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Electrochemical Studies on Complexes of Copper (II) with Anticoagulant Warfarin sodium

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ABSTRACT

Electrochemical studies on complexes of copper(II) with Warfarin sodium have been carried out by direct current polarography. Using D.M.E. electrode the comparative investigation on the electroreduction of copper(II) with Warfarin sodium in 1M KNO_3 containing 30% and 40% ethanol have been made. The voltammetric data were evaluated according to Deford and Hume's method. The reduction was reversible and diffusion controlled involving $2e^-$ in each case. Deford and Hume's treatment suggests that Warfarin forms 1 : 1 complexes at 25° and 1 : 1, 1: 2 at 35°C in 30% ethanol. Further, in 40% ethanol 1 : 1 and 1 : 2 complexes are identified at both temperatures. The values of stability constants (log) obtained are 5.0973 at 25°C, 5.0557, 5.6720 at 35°C in 30% ethanol 5.2532, 9.8783 at 25°C and 5.2222, 9.7500 at 35°C in 40% ethanol. Thermodynamic functions (G°, H°, S°) have also been reported.

Keywords: Copper(II), Warfarin sodium, reversible, electrochemical study, stability constants

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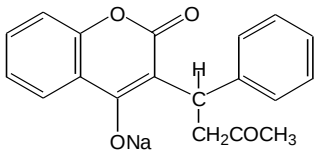
INTRODUCTION

Electrochemical studies of copper have significant value in biological field. Copper is third most abundant metallic element in human body following iron and zinc, especially bound by the protein metallothionein [1-3]. Moreover, electro analysis of copper [4, 5] finds its application in trace metal determination in the whole blood and fish's tissue.

Coordination of such biometal 'copper' with drugs can affect their homeostasis. It can be assumed, therefore, that the action of at least some of the drugs used in the treatment of metal dependent diseases can be explained on these grounds [6-11]. Earlier, complexes of Copper(II) with □-blocker[12,13] and antibiotic drug[14] have been studied. Synthesis and characterization of complexes of copper(II)-carbonic anhydrase inhibitor drugs have been reported by Ferrer et al. [15,16]. Anticoagulants are the drugs most widely used to prevent blood clotting. Warfarin sodium(I) [3-(□-acetonyl benzyl)4-hydroxy coumarin sodium salt] is an anticoagulant, used in prevention and treatment of venous thromboembolism[17,18]. Presence of coumarin ring makes Warfarin a good complexing ligand. Coumarin complexes are of significant interest because of their biological[19] and complexing ability[20-22].

Earlier, complexes of zirconium with Warfarin sodium have been investigated[23]. Further, complexes of Ce(III) with Warfarin have been studied[24]. The complexes of some lanthanides with Acenocoumarol sodium were assayed for acute intraperitoneal and peroral toxicity and for their influence on blood clotting time[25,26,27]. This paper represents further step in such investigations and is concerned with complex formation of copper(II) with Warfarin sodium. Copper(II) forms binary complexes with Warfarin. Stability constants of complexes have been determined in 30% and 40% ethanol at 25°C and 35°C by Deford and Hume's method[28]. Thermodynamic parameters (□ G°, □ H°, □ S°) have also been reported.



Warfarin sodium
(I)

EXPERIMENTAL

Apparatus

A digital D.C polarograph CL 357 was used to record current voltage data. This equipment has three electrode assembly, dropping mercury electrode as working electrode, calomel as reference electrode and platinum as counter electrode. Dropping mercury electrode had the characteristics m=1.9660mg/sec, t=4.10 sec/drop and h=40 cm. Elico digital pH meter was employed to measure the pH of solutions.

MATERIAL AND REAGENTS

Analytical grade (s.d. fine) cupric chloride was used in the study. Acetate buffer of pH 4.40 ± 0.01 was used as supporting electrolyte. 0.2M Acetate buffer was prepared by stock

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solution of sodium acetate and glacial acetic acid. Distilled ethanol was used for producing non-aqueous medium. Triton-X-100 (0.001%) was used to suppress the polarographic maxima. All solutions were made in double distilled water. In sample, solution of cupric chloride (2.5×10^{-2} M), supporting electrolyte and drug (complexing agent) were taken in definite ratio. Sample was deareated by passing inert nitrogen for 10 minutes, prior to polarographic study.

