Thermodynamic, spectral and antimicrobial activity of inclusion complexes of acridone and its oxime with β-cyclodextrin

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Abstract

One of the methods to enhance bio-accessibility of drugs like Acridone and its oxime is to form inclusion complexes with β-cyclodextrin. The formation of such complexes has been confirmed by changes in spectral characteristics and melting point data. The aqueous phase solubility studies reveal 1:1 stoichiometry between the compound and, β-cyclodextrin. The study of thermodynamic parameters like ΔG, ΔH, ΔS indicates the inclusion complex formation to be exothermic and spontaneous. The study of antimicrobial activity of these compounds indicates that the microbes like E. coli and P. aeruginosa are susceptible and the susceptibility increases significantly after formation of inclusion complex.

Keywords: Acridone; Inclusion complex; β-cyclodextrin; Thermodynamic stability; Antimicrobial study.

1. Introduction

Acridone and its derivatives are important anthracene analogue heterocyclic compounds in which a carbon in the middle ring is replaced by 'N' atom. Their DNA affinity and intercalative properties make it an important pharmacophore for the designing of several chemotherapeutic agents (anti cancer, anti bacterial, anti protozoal) [1-3]. These are pharmacologically acceptable, efficacious in preventing and treating diseases such as asthma, allergic rhinitis, atopic dermatitis, uticaria, gastrointestinal allergies etc.

Since bio-accessibility of a drug depends upon its solubility, one of the factors limiting the pharmacological activities of acridone and its derivatives is their insoluble nature in aqueous solutions [4]. The solubility of these compounds can be enhanced by forming inclusion (host-guest) complexes with cyclodextrins (CDs) which in turn increases their drug efficiency [5]. Among the natural CDs, only the more available β form has certain prospects in applications because the other forms (α, γ) are expensive. β-CD is capable of forming complexes with other compounds in both solid and liquid state. The β-CD complexes with drugs exhibit a higher stability with respect to heating and oxidation, an increase in the solubility and bio-accessibility leading to therapeutic efficacy [6].

Drug (Guest) + Cyclodextrin (Host) ⇆ Inclusion complex

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$$\text{Inclusion complex association constant} = K_T = \frac{[\text{Inclusion complex}]}{[\text{Cyclodextrin host}][\text{Guest}]}$$

Although a series of 10-N-Susbstituted acridones, bearing alkyl side chains with tertiary amine groups at the terminal position have been reported, there is no report regarding the synthesis of acridone derivatives involving the keto group [7]. In this paper an attempt has been made to synthesize acridone and its oxime derivative in their purest forms. Respective inclusion complexes of these compounds with β-CD have been synthesized and their spectral and thermodynamic properties have been studied. As these compounds contain quinolone group, they are expected to be potential drugs and hence antimicrobial activities of these compounds have been studied against microbes like E. coli and P. aeruginosa etc.

2. Experimental

2.1 Apparatus and materials

All chemicals are procured form the local market and are of suitable Anal R grade. Double distilled water is used as the solvent for dilution. Other solvents employed are redistilled before use. The elemental analysis has been performed in a CHN analyzer. Electronic spectra are recorded on Shimadzu UV-1700 spectrophotometer while IR spectra are recorded in KBr pellets in the 400- 4000 cm^{-1} region in a Shimadzu 8400 S FT- IR spectrophotometer. Melting points are recorded by open capillary method. Antimicrobial screening by Kirby-Bauer method has been done by employing Muller Hinton agar plates in normal saline medium and sterilized cotton swabs.
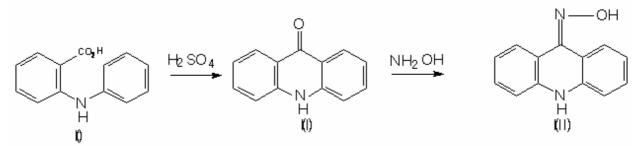
2.2 Phase solubility measurements

The aqueous phase solubility of Acridone and its oxime at various concentrating of β-CD has been studied by Higuchi-Connors method [8]. Accurately weighed sample of these compounds in quantities exceeding their aqueous solubility are shaken in a rotary flash shaker at room temperature with aqueous solution of β-CD in increasing concentration (0-10 mM L^{-1}) in a series of stoppered conical flask for a period of 48 hours till equilibrium is established. The solutions are filtered through Whatman No1 paper and are analyzed in a UV-Vis spectrophotometer at 380-420 nm range. The various values of OD at λ_{max} have been plotted against different concentration of β -CD.

