Short Communication

Studies on chelation reaction of cyclophosphamide (CPM), a valuable anti-cancer drug with carcinogenic metal ions Cu(II) and Co(II)

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Abstract

The chelation reaction of cyclophosphamide (CPM), an anti-cancer biological alkylating agent of nitrogen mustard group with Cu(II) and Co(II), has been investigated potentiometrically. The stability constants have been reported at temperatures $21°$ C and $41°$ C $\pm 1°$ C and $\mu = 0.0$ M, 0.05 M, 0.10 M and 0.20 M (KNO$_3$) $1 \cdot 1$ stoichiometry of metal ligand interaction has been confirmed by conductometry. Negative values of Δ G and positive values of Δ S indicate stable complex formation.

Key words: Cyclophosphamide, anti-cancer drug, carcinogenic metal ions Cu(II) and Co(II), stability constant

1. Introduction

Cyclophosphamide (CPM) is one of the several chemical compounds that have been introduced as cancer chemotherapeutic drugs since 1966. It was first synthesized in 1957 as diamidophosphoric acid N:N-Bis (β-chloroethyl) N'-O-propylene ester[1].

Chelating tendencies of CPM have not been studied so far, although many other anti-cancer drugs have been investigated[2-4] for their complexing behaviour towards different metal ions. It was, therefore, thought proper to choose this drug for studying its complexing tendencies towards carcinogenic[5] bivalent metal ions of cobalt and copper with the help of Bjerrum Calvin pH metric titration technique[6,7] as modified by Rossotti and Rossotti[8].

2. Experimental

The ligand CPM was obtained from M/s. Biochem Laboratories and used directly. Solutions were prepared in double-distilled air-free water. The ionic strength was maintained

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with KNO_3 (1.0 M) A Systronic 324 pH meter with a least count 0.05 pH unit was used for pH titrations in an inert atmosphere of nitrogen gas. The metal nitrate solutions were prepared and standardized by usual methods. The pH meter was calibrated with buffer solutions in acid and alkaline ranges separately. The linearity of the glass electrode was checked at intervals with standard buffers. Following mixtures were titrated against carbonate-free standard KOH (0.136 M) prepared by the method of Schwarzenbach and Biedermann[9] and metal ligand 1:5 was maintained.

   A.   5 ml of 0.011 M HNO_3
   B.   A + 5 ml of 0.002 M cyclophosphamide
   C.   B + 1 ml of 0.002 M metal nitrate.

The titrations were repeated to check the reproducibility of the results.

   Stoichiometry (1:1) of the metal ligand interaction was determined by mole ratio method by conductometry.

3. Results and discussion

Under the experimental conditions rapid equilibrium is attained and no significant change in pH occurs during 30 minutes. The ligand CPM thus does not undergo significant hydrolysis and the $> NH$ group in the ligand takes part in complexation by de-protonation.

   From the titration curves (fig. 1), $\bar{n}$ A values at various pH were calculated using solutions A and B. The protonation curve between pH and $\bar{n}$ A values was plotted (fig. 2). The values
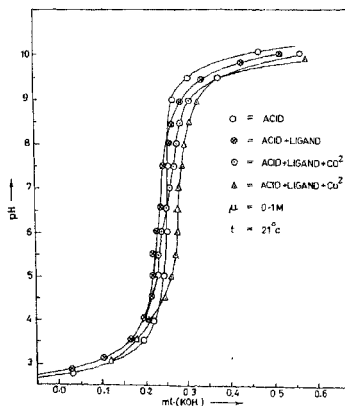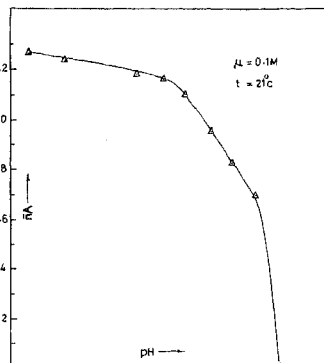

FIG. 1. Titration curves.

