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Synthesis and Biological Profiling of a Co(II) Complex Featuring 2-Acetoxybenzoic Acid as a Bidentate Ligand

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ABSTRACT

Aspirin, also known as acetylsalicylic acid, is considered the most effective and widely used anti-inflammatory drug which helps treat the majority of musculoskeletal and articular disorders. Novel Co (II) complex containing aspirin have been produced and characterized by utilizing infrared, melting point, and electrochemical studies (polarography and amperometry). The ligand has been discovered to have bidentate chelating properties. The produced compounds were formed as ML_2 based on the obtained analytical data. Aspirin binds to both oxygen of carboxyl groups. The complexes of metals were discovered to possess a range of inhibiting effects on microorganisms.

The present work the analysis of drugs and their modified forms using different physicochemical and electrochemical techniques. The Findings and Conclusion of present work is in two parts. Firstly, it attempts to investigate and developed Polarographic and Voltammetric methods for the analysis of such type of drug designing samples. On the basis of obtained results it could be concluded that Polarography has got some peculiar advantageous features and versatility. The Voltammeric and Polarographic methods have proved to be very powerful tools for the simultaneous determination of different origin samples like industrial use, pharmaceutical formulations, biological inorganic and organic compounds etc. Secondly design of modified anti-stroke medicines for better potency and safety for stroke problem.

Physiochemical, microbial and antiplatelet activity studies of 2-Acetoxy benzoic acid (Aspirin) and its complex with Co(II) have been done in aqueous and solid phase. Polarographic i.e. Direct current Polarography (DCP)& Differential Pulse Polarography (DPP) and Amperometric titration methods have been used for the qualitative and quantitative analysis of Aspirin and its complex with Co(II). Metal ligand interaction studied using Polarographic method at 25 ± 1ºC and ionic strength of μ = 1.0 (Ammonium chloride Buffer).

On the basis of results of Polarograpy, Amperometry and IR Spectral studies the probable formula of Co(II) - Aspirin has been designed and stoichiometric ratio is 1:2 (M:L). The drug co-ordinated to metal through the 2 oxygen atom of carboxyl group thus the drug act as bidentate ligand, which is finding from IR spectral analysis data.

Microbial study of Co(II)- Aspirin complex has been done against various pathogenic bacteria viz. *Staphylococcus aureus*, *Bacillus pumilus, Proteus mirabilis* and *Escherichia coli*. Using Disc diffusion method. The results revealed that the complex is more potent as compared to the parent drug.

The evaluation of Antiplatelet activity of drug metal complex was carried out on human plasma through a platelet aggregation analyser. Arachidonic acid and ADP were used to

gauge any changes in platelet shape and aggregation process. The parent drugs showed some antiplatelets activity, however their drug metal complexes demonstrated better efficacy.

Keywords: Metal ligand complex, Aspirin, Spectral studies, antimicrobial activity.

1.INTRODUCTION

Aspirin is a salicylate medication widely used worldwide. Aspirin's widespread use as an analgesic, anti-inflammatory, and antipyretic drug has significantly impacted the pharmaceutical industry. Aspirin is also used to treat rheumatic fever, pain, and other inflammatory diseases. Chemical modifications of aspirin have been widely performed, and numerous aspirin derivatives have been reported to exhibit a variety of biological actions, including antibacterial, antithrombotic, antiplatelet, and anticancer activities. Reduced-dose aspirin has also been shown to help avoid blood clot development, strokes, and heart attacks. Aspirin derivatives have been demonstrated to be antibacterial against Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli (Akinyele et al., 2019).

Various studies have been conducted to study the mechanisms involved in platelet aggregation. Several antiplatelet agents exert their effects through different mechanisms of action (Amidi et al., 2013, De Meyer et al., 2008, Maree and Fitzgerald, 2007). Among the most commonly used agents such as aspirin (by impairing thromboxane A2 synthesis through irreversible inhibition of cyclo-oxygenase I), clopidogrel (an irreversible antagonist of platelet ADP (adenosine diphosphate) receptor, P2Y12), and glycoprotein (GP) IIb–IIIa antagonists like abciximab AngiotensinII (AngII).

