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Spectroscopic, thermal and antitumor investigations of sulfasalazine drug *in situ* complexation with alkaline earth metal ions

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ABSTRACT

The complexes of sulfasalazine (H_3Suz) with some of alkaline metals Mg(II), Ca(II), Sr(II) and Ba(II) have been investigated. Sulfasalazine complexes were synthesized and characterized by spectroscopic tools; infrared spectra, electronic and mass spectra. The IR spectra of the prepared complexes were suggested that the H_3Suz behaves as a bi-dentate ligand through the carboxylic and phenolic groups. The molar conductance measurements gave an idea about the non-electrolytic behavior of the H_3Suz complexes. The thermal decomposition processes for metal(II) complexes of H_3Suz *viz*: $[M(HSuz)(H_2O)_4]$ (where M = Mg(II), Ca(II), Sr(II) or Ba(II)) have been accomplished on the basis of TG/DTG and DTA studies, and the formula conforms to the stoichiometry of the complexes based on elemental analysis. The kinetic analyses of the thermal decomposition were studied using the Coats–Redfern and Horowitz–Metzger equations. The antitumor and antimicrobial activities of the H_3Suz and their alkaline metal(II) complexes were evaluated.

1. Introduction

Sulfasalazine (Formula 1, H_3Suz) is a sulfa drug, a derivative of mesalazine (5-aminosalicylic acid abbreviated as 5-ASA), used primarily as an anti-inflammatory agent in the treatment of inflammatory bowel disease as well as for rheumatoid arthritis [1–4]. When dealing with the interaction between drugs and metal ions in living systems, a particular interest has been given to the interaction of metal ions with antibiotics. Antibiotics that interact with metal ions constitute a class of drugs which has been widely used in medicine both towards human beings and animals [5,6]. Many drugs possess modified pharmacological and toxicological properties when administered in the form of metallic complexes. Probably the most widely studied cation in this respect is Cu(II), since a host of low-molecular-weight copper complexes have been proven beneficial against several diseases such as tuberculosis, rheumatoid, gastric ulcers, and cancers [7–10]. In the literature survey, there is little attention concerning the mode of coordination of H_3Suz with metal ions. Previous studies [11–18] performed the complexation of sulfa drugs did not focus on the coordination behavior, but only dealt with the solution state and crystal structures of its metal complexes. In this article the coordination mode of (H_3Suz) chelating *via* alkaline metals such as Mg(II), Ca(II), Sr(II) and Ba(II) have been investigated. The solid products were isolated and characterized by elemental analysis CHNS, molar conductance (infrared and solid reflectance) spectra and thermogravimetric analyses TG/DTG–DTA. The free ligand and its metal complexes were tested against the bacterial species *Staphylococcus aureus*, *Bacillus anthracis*, *Pseudomonas aeruginosa*, and *Escherichia coli*, also fungal species like *Candida albican* and *Aspergillus*. An antitumor activity of Mg(II) and Ca(II) complexes was checked against liver and colon carcinoma cell lines.

2. Experimental

2.1. Chemicals and synthesized

All chemicals used are analytical grade and purchased from Aldrich and Merck companies. The complexes were prepared by mixing H_3Suz (2 mmol) and 1 mmol of metal chlorides: $MgCl_2$, $CaCl_2{\cdot}H_2O$, $SrCl_2$, or $BaCl_2{\cdot}2H_2O$ in mixed solvent 50%/50% (v/v) methanol–water (40 mL), then pH of the solution was adjusted to 8.0–9.0 with 0.1 M NH_4OH solution and the reaction mixture was continuous stirred at 60 °C for 2 h and left to stand overnight. The precipitated complexes were filtered off, washed with MeOH and double distilled water and dried under vacuum at room temperature over anhydrous P_2O_5.

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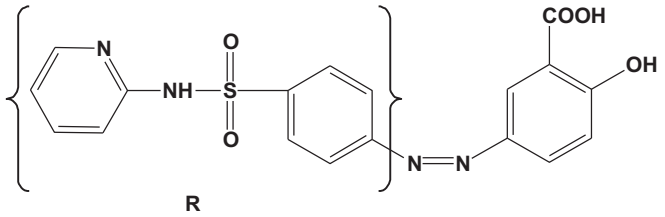

Formula 1. The chemical structure of sulfasalazine (H_3Suz) compound.

2.2. Physical measurements

Carbon, hydrogen, sulfur and nitrogen contents were determined using a Perkin-Elmer CHN 2400. The metal content was found gravimetrically by converting the complexes into their corresponding oxides. Infrared spectra were recorded on ALPHA FT-IR spectrophotometer from Bruker within range (4000–400 cm^{-1}) in KBr pellets. The electronic solid reflectance spectra were studied on the solid state for the H_3Suz and their complexes using Shimadzu UV-Vis. 3101 pc spectrophotometer within the range 800–200 nm. Molar conductance's of the freshly prepared solutions of the H_3Suz and their Mg, Ca, Sr and Ba(II) complexes with concentration of 1.0×10^{-3} M in DMSO were measured using Jenway 4010 conductivity meter. Mass spectra were scanned for the free H_3Suz and its complexes using AEIMS30 mass spectrometer. Thermo gravimetric analysis TG/DTG and differential thermal analysis (DTA) were carried out in dynamic nitrogen atmosphere (30 mL/min) with a heating rate of 10 °C/min using a Shimadzu TGA-50H and DTA-50H thermal analyzer.

