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THE ELECTROCHEMICAL SENSING OF NALBUPHINE HYDROCHLORIDE DRUG SUBSTANCE IN VITRO BY THE CYCLIC VOLTAMMETRIC AND CONDUCTOMETRIC TITRATION TECHNIQUES.

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ABSTRACT

The electrochemical sensing of Nalbuphine hydrochloride drug substance in vitro by the cyclic voltammetry technique using the activated glassy carbon electrode. The solvation of CdCl₂ alone or in the presence of Nalbuphine HCl was studied by CV technique in 0.05 M KCl supporting electrolyte and different concentrations from CdCl₂ at 305.15K. The cyclic voltammograms were preceded at different scan rates 0.1, 0.05, 0.02 and 0.01 V Sec⁻¹. Also, different Nalbuphine HCl concentrations were utilized for studying their effect as electrochemical sensors on the solvation and kinetics parameters of CdCl₂. The redox mechanism of the system was determined from the resulted data. Moreover, the Gibb's free energies of the complex formation were evaluated. The formation constants and Gibb's free energies were calculated from the conductometric titration curves. The molar ratios of the complexes were obtained indicating the formation of 1:2 and 1:1 (M:L). The formation constants of different complexes in water: methanol solvent followed the order: Kf(1:2) > Kf(1:1) for (M:L). The (∆H) and (∆S) of formation and association of complexes were also estimated and discussed. The solvation ∆G°, ∆Hs and ∆Ss were calculated from solubility measurements for nalbuphine HCl at different temperatures.

Keywords: Electrochemical sensor; Solvation; Cyclic voltammetry; Nalbuphine.

1. INTRODUCTION

Nalbuphine hydrochloride (NP.HCl) is a phenanthrene derivative. It is an opioid analgesic where it has mixed opioid agonist and antagonist activity. It is used for the relief of moderate to severe pain and as adjunct to anaesthesia. Nalbuphine has the IUPAC name (5α,6α)-17-(cyclobutyl-1-methyl)-4, 5 Exopoxymorphinan -3,6,1 4-triol hydrochloride; N-cyclobutylmethyl-14-hydroxydihydro-normorphine (Fig. 1)[1].

Cadmium is a well-known heavy metal with extra toxicity impact on human organs. It is widely exist in human's body, the main sources of cadmium toxicity being smoking and welding.

The electrochemical redox demeanor of cadmium had been determined by various electrochemical methods like hydrodynamic voltammetry, chronoamperometry, [2] coulometry [3], and polarography. There are many studies about cadmium cyclic voltammetry with many ligands.

Many literatures have been established for the sensing of Nalbuphine hydrochloride in pharmaceuticals using HPLC with UV or electrochemical detection [4].

In current work glassy carbon electrode was utilized in a sensing of Nalbuphine hydrochloride with cadmium salt using cyclic voltammetric determination and conductometric titrations.

Moreover, electroanalytical methods are less expensive, more portable and ease of handling than
specifically, cyclic voltammetry is more officially used in industrial, environmental work and in the drug detection in their dosage forms and especially in biological samples since it combines excellent sensitivity, selectivity, accuracy, and precision with low cost of instrumentation and maintenance.

Fig. 1. Structure of Nalbuphine hydrochloride.

2. OBJECTIVES

Thermodynamic study of complexation reactions of Nalbuphine hydrochloride with cadmium ions not only result an interested data on the thermodynamics of complexation reaction, but also gives a better understanding of the high sensitivity of this ligand towards cadmium ions.

This work focuses on the determination of the stability constants and thermodynamic functions of complexation reactions between Cadmium ions and nalbuphine hydrochloride using the cyclic voltammetric and conductometric techniques.

3. EXPERIMENTAL

3.1 Materials

Water used in the preparation of solutions was bidistilled with a specific conductivity of 0.07 μS cm⁻¹ at 298.15 K, all salts (CdCl₂ and KCl) were purchased from merck, The used Methanol solvent (MeOH) was obtained from CHEM-LAB nv Co.

