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Thermochemical Properties and Bioactivity of the Metallic Complexes of Levofloxacin

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ABSTRACT

The aim of this study was complexation of levofloxacin with iron, calcium, copper and ammonium salts. The formation of the metal complexes were confirmed by TLC and FT-IR. The newly formed metal complexes showed characteristics thermochemical behaviours such as differential scanning calorimetric and thermo gravimetric properties along with bioactivity. Additionally, the synthesized metal complexes have shown some extents of antimicrobial activities. It is concluded that the potentiality of metal-levofloxacin complexes are due to the action and presence of siderophores within bacterial cells.

KEYWORDS

Levofloxacin, Iron, Calcium, Copper, Ammonium, TLC, FT-IR, DSC, TGA.

INTRODUCTION

Drug-metal interaction is a modification of the effect of a drug when administered in presence of a metal. The effect may be an increase or a decrease in the action of the drug, or it may be an adverse effect that is not normally associated with the drug. The particular interaction may be the result of a chemical-physical incompatibility of the drug and the metal, or a change in the rate of absorption or the quantity absorbed in the body, the binding ability of the drug, or an alteration in the ability of receptor sites and cell membranes to bind the drug complex. Most adverse drug-metal interactions are either pharmacodynamic or pharmacokinetic in nature [1-5].

The complexation of levofloxacin with metals demonstrates a calculative prediction of their interaction in biological system and might alter the pharmacokinetics (absorption, distribution, metabolism and excretion) and pharmacodynamics of the drug. The chelation might increase its side effects or reduce the beneficial effects of the drug is intended to have. The metal drug complex might increase the drugs activity by having a synergistic effect, which might eventually cause toxicity, *e.g.*, an antihypertensive drug might reduce blood pressure to such an extent due to metal complex formation that the patient may suffer from hypotension, leading to fatal conditions [1].

Levofloxacin is a third generation fluoroquinolone antibiotic. It is used to treat a number of bacterial infections including acute bacterial sinusitis, pneumonia, urinary tract

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infections, chronic prostatitis and some types of gastroenteritis. Along with other antibiotics it may be used to treat tuberculosis, meningitis, or pelvic inflammatory disease. It is available in oral dosage form, intravenously and as eye drop [6,7].

Calcium as a mineral is highly abundant in our body as well as obtained from dietary sources. Pregnant women or one of middle age usually take calcium tablets and might therefore, be a subject of such drug metal interaction. Copper functions as a component of a number of metalloenzymes acting as oxidases to achieve the reduction of molecular oxygen. The Recommended Dietary Allowance (RDA) of copper for adult men and women is 900 µg/day [8]. If this minute amount is used in chelate formation, deficiency of copper might as well take place along with interaction effects. Similarly, iron is an essential mineral element, used in various physiological systems including blood and haemoglobin. Interaction with drug might hamper its normal physiological functions. Many drugs also contain ammonium salts, which might interact with levofloxacin in the body. Nitrogen from the body is excreted and balanced in the form of urea or ammonium salts, thus formation of chelates may retain it in the body and cause toxicity [8].

This paper describes the formation of complexes of levofloxacin with iron, calcium, copper and ammonium salts. To confirm the newly formed complexes TLC and FT-IR were carried out followed by their thermochemical properties such as differential scanning calorimetric (DSC) analysis, thermogravimetric analysis (TGA) along with bioactivity were also measured.

EXPERIMENTAL

Levofloxacin (potency 99.98 %) was a kind gift from Active Fine Chemicals Ltd., Dhaka, Bangladesh and HPLC grade methanol and chloroform were purchased from Sigma Aldrich, Germany. Nanopure water was used and collected from own source. Analytical grade ammonium hydrogen phosphate, ferric nitrate, calcium fluoride and copper(II) nitrate were purchased from local market. TLC plate was used HSF-254 from Merck, Germany.

The metal complexes of levofloxacin were synthesized according to mole ratios. Equal volumes of (20 mL) of methanol solution of 0.5 mM metal were prepared by taking 0.039 g of calcium fluoride, 0.066 g of ammonium hydrogen phosphate, 0.09375 g of copper(II) nitrate and 0.202 g of ferric nitrate separately and the drug (0.36 g) was added to each of the solutions individually with stirring followed by heated at 60 °C. The solutions were then filtered and left at room temperatures overnight. The crystals formed were filtered, washed with water and methanol and vacuum dried [9].

TLC: To observe the formation of complexes, TLC was done, where the solvent system was 50 % methanol in chloroform, the five samples being dissolved in chloroform (pure drug and four drug-metal complexes).