Currently available antiplatelet agents work through different mechanisms (Amidi et al., 2013, De Meyer et al., 2008, Maree and Fitzgerald, 2007). Among the most commonly used agents such as aspirin (by impairing thromboxane A2 synthesis through irreversible inhibition of cyclo-oxygenase I), clopidogrel (an irreversible antagonist of platelet ADP (adenosine diphosphate) receptor, P2Y12), and glycoprotein (GP) IIb–IIIa antagonists like abciximab, AngiotensinII (AngII).

Investigations are being conducted to determine the effect of metal binding on drug action. Researchers reported the synthesis of Co (II) and metformin (L) compounds with Ni (II) and Fe (II), creating an analgesic abbreviated as ML_2 (Lawal & Obaleye, 2010). Owing to their chemotherapeutic effects, more analgesic drug-metal complexes are required.

In conclusion, the successful synthesis and comprehensive characterization of complexes of novel transition metal with aspirin have been achieved and their significant antibacterial activity highlights their potential as promising candidates for antimicrobial drug development.

2. EXPERIMENTS

Materials and Methods:

The search of an appropriate, reproducible and simple analytical technique is a major task. Selection of a particular method for the estimation of a drug is usually based on consideration such as feasibility, accuracy, reproducibility, convenience and suitability for the purpose etc. Aspirin ($C_9H_8O_4$) purchased from SK Traders, Indore, India. The chemicals used were of AnalaR/BDH grade. Doubly distilled water and absolute ethanol (55:45 v/v) is used as solvent. Aspirin ($C_9H_8O_4$) solution (2.5 mM) is prepared by dissolving the requisite amount in (55:45 v/v) distilled water: ethanol. Stock solutions of 1 M potassium chloride and Ammonia-ammonium Chloride buffer were prepared by dissolving a requisite quantity of

each compound in suitable solvent. The pH of the test solution was adjusted to 8.0±0.1 pH by dilute solutions of HCl, NaOH whenever necessary.

Polarography and Amperometric titration

All the Polarographic experimental studies (DCP and DPP) were carried out in exploratory mode and peak analysis in determination mode on software connected Ω metrotism 757VA computrace (ion analyzer). The Polarographic cell consisted of a three-electrode assembly and a stirrer having a dropping mercury electrode (DME) as a working electrode, a platinum wire as an auxiliary electrode and saturated calomel electrode as a reference electrode. The nitrogen gas was bubbled for 15 minutes. A systronics digital pH meter model- 361 was used for pH measurements. The Amperometric titrations were performed on a manually operated set up equipped with a polyflex galvanometer (sensitivity 8.1×10−9 amp/div.) and an AJCO Vernier potentiometer. The capillary characteristic of the DME had a m2/3, t1/6 value of 2.5 mg2/3 s −1/2 at 60 cm effective height of mercury column.

IR Spectroscopy

The IR spectrum of solid complex was recorded using KBr pellets on a BRUKER Alpha ATR FTIR spectrophotometer of Sagar Institute of research Technology- Pharmacy, Bhopal, equipped with a Spectra-Tech Diffuse Reflectance Accessory (DRA). The spectrometer is equipped with the following: an air-cooled DTGS detector, a KBr beam splitter with a spectral range of 4000 to 650 cm-1. The instrument was run under vacuum during spectral acquisition. Spectra were recorded at a resolution of 4 cm-1, with the co-addition of 128 scans and a Blackman-Harris 3-Term apodization function was applied. Prior to analysis the samples were mixed, and lightly ground, with finely ground spectroscopic grade KBr. The spectra were then recorded using the Kubelka83 Munk mathematical function in the OPUS software to convert the spectra from reflectance into absorbance.

Antimicrobial screening

The purpose of Kirby – Bauer Disk Diffusion Susceptibility test is to determine the sensitivity or resistance of pathogenic aerobic and facultative anaerobic bacteria to various antimicrobial compounds in order to assist a physician in selecting treatment options for his /her patients the pathogenic organism is grown in Muller–Hinton Agar in the presence of various antimicrobial impregnated filter paper discs was used. The presence or absence of growth around the disks is an indirect measure of the ability of that compound to inhibit the microorganisms[9]

Polarography

The polarographic method of analysis was developed by Jaroslav Heyrovsky in 1922 which is originated by Voltammetric technique. He received noble prize in 1952 for developing this technique using dropping mercury electrode as the working electrode. The current potential curves are known as polarogram[(1)].

All compounds used were obtained commercially (Aldrich, BDH) and used without any further purification.