RESULT AND DISCUSSION

The system copper(II) - Warfarin sodium was investigated polarographically in 30% and 40% ethanol at 25°C and 35°C. Half wave potential of copper(II) (–0.120 volts vs S.C.E.) in acetate buffer (pH = 4.40 ± 0.01) has been determined.Half wave potential of copper(II) shifts towards more negative side with successive addition of Warfarin and diffusion current of metal (i_d) decreases, which suggests formation of complex. Copper(II) undergoes $2e^-$ reduction process at d.m.e. The reduction is found to be reversible and diffusion controlled. Reversible nature of the system has been revealed by the plots of $E_{d.e}$ vs $\sqrt{h}$ which were linear with slope value 28 ± 4 mV. Direct proportionality of diffusion current to the square root of mercury column ( ) indicates diffusion controlled nature.

Overall formation constant (log) of the complexes have been determined by Deford and Hume's method using polarographic measurements. The plots of $F_{(J)}(x)$ vs C_x (concentration of Warfarin sodium) were drawn and are represented in Fig. 1-4. The stability constants log $_1$ and log $_2$ were evaluated from the intercepts of these plots and their values are summarised in Table 5 at 25°C and 35°C in 30% and 40% ethanol Warfarin forms 1 : 1 complexes only at 25°C in 30% ethanol, 1 : 1 and 1 : 2 complexes at 35°C. Both 1 : 1 and 1 : 2 complexes were obtained at 25°C and 35°C in case of 40% ethanol. From the value of stability constants, thermodynamic parameters have also been evaluated.

Table 1 : Copper(II)-Warfarin sodium system in 30% ethanol

C_x = concentration of Warfarin sodium, $E_{1/2}$ (M) = –0.120 volts vs S.C.E.
$CuCl_2 = 2.5 \times 10^{-2}$ M, Temp. = 25°C ± 1°C

$C_x \times 10^{-4}$	$E_{1/2}$	log I_m/I_c	$F_0(x)$	$F_1(x) \times 10^4$
0.00	0.000	0.0000	-	-
0.05	0.005	0.0228	1.5555	11.1100
0.10	0.010	0.0469	2.4271	14.2710
0.15	0.012	0.0532	2.8767	12.5113
0.20	0.014	0.0791	3.5687	12.8430
0.25	0.015	0.0797	4.0449	12.1796
0.30	0.020	0.1139	6.1692	17.2306
0.35	0.025	0.1439	8.7545	12.1557
0.40	0.030	0.1597	14.9313	29.8282
0.45	0.031	0.1931	17.4301	38.7335

$_1 = 12.5112 \times 10^4$

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Table 2 : Copper(II)-Warfarin sodium system in 30% ethanol

C_x = concentration of Warfarin sodium $E_{1/2}$ (M) = –0.120 volts vs S.C.E.
$CuCl_2 = 2.5\times10^{-2}$ M, Temp. = 35°C ± 1°C

$C_x \times 10^{-4}$	$\Delta E_{1/2}$	$\log I_m/I_c$	$F_0(x)$	$F_1(x) \times 10^4$	$F_2(x) \times 10^5$
0.00	0.000	-	-	-	-
0.05	0.005	0.0217	1.4937	23.6920	-
0.10	0.010	0.0445	2.2329	24.6580	-
0.15	0.015	0.0687	3.4292	24.2920	-
0.20	0.020	0.0813	5.2844	28.5626	-
0.25	0.025	0.1009	5.4400	22.2000	4.8806
0.30	0.030	0.1214	12.0896	44.3584	4.8016
0.35	0.030	0.1356	18.4709	58.2363	4.6122
0.40	0.040	0.1978	27.8099	76.5997	4.6000
0.45	0.045	0.1990	46.7809	114.4522	4.7121

$\beta_1 = 11.3684 \times 10^4$

Table 3 : Copper(II)-Warfarin sodium system in 40% ethanol
C_x = concentration of Warfarin sodium, $E_{1/2}$ (M) = –0.120 volts vs S.C.E.
$CuCl_2 = 2.5\times10^{-2}$ M, Temp. = 25°C ± 1°C

