



Chenodeoxycholic acid: A Physicochemical study

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ABSTRACT

Some physicochemical properties such as dissociation constant, acoustical parameters and thermal studies of acid are studied in methanol and 1,4-dioxane at 298.15 K. The dissociation constant of Chenodeoxycholic acid is evaluated in binary mixtures (of methanol / 1,4-dioxane + water) by two methods; half-integral and average methods. The acoustical properties have been evaluated from experimental data of density, viscosity and ultrasonic velocity. It is observed that in both the solvents, strong solute-solvent interactions exist. Thermal degradation of Chenodeoxycholic acid is found to be a single step process.

Keywords: Dissociation constant; acoustical parameters; thermal analysis

1. INTRODUCTION

Chenodeoxycholic acid is a bitter-tasting white powder consisting of crystalline and amorphous particles. It is freely soluble in methanol, acetone and acetic acid and practically insoluble in water^[1,2]. It is also known as chenochoic acid, Chenodiol or Chenic Acid. Its IUPAC name is 3- α ,7- α -dihydroxy-5- β -cholanic acid; 5- β -Cholanic acid-3- α , 7- α -diol. It is one of the Bile acid, which facilitates excretion, absorption, and transport of fats and sterols in the intestine and liver^[3,4]. Bile acids have potent toxic properties (e.g., membrane disruption) and there are a plethora of mechanisms to limit their accumulation in blood and tissues^[5].

In the present work, the some physicochemical properties of acid, such as kinetics of decomposition, dissociation constants and acoustical properties in some solvents are studied.

2. EXPERIMENTAL

Chenodeoxycholic acid (CDCA) was purchased from Sigma Aldrich (CAS Number 91-56-5) and was recrystallized from methanol.

The solvents; methanol and 1,4-dioxane used in the present study was of B.D.H. Analar grade and were purified by standard procedure^[6]. Milli-Q water (Millipore Pvt. Lt. Bangalore-India) was used for dissociation studies. An electrical balance (Mettler Toledo AB204-S) with an accuracy of ± 0.1 mg was used for solution preparation.

Dissociation:

In the present work, the dissociation constant of Chenodeoxycholic acid (CDCA) drug are studied in methanol + water and 1,4-dioxane + water mixtures at 298.15 K by Calvin Bjerrum pH titration technique^[7].

- (I) 2 ml HNO₃ (1.0 M) + 4 ml water + 30 ml methanol/1,4-dioxane + 4.0 ml NaNO₃ (1.0 M).
- (ii) 2 ml HNO₃ (1.0 M) + 4 ml water + 28 ml methanol/1,4-dioxane + 2.0 ml CDCA solution (0.1 M) + 4.0 ml NaNO₃ (1.0 M).

These solutions were titrated against 0.5 M-sodium hydroxide and the corresponding pH was measured using Systronic pH meter (Model No. EQ 664). The glass electrode and a saturated calomel electrode were used as indicator and reference electrodes respectively. Before operation, the glass electrode was immersed in 0.1 M HCl for twenty minutes. Then, it was washed thoroughly with Milli-Q-water. The pH meter was calibrated with buffer solution of known pH.

Acoustical Properties:

The solutions of CDCA were prepared in methanol and 1,4-dioxane over a wide range of concentrations. At 298.15 K, for each solution, density, ultrasonic velocity and viscosity of pure solvents and their solutions were measured by a single capillary pycnometer, single crystal variable path ultrasonic interferometer (operating at 2 MHz) and Ubbelohde viscometer respectively. The accuracy of density, velocity and viscosity are ± 0.0001 g/cm³, $\pm 0.1\%$ cm/sec and 0.05%. All the measurements were carried out at 298.15 K. The uncertainty of temperature is ± 0.1 K and that of concentration is 0.0001 moles /dm³.

Thermal Analysis:

Thermal analysis was by Differential Scanning Calorimetry (DSC) and Thermo gravimetric analysis (TGA) techniques. These measurements were made on the instrument "Pyris-1, Perkin Elmer Thermal Analysis" at the heating rate of 10 °C /min in nitrogen atmosphere.

3. RESULTS AND DISCUSSION

Dissociation:

Figure 1 shows that typical titrations curve in the absence and presence of CDCA at 298.15 K for methanol/1,4 dioxane: water systems.