The complexes were then processed to find out their melting points using Gallenkemp melting equipment.

Synthesis of Metal Complex:

A preferred concentration of acetylsalicylic acid was dissolved in the smallest amount of water-DMF mixture possible, then 0.02M NaOH is added and stirred until completely dissolved. A clear solution was obtained which is aspirin sodium salt. Now an aqueous solution was prepared by adding 0.23g of 0.1M $CoC_{l2}.6H_2O$ to the aforementioned aspirin

sodium salt solution. After 3 Hours of refluxing, a purple ppt was produced, which was then filtered, then wash the ppt using ethanol, and dry, yielding 89% of yield. The same procedure was employed to generate further complexes with the compositions $[ML_2]$, $[ML_2(H_2O)_4]$ and $[M_2L_4]$, but with various molar ratios of the reactants.(Chohan et al., 2002). The excess of the solvent was removed using a rotary evaporator, followed by multiple washes with water, centrifugation, and drying (Vijayarohini et al., n.d.)

Characterization:

Using a Nicolet 6700 spectrometer, we measured the Fourier transform infrared (FT-IR) spectrum of virgin acetylsalicylic acid and acetylsalicylic acid with metal complex (Thermo, USA). UV-Vis spectroscopy with a wavelength ranging from 200 to 800 nm was used to evaluate complexed acetyl salicylic acid solutions in ethanol.

Estimation of antimicrobial activity:

Antimicrobial activity was determined for the prepared complexes against three types of bacteria: Serratia species, Escherichia coli and Bacillus subtilis. E1-sarrag5 and Abd E1-Wahab assessed the antimicrobial activity of the compounds. Antimicrobial activity was tested on seeded NA that had been pierced with 0.9cm diameter wells. Using methanol as the solvent, various concentrations i.e.0.10 percent and 01.00 percent (w/v) of sterile solution of the complexes and ligands was prepared. Wells were fiiled with 0.1 ml of solution taken from each concentration range and incubation done at 370 C for about 3 days. Antimicrobial activity was determined by measuring the size of the inhibition zone in terms of its diameter, and inhibition in growth was determined in percentage using the avg diameter of the bacteria colony grown on desired medium in comparison to the respective control cultures, as shown in the below equation.

Percentage of Inhibition = [(B – A)/B]100

Where B= Avg diameter of bacterial colony on the control and A= Avg diameter of bacterial colony on the test plate (Lawal & Obaleye, 2010)

3. RESULT AND DISCUSSION

Physical Measurements:

Because the complexes and ligands were soluble in ethanol and methanol in trace amounts but insoluble in water, the molar conductance were determined in DMF at temperature 27°C. The result observed ranged from 2.58 to 35.64 ($\Omega^{-1}cm^2mol^{-1}$). This is characteristic for metal complexes that are covalent in nature. The metallic complexes formed are pigmented and they possess a higher range of melting point, making them extremely stable until approximately 300°C of temperature. The analysis of metals was carried out experimentally. The observed results were extremely similar to theoretically obtained values. (Akinyele et al., 2019).

Polarography

For Polarographic experimental set were prepared keeping overall Ammonia-Ammonium Chloride buffer concentration fixed at 0.1M, the total volume of the test solution was made upto 100ml with 5% of 95% ethanol. The Aspirin concentration was varied from 0.0mM to 10mM. The pH of test solution was adjusted to 8.0±0.1. Polarographic reduction of Aspirin in 0.1M Ammonium- Ammonium Chloride buffer at pH 8.0±0.1 buffer (pH8.0±0.1) containing 5% ethanol exhibits well defined polarographic wave/peak at potentials $E_{1/2}$ and Ep -0.90 and -0.92V vs SCE. Its wave height and peak height was found to be proportional to the concentration of Aspirin. The plot of E vs log i/id–i was a straight line showing the reduction to be reversible.

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The quantitative determination of Aspirin is done by calibration and standard addition method. The direct current polarogram and differential pulse polarogram of Aspirin are depicted in Figure 4.11 and 4.12.

Co(II) give a well-defined polarographic waves and peaks with Half wave potential $E_{1/2}$--0.56 V. The metals and its complexe with the ligand understudy were found to be reversibly reduced involving two and three electrons. Which was evidenced by the plots of log i/(id-i) versus Potential (E). The shift in half wave potentials towards a more negative value a with increasing concentration of ligand and decrease in diffusion current indicated complex formation between bio metal ions to be used i.e Co(II) with drug Aspirin. The curves are shown in Figures 1-3.

