Asian Journal of Chemistry; Vol. 33, No. 10 (2021), 2301-2305

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2021.23318

Determination of Protonation Constants of Viral Inhibitor, Aurintricarboxylic Acid in SDS and CTAB Micellar Media: A Potentiometric Study

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Received: 18 May 2021; *Accepted*: 6 July 2021; *Published online*: 20 September 2021; AJC-20498

Protonation constants of a viral inhibitor, aurintricarboxylic acid were determined using potentiometric method of data acquisition followed by chemometric modelling methods of analysis in the presence of two different kinds of micellar media, CTAB, a cationic micelle and SDS, an anionic micelle. MINIQUAD75 program was used for the determination of the plausible species and their corresponding formation constants at 303 ± 0.1 K and 0.01 M ionic strength. Five formation constants were identified corresponding to five ionizable hydrogens. Species concentration distribution diagrams were generated using Origin software. Best-fit chemical models were selected on the basis of statistical parameters like standard deviation (SD), U (sum of the squares of the residuals in mass balance equations) and chi-square test. It was found that the formation constants are lower in CTAB micellar medium while there is no significant change in the presence of SDS compared to aqueous medium.

Keywords: Aurintricarboxylic Acid, Sodium dodecyl sulfate, Cetyltrimethylammonium bromide, Protonation constant.

INTRODUCTION

Surfactants are known to have a large influence on the properties of physiological systems [1]. They can solubilize, concentrate and categorize many ions and molecules, modify various equilibria (complex and acid-base), redox properties and reaction rates [2]. To understand the influence of micelles on acid-base equilibria, aurintricarboxylic acid was chosen as a probe and a pH metric study of the determination of protonation constants was carried out in the presence of different surfactants, cetyltrimethylammonium bromide (CTAB) a cationic and sodium dodecyl sulfate (SDS) an anionic surfactant. The effect of micelles on the protonation equilibria has been well established [3]. The values of protonation constants in the presence of micellar media change due to two factors; one is the lower dielectric constant of the micellar medium which has an effect on protonation-deprotonation equilibria and second is the difference in the concentration of protons at the micellar phase and bulk solutions *i.e.* microenvironment factor. For example, in case of anionic micelles, alkyl amines and carboxylic acids in their deprotonated state (RNH_2 and $RCOO^-$) stay in the bulk of the solution while the protonated amine (RNH_3^+) will be located both at the interface and bulk solution of anionic micelles [4].

Aurintricarboxylic acid (ATA) is an inhibitor of protein-nucleic acid interactions and this dye was first used for the quantitative determination of aluminium [5]. Molecular biologists use it for the inhibition of important cellular processes, which involve the formation of a protein-nucleic acid complex. Studies employing both prokaryotic [6,7] and eukaryotic systems [8-11] have shown that the initiation phase of protein synthesis is inhibited by ATA, which has been used as a marker stain for protein in gel electrophoresis experiments because it interacts strongly with proteins [12]. The biological [13-15] and anti-HIV [16,17] activities of ATA have also been well studied. Aurintricarboxylic acid (ATA) has been shown to inhibit the replication of viruses including human immunodeficiency virus, vesicular stomatitis virus and the corona virus causing severe respiratory problems [18]. Aurintricarboxylic acid also has antiviral features against SARS-CoV virus [19] and other pathogenic RNA viruses [19,20], influenza viruses [21] and Enterovirus 71 (EV71) [22].

Three aromatic moieties present in ATA contain two hydroxyl groups and three carboxyl groups. Depending on the pH of the solution ATA exists in five protonated forms, *viz.* LH, LH_2, LH_3, LH_4 and LH_5 (Fig. 1). Since ATA forms a number of complexes in biological systems, determination of protonation constants is important for studies on ATA. Micellar media as solvents are closer in properties to biological fluids and therefore, we have taken up the study of acid-base equilibria of ATA in cationic and anionic micellar media.

