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Enhancement of Bioavailability of Pharmaceutically Active Acridone Derivatives by Inclusion Complex Formation with β-Cyclodextrin as Encapsulate

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ABSTRACT

In the present work, we synthesized some acridone derivatives and formed inclusion complex with cyclodextrin to improve the solubility. Thermodynamic parameters of the complexes were evaluated. Antimicrobial test was performed to evaluate the potentiality of the synthesized compounds or complexes as drugs.

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KEYWORDS

Acridone, β-Cyclodextrin, Bioavailability, Inclusion complex.

INTRODUCTION

Acridones, the oxidized products of acridines are the scaffold of many compounds with pharmaceutical importance. Certain acridone alkaloids (extracted from plants) have been found to have antimicrobial activities [1]. The presence of quinolone group in the acridone and their derivatives makes them a potential drug. Works related to acridone establish its anticancer, antimalarial, antiviral, antibacterial and anti-inflammatory activities [2-5]. Although several substituted acridones have been synthesized or extracted from plants to study their biological activity, no work related to substituted acridones presented in this paper is reported in literature. The present work deals with the synthesis, characterization of acridone derivatives and evaluation of thermodynamic parameters.

In recent times, significant number of therapeutically active drugs has been discovered but the efficacy remains in its water solubility, dissolution rate and bioavailability. As the compounds, we synthesized are hydrophobic in nature, the bioaccessibility and efficiency can be improved by inclusion complex formation, a solubility enhancement technique out of many such approaches [6,7]. Hydrophilic β-cyclodextrin (β-CD) has been used as complexing agent to increase the bioavailability of poorly soluble drugs forming inclusion complex [8]. The physico-chemical properties of drug (guest) bound to β-CD (host) in the complex changes so also the water solubility parameters. Inclusion complexes with β-CD have been synthesized by co-precipitation method [9,10]. The structure of acridone, its derivatives and the complexes has been confirmed by their spectral studies.

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EXPERIMENTAL

All AR grade chemicals were procured, solvents were redistilled before use. The percentage composition of the elements (CHN) of the compounds was determined using an elemental analyzer, UV-VIS electronic spectra were recorded on a Shimadzu UV 1700A spectrophotometer. IR spectra were recorded in KBr pellets in the region of 4000-400 cm^{-1} using Shimadzu 8400S FTIR spectrophotometer. Antimicrobial susceptibility was assessed by employing Kirby-Bauer disc diffusion method [11].

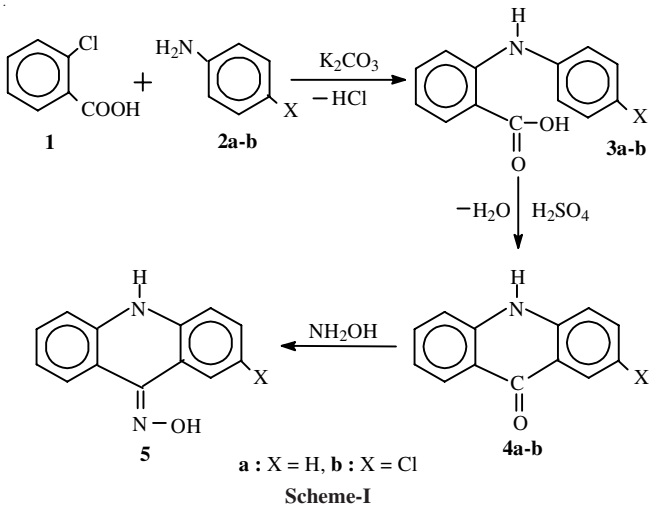
Synthesis of 9(10H)-acridone (4a): Mixture of 8 g (0.05 M) of *o*-chloro benzoic acid (**1**), 5 mL (0.05 M) of substituted aninline (**2**), 8 g of anhydrous K_2CO_3 and 0.4 g of copper oxide were refluxed under boiling condition for 2 h. After steam distillation, 2 g of animal charcoal was added to the solution and heated to boiling for 15 min and filtered. On acidification of hot filtrate with 6 mL of conc. HCl precipitates of compound **3a** was obtained. This was washed with water and recrystallized from ethyl alcohol and dried in an oven at 100 ºC. A mixture of compound **3a** (4 g) and 20 mL of conc. sulphuric acid was heated on a steam bath for 3 h. The resultant solution was cooled to room temperature, on adding ice-cold water with shaking crystals of compound **4a** was obtained.This was filtered and purified by boiling with 5 % solution of sodium carbonate. Filtered, washed and dried in an oven at 100 ºC to get pure crystals of compound **4a** [12]. Yield 90 %, m.p. 354 ºC. IR (KBr, ν_{max}, cm^{-1}): 3078 (N-H), 3021 (C-H arom.), 1623 (C=C), 1685 (C=O), 1157 (C-N). Elemental analysis: calcd (found) %: C 80.00 (80.29), H 4.61 (4.52), N 7.18 (7.01).