Amperometric Experiment

A fully manually operated set up, a polyflex galvanometer (sensitivity 8.1×10^{-9} amp./Div.) and an AJCO vernier potentiometer was used for the amperometric estimation of the divalent Co(II) with antistroke drug Aspirin. DME was used as an indicator electrode, saturated calomel electrode (SCE) as reference electrode. The capillary characteristic of DME had a $m^{2/3}$, $t^{1/6}$=2.13 $mg^{2/3}$ $Sec^{-1/2}$ at 50cm effective height of mercury column.

To confirming the drug metal ratio, amperometric titrations were performed. Experimental sets of different but known concentration of aspirin were prepared in appropriate amount of supporting electrolyte i. e. potassium chloride and maximum suppressor i.e., 0.001% gelatin at pH 7.0 ± 0.1 and titrated separately against the standard solution of the iron ions whose pH has adjusted to that of the titrate (7.0 ± 0.1).

Co (II) gives a well-defined polarographic wave with half wave potential -0.56 V vs SCE in 0.1M KCl at pH 7.0 ± 0.1. The diffusion current was found proportional to the concentration of Co (II). The aspirin drug does not produce any wave under the said experimental conditions. The platue potential for the polarographic wave of Co (II) was -0.64 V versus Hg pool was applied for carrying out titration. On performing the amperometric of aspirin solution with standard solution of Co (II), the current volume plots resulted in “L” shaped curve. The end point is determined by graphical method revealed metal to drug ratio of 1:2. The result is shown in Figure 4.

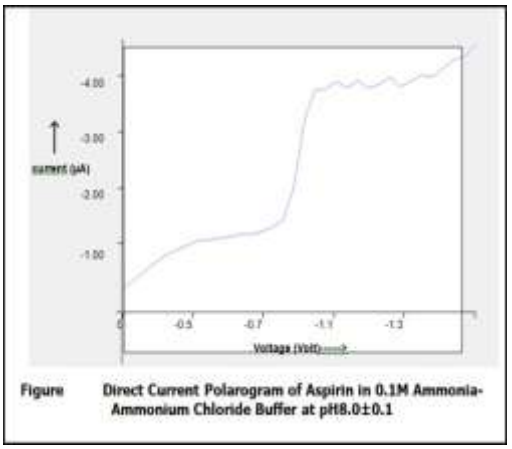

Figure Direct Current Polarogram of Aspirin in 0.1M Ammonia-Ammonium Chloride Buffer at pH8.0±0.1

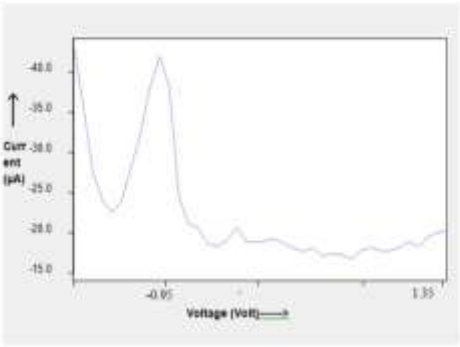

Figure Differential Pulse Polarogram of Aspirin in 0.1M Ammonia-Ammonium Chloride Buffer at pH 8.0±0.1

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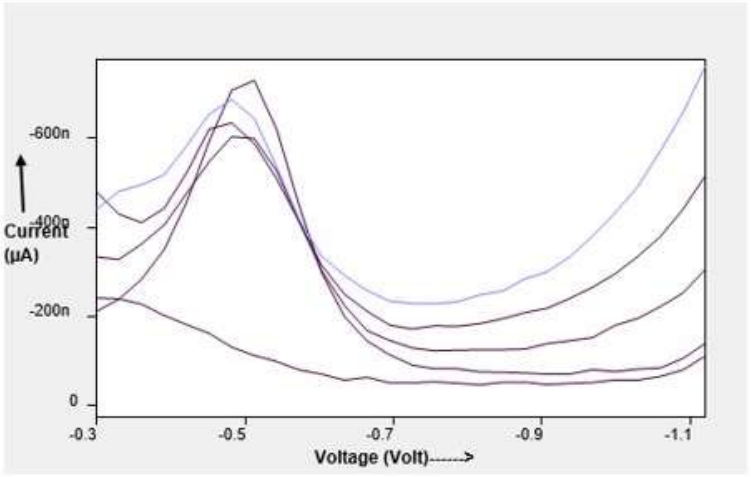