DSC thermogram: DSC thermograms were obtained from the DSC instrument, model: DSC 60, Shimadzu, Japan. The thermograms were taken in aluminium seal pan at the temperature range of 30-300 °C where temperature rising rate was 10 °C/min in nitrogen gas at a flow rate of 20 mL/min. All the drug metal complexes along with pure levofloxacin were studied and the results recorded.

TGA: TGA were carried out in TGA 50H, Shimadzu, Japan. The thermograms were taken in aluminium pan at the temperature range of 21-600 °C with-hold time 5 min where temperature rising rate was 10 °C/min in nitrogen gas at a flow rate of 10 mL/min. All the drug metal complexes along with pure levofloxacin were studied and the results recorded. All the samples were studied under same condition and results recorded.

FT-IR: Appropriate quantity of KBr and standard drug and samples (in the ratio 100:0.1) were mixed by grinding in an agate mortar. Pellets were made with about 100 mg mixture. FT-IR Spectra were recorded with FT-IR 8400S Shimadzu spectrophotometer in the range of 4000-400 cm^{-1}, resolution: 4 cm^{-1}, number of scans: 30 times.

Antimicrobial study: The antimicrobial study was done according to disc diffusion method [10]. Dried and sterilized filter paper discs (6 mm in diameter) containing test samples and standard ciprofloxacin (30 µg/disc) was inserted in the petridishes containing test microorganisms on nutrient agar medium. They are incubated at 40 °C for 24 h, followed by inversion and incubation at 37 °C for 24 h. The diameter of the zones of inhibition was measured in millimetres.

RESULTS AND DISCUSSION

Drug and metals formed complexes and both crystals and amorphous solids were obtained. To prove complexation, TLC was carried out fewer than 50 % methanol in chloroform solvent system we found five different single spots from the five complexes, which were also different from their parent drug (Table-1). Each spot indicates presence of a new complex.

TABLE-1
R_f VALUES OF LEVOFLOXACIN AND ITS COMPLEXES

Item	R_f value at the same condition
Pure levofloxacin	0.30
Ca-levofloxacin complex	0.50
Cu-levofloxacin complex	0.60
Fe-levofloxacin complex	0.70
NH_4-levofloxacin complex	0.75

Pure levofloxacin showed melting endotherm at 224.8 °C and that of copper complex and iron complex showed exothermic thermograms indicating the complex formation between copper and levofloxacin as well as iron and levofloxacin. The melting endotherm of calcium-levofloxacin and ammonium-levofloxacin complex was at 231.90 and 233.29 °C, respectively which were different from levofloxacin melting endotherm. It also indicated the formation of new complex between drug and metals respectively (Fig. 1).

For pure levofloxacin, we found 5 % degradation at 280 °C. Most probably, a water molecule is removed at this point. Similarly, at 402 °C, it degraded 35 %, correspondingly which accounts for releasing a water molecule and a hydroxyl molecule. At 599 °C, we found 23 % degradation, which results from the loss of a methyl group and a nitrogen.