EXPERIMENTAL

Analytical grade reagents were used throughout the work. Aurintricarboxylic acid (ATA) was obtained from the TCI, India. Aqueous ATA solution (0.005 mol L^{-1}) was prepared by maintaining 0.05 mol L^{-1} HCl which is required to increase the solubility. Sodium dodecyl sulphate (SDS) and cetyltrimethylammonium bromide (CTAB) were purchased from Merck, India. Hydrochloric acid (0.2 mol L^{-1}, Merck, India) and sodium hydroxide (0.4 mol L^{-1}, Merck, India) solution were prepared and standardized. The strengths of alkali and mineral acid were determined using the Gran plot method [23,24].

Analysis of data: Calvin-Wilson titrations were carried out at different concentrations (0.0-2.5 % w/v) of SDS and CTAB. An ionic strength of 0.16 mol L^{-1} was fixed with sodium chloride at 303 ± 0.05 K. A Metrohm (Titrino plus 877) auto titrator, connected with pH sensor was used. The amount of ATA in the titrations was changed from 0.25 to 0.50 mmol in different experiments. The glass electrode was equilibrated in SDS-water and CTAB-water mixtures for several days. The data from titrations is given as input to the SCPHD software to get the approximate protonation constants of ATA. The data obtained from titrations was subjected to MINIQUAD75 (a non-linear least-squares computer program developed in this laboratory), which makes use of the advantage of constrained least-squares method in the initial refinement and reliable convergence of Marquardt algorithm [25]. MINIQUAD75 gives best fit models, which are chosen based on some important statistical parameters (Table-1). The best-fit models were selected on the basis of U/NP (U = sum of the squares of residuals