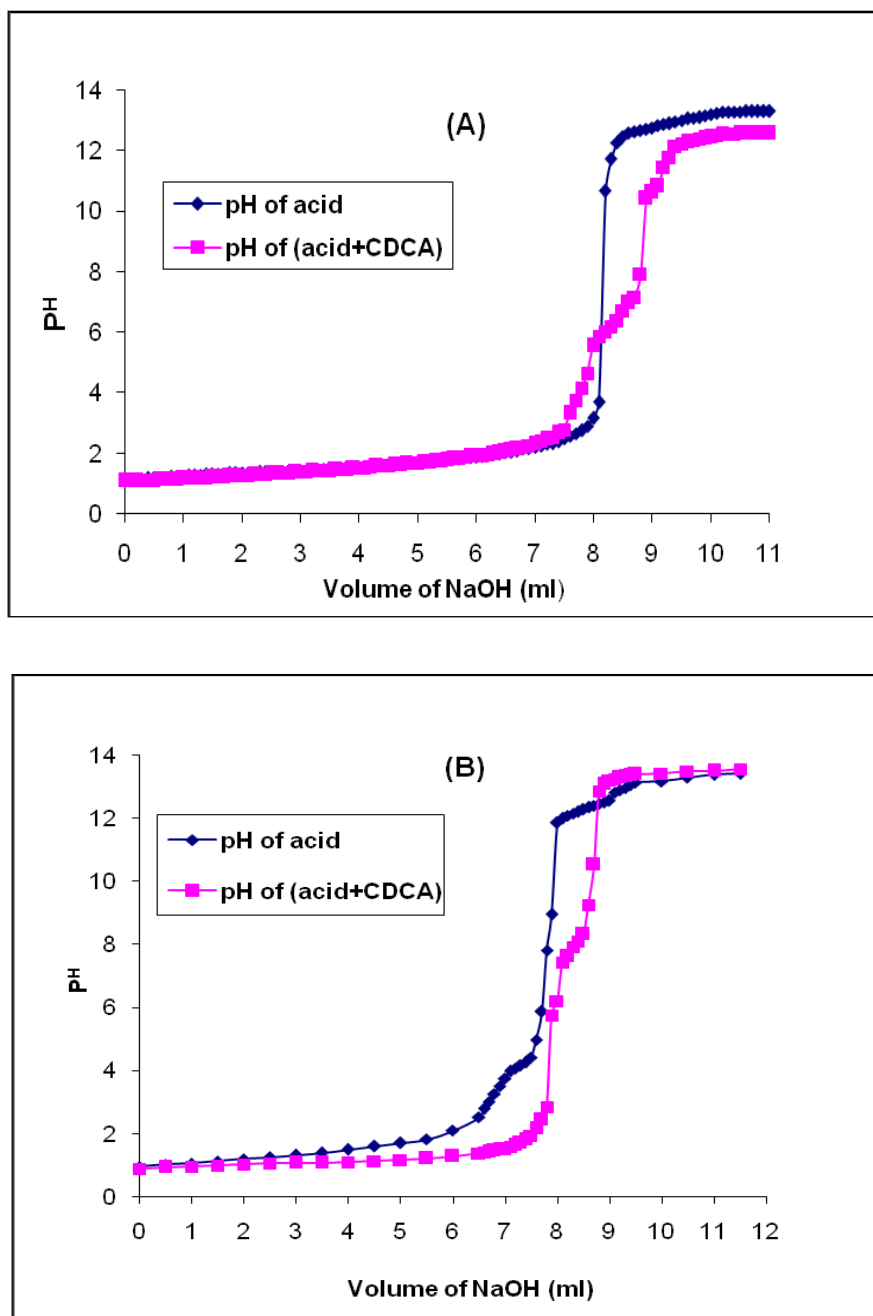


Figure 1. The plot of pH against volume of alkali in [A] methanol + water and [B] 1,4-dioxane+ water at 298.15 K.

From these titration curves, the average number of protons associated with the CDCA (\bar{n}_H) can be calculated by the equation given by Irving and Rossotti [7].

$$\bar{n}_H = Y - \frac{(V'' - V')(N^0 + E^0)}{(V^0 + V')T_L^0}$$

where Y is the number of displaceable protons. V' and V'' are the volume of alkali required at the same pH for both HNO_3 and Chenodeoxy Cholic acid titration curves respectively. V^0 is the initial volume of the test solution. N^0 , E^0 and T_L^0 are the initial concentration of the alkali, nitric acid and CDCA respectively. It is observed that the value of \bar{n}_H are found to be between zero to two suggesting there by that CDCA has two replaceable protons.

The dissociation constants in both the solvent systems are evaluated by half integral and average methods. In half integral method, pK_1^H and pK_2^H value was evaluated at $\bar{n}_H = 0.5$ and $\bar{n}_H = 1.5$. Whereas in average method, for all the points below $\bar{n}_H = 1$, the following equation was used to determined pK_1^H

$$\log pK_1^H = pH + \log \bar{n}_H / (\bar{n}_H - 1)$$

From these evaluated various values of pK_1^H , average value of pK_1^H was calculated.