$C_x \times 10^{-4}$	$\Delta E_{1/2}$	$\log I_m/I_c$	$F_0(x)$	$F_1(x) \times 10^4$	$F_2(x) \times 10^9$
0.00	0.000	0.0000	-	-	-
0.05	0.007	0.0112	1.7697	12.3940	-
0.10	0.012	0.0469	2.8356	18.3560	0.4414
0.15	0.015	0.0595	3.6872	17.9146	-
0.20	0.020	0.0791	5.6824	23.4870	2.7862
0.25	0.025	0.0997	8.8117	31.2468	5.3288
0.30	0.030	0.1139	13.4369	41.4563	7.8472
0.35	0.032	0.1362	16.5272	44.3634	7.5568
0.40	0.035	0.1597	22.0394	52.5985	8.1459
0.45	0.035	0.1760	22.8823	48.6273	6.8250

$\beta_1 = 17.9143\times 10^4$ $\beta_2 = 7.5561\times 10^9$

Table 4 : Copper(II)-Warfarin sodium system in 40% ethanol
C_x = concentration of Warfarin sodium, $E_{1/2}$ (M) = –0.120 volts vs S.C.E.
$CuCl_2 = 2.5\times10^{-2}$ M, Temp. = 35°C ± 1°C

$C_x \times 10^{-4}$	$\Delta E_{1/2}$	$\log I_m/I_c$	$F_0(x)$	$F_1(x) \times 10^4$	$F_2(x) \times 10^9$
0.00	0.000	-	-	-	-
0.05	0.007	0.0222	1.7831	15.6620	-
0.10	0.012	0.0457	2.7434	17.4340	-
0.15	0.015	0.0579	3.5371	16.9140	-
0.20	0.020	0.0705	5.3068	21.5340	0.2670
0.25	0.025	0.0969	8.2186	28.8744	3.1497
0.30	0.030	0.1106	12.3623	37.8743	5.6242
0.35	0.032	0.1322	15.1042	40.2977	5.5136
0.40	0.035	0.1627	20.3095	48.2737	5.8181
0.45	0.036	0.1706	22.3048	47.3440	5.8542

$\beta_1 = 16.6801 \times 10^4$ $\beta_2 = 5.6234\times 10^9$

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Table 5 : Stability constants of copper(II)-Warfarin complexes in 30% ethanol.

System	Composition of complexes	Stability constant	
		25°C	35°C
$[Cu(Warf.sod)]^{+2}$	1 : 1	5.0973	5.0557
$[Cu(Warf.sod)_2]^{+2}$	1 : 2	-	5.6720

Table 6 : Stability constants of copper(II)-Warfarin complexes in 40% ethanol.

System	Composition of complexes	Stability constant	
		25°C	35°C
$[Cu(Warf.sod)]^{+2}$	1 : 1	5.2532	5.2222
$[Cu(Warf.sod)_2]^{+2}$	1 : 2	9.8783	9.7500

Table 7 : Thermodynamic parameters for copper(II)-Warfarin complex at 25°C in 30% ethanol.

System	Composition	Thermodynamic parameters		
		$\Delta G°$ Kcal mol^{-1}	$\Delta H°$ Kcal mol^{-1}	$\Delta S°$ Cal K^{-1} mol^{-1}
$[Cu(Warf.sod)]^{+2}$	1 : 1	–6.9283	–17.4894	–35.4222

Table 8 : Thermodynamic parameters for copper(II)-Warfarin complex at 25°C in 40% ethanol.

System	Composition	Thermodynamic parameters		
		$\Delta G°$ Kcal mol^{-1}	$\Delta H°$ Kcal mol^{-1}	$\Delta S°$ Cal K^{-1} mol^{-1}
$[Cu(Warf.sod)]^{+2}$	1 : 1	–7.1402	–13.0330	–19.7645
$[Cu(Warf.sod)_2]^{+2}$	1 : 2	–13.4267	–53.9398	–135.8817