3.2 Cell and Instrument

For cyclic voltammetry, the cell used containing three electrodes connected to potentiostat DY 2000, Ag/AgCl in saturated KCl solution was used as the reference electrode, glassy carbon electrode (GCE) used as the working electrode and Platinum wire electrode was used as the auxiliary electrode. The glassy carbon electrode was prepared in the laboratory from pure carbon piece and polished with fine aluminum oxide on the wet woolen piece and its surface area is 0.0314 cm². The conductometric titration of the CdCl₂ (1x10⁻⁴ mole/L against the ligand (Nalbuphine HCl 1x10⁻³ mole/L) in Methanol: water 30: 70 v/v was performed with 0.2 ml interval addition of Nalbuphine HCl solution. The specific conductance values were recorded using conductivity bridge ADWA, AD 3000 with a cell constant equal to 1 cm⁻¹. The temperature was adjusted at 293.15 K, 298.15 K, 303.15 K and 308.15 K respectively.

3. RESULTS AND DISCUSSIONS

4.1 Cyclic voltammetry measurements

4.1.1 Cyclic voltammetry of CdCl₂ with Nalbuphine

4.1.1.1 Oxidation and reduction mechanism of CdCl₂ in absence of Nalbuphine HCl.

The redox behavior of Cd (II) in CdCl₂ was examined in 0.05 M of KCl as a supporting electrolyte by cyclic voltammetry on GCE at temperatures (305.15 K). This process was measured from 1.5 to -1.5 V of potential window, the resulted current measured in Amp. and 0.1 V/S scan rate. The CdCl₂ solution is added step wisely to reach the final concentration (3.85x10⁻³ M) as shown in Fig.2 which illustrate that Cd²⁺ solution is electroactive since it gives one anodic peak (Cd²⁺ / Cd¹⁺). At 305.15 K the anodic peak current of the anodic peak is 4.91 x10⁻⁴ Amp., and its anodic potential is -0.4975 V. These results indicate that the Cd²⁺ ion system is irreversible system involving the transfer of one electron.

Cyclic voltammetry behavior of Cd (II) scan between 1500 mv to –1500mv is introduced in (Fig.2).
The diffusion coefficient was estimated by using Randles-Sevcik equation (1) [5] which indicates the relation among the peak current, scan rate and diffusion coefficient.

\[ i_p = 0.4463 n F A C \left( \frac{n F D v}{RT} \right)^{1/2} \]  

(1)

Where \( i_p \) is the peak current in Ampere, \( n \) is number of electrons transferred in the redox event (usually 1), \( F \) is Faraday’s constant which equals 96485.33 C.mol\(^{-1}\), \( A \) is the area of the working electrode in cm\(^2\), \( C \) is the bulk concentration of metal ion (mol.L\(^{-1}\)), \( D \) is the diffusion coefficient in cm\(^2\)/sec, \( v \) is the scan rate in volts/sec, \( R \) is the gas constant which equals 8.314J.mol\(^{-1}\).K\(^{-1}\) and \( T \) is the absolute temperature.

Additionally, the surface coverage \( \Gamma \) (mol.cm\(^{-2}\)) and the Quantity of charge \( Q \) were estimated from equations (2, 3).

\[ \Gamma = \frac{i_p 4RT}{n^2 F^2 A v} \]  

(2)

\[ Q = n F A \Gamma \]  

(3)

4.1.1.2 Effect of different concentrations of CdCl\(_2\) at 305.15 K

The impact of several concentrations of CdCl\(_2\) (1.61, 2.38, 2.68, 3.13 & 3.85) x10\(^{-3}\) M have been studied in 0.05 M KCl at 305.15 K as displayed in Fig.3. The anodic peak gradually increased by raising the concentration of Cd ions which gives a proof that the reaction is governed by diffusion processes. Moreover, the solvation parameters like (\( \Gamma_a \) & \( Q_a \)) increased as reported in Table (1). This behavior could be explained as the increase in concentrations of the electroactive species.

Increase in the anodic diffusion coefficient \( D_a \), anodic surface coverages and Quantity of charge for anodic parts was increased by increasing cadmium chloride concentration indicating diffusion controlled reactions.