2.3 Syntheses of acridone and its oxime

0.2 mole of N-phenylanthranilic acid (I) in 100 ml of conc. H$_2$SO$_4$ is refluxed in a 500 ml flask on a boiling water bath for four hours and then poured into a 1L flask containing hot water slowly and carefully. The yellow precipitate formed is filtered after boiling for few minutes and then the moist solid is again boiled for five minutes with a solution of 0.28 mole Na$_2$CO$_3$ in 400ml of distilled water. The precipitate is collected with suction and washed well with water. After drying, the crude acridone (II) obtained is then recrystalised form a mixture of aniline and acetic acid.

1 gm of Hydroxylamine hydrochloride and 1.5 gm of crystallized sodium acetate are dissolved in 10 ml water to which 0.5 gm of acridone is added and shaken. Alcohol is added till turbidity disappeared to give a clear solution. Then the solution is refluxed for 2 hours on a water bath with condenser. The resulting solution is poured carefully into ice-cold water where the crystals of acridoxime (III) are obtained. These are recrystallised from alcohol and water mixture and finally dried. The synthesis of acridone & its oxime is shown in scheme 1.

Scheme 1 Synthesis of acridone [II] and acridoxime [III]

2.4. Synthesis of inclusion complexes

The inclusion complexes of acridone and its oxime have been synthesised as per co-precipitation method [10, 11]. The solution of the synthesized compounds is prepared in required concentrations (0.03 mol L^{-1}) and is added drop wise to previously stirred β-CD solution. The mixtures are stirred at room temperature for 48 hours and filtered. Then the content is cooled for another 48 hours in refrigerator. Finally the precipitate obtained is filtered through G-4 crucible, washed with distilled water and dried in air for 24 hours.

2.5. Study of thermodynamic properties

The thermodynamic stability constant (K_T) at room temperature of the complexes are calculated using Benesi-Hilderbrand relation. The stability constant K (during de-encapsulation) of each complex has been calculated with increasing temperature. The slope of the linear plot of ln K vs. I/T gives rise to the calculation of ΔH (change in free enthalpy) and then ΔS (change in entropy) was calculated using the integrated from of the van't Hoff equation.

$$\ln K = \left(\frac{-\Delta H}{RT} \right) + \frac{\Delta S}{R}$$

The value of ΔG was calculated from the value of K_T at 298 $^\circ$K using the equation: $\Delta G = -RT \ln K_T$

2.6. Study of antimicrobial activity

The disk diffusion method for antimicrobial susceptibility test is the Kirby-Bauer method [12, 13]. Muller-Hinton agar plates with normal saline medium have been used for this test. The bacterial inoculums are prepared by making a direct saline suspension of colonies of same morphological type that are selected from an 18-24 hour agar plate. The turbidity with sterile saline is adjusted. Within 15 minutes after adjusting the turbidity, a sterile non-toxic swab is dipped on an applicator into the adjusted suspension. A maximum of 5 disks on a100 mm plate are placed on the surface of the agar plate. The plates are inverted and are placed in an aerobic incubator at 35 °C. After 16-18 hours of incubation, the diameters of zones of complete inhibition are measured. The zone sizes are interpreted by referring to standard antibiotics (manufacturer provided standard table) and the report on the organism to be susceptible (S), intermediate (I) or resistant (R) are made.

3. Results and discussion

3.1. Synthesis of acridone and acridoxime

The synthesis of acridone and acridoxime are confirmed from elemental analysis and IR-data as shown in (Table-1). The elemental composition nearly matches with theoretical data.

Infrared data of C=O$_{str}$ at 1674cm^{-1}, N-H$_{str}$ at 3274 cm^{-1}, C-N$_{str}$ at 1161cm^{-1} etc. suggest formation of acridone. Similarly, C=N $_{str}$ at 1641 cm^{-1}, N-H$_{str}$ at 3321 cm^{-1}, O-H $_{str}$ (oxime) at 3240 cm^{-1} etc suggest the formation of acridoxime. In addition, both acridone and its derivatives differ significantly in their melting points (Table 1).

Table 1
Analytical data of acridone and its oxime with and without inclusion complex.