The dissociation constants of CDCA by both average and half integral methods are given in Table 1 in methanol + water and 1,4-dioxane + water systems. It is observed that pK_1^H and pK_2^H values maximum in methanol and minimum in 1,4-dioxane. The higher pK_1^H and pK_2^H values suggest that dissociation decreases in methanol. Further, comparison of pK_1^H and pK_2^H values in the two solvent systems suggests that CDCA is more acidic in 1,4-dioxane + water system.

Table 1. The pK^H values for Chenodeoxy Cholic acid evaluated by average and half-integral methods in methanol and 1,4-dioxane.

Solvent	Half-integral method		Average method	
	pK_1^H	pK_2^H	pK_1^H	pK_2^H
Methanol	6.38	9.20	6.98	8.72
1,4-dioxane	6.24	8.69	6.78	8.13

Acoustical Properties:

The experimental data of density (ρ), viscosity (η), and sound velocity (U) for pure solvents and solutions of Chenodeoxy Cholic acid are reported. In Table 2 in methanol and

1,4-dioxane. It is observed that all these experimental values increase with concentration in both the solvents.

Table 2. Experimental values of density (ρ), viscosity (η), ultrasonic velocity (U) of Chenodeoxy Cholic acid in methanol and 1,4-dioxane at 298.15 K.

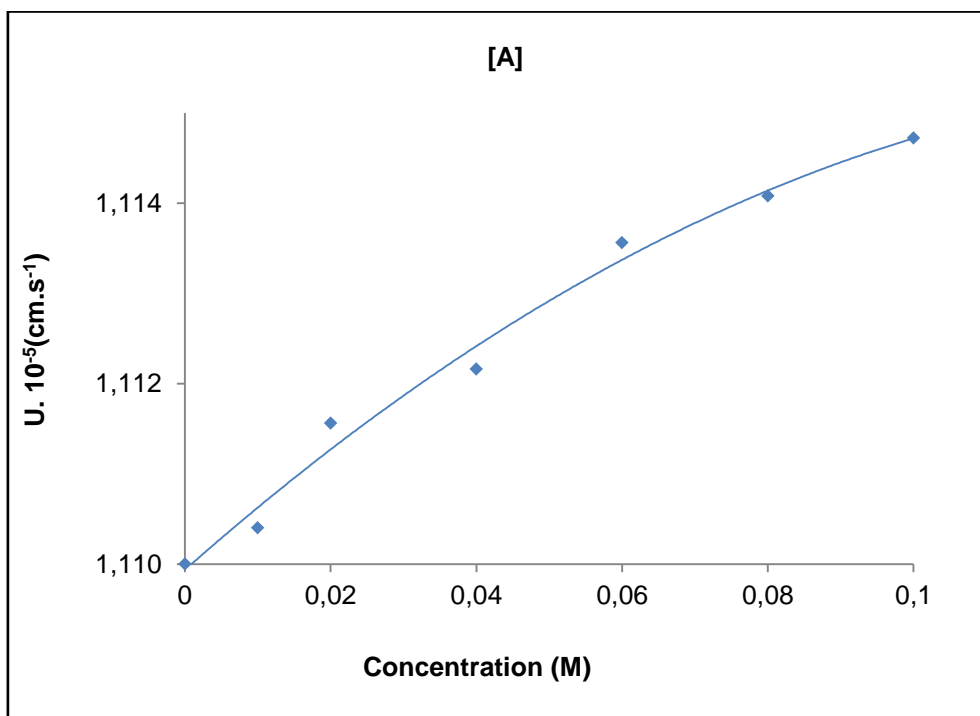
Conc (mol/l)	Methanol			1,4-dioxane		
	ρ (g/cm ³)	U (cm/s)	η (poise)	ρ (g/cm ³)	U (cm/s)	η (poise)
0.00	0.786	1.1100	5.5044	1.02409	1.3458	13.3814
0.01	0.7958	1.1104	5.7812	1.02474	1.3469	14.0248
0.02	0.7969	1.1112	5.9006	1.02606	1.3479	14.2861
0.04	0.799	1.1124	6.1154	1.02735	1.3487	14.7303
0.06	0.8009	1.1132	6.3163	1.02937	1.3496	15.0908
0.08	0.8029	1.1144	6.5269	1.03125	1.3509	15.3652
0.10	0.8056	1.1156	6.7426	1.03182	1.3520	15.7452

From these experimental data, various acoustical parameters like specific acoustical impedance (Z), Adiabatic compressibility (κ_s), intermolecular free path length (L_f), Vander waal's constant (b), relaxation strength (r), internal pressure (π), solvation number (S_n), etc. were evaluated using standard equations reported^[8]. Some of these values are given in Table 3.

Table 3. Some evaluated acoustical parameters for solution of CDCA in methanol and 1,4-dioxane at 298.15 K.