4.1.1.3 Effect of different scan rates for CdCl\(_2\)

The influence of scan rates (0.1, 0.05, 0.02 & 0.01) V.S\(^{-1}\) was determined at 305.15 K as shown in Fig.4. The different solvation parameters increased by the decreasing of the scan rate supporting the diffusion mechanism of solvation as reported in Table (2). Also, the redox peak current decreased by decreasing the scan rates. The linear relation between \(-i_{pa}\) and the square root of scan rate in Fig. (5). This demonstrated that the reaction was governed by the surface diffusion process [7].

**Fig. 3.** Cyclic voltamograms of different of concentration for CdCl\(_2\) in absence of Nalbuphine HCl.

**Table 1:** Solvation and kinetic parameters (D, \( \Gamma \) and Q) of CdCl\(_2\) in the absence of Nalbuphine HCl at 305.15 K, scan rate 0.1 V/Sec

| [M] x10\(^{-3}\)mol.L\(^{-1}\) | \( E_{pa} \) Volt | \( I_{pa} \) x10\(^{-4}\) Amp | \( D_a \) x10\(^{-10}\) cm\(^2\).s\(^{-1}\) | \( \Gamma_a \) x10\(^{-4}\) mol.cm\(^{-2}\) | \( (-) Q_a \) x10\(^{-4}\) C |
|-------------------------------|------------------|-----------------|------------------|-----------------|------------------|
| 1.61                          | -0.434           | 0.42            | 0.93             | 1.45            | 0.44             |
| 2.38                          | -0.501           | 2.01            | 10.00            | 6.99            | 2.12             |
| 3.13                          | -0.512           | 4.36            | 27.32            | 15.13           | 4.58             |
| 3.85                          | -0.498           | 4.91            | 22.81            | 17.03           | 5.16             |
Effect of Nalbuphine HCl on the redox behavior of CdCl$_2$

The influence of Nalbuphine HCl different concentrations ($0.31, 0.76, 1.49, 2.21, 2.90, 3.57$) x 10$^{-3}$ M on the electrochemical behavior of CdCl$_2$ was determined at 305.15 K and potential range from 1.5 V to -1.5 V as shown in Fig.6.

As reported in Table (3) the addition of Nalbuphine hydrochloride to the solution and stepwisely increasing its concentration, the redox peaks current decreased than observed with CdCl$_2$ alone as mentioned in Table (1).

| Scan rate V/sec | Ep,a volt | ipa x10$^{-4}$ Amp | Da x10$^{-9}$ cm$^2$/Sec | Γa x10$^{-2}$ mol/cm$^2$ | (-)Qa x10$^{-4}$ |
|-----------------|-----------|--------------------|--------------------------|-------------------------|-----------------|
| 0.10            | -0.498    | 4.91               | 2.28                     | 1.70                    | 5.16            |
| 0.05            | -0.529    | 4.18               | 3.31                     | 2.90                    | 8.80            |
| 0.02            | -0.533    | 1.40               | 0.94                     | 2.44                    | 7.39            |
| 0.01            | -0.571    | 1.12               | 1.19                     | 3.89                    | 11.8            |

Effect of different scan rates in presence of Nalbuphine HCl

The influence of different scan rates (0.1, 0.05, 0.02, 0.01) V.S$^{-1}$ for 1:1 molar ratio between cadmium and nalguphine had been studied as shown in Fig. 7. The decrease of the scan rate was followed by the decrease of the redox peaks currents, but the solvation parameters like ($Γ_a$ & $Q_a$) increased as shown in Table (4).