Synthesis of 2-chloro-9(10H)-acridone (4b): Mixture containing compound **2b** (7 g), compound **1** (8 g), anhydrous potassium carbonate (8 g) and copper oxide (0.4 g) were refluxed under similar condition and same process was followed as for the synthesis of compound **4a**. Yield 76 %, m.p. 327 ºC. IR (KBr, ν_{max}, cm^{-1}): 3092(N-H),3033 (C-H aromatic),1637 (C=C), 1702 (C=O), 1169 (C-N). Elemental analysis: calcd (found) %: C 67.97 (67.80), H 3.48 (3.57), N 6.10 (6.02).

Synthesis of 9(10H)-acridone- 2-chlorooxime (5b): Compound **4b** (1 g) in alcohol was dissolved till no turbidity was observed. Hydroxylamine hydrochloride (1 g), sodium acetate (2 g) and 25 mL of water were added to alcoholic solution. The mixture was refluxed under boiling condition for an hour on a water bath. The resulting solution was cooled and filtered. The residual compound was again washed with ice cold water, filtered, recrystallized from ethyl alcohol and dried in hot air oven at 100 ºC (**Scheme-I**). Yield 85 %, m.p. 305 ºC. IR (KBr, ν_{max}, cm^{-1}): 3310 (N-H), 1623 (C=N), 3218 (O-H). Elemental analysis: calcd (found) %: C 63.80 (63.63), H 3.68 (3.81) N 11.45 (11.57).

Synthesis of inclusion complex: Inclusion complex of β-CD with acridone and its derivatives were prepared by co-precipitation method [13]. To the aqueous solution of β-CD, the synthesized compounds were added slowly maintaining 1:1 molar proportions, stirred using a mechanical shaker for 24 h and kept overnight at room temperature to promote complete precipitation of complexes. The suspensions were filtered and refrigerated for 24 h.The precipitates were washed and air dried for 24 h.

Compound 4a complex: Yield 83 %, m.p. 365 ºC. IR (KBr, ν_{max}, cm^{-1}): 3067 (N-H), 1615 (C=C), 1671 (C=O), 1142 (C≡N).



Scheme-I

Compound 4b complex: Yield 71 %, m.p. 340 ºC. IR (KBr, ν_{max}, cm^{-1}): 3081 (N-H), 1629 (C=C), 1690 (C=O), 1154 (C≡N).

Compound 5b complex: Yield 74 %, m.p. 316 ºC. IR (KBr, ν_{max}, cm^{-1}): 3296 (N-H), 1611(C≡N), 3207 (O-H).