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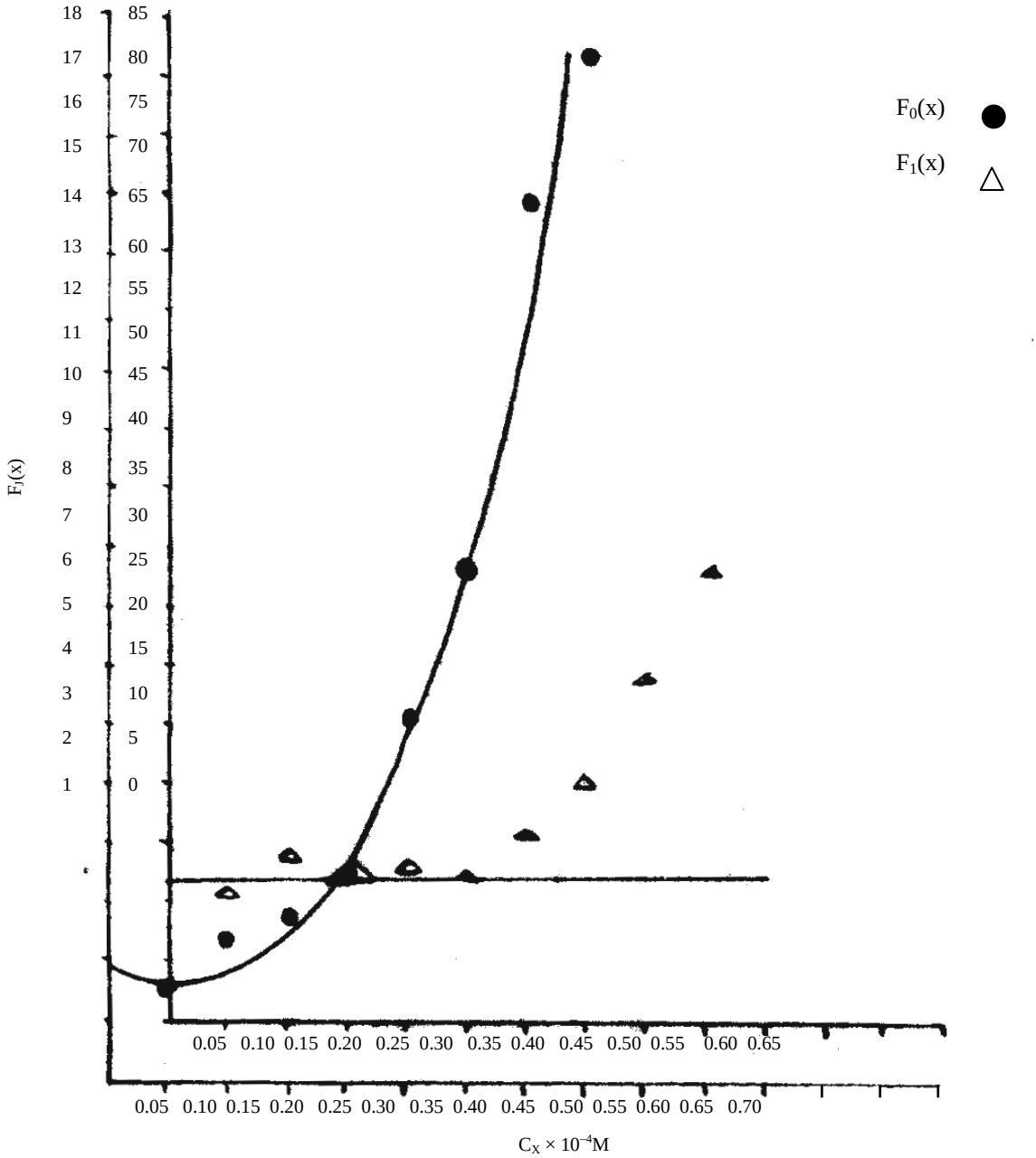
Fig. 1 : Plot of F_j (x) Vs C_x
Copper(II)-Warfarin sodium system in 30% ethanol at 25°C