Methanol				
Conc. (mol/l)	Z (gm ⁻² ·s ⁻¹)	r	$\pi * 10^{-8}$ Pas	b cm ³ ·mol ⁻¹
0.00	0.8725	0.5187	1078.0665	40.7360
0.01	0.8837	0.5184	1045.7354	42.4693
0.02	0.8855	0.5177	995.8381	44.6364
0.04	0.8888	0.5166	908.8566	48.9415

0.06	0.8916	0.5159	836.3747	53.2176
0.08	0.8948	0.5149	776.3077	57.4433
0.10	0.8987	0.5138	726.2925	61.5539
1,4-dioxane				
0.00	1.3782	0.2925	559.0531	86.0354
0.01	1.3802	0.2914	563.6333	87.1190
0.02	1.3830	0.2903	560.6054	88.1407
0.04	1.3856	0.2895	552.9509	90.2921
0.06	1.3892	0.2885	544.3582	92.3620
0.08	1.3931	0.2871	534.5251	94.4290
0.10	1.3950	0.2860	526.4778	96.6171



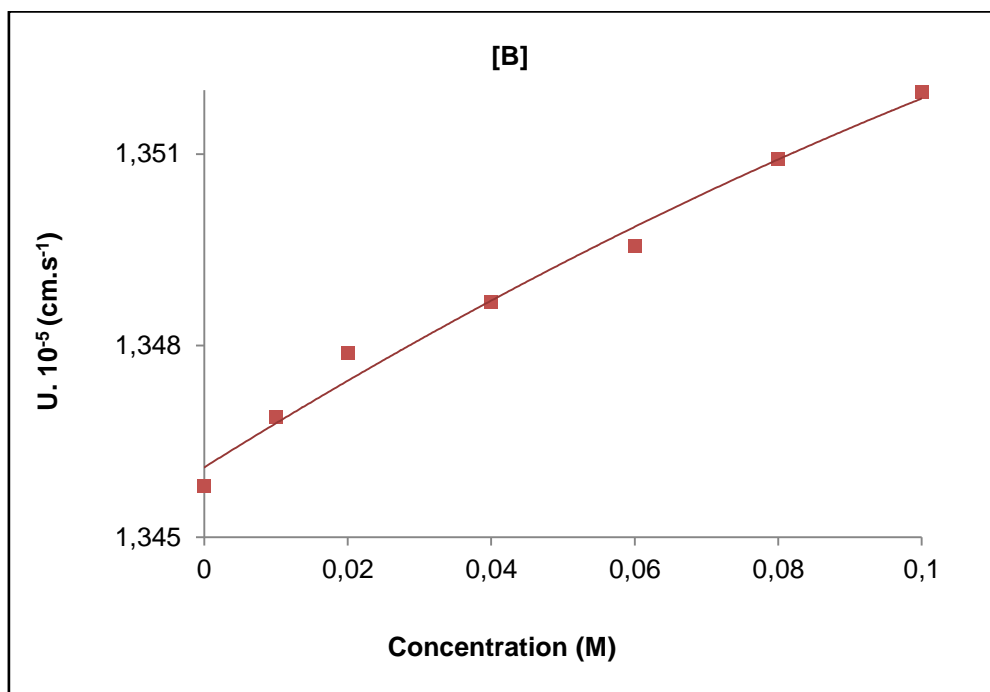


Figure 2. The variation of velocity (U) with concentration for CDCA in [A] methanol and [B] 1,4-dioxane.

It is observed from Figure 2 that ultrasonic velocity increases non-linearly with concentration. The velocity (U) depends on intermolecular free path length (L_f). Larger the intermolecular free path length, smaller will be the velocity and vice versa. It is evident from Figure 3 that intermolecular free path length decreases non-linearly with concentration. The increase of velocity and decrease of intermolecular free path length suggests close association between CDCA and solvent molecules.

Further, Table 3 shows that relaxation strength (r) decreases whereas Z increases with concentration. This again suggests that solute molecules interact strongly with solvent molecules. This is further supported by the decrease of adiabatic compressibility with concentration in both solvents, as shown in Figure 4. The decrease of adiabatic compressibility (κ_s) is due to aggregation of solvent molecules around solute molecules. The internal pressure π is a measure of cohesive forces which is found to increase with concentration (Table 3). This confirms the existence of solute-solvent interactions also in the studied solutions.

The type of interactions in a solution can also be confirmed by the solvation number, which is a measure of structure forming or structure breaking tendency of a solute in a solution. Figure 5 shows that for CDCA, solvation number (S_n) increases with concentration and are positive in both the solvents. The positive S_n values suggest structure forming tendency of CDCA in solutions. This again proves existence of strong solute-solvent interactions in the studied solutions.

Thus, it is concluded that in both the solvents, solute-solvent interactions exist for CDCA.