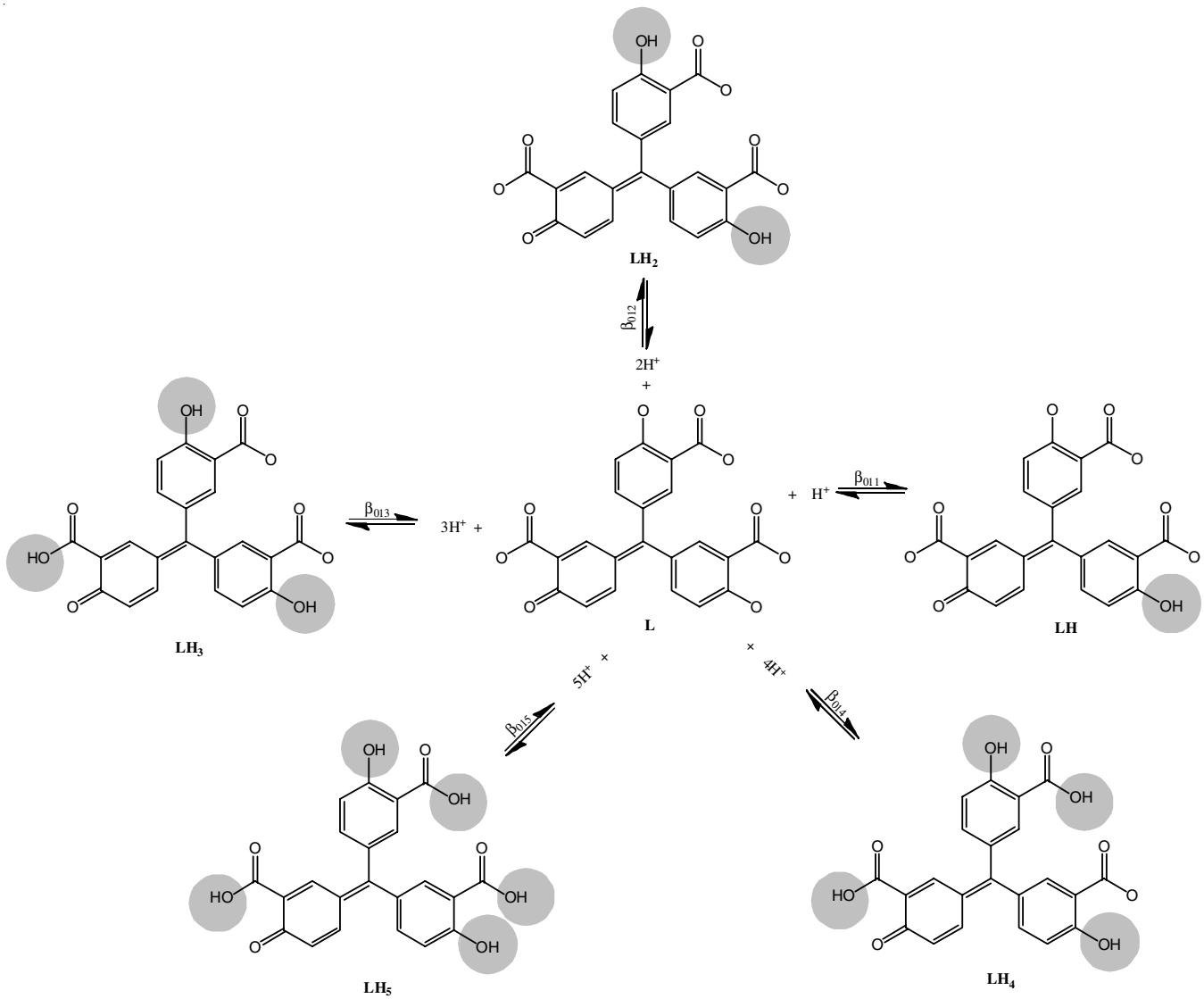

Fig. 1. Protonation and deprotonation equilibria of aurintricarboxylic acid

TABLE-1
BEST FIT MODELS FOR ACID-BASIC EQUILIBRIA OF AURINTRICARBOXYLIC ACID IN MICELLAR MEDIUM
[Temp. = 25 ± 0.1 °C, Ionic strength = 0.16 mol dm^{-3}]

% v/v	log β_{MLXH}(SD)					NP	$U_{Corr} \times 10^8$	Skewness	Kurtosis	χ^2	R-Factor
	LH_5	LH_4	LH_3	LH_2	LH						
CTAB pH Range: 1.5-10.50											
0.0	31.82	28.80	24.83	20.51	10.95	162	2.02	0.95	5.25	38.78	0.01
0.5	31.25	28.30	24.46	20.28	10.86	150	3.06	-0.03	3.25	10.93	0.017
1.0	29.96	27.10	23.42	19.40	10.20	191	6.10	-1.88	18.51	161	0.038
1.5	29.53	26.81	23.19	19.28	10.18	159	2.90	1.87	6.40	56	0.015
2.0	28.65	26.00	22.56	18.74	9.92	159	2.37	1.69	6.53	58	0.012
2.5	28.01	25.40	22.05	18.34	9.62	136	2.50	-1.94	21.33	66.71	0.016
SDS pH Range: 1.5-10.80											
0.0	29.99	26.97	23.27	19.95	10.95	162	2.02	0.95	5.25	38.78	0.01
0.5	27.44	24.40	22.18	19.96	10.95	150	3.06	-0.03	3.25	10.93	0.017
1.0	29.57	25.57	23.31	19.96	10.95	310	2.31	1.30	10.23	106	0.012
1.5	29.56	25.56	23.30	19.95	10.95	248	1.18	1.15	10.29	93	0.006
2.0	29.54	25.55	23.30	19.95	10.95	316	1.92	1.11	6.66	65.92	0.01
2.5	29.59	25.53	23.30	19.95	10.95	284	2.44	-1.18	11.52	64	0.01

in mass balance equations), standard deviations and other statistics like χ^2 test. Mean, standard deviation and mean deviation for the systems are found to be very low. The values of kurtosis (Table-1) were between 21.33 and 3.25. The values of skewness are between -0.03 and 1.87. Low crystallographic R-values also indicate that the model is good. These statistical parameters thus show that the best fit models portray the acid-base equilibria of ATA in SDS-water and CTAB-water mixtures.