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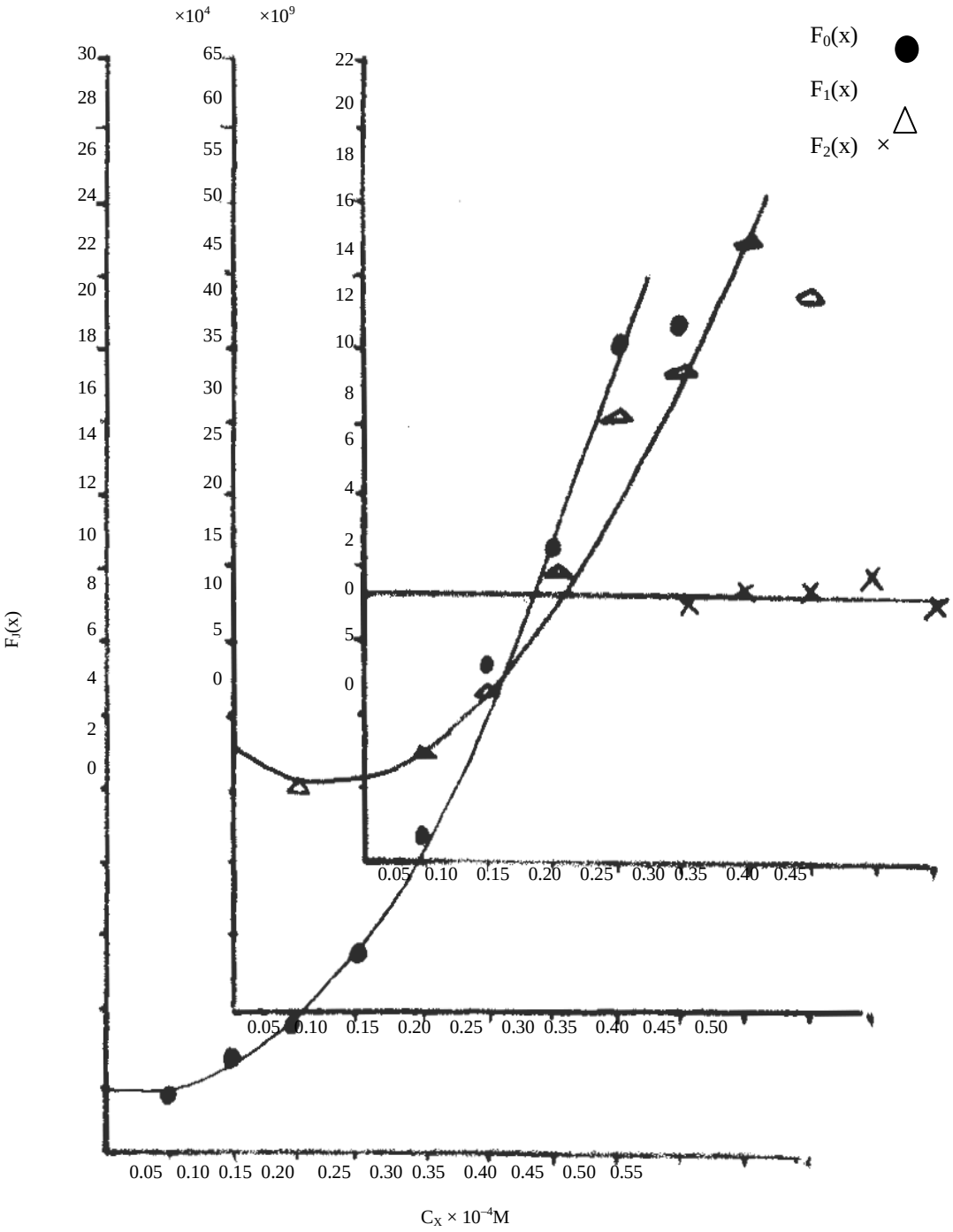

Fig. 2 : Plot of F_j (x) Vs C_x
Copper(II)-Warfarin sodium system in 30% ethanol at 35°C

