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Short communication

Electrochemical analysis and analgesic behavior of Zn(II)–baclofen complex

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Abstract

The electroanalytical behavior of Zn(II)–baclofen complex has been described by direct current polarographic (DCP) method. The complex of transition metal Zn(II) with baclofen, [4-amino-3(4-chlorophenyl) butanoic acid, has been prepared and characterized on the basis of elemental analysis, IR-spectral, polarographic and amperometric studies. The metal–ligand interaction in aqueous medium has been studied polarographically at 25 °C and at an ionic strength of $\mu = 1.0$ M KCl. The reduction of the complex was found to involve two electrons and of diffusion controlled nature. The analytical results indicated a 1:1 (M:L) stoichiometry for Zn(II)–baclofen complex. The results on the drug and drug–metal complex revealed that the complex is more potent as compared with the parent drug.

Keywords: Baclofen; Analgesic drug; Zn(II)–baclofen complex; Polarography

1. Introduction

Baclofen [4-amino-3 (4-chlorophenyl)] butanoic acid, is a derivative of inhibitory neurotransmitter gamma-aminobutyric acid (GABA). Its efficacy in the treatment of neuralgias, central pain following spinal lesions or painful strokes, migraine and medication misuse, chronic daily headache, suggested that it may prevent cluster headache attacks [1]. A number of analytical methods for determination of baclofen viz. GLC, enantioselective method, mass spectrometry, solid-phase extractions, capillary electrophoretic separation [2–4] etc. have been reported in the literature.

However, formation of the Zn–baclofen complex and its characterization by polarographic methods which are far better than the existing methods in the field as regards to their extra ordinary detection sensitivity, oligo determination capability, minimum detection limit, low cost, rapidity, accuracy, simplicity and non destructive nature, have not been used for the said purpose.

Several metals such as Fe, Co, Zn, Cd and Ni in trace amount are essential for health and for the maintenance of human biological system. The complexes of life essential metals play significant role in biological system, in which enzymes are

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know to be activated by metal ions [5]. The advantage of complexes of Ni, Fe, Co, Zn, etc. with different types of drugs have been discussed by number of workers [6–9]. In continuation of the work reported from our laboratory on the study of electrochemical and bioinorganic behavior of some drugs and metal–drug complexes [10–13], the present paper describes the characterization of Zn(II)–baclofen complex using polarographic and spectral methods. Analgesic studies on the drug and its complex have also been reported in the paper.

2. Experimental

2.1. Apparatus

All the polarograms/voltammograms were recorded on an Elico (India) DC polarograph, model CL-357, coupled with an x-y polarocard model LR-101. The polarographic cell consisted of an electrode assembly having a dropping mercury electrode (working electrode), a saturated calomel electrode (reference electrode), and a coiled platinum wire (auxiliary electrode). A Systronics digital pH meter-335 was used for the pH measurements. The amperometric titrations were performed on a manually operated set up, equipped with a polyflex galvanometer (sensitivity 8.1×10^{-9} amp. per div.) and an AJCO vernier potentiometer. The capillary characteristics of the DME had a $m^{2/3}t^{1/6}$ value of 2.5 $mg^{2/3}$ $s^{-1/2}$ at 60 cm effective height of mercury column. The IR spectrum of solid complex was recorded using KBr pellets on an Shimadzu, Japan. Model 470, IR spectrophotometer.

2.2. Chemicals and reagents

The chemicals used were of AnalaR/BDH grade. Free gift baclofen ($C_{10}H_{12}ClNO_2$) sample was procured from Novarartis Pharma AG Basel, Switzerland. Doubly distilled water was used as solvent. Baclofen solution (5 mM) was prepared by dissolving the requisite amount in distilled water. Stocks solutions of 1 M potassium chloride was prepared by dissolving a requisite quantity of compound in distilled water. pH adjustments were made using dilute solutions of HCl, NaOH whenever necessary. The test solutions were deaerated by bubbling nitrogen gas for 10 min before recording the polarogram/voltammogram.