RESULTS AND DISCUSSION

Acid-base equilibria of aurintricarboxylic acid (ATA): The protonation and deprotonation equilibria of ATA is shown in Fig. 2. The best fit model obtained contains five formation constants β_{011}, β_{012}, β_{013}, β_{014} and β_{015} corresponding to the formation of LH, LH_2, LH_3, LH_4 and LH_5 species, respectively (Table-1). The stepwise protonation constants of ATA determined in various SDS-water and CTAB-water mixtures are listed in Table-2. Calvin-Wilson titration technique involves the titration of fully protonated form of the ligand LH_5 to most anionic form of the ligand L. The successive deprotonation takes place from LH_5 to LH_4, LH_4 to LH_3, LH_3 to LH_2, LH_2 to LH and LH to L, when ligand solution in CTAB-water mixture and SDS-water mixture is titrated with an alkali. The first three sequential deprotonations are observed due to the releasing of H^+ ions from the three carboxylic acid groups present in ATA. These occurs in the pH region of 2.61 ± 0.5 for K_5, 3.35 ± 0.6 for K_4 and 3.71 ± 0.6 for K_3 in CTAB-water mixture and 3.02 ± 1.0 for K_5, 2.22 ± 1.0 for K_4 and 3.32 ± 0.1 for K_3 in SDS-water mixture, respectively. Carboxylic acid groups are more prone to release of H^+ ions compared to phenol groups within the acidic pH region because the carboxylate ion is more stable by delocalizing the negative charge between the two oxygen ions along with carbon, whereas phenol groups have delocalization of negative charge only on one oxygen atom with carbon. Therefore, last two deprotonation steps observed in higher pH region corresponding to the releasing of two phenolic H^+ ions. These are observed in the pH region of 8.72 ± 0.8 for K_2 and

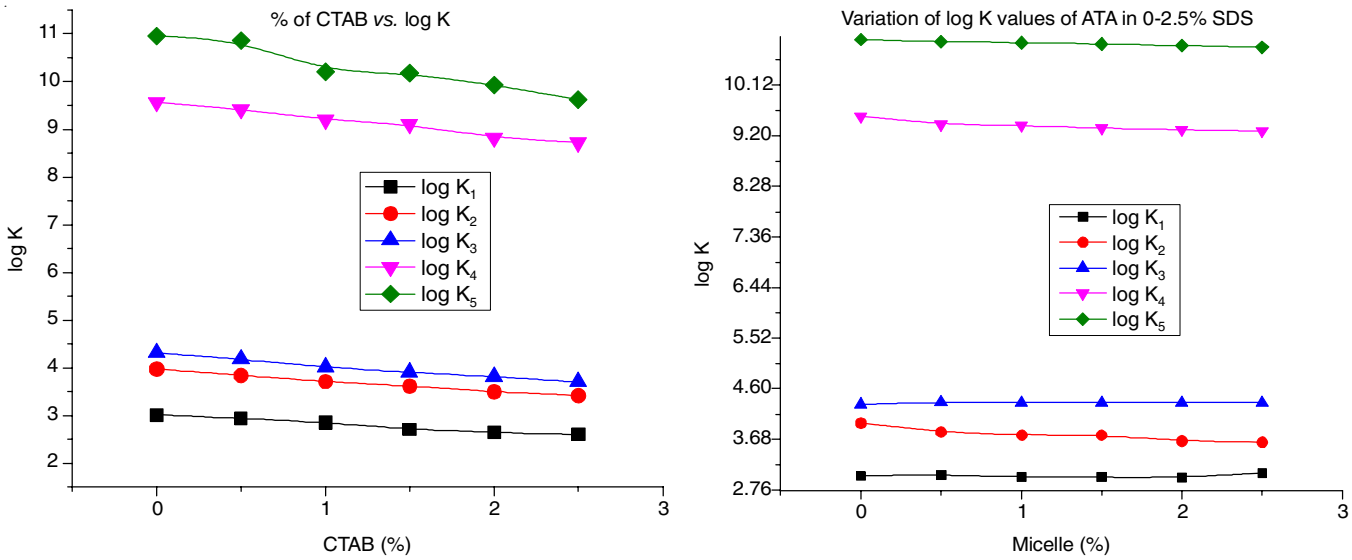

Fig. 2. Variation of log K values of aurintricarboxylic acid (ATA) with % concentration of surfactant