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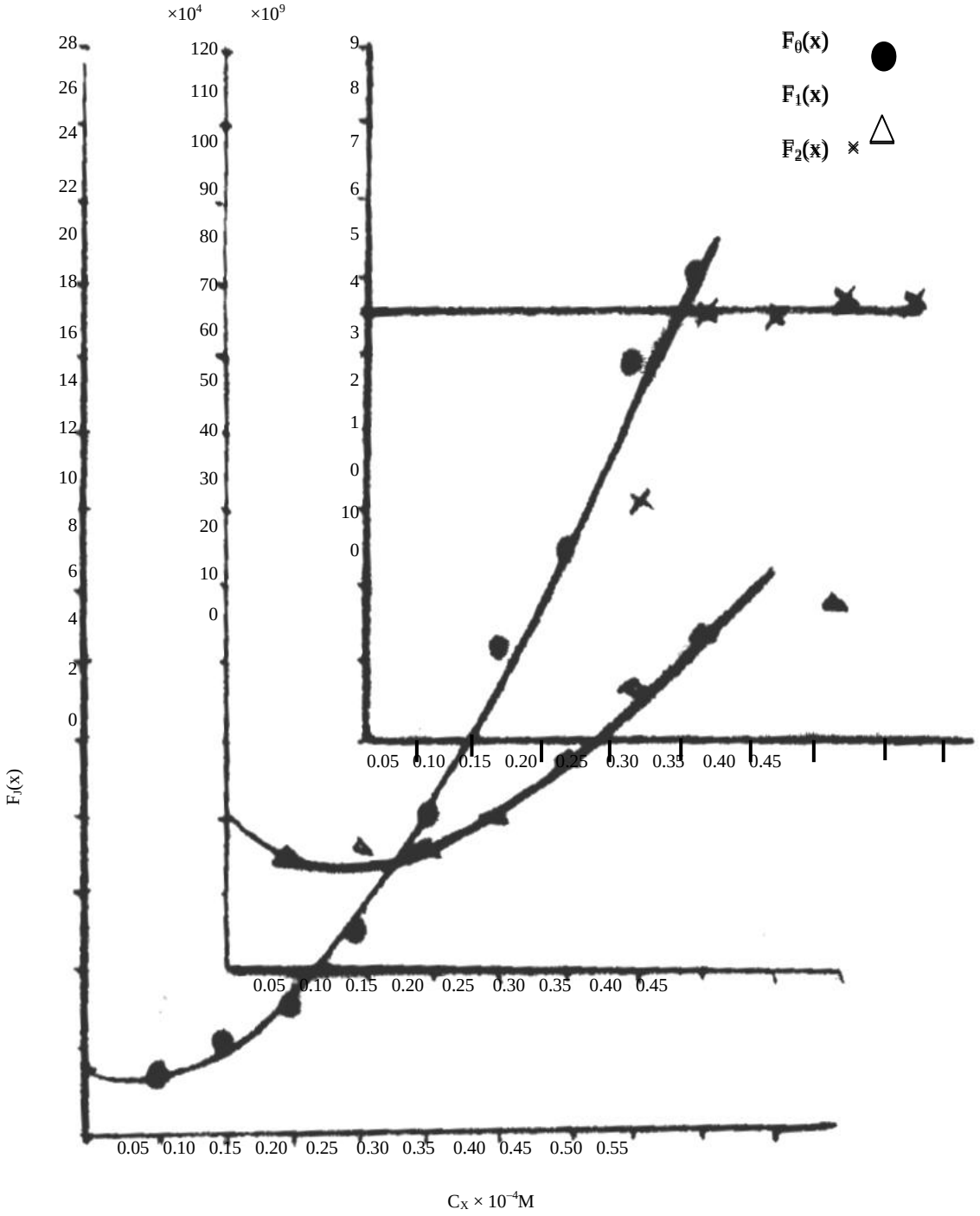

Fig. 3 : Plot of F_j (x) Vs C_x
Copper(II)-Warfarin sodium system in 40% ethanol at 25°C

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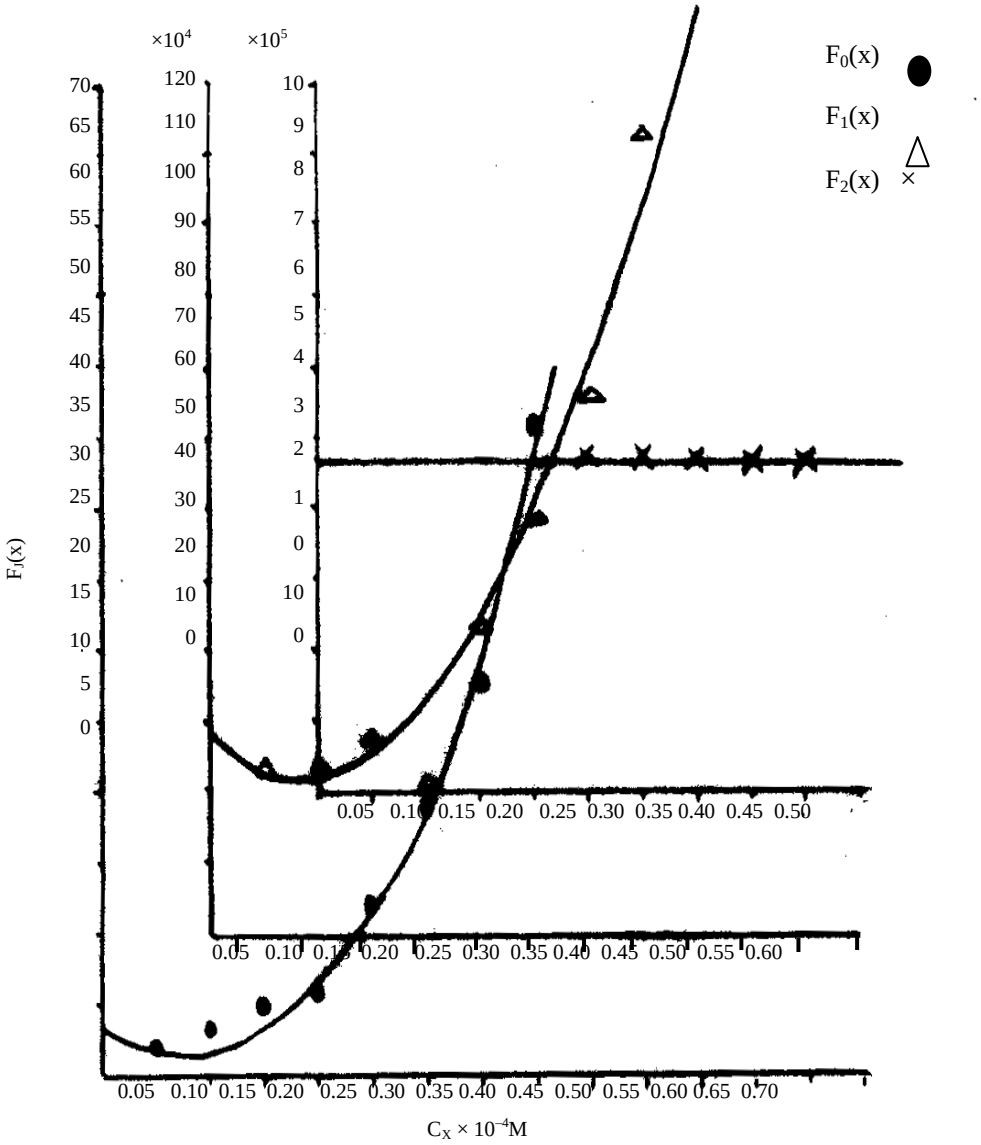