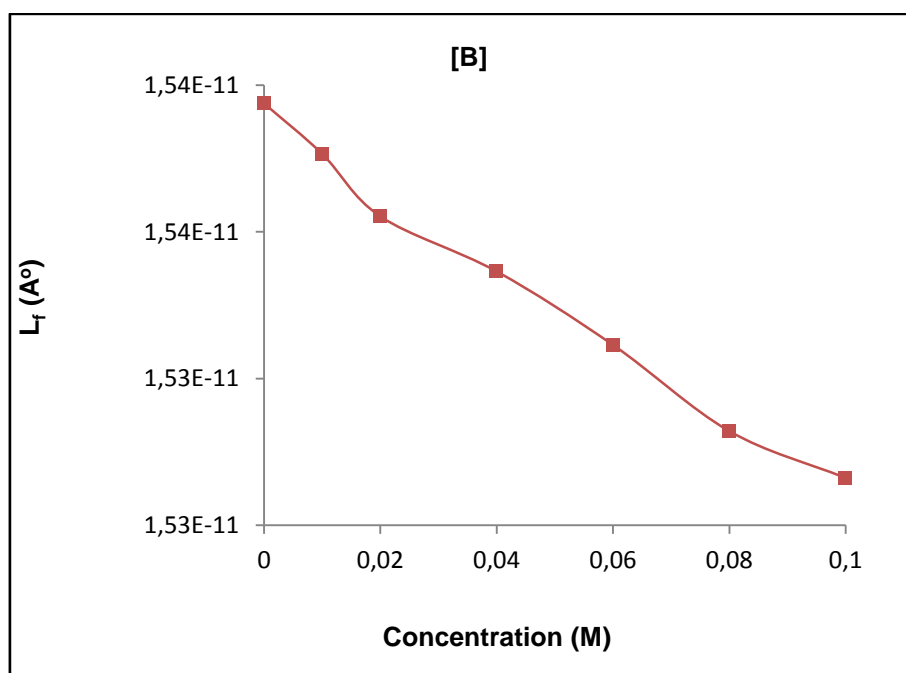
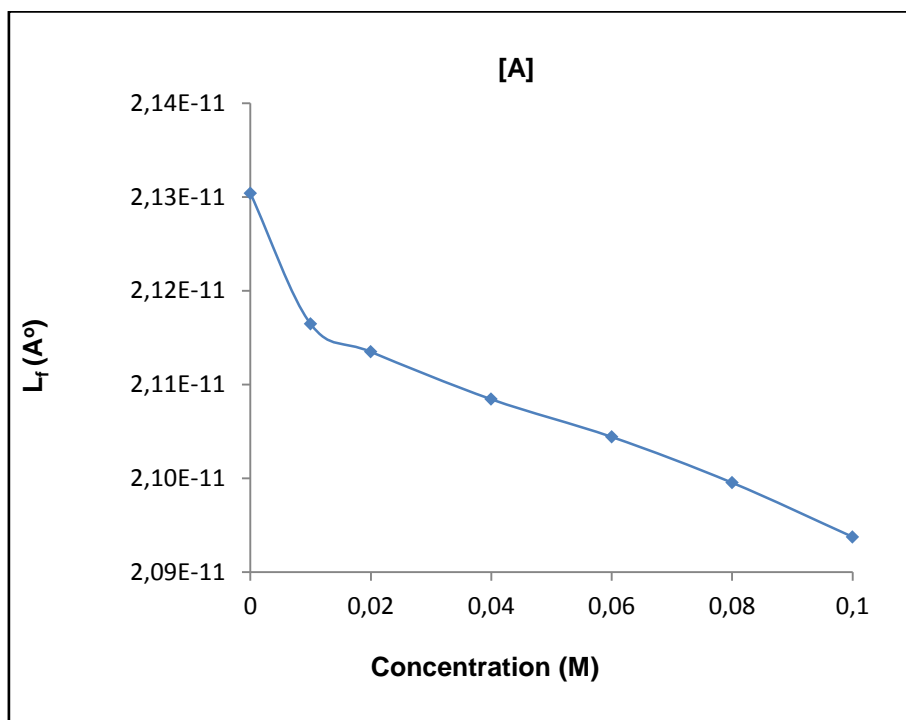


Figure 3. The variation of intermolecular free path length (L_f) against concentration for CDCA in [A] Methanol and [B] 1,4-dioxane.

