Gran G. *The Analyst* 1952; 77: 661-671.
- [9] Rao R S and Rao G N, "Computer Applications in Chemistry", (Himalaya Publishing House, Mumbai) 2005, pp. 277.
- [10] Ramanaiah M, Goutham Sri S and Sailaja B B V. *Bull Chem Soc* 2014; 28: 383-391.
- [11] Ramanaiah M, Seetharam P and Sailaja B B V. *J Indian Chem Soc* 2014; 91: 1011-1020.
- [12] Ramanaiah M and Sailaja B B V. *Chem Speciat Bioavail* 2014; 26: 119-125.
- [13] Rao R S and Rao G N, "Computer Applications in Chemistry", (Himalaya Publishing House, Mumbai) 2005, pp. 277.
- [14] Ramanaiah M, Goutham Sri S and Sailaja B B V. *Chem Speciat Bioavail* 2013; 25: 285-290.
- [15] Ramanaiah M, Goutham Sri S and Sailaja B B V. *J Indian Chem Soc* 2014; 91: 351-357.
- [16] Ramanaiah M and Sailaja B B V. *J Indian Chem Soc* 2014; 91: 639-645.
- [17] Gans P and Sabatini A, Vacca A. *Inorg Chim Acta* 1976; 18: 237-239.
- [18] Ramanaiah M, Goutham Sri S and Sailaja B B V. *Chem Speciat Bioavail* 2014; 26: 231-239.
- [19] Nageswara Rao CH, Ramanaiah M and Sailaja B B V. *Chem Speciat Bioavail* 2014; 26: 266-272.
- [20] Rao R S and Rao G N, "Computer Applications in Chemistry", (Himalaya Publishing House, Mumbai) 2005, p. 277.
- [21] Hamilton W C. *Acta Crystallog* 1965; 18: 502-510.
- [22] Zekarias M T, Hirpaye B Y and Rao G N. *Der Pharm Chem* 2011; 3(4): 69-77.
- [23] Born M. *Z Phys* 1920; 1: 45-48.
- [24] Ramanaiah M, Nageswara Rao CH and Sailaja B B V. *Proc National Acad Sci* 2014; 84: 485-494.
- [25] Ramanaiah M and Sailaja B B V. *J Indian Chem Soc* 2014; 91: 1649-1660.
- [26] Rama Raju B, Santhee Devi K V and Rao G N. *Chinese J Inorg Chem* 2012; 28: 785-794.
- [27] Rama Raju B, Santhee Devi K V, Padmaja N and Rao G N. *Chem Speciat Bioavail* 2012; 24: 89-96.