For phase solubility studies, Higuchi-Connor [14] method was employed. Equal quantities of all the compounds were added to β-CD solutions of increasing concentrations and the resulting solution was equilibrated in thermostatic shaking water at 20, 25 and 30 ºC for 48 h and filtered. The absorbance values of all compounds in different β-CD solutions were recorded spectrophotometrically and plotted against [β-CD]. The apparent stability constant, K of the complexes was calculated from the A_L type phase-solubility diagram using eqn. 1. Antimicrobial susceptibility was assessed by employing Kirby-Bauer disc diffusion method.

$$K = \frac{\text{Intercept}}{\text{Slope}} \qquad (1)$$

RESULTS AND DISCUSSION

The spectral studies (electronic & IR), elemental analysis and melting point measurements ensure the formation of acridone and its derivatives.The percentage composition of the elements(CHN) of the samples almost matches to the calculated values. The IR data suggested the formation of compounds **4a** and **4b**. Inclusion complex formation of the compounds with β-CD is confirmed from the rise in melting point of the complexes from the parent molecule and spectral data (UV-vis and IR).The rise in melting point of the complex can be attributed to the binding of guest molecule (acridone and its derivatives) with host β-CD by van der Waal′s forces [15]. The electronic spectra show a blue shift after complex formation with broader and weaker peaks (Fig. 1). Downward shift (lower energy) in IR frequencies of the complexes is observed compared to free drug molecules.These observations led to the conclusion that encapsulation of the compounds occured with β-CD, *i.e.*, the drug molecule have moved to a less protic environment, *i.e.*, into the cavity of host β-CD molecules.

Fig.2 shows the linear increase in [acridone] with increasing β-CD molar concentration. From the saturation aqueous

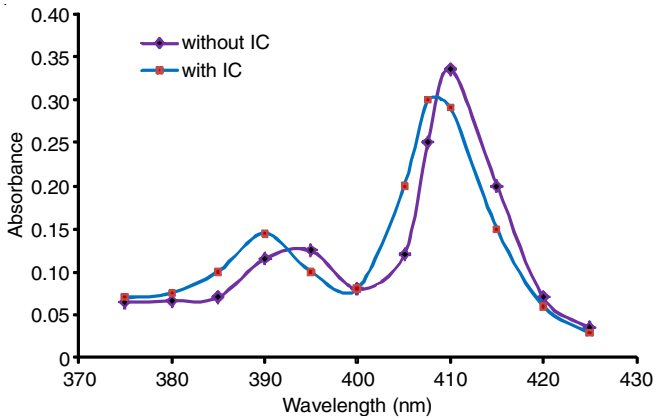


Fig. 1. Visible spectra for 9(10*H*)-acridone-2-chloroxime with and without inclusion complex (IC)

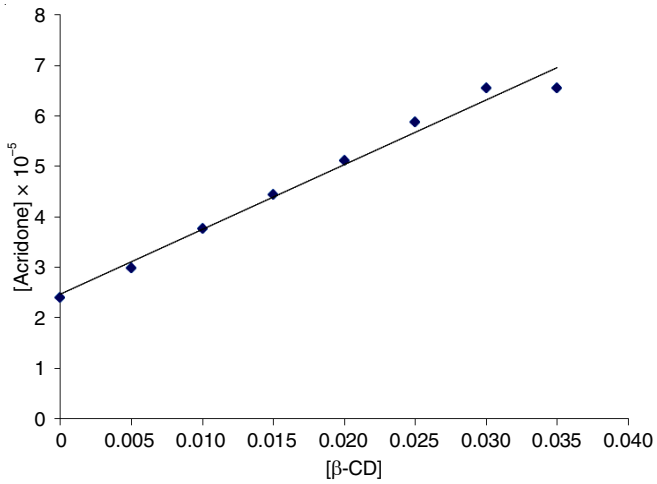


Fig. 2. Solubilization of acridones as a function of [β-CD] at 25 °C

solubility (4.7 mg/mL) of acridone and electronic spectra recorded, the extinction coefficient of acridone is found to be 5192.02 mol^{-1} dm^3cm^{-1}.The concentration of acridone in each saturated solution in β-CD was determined from its absorbance value at 406 nm. Fig. 3 shows the phase solubility measurements of the compounds with β-CD. The linear rise in absorbance of acridone and its derivatives with concentration of β-CD suggest that there is an increase in solubility of these compounds with increasing β-CD concentration. The solubility reaches a maximum then becomes saturated for [β-CD] = 0.03 mol dm^{-3}.