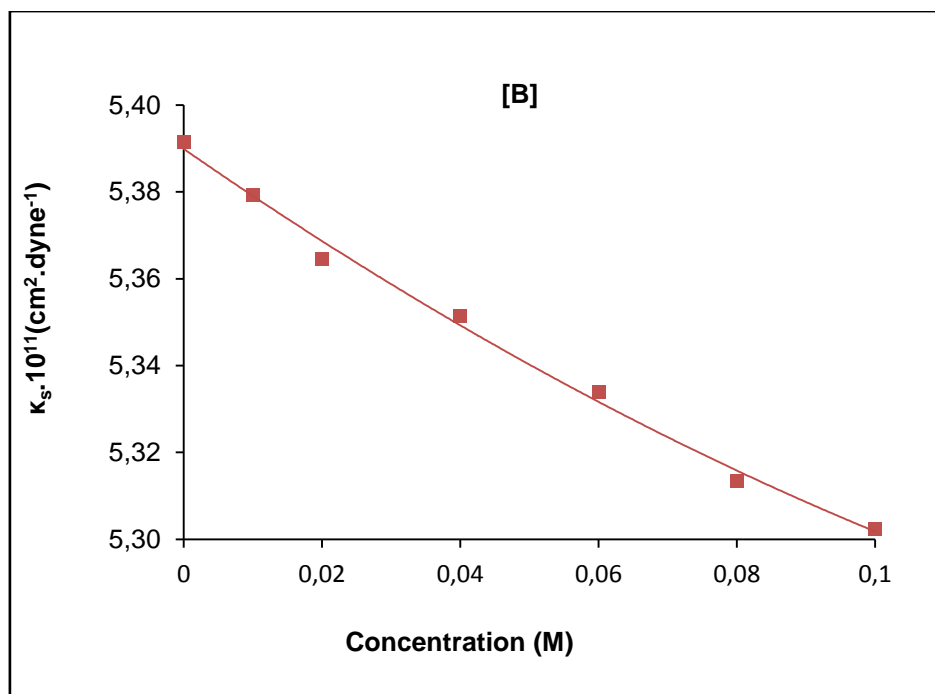
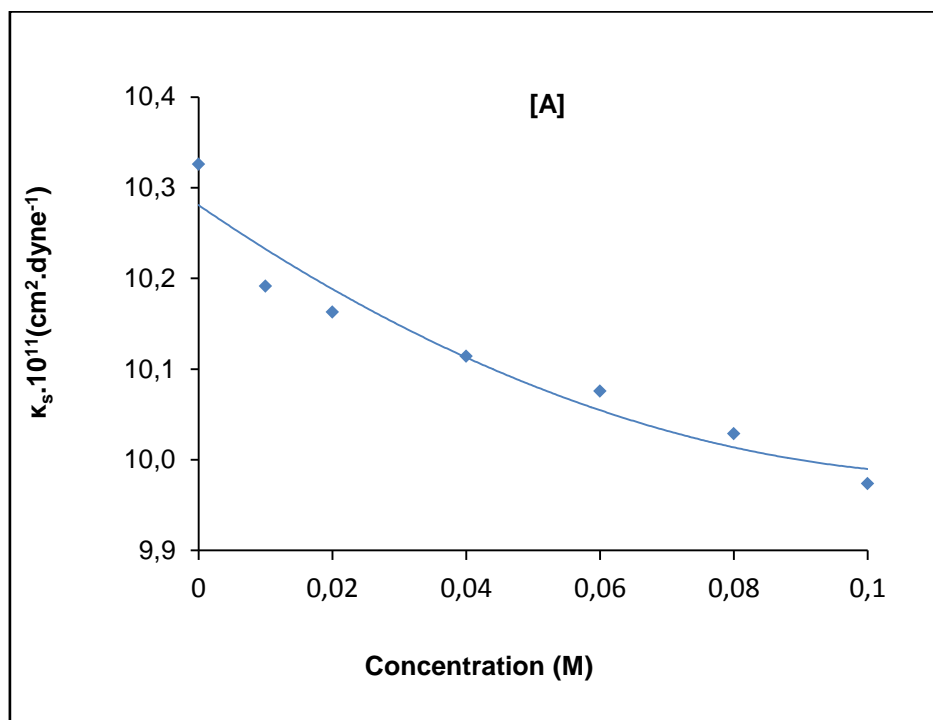


Figure 4. The variation of adiabatic compressibility (κ_s) with concentration in [A] methanol and [B] 1,4-dioxane.