Figure DP Polarograms of Co (II) in 0.1 M Ammonium-Ammonium Chloride at pH 8.0±0.1 and without and with Aspirin, (i) 0.25 mM Aspirin (ii) 0.5 mM Aspirin (iii) 1.0 mM Aspirin and (iv) 1.5mM Aspirin

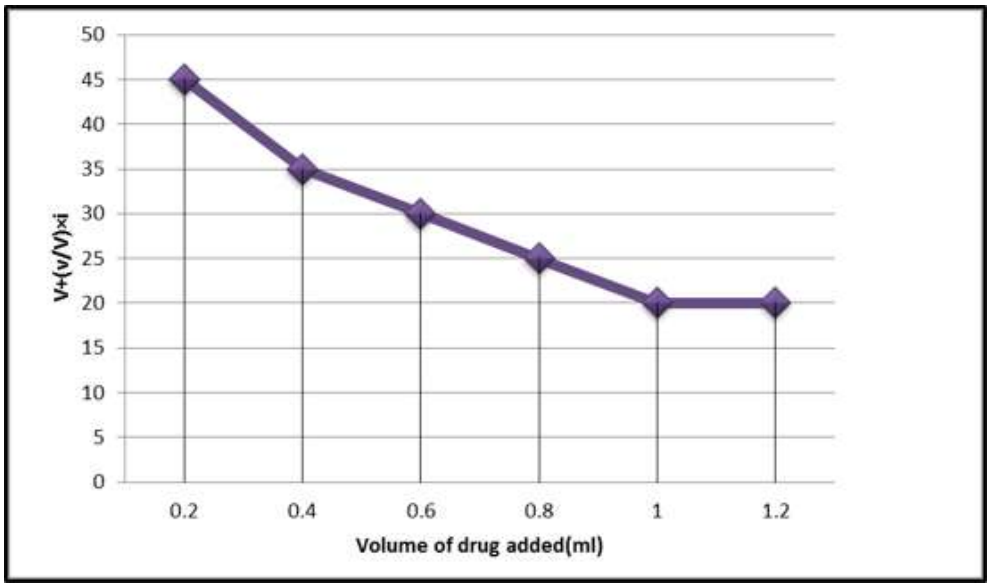

Figure 4: Amperometric titration of Aspirin (2mM/10ml) with Co (II) (1mM/ l) solution in 0.1 M KCl Supporting Electrolyte at pH 7.0±0.1.

Infrared Spectroscopy:

The IR bands of complexes and ligands that were structurally relevant. In the presence of carboxylic acid, the aspirin ligand exhibited two distinct bands, one at 1753 cm^{-1} for the (C=O) bond, and the second at 3489 cm^{-1} for the (O-H) bond in the presence of ester and carboxylic acid, respectively. Spectra of the complex of Co (II) show that two bands are present, which are (C=O) and (O-H) bands, which confirms that this ligand is in keto form. Also, it is inferred that the ligand is not in enolic form as v (C-O) bands are absent. According to the reduction in the (C=O) band to 1645 cm^{-1}, it has been determined that the carbonyl groups have been attached to the metal ion. The decrease in intensity and frequency of the (C=O) bond of the ester group is indicative of the ester of aspirin being involved in

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complexation, as indicated by the decrease in frequency and intensity of the (C=O) bond of the ester. Also discovered as novel bands in the spectrum for the metal complexe ranging from 520 to 680 cm^{-1} are (M-O) , while Co (II) complexes contain one more band at 462 cm^{-1} that has been ascribed to (M-O) as well (M-Cl). The Spectra shown in Figure 5.