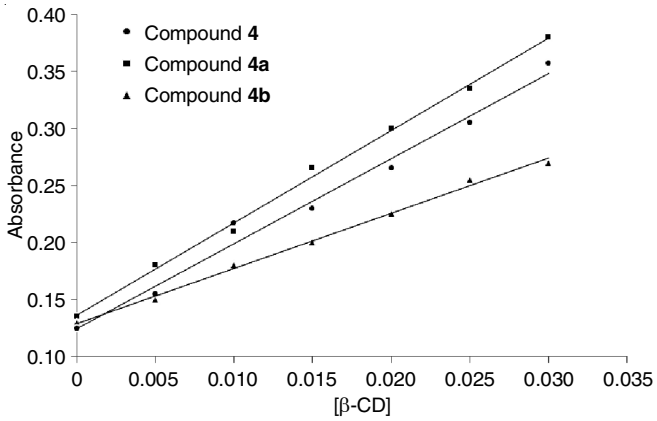


Fig. 3. Extent of phase-solubility of acridone compounds as a function of [β-CD] at 25 °C

This is a typical A_L type [14] diagram, consistent with 1:1 stoichiometry of molecular inclusion complex formation. The binding constant, K of the complexes at 298 K are calculated using eqn. 2 [16] and are presented in Table-1.

$$\frac{1}{\Delta A} = \frac{1}{\Delta \varepsilon} + \frac{1}{K[Guest]_o \Delta \varepsilon} \times \frac{1}{[\beta\text{-CD}]} \quad (2)$$

TABLE-1
THERMODYNAMIC VALUES FOR INCLUSION COMPLEX FORMATION OF ACRIDONE AND ITS DERIVATIVES WITH β-CD AT 298 K (ΔG: FREE ENERGY CHANGE, ΔH: ENTHALPY CHANGE, ΔS: ENTROPY CHANGE AND K: STABILITY CONSTANT)

Compd. No.	K (M^{-1})	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (J/K mol)
4a	119.4	-11.84	-44.10	-108.01
4b	175.6	-12.80	-51.37	-129.42
5b	207.9	-13.22	-58.63	-152.38

Fig. 4 shows the linear co-relations for a plot of 1/absorbance against 1/[β-CD] for acridone and its derivatives. The K values at 298 K are computed from the slope and intercept values of the plots in Fig. 4 using eqn. 1 and are found well within the acceptable range of 100-1000 M^{-1} [17], indicating the stability of inclusion complex. The stability constant during de-encapsulation of each complexes with varying temperature conditions are calculated and found to decrease with rise in temperature. Fig. 5 presents vant-Hoff's plot of log K *vs.* 1/T for all the complexes. Slope (-ΔH/R) of the linear plots gives ΔH (enthalpy change during complexation), ΔG (free energy change) at 298 K is calculated using eqn. 3:

$$\Delta G = -RT \ln K \quad (3)$$

The entropy change for the complexation reaction (ΔS) is calculated from ΔH and ΔG values at 298 K. Various thermodynamic data are presented in Table-1.The decrease in stability constant values with increasing temperature during de-encapsulation is quite expected for exothermic processes [18]. The negative values of ΔG suggested the spontaneous formation of complexes [19]. A relatively low negative ΔH obtained from the plots may be attributed to the stabilization of drug molecules within the protic environment of β-CD cavity not by any permanent chemical binding but most likely by weak intermolecular binding forces such as hydrogen bonding, van der Waal's