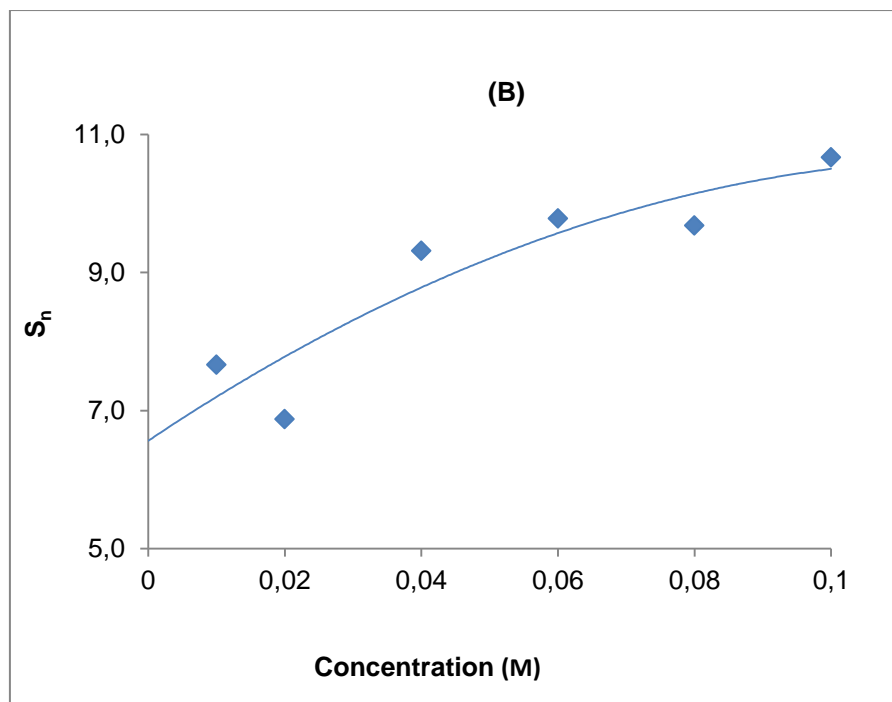
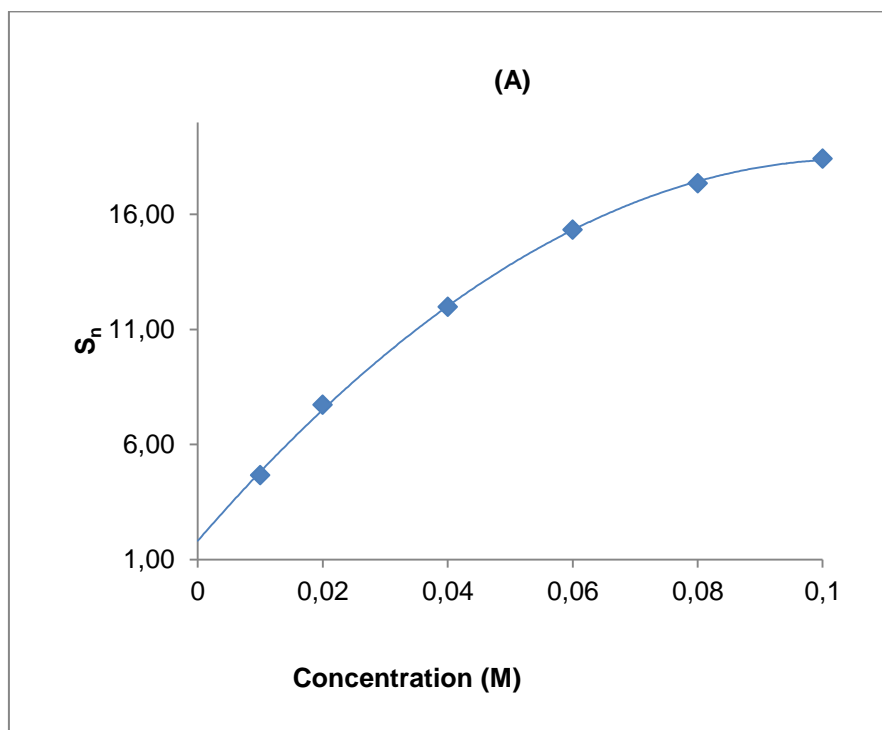


Figure 5. The variation of solvation number (S_n) with concentration in [A] Methanol and [B] 1,4-dioxane.

Thermal studies:

The TGA thermo gram of Chenodeoxycholic acid is given in Figure 6. The degradation is single step process. The decomposition temperature range is found to be approximately from 340-400 °C and the maximum degradation temperature is 378.32 °C.

