

**THE ROLE OF Co (II) IN THE INCREASED MEDICINAL POTENCY OF
GLIMEPRIDE ANALYZED BY ELECTROCHEMICAL METHODS**

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ABSTRACT: The formation of complexes of glimepride and Co(II) was studied by polarography. The catalytic hydrogen wave of glimepiride and glimepride complex was measured at -1.36v (vs SCE) in 0.09 mol x L(-1) Na₂B₄O₇-KH₂PO₄ (pH 6.24 +/- 0.1) supporting electrolyte. When 1.0 x 10(-2) mol x L(-1) K₂S₂O₈ was present.. The polarogram indicated formation of complexes between glimepride and Fe (II). Glimepride produces a well-defined direct current polarogram and differential pulse polarogram in 0.09 mol x L(-1) Na₂B₄O₇-KH₂PO₄ (pH 6.24 +/- 0.1) supporting electrolyte. When 1.0 x 10(-2) mol x L(-1) K₂S₂O₈ was present. The stoichiometry of the Fe(II)-curcumin complex is 1 : 1. Antidiabetic studies on the drug and its metal complex have been performed more potent in antidiabetic activity compared to the parent drug.

Keywords: Polarography; Glimepride, Cobalt complex; DCP; DPP

INTRODUCTION

Glimepiride is a sulphonylurea that is used as an antihyperglycaemic agent for the oral therapy of type 2 diabetes mellitus. Glimepiride lowers the blood glucose level by stimulating pancreatic beta cells to produce more insulin and by inducing increased activity of intracellular insulin receptors. It is considered a secretagogue.^[1] Glimepiride specifically binds to a certain membrane protein close to the potassium channel of the β -cell membrane and reduces the opening probability of this channel. The resulting depolarisation opens voltage-dependent calcium channels and leads to calcium influx into the cell. In the presence of glucose, the elevated intracellular calcium levels trigger insulin secretion.

Extraparacrine actions have also been demonstrated for glimepiride.^[2-5] The drug improves the insulin sensitivity of peripheral tissue.^[4] Glimepiride also increases the number of glucose transporter molecules in the plasma membrane of peripheral muscle and adipose tissue and enhances their glucose uptake. This agent activates insulin-mediated glycogen synthesis and lipogenesis, and it inhibits hepatic gluconeogenesis. Both the increase in insulin secretion (the main mechanism of action), and the improvement of glucose utilisation (an additional beneficial effect), are responsible for the glucose-lowering properties of this agent.

Following oral administration, glimepiride is rapidly and completely absorbed. Maximum plasma concentrations are achieved at approximately 2.5 hours. With continuous administration the half-life is 5 to 8 hours. The physiological response to physical exertion, including a reduction of insulin secretion, is maintained on therapy with glimepiride.^[6]

Cobalt is needed for the body and is an essential trace element found in small amounts in different organs and bones in the body. cobalt is an integral part of vitamin B12, which is vital to the formation of red blood cells. cobalt is currently being used in biomedical research for cancer treatment methods.

In this study we elucidate the active chelating site of glimepride ligands and their complexing ability towards cobalt(II) by polarography.

Authentic drug: glimepride, would be dissolved in a suitable solvent preferably water. The drug complex formation during the reaction involve modification of drug. The change in drug structure conformation during the reaction can be detected polarographically, which will help in understading the mechanism of drug action.

Experimental

Chemical and Reagents:

The chemicals used were of Anal-R grade. The Glimepiride was from Sigma chemical Company. Double –distilled water and absoult ethanol were solvents; pH adjustment was made using dilute solutions of HCl or NaOH whenever necessary. Whereas, $\text{Na}_2\text{B}_4\text{O}_7\text{-KH}_2\text{PO}_4$ were of Loba Chemical Pvt. Ltd. Mumbai.

Apparatus:

The direct current polarographic (DCP) and differential pulse polarographic (DPP), studies were carried out on an Elico (India) micro processor based polarographic analyzer, model CL-362. The polarographic cell consisted of an electrode assembly having a dropping mercury electrode (DME), a coiled platinum wire electrode and a saturated calomel electrode (SCE).

The capillary characteristics of the DME had a $m^{2/3} t^{1/6}$ value of $2.5\text{mg}^{2/3} \text{sec}^{-1/2}$ at 60 cm effective height of mercury column.

A systronics digital μpH meter model-361 was used for the pH measurements.

For the study of complex formation:

Qualitative and Quantative studies on Glimepride were carried out using direct current polarography (DCP) and differential pulse polarography (DPP).

Glimepride was dissolved in 60:40 ethanol:water and a set of solutions containing varying concentration of glimepride were prepared in 1M overall concentration of $0.09 \text{ mol x L}^{-1}$ $\text{Na}_2\text{B}_4\text{O}_7\text{-KH}_2\text{PO}_4$ (pH 6.24 +/- 0.1) used as supporting electrolyte. When $1.0 \times 10^{-2} \text{ mol x L}^{-1}$ $\text{K}_2\text{S}_2\text{O}_8$ was present.