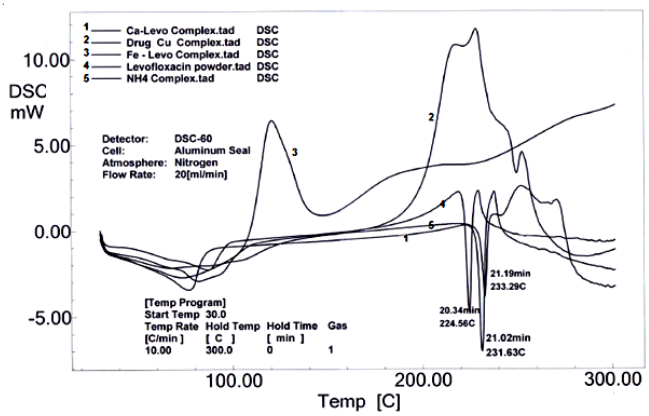

Fig. 1. Overlaid DSC thermograms of levofloxacin and its complexes

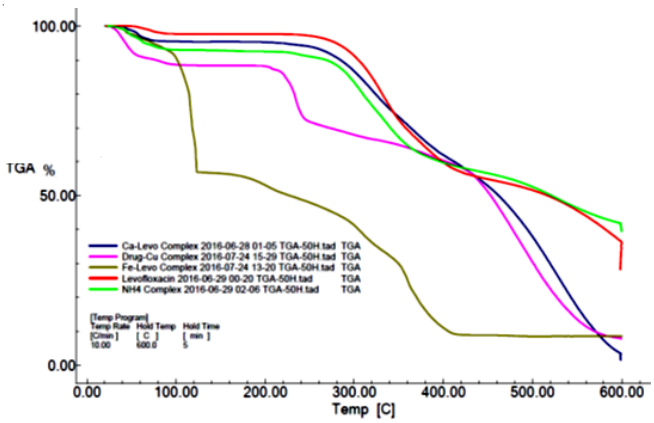

Fig. 2. Overlaid TGA of levofloxacin and its complexes

The drug complexes show, however, a completely different degradation pattern. For iron-drug complex, 43.15 % was seen to degrade at 123.90 °C, whereas in copper-drug complex only 14.90 % degraded at 219.32 °C. For drug complex with calcium, we found steep degradation pattern where only 8 % degraded within 267 °C, while ammonium-drug complex, 11.68 % was broken down at 274 °C. The pattern suggests a complete different nature for the parent drug and its complexes (Fig. 2). FT-IR is most useful in providing information about the presence or absence of specific functional groups. If two pure samples display the same IR spectrum it can be argued that they are the same compound. Similarly, any shifts or disappearance of peaks indicate presence of a new compound. The IR spectrum for iron-drug complex was seen to demonstrate a new pattern of peaks compared to pure levofloxacin powder. The peaks from 3080 to 2690 was seen to be abolished and a single peak was seen in place of double at 3435.22 cm^{-1} for drug-iron complex. For calcium complex of the drug, we see a broad peak at 3437 cm^{-1}, instead of the sharp one in the parent drug and for both copper-drug and ammonium-drug complex, we see a single peak at 3431 and 3435 cm^{-1}, respectively instead of the two peaks in levofloxacin powder at 3423 and 3273 cm^{-1} respectively (Fig. 3).

The drug-metal complexes were screened for antibacterial activity against five Gram-positive and eight Gram-negative bacteria. The test samples of the metal complexes revealed significant inhibitory activity against the test pathogens having zone of inhibition ranging from 35.0-45.0 mm, with the highest inhibition of bacterial growth by the calcium and copper complexes (45.0 mm) against *Bacillus cereus* (Table-2). The inhibitory activity of the extractives was compared with ciprofloxacin as standard. The minimum activity was obtained by iron-drug complex against *Salmonella typhi* (35.0 mm). Thus, the complexes itself had more or less significant antimicrobial activity against the pathogens and can be also used as an antimicrobial agent. Bacteria express a wide variety of siderophores and siderophore receptors, which are known to be involved in identification and uptake of bacterial catechol-containing siderophores such as enterobactin [11,12]. Enterobactin is a well-characterized siderophore which is produce by *Escherichia coli* and other *Enterobacteriaceae* and is utilized by a number of bacteria (including *P. aeruginosa*) to sequester iron [13-15]. Zaniewski *et al.* [16] have described a series of pyridone-conjugated monobactam analogues with *in vitro* antibacterial activity against Gram-negative species including *P. aeruginosa*, *Klebsiella pneumoniae* and *E. coli*, where, it has proven that the siderophore receptors PiuA and PirA play a role in drug uptake in *P. aeruginosa* strain PAO1. Thus, it can be hypothesized that previously studied analogues/complex compounds are playing similar type of biological functions within bacterial

TABLE2
ANTIMICROBIAL ACTIVITY OF LEVOFLOXACIN AND ITS COMPLEXES

Test microorganisms	Diameter of zone of inhibition (mm)				
	Levofloxacin	Calcium complex	Copper complex	Iron complex	Ammonium complex
Gram-positive bacteria					
Bacillus cereus	45.0	45.0	45.0	40.0	40.0
B. megaterium	42.0	43.0	41.0	42.0	38.0
B. subtilis	44.0	44.0	38.0	38.0	40.0
Staphylococcus aureus	45.0	40.0	42.0	43.0	41.0
Sarcina lutea	44.0	43.0	39.0	44.0	40.0
Gram-negative bacteria					
Salmonella paratyphi	41.0	38.0	45.0	43.0	40.0
S. typhi	42.0	41.0	43.0	35.0	42.0
Vibrio parahemolyticus	44.0	41.0	41.0	44.0	44.0
V. mimicus	45.0	41.0	45.0	43.0	42.0
Shigella dysenteriae	43.0	44.0	41.0	45.0	43.0
S. boydii	45.0	44.0	41.0	42.0	42.0
E. coli	43.0	43.0	43.0	43.0	42.0
Pseudomonas aeruginosa	44.0	44.0	40.0	41.0	44.0