The linear relation between the peak current and square root of scan rate confirmed that the reaction was governed by diffusion processes as shown in Fig. (8).
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Fig. 6 Cyclic Voltammogram of CdCl$_2$ in the presence of Nalbuphine HCl at 305.15K

Table 4: Solvation and kinetic parameters (D, $\Gamma$ and Q) of CdCl$_2$ in the presence of Nalbuphine HCl at 305.15 K, scan rate 0.1 V/Sec

| $[L]$ x10$^{-3}$ mol.L$^{-1}$ | $E_{p,a}$ Volt | $I_{p,a}$ x10$^{-4}$ Amp | $D_{a}$ x10$^{-10}$ cm$^2$.s$^{-1}$ | $\Gamma$ a x10$^{-8}$ mol.cm$^{-2}$ | $Q$ a x10$^{-4}$ C |
|-----------------------------|--------------|-----------------|-----------------|-----------------|-----------------|
| 0.31                        | -0.406       | 1.35            | 1.74            | 4.68            | 1.42            |
| 0.76                        | -0.461       | 1.24            | 1.51            | 4.32            | 1.31            |
| 1.49                        | -0.465       | 1.27            | 1.61            | 4.39            | 1.33            |
| 2.21                        | -0.482       | 0.74            | 0.57            | 2.57            | 0.78            |
| 2.90                        | -0.491       | 1.01            | 1.09            | 3.51            | 1.06            |
| 3.57                        | -0.495       | 0.95            | 0.98            | 3.29            | 0.997           |

Most of the analysis of the redox processes data are smaller in case of CdCl$_2$ plus Nalbuphine HCl ligand than CdCl$_2$ alone indicating the complex reaction between metal and ligand, hence the method is sensitive to detecte Nalbuphine hydrochloride.

Fig. 7. Effect of different scan rate of 1:1 rate of scan rate for (Cd-Nalbuphine) complex.

Fig. 8. The relation between $-I_{p,a}$ and square root of scan rate for (Cd-Nalbuphine) complex.

Table 5: Correlation coefficient measurements:

| Number of Points | 3 |
|------------------|---|
| Degrees of Freedom | 1 |
| Residual Sum of Squares | 1.25E-10 |
| Adj. R-Square    | 0.84419 |

Table 6: Solvation and kinetic parameters (D, $\Gamma$ and Q) of effect of scan rate on complex at 305.15 K.

| Scan rate V/Sec | $E_{p,a}$ Volt | $I_{p,a}$ x10$^{-5}$ Amp | $D_{a}$ x10$^{-10}$ cm$^2$.Sec | $\Gamma$ a x10$^{-8}$ mol.cm$^{-2}$ | $Q$ a x10$^{-4}$ C |
|-----------------|--------------|-----------------|-----------------|-----------------|-----------------|
| 0.10            | -0.49        | 9.48            | 0.987           | 3.29            | 0.997           |
| 0.05            | -0.47        | 11.4            | 2.85            | 7.91            | 2.40            |
| 0.02            | -0.51        | 9.03            | 4.48            | 15.70           | 4.75            |
| 0.01            | -0.55        | 5.75            | 3.63            | 20.00           | 6.05            |
4.1.1.6 The stability constant for (Cd-Nalbuphine) complex

The values of stability constant ($\log \beta_{MX}$) and Gibbs free energy ($\Delta G^\circ$) increased by increasing the $j$ (L/M) ratio, indicating the tendency towards the formation of the complex as reported in Table (5). The decrease of scan rate was followed with the decrease of the stability constant of the complex ($\log \beta_{MX}$) and the Gibbs free energy as reported in Table (6).

The stability constant ($\log \beta_{MX}$) of the Cd-Nalbuphine complex was calculated from equation (4) [8].

$$\Delta E'=E'_c - E'_M = 2.303 \frac{RT}{nF} \times (\log \beta_{MX} + j \log C_x)$$

Where ($E'_c$) is the potential of the complex after every addition of (ligand) Nalbuphine HCl, ($E'_M$) the potential of the last addition of metal (cadmium) before adding the ligand (Nalbuphine HCl), ($C_x$) the ligand concentration in the solution, $j$ (the coordination number of the complex).

The Gibbs free energy for the interaction between cadmium and the Nalbuphine HCl was determined from the stability constant by applying equation (5) [9].

$$\Delta G^\circ = -2.303 RT \log \beta_{MX}$$

4.2 Conductometric measurements

The specific conductance values ($K_s$) of the solutions of different concentrations of CdCl$_2$ solution in (MeOH-H$_2$O) mixtures were measured experimentally in absence and in presence of ligand at different temperatures (293.15, 298.15, 303.15 and 308.15 K).