Fig. 4 : Plot of F_j (x) Vs C_x
Copper(II)-Warfarin sodium system in 40% ethanol at 35°C

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CONCLUSION

Warfarin forms 1 : 1 and 1 : 2 complexes with copper(II) in different experimental conditions. One sodium is liberated at each step of complexation. Therefore, following mechanism can be assumed in different experimental conditions[25].

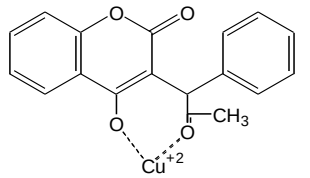
$$Cu^{+2} + NaR \xrightarrow{K_1} CuR^{+} + Na^{+} \quad (1)$$

$$CuR^{+} + NaR \xrightarrow{K_2} CuR_2 + Na^{+} \quad (2)$$

Observing the stability constants data, stability constants for Warfarin-copper(II) complexes are more in case of 40% ethanol for 1:2 complex, which shows that 1:2 complexes are more stable in 40% ethanol. This might be due to the increase in the pH. More basic is the solution, easy availability of e^{-} to central metal ion, hence the formation of complexes of greater stability constants [29,30]. Comparative observations of thermodynamic parameters for 1:1 complexes reveals that value of free energy ($\Delta G°$) and enthalpy ($\Delta H°$) are almost similar in 30% and 40% ethanol but entropy factor ($\Delta S°$) favours the 1:1 complexes in 30% ethanol than compare to 40% ethanol. This might be due the reason that more negative will be the entropy, more stable will be the complexes.

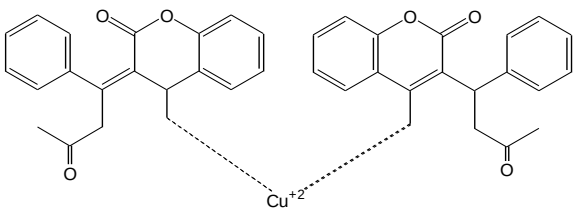
Proposed structure of complexes[25]

Copper(II) forms two type complexes with Warfarin i.e. 1:1 and 1:2 their structure can be proposed on the basis of literature collected.



Proposed structure of [Cu-(Warf sod)]$^{+2}$

(II)



Proposed structure of [Cu-(Warf sod)$_2$]$^{+2}$

(III)

Survey of literature reveals that complex of zirconium with Warfarin showed cytotoxic activity against human promyclocytic leukemic cells[23] cerium complexes of Warfarin sodium also possess biological activity[24]. Hence copper complexes of Warfarin sodium are also supposed to possess more anticoagulant effect than Warfarin itself.

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