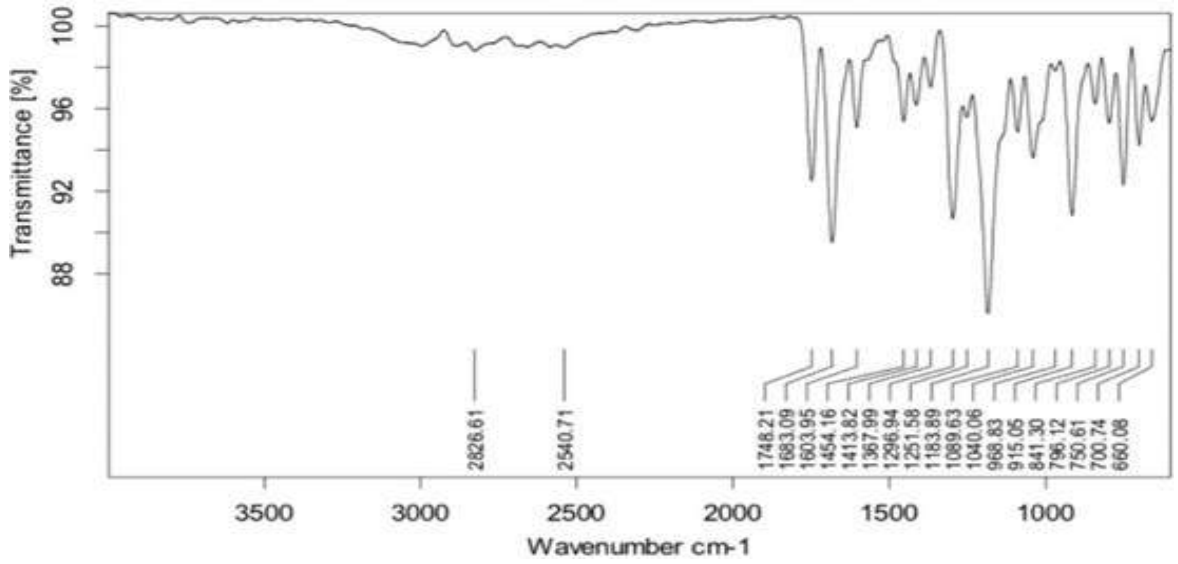

Figure 5: Infrared spectra of the Co(II) Aspirin complex

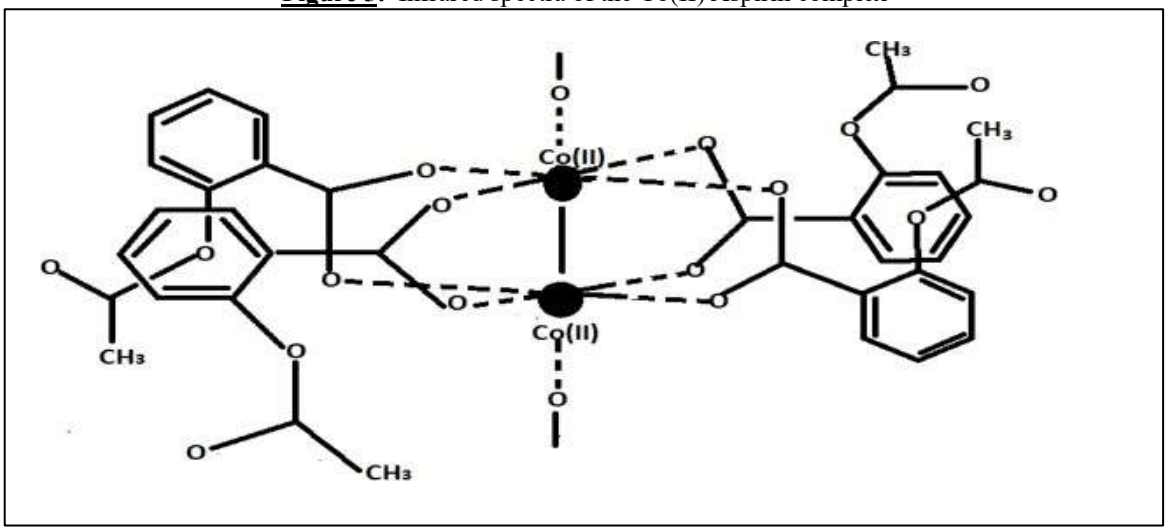

Figure 6: Speculative Co(II) complex of Aspirin

Antimicrobial Activities

The prepared drug metal complexes of Aspirin with life essential metal Co(II) was tested against gram positive and gram negative bacteria viz. *Staphylococcus aureus, Bacillus pumilus, Proteus mirabilis* and *Escherichia coli* respectively by disk diffusion method [8-10] on the basis of results obtained by this study it could be concluded that prepared drug metal complexes were found to have more antibacterial activity against gram positive and gram negative bacteria and compare this with the standard antibiotic *Penicillin*. The range of concentrations of the drug metal complexes used for microbial activity against various micro-organisms is in between 100-500 mg/ml and the 37^0C temperature was selected and time duration was 36 hours. A good number of reproducible results have been observed. On the

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basis of obtained results by antimicrobial activity against various bacteria it could be concluded that very good results show (Table 1) by Co(II)-Aspirin complex about 50% inhibition against *Staphylococus aureus*.