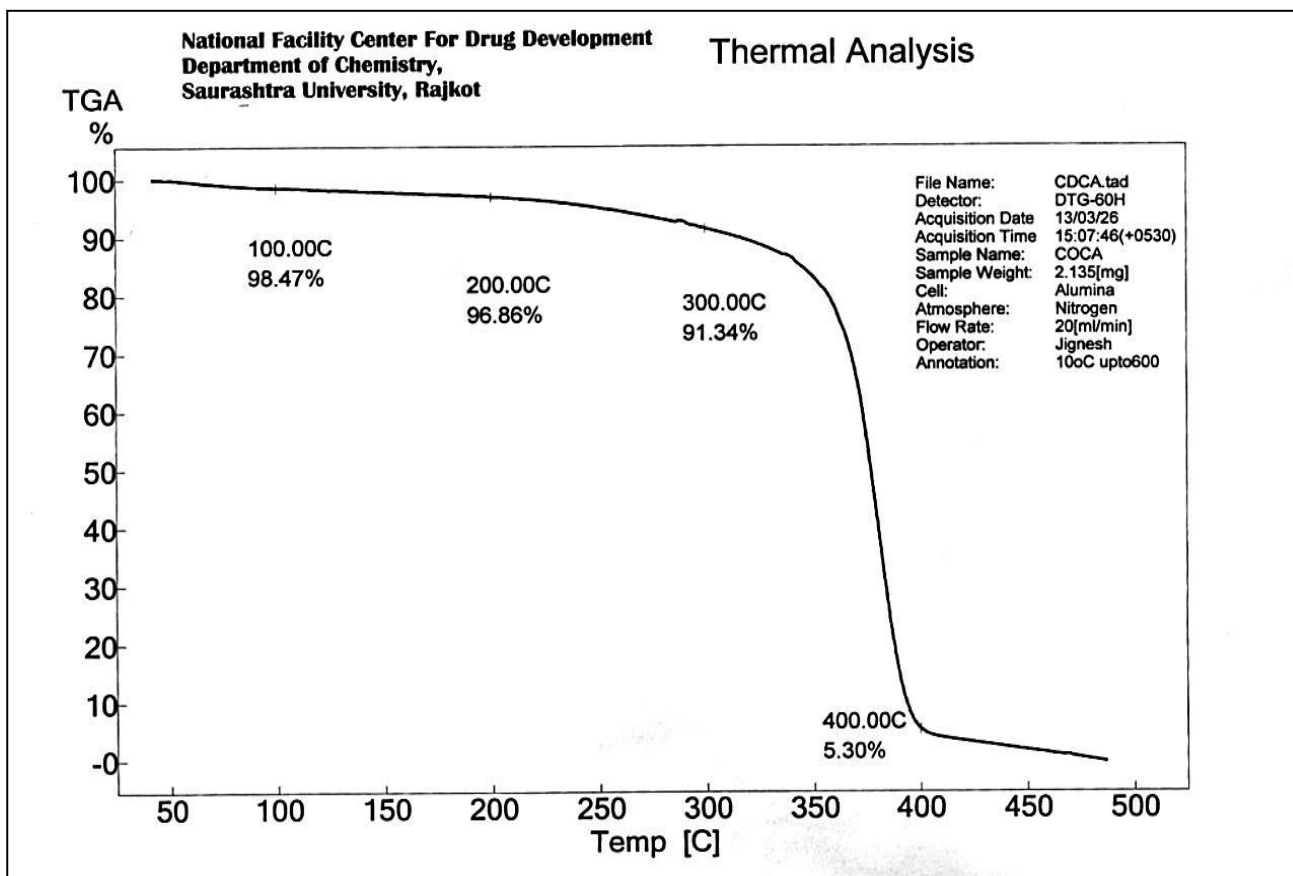


Figure 6. The Thermogram of Chenodeoxy Cholicacid.

Further, various kinetic parameters, such as order of the degradation (n), energy of activation (E), frequency factor (A) and entropy change (ΔS) have also been calculated from the thermogram using the following Freeman-Anderson equation [9]:

$$\Delta \ln dW/dt = n' \Delta \ln W - (E/R) \Delta (1/T)$$

where W is residual mass evaluated from thermogram, n' is order of reaction, E^* is energy of activation, T is temperature and R is gas constant.

From the slope of Freeman-Anderson plot, energy of activation (E) was evaluated whereas intercept gives the order of reaction (n').

The frequency factor A' is calculated by the following equation:

$$A' = (E\beta/RT^2) e^{E/RT}$$

where β is heating rate. The entropy change (ΔS^*) is also evaluated using equation:

$$\Delta S^* = R \ln (A/h/kT)$$

where h is Planck's constant and k is Boltzmann constant.

These evaluated parameters are listed in Table 4. The order of single step decomposition kinetics is less than one and entropy is positive. The positive change in entropy (ΔS) indicates that the transition state is less ordered than the original compound [10].

Table 4. The kinetic parameters of Chenodeoxy Cholic acid.

n	E kJ·mol ⁻¹	A Sec ⁻¹	ΔS° J·mol ⁻¹ ·K ⁻¹
0.588	330.897	2.254x10 ⁴⁴	602.2115

4. CONCLUSION

The dissociation constant of CDCA is determined in methanol + water and 1,4-dioxane + water systems and the observed pK_1^H and pK_2^H values are found to be maximum in methanol and minimum in 1,4-dioxane. Thus, dissociation decreases in methanol and CDCA is more acidic in 1,4-dioxane + water system. The studied acoustical parameters suggest that in both methanol and 1,4-dioxane solutions of CDCA, solute-solvent interactions exist. The degradation of CDCA is single step process. The decomposition temperature range is approximately 340-400 °C and the maximum degradation temperature is 378.32 °C. The order of decomposition reaction is less than one. The positive entropy indicates that the transition state is less ordered than the original compound CDCA.

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