FIG. 2. Protonation curve.

of pk_1^H and pk_2^H were calculated by intrapolation at half $\bar{n}$ A value method. The values were refined by pointwise method and they agree well.

$\bar{n}$ and pl values were computed using titration curves of solutions B and C (fig. 1). Metal complex formation curve (fig. 3) was obtained by plotting n values against corresponding pL values. Log k_1 and Log k_2 were obtained by intrapolation at half $\bar{n}$ value method and refined by pointwise method. Representative values are recorded in Table I. Limit of error lies between $\pm$ 0.025.

Cyclophosphamide showed a maxima lesser than 1.5 for $\bar{n}$ A indicating the presence of only one replaceable proton in the molecule which ionises as follows

Cl. CH₂. CH₂   NH – CH₂
        \   /   \
         N – P = 0   CH₂   ⇌
        /   \   /
Cl. CH₂. CH₂   O – CH₂

Cl. CH₂. CH₂   N̄ – CH₂
        \   /   \
         N – P = 0   CH₂ + H⁺
        /   \   /
Cl. CH₂. CH₂   O – CH₂

It is clear from pH measurements that $\bar{n}$ increases with increase in pH showing that anionic form of ligand takes part in complexation[10].

The close values of successive stability constants indicate spontaneous formation of 1 : 1 metal: cyclophosphamide chelate[11].

The thermodynamic parameters (Table II) were calculated from the well known thermodynamic relations. Negative values of Δ G and Δ H suggest metal: drug interaction and the positive values of entropy change Δ S indicate the formation of stable chelates[12]. The reaction is exothermic in nature.

The relative chelating tendencies of the CPM follow the well-known Irving William order[13] as Cu(II) > Co(II) at 0, 0.05, 0.10 and 0.20 M at 21°C. The results are also in agreement with the results of other workers[14-17]

FIG. 3. Complexation curves.

Table I

Chemical stability constants of CPM: Cu^{2+} and Co^{2+} chelates

Standard deviation $= \pm 0.025$

Ionic strength (μ) M	$t\,°C$ $\pm 1\,°C$	Cation	$\log K_1$	$\log K_2$	$\log \beta^n$
0.00	21	H^+	11.05	--	11.05
		Cu^{2+}	3.72	3.89	7.56
		Co^{2+}	3.72	3.72	7.44
0.05	21	H^+	11.40	—	11.40
		Cu^{2+}	3.73	3.87	7.60
		Co^{2+}	3.74	3.82	7.56
0.10	21	H^+	10.13	-	10.13
			(10.13)	--	(10.13)
		Cu^{2+}	3.75	3.86	7.61
			(3.76)	(3.86)	(7.62)
		Co^{2+}	3.75	3.85	7.60
			(3.75)	(3.85)	(7.60)
0.10	41	H^+	9.34	--	9.34
			(8.95)	--	(8.95)
		Cu^{2+}	3.21	3.56	6.77
			(3.17)	(3.56)	(6.73)
		Co^{2+}	3.74	3.85	7.59
			(3.74)	(3.85)	(7.59)
0.20	21	H^+	9.78	.-	9.78
			(9.42)	.-	(9.42)
		Cu^{2+}	3.76	3.85	7.61
			(3.75)	(3.86)	(7.61)
		Co^{2+}	3.76	3.85	7.61
			(3.75)	(3.84)	(7.59)

Figures in brackets are obtained from point wise method.

Table II

Change in thermodynamic parameters during complexation

$\mu = 0.1$ M, $t_1 = 21\,°C$ and $t_2 = 41\,°C \pm 1\,°C$

Cation	$-\Delta H$ (KJ/mol)	$-\Delta G$ (KJ/mol)		ΔS KJ degree^{-1}mol^{-1}	
		$21\,°C$ (294 K)	$41\,°C$ (314 K)	$21\,°C$ (294 K)	$41\,°C$ (314 K)
H^{+2}	13.2	57.05	56.17	0.14	0.13
Cu^{+2}	16.13	42.84	40.71	0.09	0.07
Co^{+2}	0.2	42.80	45.64	0.10	0.10

Acknowledgement

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