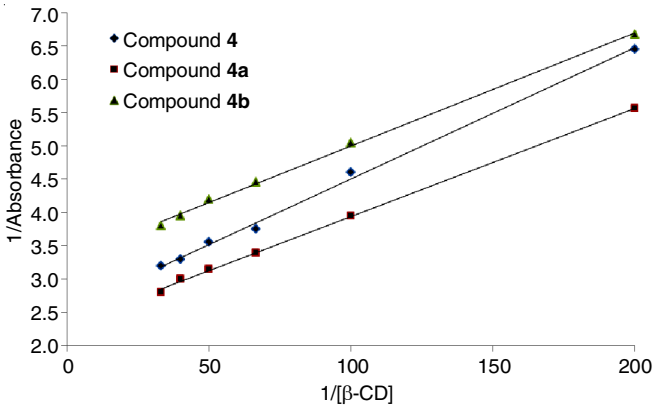


Fig. 4. Higuchi-Connor plots for inclusion complex of acridone compounds

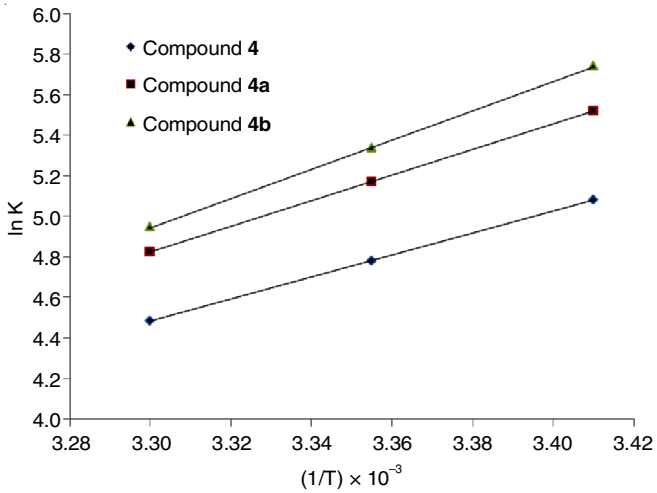


Fig. 5. vant Hoff's plot for the inclusion complex of acridone and its derivatives with [β-CD]

interaction. This is indicative of the fact that the reaction is exothermic and enthalpy controlled as well. The observed values of entropy changes can be explained considering the restricted movement of free drug molecules after complex formation [20].

Kirby- Bauer disk diffusion method [11] was followed using Muller-Hinton agar plates to test the antimicrobial suscepti-bility. The zone of inhibition (ZI) for pure drug and their inclusion complex of each species are measured (Table-2). Considerable increase in zone of inhibition is seen with the compounds in complex formed with β-CD. The obvious reason of enhanced performance is because of improved bioaccessibility of the drug molecules in inclusion complex.

TABLE-2
ANTIMICROBIAL SUSCEPTIBILITY TEST FOR COMPOUNDS **4a, 4b & 5** AND THEIR INCLUSION COMPLEX (ZONE OF INHIBITION IN mm)

Compound	*E. coli*	*P. aeruginosa*
4a	13	24
4a/β-CD complex	16	27
4b	16	26
4b/β-CD complex	19	29
5b	14	32
5b/β-CD complex	18	36

Conclusion

Acridone and its derivatives are potential drugs for pharmaceutical applications as they showed antibacterial activity. Because of low solubility in aqueous medium and less bioavailability, an attempt has been made in this work to enhance their solubilization properties. β-Cyclodextrin is found to enhance the solubility of these drugs by modifying the absorption properties. The hydrophilic outer surface helps β-CD to be soluble in aqueous medium while the hydrophobic interior (cavity of the molecule) forms the inclusion complex with drugs chosen for the study. Various thermodynamic parameters calculated from the phase solubility studies at different temperatures explain well about the complex formation between β-CD and drug molecules. Negative values for free energy and enthalpy changes suggested the spontaneous and exothermic nature of the complex formation process. The negative values of entropy change for the process is due to the hindered mobility of the molecules as compared to free molecules.

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