The molar conductance ($\Lambda_m$) values were calculated [10] using equation (6):

$$\Lambda_m = \frac{(K_s - K_{solv}) \times K_{cell} \times 1000}{C}$$

Where $K_s$ and $K_{solv}$ are the specific conductance of the solution and the solvent, respectively; $K_{cell}$ is the cell constant and $C$ is the molar concentration of the metal salt solution.

4.2.1 Formation constant for the complexation of the CdCl$_2$ with Nalbuphine HCl

The experimental data of ($\Lambda_m$) were analyzed for the determination of formation constants for each type of the stoichiometric complexes. The formation constants ($K_f$) for CdCl$_2$ complex were calculated for each type of complexes (1:2) and (1:1) (M:L) [11] by using the equations:

$$K_f = \frac{[\text{ML}]}{[\text{M}] [\text{L}]} = \frac{\Lambda_{M,\text{obs}} - \Lambda_{\text{ML}}}{\Lambda_{\text{obs}} - \Lambda_{\text{ML}}} \quad (7)$$

$$[\text{L}] = \frac{C_M \times (\Lambda_{M,\text{obs}} - \Lambda_{\text{ML}})}{\Lambda_{M} - \Lambda_{\text{ML}}} \quad (8)$$

Where $\Lambda_m$ is the molar conductance of the metal before adding the ligand, $\Lambda_{\text{obs}}$ is the molar conductance of solution during titration and $\Lambda_{\text{ML}}$ is the molar conductance of the complex. The obtained values of log ($K_f$) for the metal-ligand stoichiometric complexes are presented in Table (7) for CdCl$_2$ in (MeOH-H$_2$O) mixture.

The relation between $\Lambda_m$ and the [M]/[L] molar ratio for CdCl$_2$ in presence of Nalbuphine HCl in 30 % (MeOH-H$_2$O) mixture as shown in Fig. (9).

Increasing temperature is followed by decrease in log $K_f$ favouring less solvation for interaction of CdCl$_2$ with Nalbuphine HCl indicating migration of ions away from the collecting area.

It was obviously that complex formation between CdCl$_2$ and Nalbuphine HCl in (Methanol: Water) as a solvent:
- 293.15 K is favorable temperature in the formation of 1:1 [M]/[L]
- 298.15 K is favorable temperature in the formation of 1:2 [M]/[L]
- 303.15 K is favorable temperature in the formation of 1:2 [M]/[L]
- 298.15 K is the same in the formation of 1:2 and 1:1 [M]/[L]

The Gibbs free energies of formation for 1:1 and 1:2 (M:L) stoichiometry complexes ($\Delta G_f$) were calculated [12] by using the equation (9):
\[ \Delta G_f^{\circ} = -2.303 \, RT \log K_f \quad \ldots \ldots \ (9) \]

The enthalpy (\(\Delta H_f\)) for the metal salt complexes were calculated for each type of complexes, (1:2) and (1:1) (M: L) by using van’t Hoff equation:

\[
\Lambda_m = \frac{\Delta H_f - R \, T \, \log K_f}{2.303} \]

Where \(R\) is the gas constant and \(T\) is the absolute temperature. On plotting of \(\log K_f\) versus \(1/T\) different lines are obtained for the formation of 1:2 and 1:1 (M:L) stoichiometric complexes for CdCl₂ with Nalbuphine HCl as shown in Fig. 10.

**Fig 9.** The relation between \(\Lambda_m\) and the [M]/[L] molar ratio for CdCl₂ in presence of Nalbuphine in 30 % (MeOH-H₂O) mixture.