No	Compound	m.p. °C	Colour	Elemental Analysis Found (calculated) %				λ_{max} nm	IR (KBr)
				C	H	N	O		
1	Acridone	350	Greenish yellow	80 (80.2)	4.8 (4.6)	8.4 (8.2)	7.0 (7.18)	403,385	1674 (C=O) 3274(N-H) 1161 (C-N) 1633 (C=C) 1572 (ring)
2	Acridone/ ß-CD complex	359	Yellow	--	--	--	---	401,383	1662 (C=O) 3270 (N-H) 1155 (C-N)
3	Acridoxime	322	Bright yellow	74.31 (74.29)	4.8 (4.76)	13.3 (13.33)	7.59 (7.62)	406,383	1641 (C=N) 3240 (O-H) 3321 (N-H)
4	Acridoxime / ß-CD complex	334	Yellow	-	-	-	-	403,381	1640 (C=N) 3234 (O-H) 3315 (N-H)

3.2. Synthesis of inclusion complex

The synthesis of inclusion complexes of acridone and acridoxime are confirmed from melting point data, colour and spectral characteristics. (UV-Vis and IR) (Table 1). The melting point of acridone is 350 °C while that of inclusion complex with β-CD is 359 °C. A higher melting point of inclusion complex than acridone itself is due to the fact that extra amount of thermal energy is required for the later to bring it out of β-CD cavity. Similarly melting point of acridoxime is 322 °C, but that of its inclusion complex is 334 °C.

3.3. Study of spectral characteristics

The drug recipient interaction is better identified by employing IR spectrophotometer as a useful tool [14]. The absorption maxima are shown to undergo a distinct blue shift after their inclusion complex formation with β-CD (Table 1). This observation clearly demonstrates transference of the compound from a more protic environment (aqueous media) to a less protic environment (cavity of β-CD). The compound and β-CD interaction leading to inclusion complex formation is further supported by IR data (Table 1). It is seen that the IR- stretching frequencies due to different bonds (C=O, N-H, C-N etc. in case of acridone and C=N, N-H, O-H etc. in case of acridoxime) undergo a downward shift towards lower energy and the peaks become broader, weaker and smoother. Such changes in IR- spectral characteristics due to inclusion complex formation may be attributed to development of weak interaction like H-bonding, vander-Waal forces and hydrophobic interactions between host and guest molecules [15].

3.4. Phase solubility studies

   The phase solubility plots of acridone and acridoxime with and without inclusion complex formation with β-CD are shown in Fig. 1. In both the cases it is seen that there is a linear increase in solubility of these compounds with increasing concentration of β-CD. At a higher concentration of β-CD, a small negative deviation is observed. Since the slopes of both plots are less than unity, the stoichiometry of the inclusion complexes is 1:1 [16].
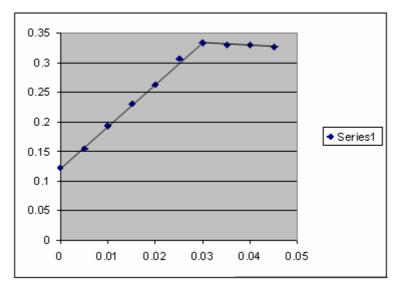

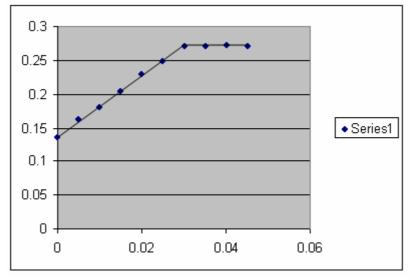**Fig. 1a.** Phase solubility plot (OD vs. [B-CD]) of acridone.

Fig. 1b. Phase solubility plot (OD vs. [B-CD]) of acridoxime.

   The thermodynamic stability constants (K_T) of inclusion complexes are determined by following Benesi-Hilderbrand relation [17].

$1/\Delta A = 1/\Delta\epsilon + 1/K[guest]_0 \Delta\epsilon . 1/[\beta\text{-CD}]_0$

   Good linear correlations (Fig. 2) are obtained for a plot of $1/\Delta A$ verses $1/[\beta\text{-CD}]_0$ for acridone and acridoxime. The values of K_T for both the complexes are calculated using the relation:

K_T = Intercept / Slope

The K_T values for these inclusion complexes i.e. acridone- β-CD and acridoxime - β-CD are found to be 104 M^{-1} and 155.5 M^{-1}, respectively. The data obtained are within 100 to 1000 M^{-1} (ideal values) indicating appreciable stabilities for the inclusion complexes [18].
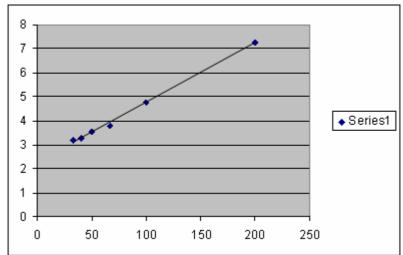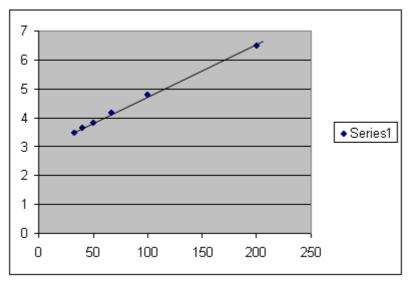

Fig. 2a. Plot (1/OD vs. 1/[B-CD]) of acridone.