2.3. Preparation of complex

For the study of metal:ligand (M:L) complexation equilibrium experimental sets were prepared by keeping overall Zn(II) and potassium chloride (supporting electrolyte) concentration fixed at 1.0 mM and 0.1 M, respectively. The ligand concentration was varied from 0.0 to 25 mM. The volume was made to 100 ml with distilled water and the pH of each set was adjusted to 5.0 ± 0.1 using HCl/NaOH solution. The test solution was deaerated for 10 min before recording the polarogram/voltammogram.

2.4. Amperometric titration

Experimental sets, each having different but known amount of Zn(II) were prepared in appropriate quantity of supporting electrolyte (potassium chloride) and pH was adjusted to 5.0 and titrated separately against the standard solution of the titled baclofen whose pH was also adjusted to that of the titrate (5.0 using HCl/NaOH) at -1.20 V versus SCE (the plateau potential of Zn(II)). The current after each addition of the titrant was read and a curve was plotted between current against volume of titrant added.

2.5. Synthesis procedure of solid complex

Zinc sulphate and baclofen solutions were prepared separately in water and were mixed in 1:1 molar ratio. The mixture was then refluxed in a round bottom flask for 2 h. The residue (complex) was filtered and washed thoroughly to remove any unreacted material. The complex was dried at low temperature and stored over P_4O_{10}.

The results of elemental (C, H, and N) analysis on the drug and Zn(II)–baclofen complex were furnished by the CDRI, Lucknow, India. The complexometric titration method was used for the estimation of zinc in the complex [14].

2.6. Analgesic activity

Analgesia is defined as a state of reduced awareness to pain, and analgesics are substances, which decrease pain sensation by increasing threshold to painful stimuli.

Painful reaction in experimental animal can be produced by applying noxious (unpleasant) stimuli such as thermal (radiant heat as source of pain), chemical (irritants such as acetic acid, bradykinin etc.) and physical pressure (tail compression).

Tail flick responses [15] with albino rats were adopted for evaluation of analgesic activity of synthesized compounds. Fifteen rats both male and female were used for the present study. They were weighted and divided into three groups. Each rat was placed in the rat holder and tail was protruded out through the slot in the lid and placed on a hot wire (3 A). Time taken to flick the tail was noted, known as the normal reaction time. Test dosage 20 mg kg^{-1} was administered orally to albino rats of respective group excluding control group. The response was again noted after subsequent intervals of 30, 60, 90 and 120 min.

3. Results and discussion

3.1. Polarographic study of M:L complexation equilibrium

Zn(II) and its complex with baclofen ligand were found to be reversibly reduced in 0.1 M KCl at pH 5.0 involving two electrons which was evidenced by the plot of $\log(i/i_d - i)$ versus E. The reduction was found to be diffusion controlled, as revealed by plot of i_d versus $\sqrt{h}$ corr. On gradual increase of baclofen concentration, the half wave potential of Zn(II) metal ion shifted to more electronegative value and the diffusion current also decreased, there by showing complex formation between Zn(II) with baclofen.

The composition and formation constant of the complex was studied by the plots of $\Delta E_{1/2}$ (shift in the $E_{1/2}) = (E_{1/2})_c - (E_{1/2})_s$ against $\log C_x$ (logarithm of the concentration of the ligand). The plots were linear showing the formation of single complex species in solution. Lingane [16] method was, therefore, applied, which showed 1:1 (M:L) complex formation with formation constant, $\log \beta_1 = 3.3$.

The analyte was found to be fairly stable, as indicated by the reproducibility of the polarogram. The presented data has been compared with that observed using spectrophotometric method and was found in good agreement.

3.2. Amperometric determination of baclofen with Zn(II)

Under the above mentioned experimental conditions, Zn(II) gives a well defined polarographic wave in 0.1 M KCl at pH 5.0. The diffusion current was found to be proportional to its concentration. Baclofen does not produce any wave under the said experimental conditions. The plateau potential for the polarographic wave of Zn(II) i.e. −1.2 V versus SCE, was applied on the potentiometer for carrying out amperometric titration. Zn(II) was taken as the titrate and the drug solution was taken as titrant. The current volume plots resulted in L shaped curve. The end point as located by graphical method revealed metal to drug ratio of 1:1, which is in agreement with author's observations on the metal:ligand complexation equilibrium using polarographic method. The standardized method was found to be accurate for the analysis of complexes.