TABLE-2
STEP-WISE PROTONATION CONSTANTS OF AURINTRICARBOXYLIC ACID IN MICELLAR MEDIA

% v/v	CTAB					SDS				
	log K_1	log K_2	log K_3	log K_4	log K_5	log K_1	log K_2	log K_3	log K_4	log K_5
0	3.02	3.98	4.32	9.56	10.95	3.020	3.610	3.320	9.00	10.95
0.5	2.95	3.85	4.18	9.42	10.86	3.040	3.220	3.352	9.01	10.91
1	2.86	3.72	4.02	9.20	10.20	3.000	3.260	3.350	9.01	10.89
1.5	2.72	3.62	3.91	9.10	10.18	3.000	3.260	3.350	9.00	10.87
2	2.65	3.50	3.82	8.82	9.92	2.990	3.253	3.350	9.00	10.84
2.5	2.61	3.42	3.71	8.72	9.62	3.068	3.230	3.350	9.00	10.81

9.62 ± 1.0 for K_1 in CTAB-water mixture and 8.95 ± 0.06 for K_2 and 10.95 for K_1 in SDS-water mixture, respectively.

Distribution diagrams: Typical distribution plots were drawn using Origin 8.5 software with protonation constants as inputs from the best fit models. These diagrams show the existence of LH_4^-, LH_3^{2-}, LH_2^{3-}, LH^{4-} and L^{5-} as a function of pH (Figs. 3 and 4, one of the plots is shown for each system at a particular ATA concentration in SDS and CTAB media, respectively).