**Table 7:** \(\Lambda_{ml}\) is the molar conductance of the complex, formation constant (K₁), for Cd\(^{2+}\) – Nalbuphine HCl complex formation in (MeOH-H₂O) mixture at different temperatures.

| T (K)  | M:L | [L] \(\times 10^5\) | [M] \(\times 10^5\) | \(\Lambda_m\) (S cm\(^2\).mol\(^{-1}\)) | \(\Lambda_{obs}\) (S cm\(^2\).mol\(^{-1}\)) | Log \(K_f\) |
|-------|-----|------------------|------------------|----------------|----------------|-----------|
| 293.15| 1:2 | 9.524           | 4.762            | 176.645        | 160.965        | 3.076     |
|       | 1:1 | 9.091           | 9.091            | 160.970        | 144.375        | 3.208     |
| 298.15| 1:2 | 9.524           | 4.762            | 212.695        | 183.020        | 3.345     |
|       | 1:1 | 9.091           | 9.091            | 183.000        | 162.860        | 3.249     |
| 303.15| 1:2 | 9.524           | 4.762            | 234.300        | 198.500        | 3.406     |
|       | 1:1 | 9.091           | 9.091            | 198.500        | 177.900        | 3.211     |
| 308.15| 1:2 | 9.524           | 4.762            | 237.900        | 211.680        | 3.200     |
|       | 1:1 | 9.091           | 9.091            | 211.700        | 189.420        | 3.220     |

\[
\frac{d \ln K_f}{dT} = \frac{\Delta H_f^0}{RT^2} \quad \ldots \ldots \ (10)
\]

From the relation between \(\log K_f\) and \(1/T\), \(\Delta H_f^0\) can be calculated for each type of complexes from
the slope of each line (-ΔH/2.303R). The entropy values (ΔSf) for complexes were calculated for each type of complexes (1:2) and (1:1) (M:L) by using the equation:

\[ ΔG_f^° = ΔH_f - TΔS_f \]  

Where (S) is the entropy of system.

Formation thermodynamic parameters (ΔGi, ΔHf, TΔSf, ΔSf) were collected in Table (8).

From the previous Table decreasing in ΔGi° by increasing in temperatures indicating more spontaneous process.

Most complex formation data are bigger in forming 1:2 complex than that of 1:1, indicating that the formation of 1:2 complex is favorable than 1:1 complex.

5 CONCLUSION

- Most of the analysis of the redox processes data are smaller in case of CdCl2 plus Nalbuphine HCl ligand than CdCl2 alone indicating the complex reaction between metal and ligand, hence the method is sensitive to detect Nalbuphine hydrochloride.
- All scan rate measurements proved the diffusion controlled reactions.
- On drawing the relation between molar conductance and the ratio of metal to ligand concentrations, Different lines are obtained indicating the formation of 1:1 and 2:1 [L]/[M] stoichiometric complexes.
- The formation constants and Gibbs free energies of different complexes were determined. Negative values of Gibbs free energy of complexation indicate that the reaction is spontaneous.

Declaration:

The authors declare that they have no conflict with anybody.

Fig. 10. log Kf v.s 1/T for Cd-Nalbuphine complexes (1:2) and (1:1)

Table 8: Complex formation thermodynamic parameters for Cd^{2+} – Nalbuphine HCl complex formation in (MeOH-H2O) mixture at different temperatures.

| T(K)   | M:L   | ΔGi°  (kJ mol⁻¹) | ΔHf  (kJ mol⁻¹) | TΔSf (kJ mol⁻¹) | ΔSf (kJ mol⁻¹ K⁻¹) |
|--------|-------|-----------------|----------------|----------------|-------------------|
| 293.15 | 1:2   | -17.030         | 7.973          | 25.003         | 0.086             |
|        | 1:1   | -17.759         | -0.025         | 17.735         | 0.061             |
| 298.15 | 1:2   | -19.094         | 7.973          | 27.067         | 0.091             |
|        | 1:1   | -18.547         | -0.025         | 18.522         | 0.062             |
| 303.15 | 1:2   | -20.097         | 7.973          | 28.069         | 0.091             |
|        | 1:1   | -18.949         | -0.025         | 18.924         | 0.061             |
| 308.15 | 1:2   | -19.493         | 7.973          | 27.466         | 0.086             |
|        | 1:1   | -19.616         | -0.025         | 19.591         | 0.062             |
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