3.3. Characterization of Zn(II)–baclofen complex

The results of elemental analysis on the drug and its complex with Zn(II) are depicted in Table 1. The table revealed 1:1 metal:drug ratio in the complex, which supports author's finding using polarographic and amperometric methods.

3.4. IR spectral analysis of Zn(II)–baclofen complex

A critical comparison of the IR spectra of baclofen and its complex with metal shows (Table 2) that the broad band at between 3200 and 3400 cm^{-1} due to N–H stretching vibrations in the spectrum of baclofen, appears as a reduced and broad band between 3100 and 3300 cm^{-1} [17,18]

Table 1
Analytical data of baclofen and Zn(II)–baclofen complex

Compound	Found (calculated) (%)					
	M(Zn)	C	H	N	O	Cl
Baclofen	0	56.21 (56.21)	5.66 (5.70)	6.56 (6.52)	14.98 (14.91)	16.59 (16.53)
Zn(II)–baclofen complex	20.44 (21.35)	44.7 (44.22)	4.47 (4.40)	5.22 (5.15)	11.9 (11.3)	13.22 (13.12)

O% = 100 − % of other elements present in compound.

Table 2
Principal IR signals (cm^{-1}) and their assignments for baclofen and its Zn(II) complex

Baclofen	Assignments	Complex
730	C–Cl halogen compound	730
–	Stretching vibration	–
1620	N–H bending vibration	1620
–	C–O stretching vibration	1650
3200–3400	Symmetrical N–H stretching	3100–3300

and a new broad band appears at 1650 cm^{-1} in the IR spectrum of Zn(II)–baclofen complex which may be due to the involvement of COOH group in complexation. These observation indicate the involvement of nitrogen of N–H group and oxygen of carboxylic group in complex formation.

However, band due to C–Cl stretching vibration at 730 cm^{-1} is observed at the same frequencies in the IR spectrum of both baclofen and its Zn(II)-complex.

3.5. Analgesic activity

Analgesic activity of baclofen and its complex on the rat groups was studied. It was observed that the tail flick time without administration of baclofen drug or its Zn(II) complex is 6.82 s whereas on administration of 20 mg kg^{-1} of the drug (baclofen), the tail flick time increases from 7.0 to 14.7 s after 0.0–60.0 min, after administering the drug. The tail flick time decreases to 10.60 s after 120 min of the drug administering. However, increased effect is observed by administering 20 mg kg^{-1} of the Zn(II)–baclofen complex, whereas the tail flick time increased to 16.78 s after 60.0 min of administering the complex. But it looses its effectiveness with due course of time. The activity was found to be in the order: Baclofen < Zn(II)–baclofen complex Table 3.

Table 3
Data for analgesic activity of baclofen and Zn(II)–baclofen complex

Group	Average tail flick time (s)	Time (min)
Control	6.82	0
	6.90	30
	6.84	60
	6.82	90
	6.82	120
Baclofen	7.0	0
	10.82	30
	14.75	60
	13.20	90
	10.60	120
Zn(II)–baclofen complex	7.23	0
	12.60	30
	16.86	60
	13.40	90
	10.79	120

4. Conclusion

Though, some analytical methods viz. GLC, mass spectroscopy, capillary electrophoretic separation methods, etc. are in use for the analysis of baclofen, but the use of sensitive polarographic and amperometric methods makes it possible to analyze baclofen individually and in pharmaceutical formulation with high accuracy and precision of determination.

The standardized methods were found to be highly accurate for the study of Zn(II)–baclofen complex. On the basis of the analgesic studies on

baclofen and Zn(II)–baclofen complex against the rats, it could be concluded that the baclofen complex with Zn(II) is more potent as compared with parent analgesic drug.

Thus Zn(II)–baclofen complex may be recommended to the therapeutic expert as a more potent analgesic drug in lieu of the drug taken for present study i.e. baclofen.

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