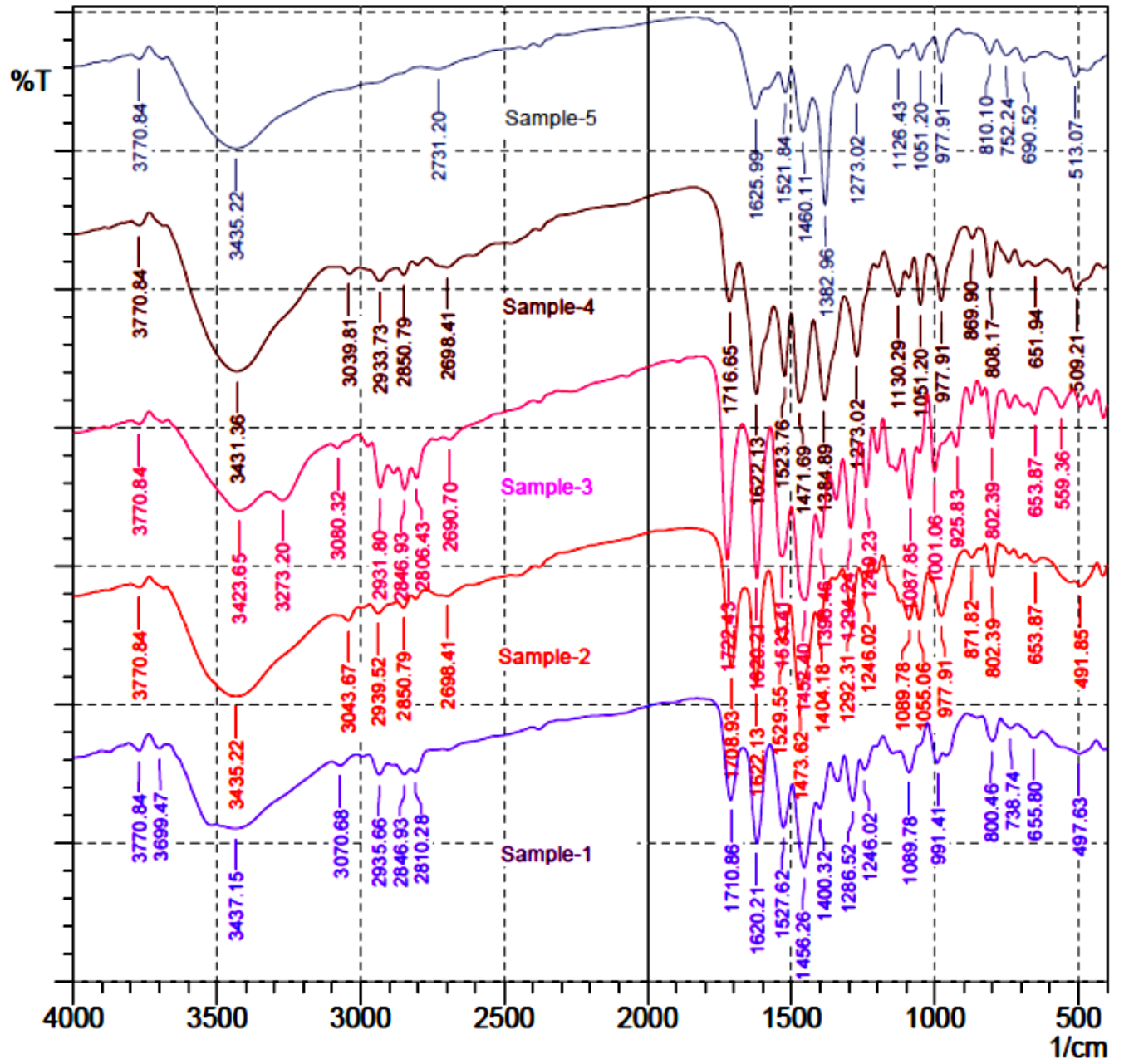

Fig. 3. Overlaid FT-IR of levofloxacin and its complexes (Sample 1 = Ca-levofloxacin complex, Sample 2 = NH_4-levofloxacin complex, Sample 3 = Pure levofloxacin powder, Sample 4 = Cu-levofloxacin complex and Sample 5 = Fe-levofloxacin complex)

cell and showing antimicrobial activity, which needs further preliminary biological assays to justify the idea.

Conclusion

It were demonstrated the different metal complexes of levofloxacin formed with calcium, iron, copper and ammonium. All these metals are elements of body compositions, where calcium is found in bones and teeth, iron in haemoglobin, copper as coenzymes and ammonium as nitrogenous compounds in our body. Thus, when the drug is ingested, it might interact with these elements from the body and result into alteration of potency of itself. We have studied the *in vitro* interaction of the drug and the metals where the drug forms complexes with calcium, iron, copper and ammonium as revealed by DSC, TGA, TLC, FT-IR. It is also justified that the antimicrobial activity of the complexes against five Gram-positive bacteria and eight Gram-negative bacteria were found that the metal complexes showed significant antimicrobial activity.

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