Fig. 2b. Plot (1/OD vs. 1/[B-CD]) of acridoxime.

3.5. Thermodynamic properties

The thermodynamic parameters associated with binding of acridone and its oxime with β-CD for 1:1 stoichiometry have also been calculated by determining the K values at different temperatures. The K values are found to decrease with increasing temperature (de encapsulation) as expected for an exothermic process [19, 20]. The plot of ln K as a function of inverse absolute temperature produced linear plots (Fig. 3). In each case, the slope corresponds to (-ΔH / R). From this value and value of K_T at 298 K, ΔG, ΔS and ΔH have been calculated (Table 2) [21].
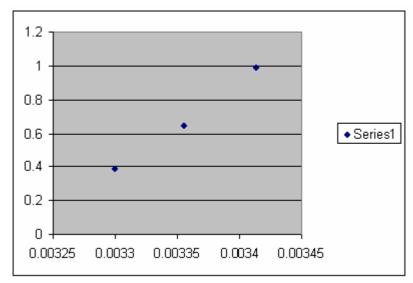
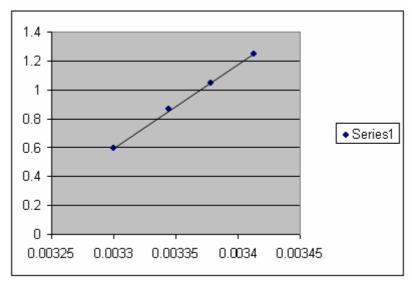**Fig. 3a.** Plot (ln K vs. 1/T) of acridone.

Fig. 3b. Plot (ln K vs. 1/T) of acridoxime.

Table 2
Thermo dynamical data of acridone and its oxime with and without inclusion complex at 298 K.

Sl No.	Compound	K (mol L^{-1})	ΔG (kJ / mol)	ΔH (kJ / mol)	ΔS (kJ / mol)
1	Acridone / ß-CD complex	104	-11.5	-43.7	-0.11
2	Acridoxime / ß-CD complex	155.5	-12.5	-48.25	-0.12

As can be seen from the table, ΔG values are negative for both the complexes. These data clearly demonstrate the spontaneous formation of inclusion complexes. Secondly, the ΔH and ΔS values are negative at 298 K which suggests that the complex formation is an exothermic and enthalpy controlled process. The negative enthalpy change is due to stabilization of the compound within the cavity of β-CD by weak intermolecular forces as suggested earlier. The small negative entropy change is due to steric barrier caused by less free movement of guest

molecules. The study further suggests that change in entropy (ΔS) in destabilizing inclusion complexes is compensated by change in enthalpy (ΔH) [22].

3.6. Antimicrobial Screening

The result obtained in the antimicrobial susceptibility test (Table 3) shows that acridone and its oxime are susceptible to Gram negative bacteria P.aeruginosa and Gram positive bacteria E. coli. However, these compounds are resistant to fungi like Candida and Aspergillus. This may be due to specificity of the drug towards a particular microbe. When the inclusion complexes of these compounds are tested against the above microbes, the antimicrobial susceptibility increases significantly for the microbe P.aeruginosa and E.coli. Since inclusion complex formation enhances the solubility of the drug, it becomes more available to specific tissues leading to increased antimicrobial activity.

Table 3
Antimicrobial Susceptibility test of acridone and its oxime with & without inclusion complex (**A**= Acridone, **AA**=Acridone/ ß-CD, **B**=Acridoxime, **BB**= Acridoxime / ß-CD, sample 1 mmol L^{-1}).

Organism	A	AA	B	BB
E. coli	S (10)	S(15)	S(15)	S (17)
Staph. aureus	R	R	R	R
Pseudomonas	S(21)	S (27)	S (31)	S (35)
Candida	R	R	R	R
Aspergillus	R	R	R	R

4. Conclusion

From the above results and discussions it is clear that the solubility of acridone and its derivatives can be improved by inclusion complex formation with β-CD which is a very good analytical tool for enhancing the bio-availability of drugs. The study of these complexes furnishes information about non-covalent intermolecular forces binding the "host – guest" molecules. The negative ΔG, ΔH, ΔS values support the formation of such complexes. Cyclodextrins are now widely used for the stabilization of many drugs [23]. Acridone and its derivatives show antibacterial activity which can further be enhanced by forming their inclusion complexes.

Acknowledgement

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