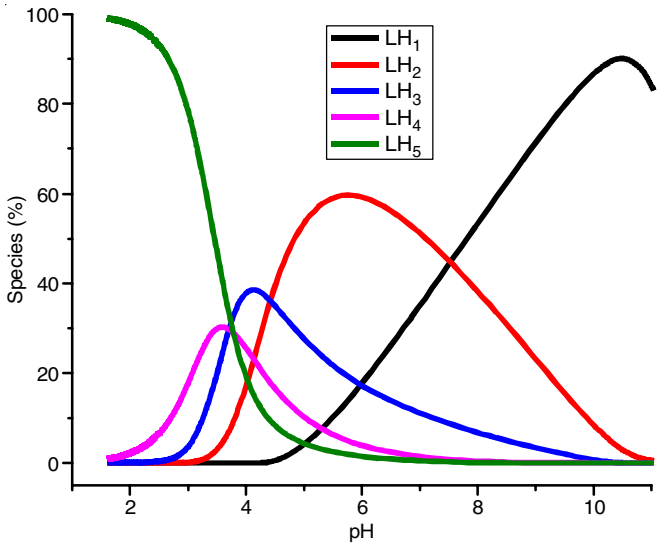

Fig. 3. Species distribution diagram of aurintricarboxylic acid in 2% CTAB

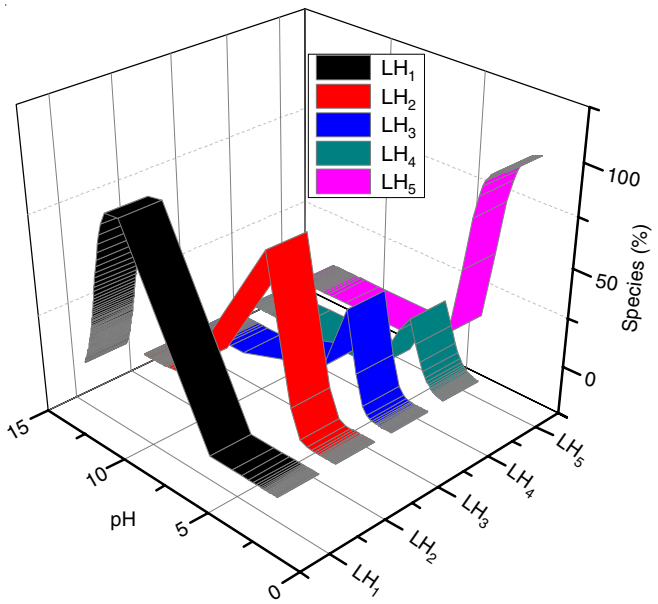

Fig. 4. Species distribution diagram of aurintricarboxylic acid in 2% SDS

Aurintricarboxylic acid (ATA) in CTAB medium: LH_5 of ATA in presence of CTAB exists in the pH range of 2-11 and has an extent of formation up to 98%. With gradual rise in pH upon titrating with an alkali, various species such as LH_4, LH_3, LH_2 and LH were formed due to the deprotonation of LH_5 in the solution. LH_4 has the extent of formation up to 32% within the pH region of 2-7, LH_3 is extended to 40% in the pH region of 3-10, LH_2 is distributed with a wide range of pH from 2.0-11.0 with maximum 60% of formation and LH species is observed to be with a maximum of 92% and is distributed in the pH range of 4.5-11.0.

Aurintricarboxylic acid in SDS medium: The completely protonated state LH_5 in SDS medium has more percentage of formation in the acidic pH region from 0-4 range. LH_4 is distributed from 0-8 pH with a maximum formation of 38%, LH_3 has 30% of maximum formation within the region of pH from 2-9, a wide range of distribution of LH_2 species from 2.5-11 is observed with an extent of maximum formation 58% and LH is observed to be with 60% of maximum in the pH range of 3.5-11.

Effect of surfactants: The values of protonation constants are much less in the presence of CTAB micellar media compared to aqueous medium while there is no significant change in the presence of SDS micellar media (Table-2). The interpretation of the results in the presence of CTAB is because the cation micelle stabilizes the negatively charged species more than the neutral species or positively charged species. In case of the first four protonation constants both the left-hand side and right-hand side are negatively charged species and the electrostatic effect is less (Fig. 5). But in the case of 5th equilibrium, the left-hand side is negatively charged and more stabilized by the cationic micelle than the right-hand side which is a neutral species. The electrostatic effect is also reflected in the large difference in log K values with percentage of surfactant concentration for the fifth equilibrium compared to first four equilibria. With increase in percentage of CTAB, there is a lowering of protonation constant values for all the five formation constants. In the presence of negatively charged surfactant (SDS), no significant change compared to aqueous medium is observed, since both the pronated and deprotonated species are negatively charged and SDS does not influence the equilibrium due to electrostatic effect. Similar explanation was given by Hartley [26]. There is no change in K_1, K_2, K_3, K_4 and K_5 in case of SDS with increase in surfactant concentration.

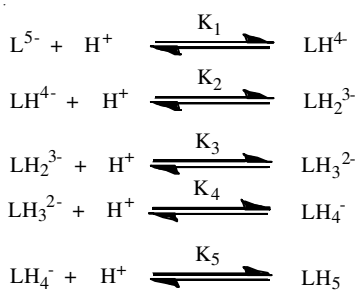

Fig. 5. Protonation and deprotonation equilibria

Conclusion

In present study, protonation constants of a viral inhibitor, aurintricarboxylic acid were determined using potentiometric method. Aurintricarboxylic acid (LH_5) has five protonation constants, three of which due to the protons of the carboxyl acid groups (K_1, K_2 and K_3) and two are due to phenolic groups (K_4, K_5). There is a decrease in the value of protonation constants in the presence of CTAB micellar media while there is no significant change in the presence of SDS micellar media. The values of protonation constants of ATA decrease with increase in concentration of CTAB in CTAB-water mixtures and this effect is absent in SDS micellar media indicating the presence of electrostatic interactions in CTAB micellar media.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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