Table 1:

S. N	Test Organism	Inhibition zone (mm) Con. of complex 02mM/10 ml (B)	Control Co(II) metal (A)1.0 mM/10ml	% Change over Metal (A-B/A) ×100	Control(Y) Aspirin drug 1.0mM/10ml	% Change Over Drug (Y-B/Y) ×100
1	***Staphylococus aureus***	09	18	50	33	30
2	***Bacillus pumilus***	29	34	14	27	7.4
3	***Proteus mirabillis***	--	25	--	17	--
4	***Escherichia coli***	14	17	18	11	-27

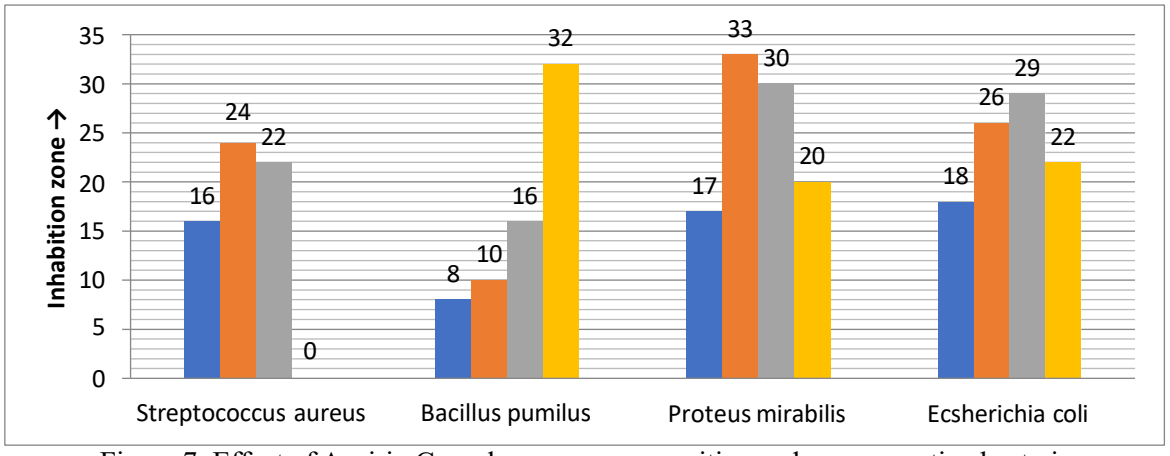
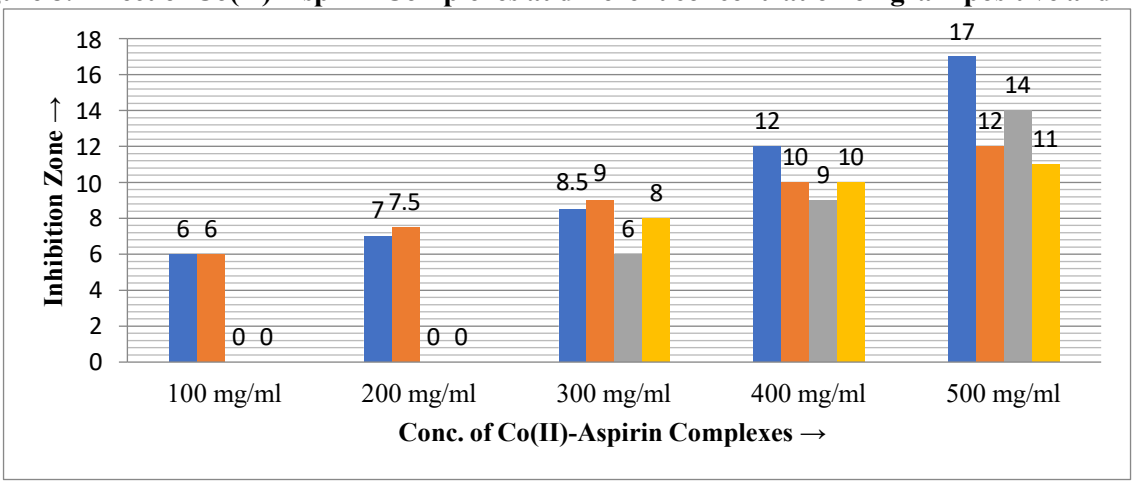