For study of stiochiometry and formation of the complex, Lingane's polarographic method was used, a simple for study of metal ligand equilibria in casea where only one complex is formed over the entire range of ligand concentration

Experimental solution were prepared by keeping overall cobalt (metal ion) and $\text{Na}_2\text{B}_4\text{O}_7\text{-KH}_2\text{PO}_4$ concentration fixed at 1mM and 0.1M, respectively, while varing the ligand concentration 0 to 15mM. The pH was adjusted to 6.24 ± 0.1 , and the solution was deaerated with purified H_2 gas. Polarogram was recorded keeping the initial potential set to -1.36v

For polarography analysis of antidibatic Glimepride and its complex with Cobalt , $0.09 \text{ mol x L}^{-1}$ $\text{Na}_2\text{B}_4\text{O}_7\text{-KH}_2\text{PO}_4$ (pH 6.24 +/- 0.1) used as supporting electrolyte. When $1.0 \times 10^{-2} \text{ mol x L}^{-1}$ $\text{K}_2\text{S}_2\text{O}_8$ was present.. On gradual increase of the drug concentration the half wave potential of Co shifted to more negative value with decreasing diffusion current , there by revealing complex formation Co with drug.

Ethanol:water (1:1) solution of glimepride gives an absorbance at 254nm. for spectrophotometric study of M:L equilibrium, job's method of continuous variation was performed.

Methodology for the study of Pharmacology:

The complexes used in the present study were found to be effective with albinorats. Healthy Albino wistar rats of either sex between 100-180g were selected for the studies. Rats were allowed to take standard lab feed and water in the animal house and were maintained in clean and hygienic condition. For antidiabetic study rats were divided into three groups six animal per group.

For antidiabetic studied six albino rats were selected. Three groups each with six animal rats, each rats weighing about 150-180g were selected and their fasting blood glucose level were measured on the Glucometer using sugar scan blood glucose test strips then they were injected interavenously (on tail) with solution 5mg/100g. Streptozotocin in normal saline. The albino rats were allowed to develop diabetes for about 15 days. At this stage this blood glucose level was again measured and checked whether it was higher than 180mg/dl. A glucose level higher than this indicates diabetic condition and the albino rats were ready for in vivo evaluation. The albino rats were allowed a free access to food and water. An appropriate amount 0.035gm/kg of drug and complexes were separately given orally in cannula to the rats. The response was noted after subsequent intervals of 1,2,3,4,5,6,7,8,9,10,11 hours.

RESULT AND DISCUSSION:

Glimepride in $0.09 \text{ mol} \times \text{L}^{-1}$ $\text{Na}_2\text{B}_4\text{O}_7\text{-KH}_2\text{PO}_4$ (pH 6.24 \pm 0.1) used as supporting electrolyte. When $1.0 \times 10^{-2} \text{ mol} \times \text{L}^{-1}$ $\text{K}_2\text{S}_2\text{O}_8$ was present produce a well-defined DC polarographic curve with $E_{1/2}$ 1.36mV vs SCE, where as the DPP response of the solution resulted in two well-define peaks with $E_p = -1.38\text{mV}$ vs SCE. Both peak heights for DPP were proportional to Glimepride concentration.

Polarographic study of M:L complexation equilibrium

Both Co and its complex with Glimepride produce a reversible two-electron reduction wave in $0.09 \text{ mol} \times \text{L}^{-1}$ $\text{Na}_2\text{B}_4\text{O}_7\text{-KH}_2\text{PO}_4$ (pH 6.24 \pm 0.1) used as supporting electrolyte. When $1.0 \times 10^{-2} \text{ mol} \times \text{L}^{-1}$ $\text{K}_2\text{S}_2\text{O}_8$ was present. complex formation between Co(II) and Glimepride (Supplementary material) was revealed by the shift in half-wave potential and peak potential to a more negative value and decrease in the height of the diffusion current with gradual increase of the glimepride concentration. plot 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 ligand) resulted in a linear plot. Showing formation of a single complex in solution. Lingane treatment of the observed polarographic data revealed 1:1 Co(II)-Glimepride complex.

Pharmacology study

The normal fasting glucose level of albino rat is in the range of 100-130mg/dl. In case of normal albino rat, the glucose level shot up immediately after the dose of Glimepride was given orally to the rat. However the effect lasted only about 10-12 hours. However in case of the diabetic rats. Fasting glucose level was in the range of 180-200 mg/dl using streptozotocin treatment within 15 days, interavenously. An appropriate amount 0.036g of glimepride and their complexes were separately given orally in cannula to the rats. The blood sugar levels were than noted at different time interval.

S.no.	Time Interval (hrs.)	Initial blood glucose level(mg/dl)	Pure Drug blood glucose level(mg/dl)	Complex with Co	Complex with Ni
1	0	242	242	241	241
2	1.5	-	217	190	202
3	3	-	182	175	179
4	4.5	-	158	142	152
5	6	-	147	129	135
6	7.5	-	87	76	81
7	9	-	72	58	68
8	11.5	-	90	81	76

Table 1: show that the intial blood sugar level is 242 mg/dl which on administration of the glimepride drug and its complexes show a decrease in the blood glucose level with time. It it could be concluded from the table complex of Co complex with glimpride is seen to more effective bringing down the blood glucose level in 9 hrous.

Conclusion

The data show stoichiometric ratio of 1:1 for the Co(II) glimepride complex. Phamacology studies on the metal-drug complex are more potent than glimepride. The polarographic methods are used for qualitative and quantitative analysis of glimepride and are recommended for quality control in the drug industry. The increased potency of the complex may allow use as a potent antidibatic drug.

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