Figure 7: Effect of Aspirin Complexes on gram positive and gram negative bacteria

Figure 8: Effect of Co(II)-Aspirin Complexes at different concentration on gram positive and gram-negative bacteria



4. CONCLUSION

The structure and behaviour of an Important anti-stroke medicine Aspirin complexe with life essential metal ions Co(II) has studied. To know the change in physical and chemical parameters of these medicines, some electrochemical methods i.e. Polarography, Amperometry and Spectral methods viz FTIR and Elemental methods have been used. To knowing the change in biological behaviour of synthesized anti-stroke complexes, Microbial activity against various pathogenic bacteria and viruses have been done. To investigate pharmacological activity of prepared complexes Antiplatelet aggregation test have also been done against both pure and prepared complexes.

The polarographic (Direct current polarography (DCP) Differential pulse polarography (DPP) and amperometry were used for qualitative and qualitative estimation of Aspirin and Clopidogrel medicines in pharmaceutical formulations.

In 0.1M Ammonium- Ammonium Chloride buffer at pH 8.0±0.1 produce well defined polarographic wave/peak at $E_{1/2}$ and Ep -0.90 and -0.92V vs SCE. Its wave/peak height was found to be proportional to the concentration of Aspirin. The plot of ΔE vs log i / id – i was a straight line showing the reduction to be reversible.

The quantitative determination of Aspirin is done by calibration and standard addition methods. The results of which showed the presence of 99.01 mg of Aspirin, which is in good agreement as claimed by the manufacturer (100mg).

The data obtained by Polarography, Amperometric, Elemental analysis and IR measurements it is quite clear that metal complexes of Aspirin show stoichiometric ratio of 1:2

To Study the concentration and the formation constant of the complexes plot of $\Delta E_{1/2}$ = Shift in the $E_{1/2}$ = $(E_{1/2})c$ - $(E_{1/2})s$ against log Cx logarithms of concentration of the ligand was drawn.

The plot was linear showing the formation of single complex species in solution. Lingan's treatment of the observed polarographic data revealed 1:2 Metal: Aspirin complex formation with formation constant log β_1 (Co) = 5.67.

Co(II) metal ion gives a well-defined polarographic wave in 0.1 M potassium chloride (KCl) at pH 7.0±0.1. With 5% of 95% of ethanol were selected. The diffusion current was found to be directly proportional to concentration. All the drug are does not produce any wave under said experimental condition. The plateau potential for the polarographic wave of metal is as Co(II) -1.30V Vs SCE was applied for carrying out amperometric titration. Metal was taken as the titrate and the drug solution was taken as titrant. The current volume plots resulted in L shaped curve for Aspirin. The end point as located by graphical method revealed metal to drug ratio of 1:2 ratio, which is in agreement with the author's observation on the metal: ligand complex equilibrium using polarographic method.

The IR spectra of the titled drugs and their complexes with some bio metal Co(II) was recorded in 4000-500 cm^{-1} region. The observation of IR peaks clearly indicates the involvement of the carboxylic group in complex formation in Aspirin.

The biological activity of this drug was tested by the antimicrobial study of the above-mentioned bio metal drug complexe of Aspirin against various pathogenic bacteria and fungi viz. *Staphylococcus aureus, Bacillus pumilus, Proteus mirabillis* and *Escherichia coli*. Disc diffusion method is employed for the present work. The complexes used in the present study were found effective with promising results against a microbes. The Co(II)-Aspirin complex show very good inhibition over 50% against *Staphylococus aureus*.

The antiplatelet aggregation activity of the prepared metal- drug complexes were measured by using human plasma by antiplatelet aggregation method.

On the basis of obtained results by Physicochemical and Electrochemical methods, Microbial study and Pharmacological analysis it could be concluded that prepared metal complexes are found to be medicinally effective as compared to the respective parent drugs, it would also be possible to recommend the prepared Co(II) - Aspirin complex to ascertain their possible use as more potent drugs in lieu of the parent lead compound.

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