2.3. Biological evaluation

2.3.1. Antimicrobial activity test

According to Gupta et al. [19], the hole well method was applied. The investigated isolates of bacteria were seeded in tubes with nutrient broth (NB). The seeded NB (1 mL) was homogenized in the tubes with 9 mL of melted (45 °C) nutrient agar (NA). The homogeneous suspensions were poured into Petri dishes. The holes (diameter 4 mm) were done in the cool medium. After cooling, 2 mL of the investigated compounds were applied using a micropipette. After incubation for 24 h in a thermostat at 25–27 °C, the inhibition (sterile) zone diameters (including disc) were measured and expressed in mm. An inhibition zone diameter over 7 mm indicates that the tested compound is active against the bacteria and fungi under investigation. The antibacterial activities of the investigated compounds were tested against bacteria *S. aureus*, *E. coli*, *B. anthracis* and *P. aeruginosa* and fungi *C. albican* and *Aspergillus niger*.

2.3.2. Antitumor activity test

The cytotoxic effect of the chemical synthesized of H_3Suz complexes was expressed by the cell viability as estimated by the sulfo-rhodamine B assay [20]. Sulfasalazine was used as an inhibitor of GSTs (glutathione S-transferase), to evaluate the relative role of GSTs in mediating *cis*-platinum resistance in two human small-cell lung cancer cell lines, NCI H-69 and H-2496 by modulation of *cis*-diamminedichloroplatinum(II) cytotoxicity [21]. The antitumors tests were conducted by the pharmacology unit, National Cancer Institute, Cairo University, Egypt, regarding to doxorubicine as standard anticancer agent. In briefly, the carcinoma cell lines (10^4 cells/well) were plated in the bottom of 96-multiwell plate containing serum free media, then treated with 20 μg from different concentrations (0–10 μg) for each tested compound. Triplicates were prepared for each concentration. After incubation at 37 °C for 48 h in humidified 5% CO_2 atmosphere, the cells were fixed, washed and stained with sulfo-rhodamine B-stain. The excess of sulfo-rhodamine was removed by washing using acetic acid. The adherent stain was recovered using Tris–EDTA buffer and the intensity of the stain color was measured by ELISA reader at 595 nm. The relation between the viability of tumor cell lines and concentration of the tested compound was plotted. The cytotoxicity of each compound was reflected from the calculation of the half maximal inhibitory concentrations (IC_{50}).

Table 2
Main infrared data of H_3Suz complexes.

Complexes	υ(O–H)	υ_{as}(COO)	υ_s(COO)	Δυ(COO)	δ(OH)	υ(C–O)
Mg(II)	3425	1600	1354	246	1389	1260
Ca(II)	3430	1596	1354	242	1390	1266
Sr(II)	3448	1589	1354	235	1389	1261
Ba(II)	3441	1591	1356	245	1388	1269

3. Results and discussion

The elemental analysis (C, H, N and S) agrees quite well with the speculated structure of the colored H_3Suz complexes (Table 1). The prepared complexes have an orange color. They are thermally stable above >250 °C, soluble in DMSO and DMF. The conductivity data were measured in DMSO at room temperature and found to be in the range of non-electrolytes [22,23]. The interpretation concerning the decreasing of conductivity values resulted to the de-protonation of both OH of carboxylic and phenolic groups for the H_3Suz ligand. This assumption proves that free ligand acts as a bi-dentate fashion *via* carboxylic and phenolic groups and also attributed to the participation of carboxylic group as a monodentate chelate.

3.1. Infrared spectra

Fig. 1A–E gave the infrared spectra of the free H_3Suz and their metal Mg, Ca, Sr and Ba(II) complexes. The essential region 1700–1200 cm^{-1} in the infrared spectra of all complexes comparison with the free H_3Suz ligand was identified and assigned in Table 2. The interpretation data of Mg(II), Ca(II), Sr(II), and Ba(II) H_3Suz complexes are not recorded absorption band at 1676 cm^{-1} which distinguished to the υ(C=O) vibration of the carboxylic group in the free H_3Suz ligand, that is meaning the involvement of the carboxylic group in the chelation with metal(II) ion. The asymmetric stretching vibration of the carboxylate group, $\upsilon_{as}(COO^-)$, which appears at 1600, 1596, 1589 and 1591 cm^{-1} for Mg(II), Ca(II), Sr(II), and Ba(II) H_3Suz complexes, respectively, are absent in the spectrum data of the free H_3Suz ligand. The infrared

Table 1
Elemental analyses and physical data of H_3Suz complexes.

Complexes	Mwt	mp (°C)	Color	%Content found (calc.)				Λ (S cm^2 mol^{-1})
				C	H	N	S	
$[Mg(HSuz)(H_2O)_4]$	494.31	275	Orange	(43.69) 44.29	(4.45) 5.22	(11.33) 11.89	(6.47) 6.21	24.84
$[Ca(HSuz)(H_2O)_4]$	510.08	300	Orange	(42.35) 43.63	(4.31) 3.96	(10.98) 11.00	(6.27) 5.82	27.41
$[Sr(HSuz)(H_2O)_4]$	557.62	251	Orange	(38.74) 38.55	(3.94) 4.43	(10.04) 9.94	(5.74) 5.49	23.59
$[Ba(HSuz)(H_2O)_4]$	607.33	243	Orange	(35.56) 36.08	(3.62) 3.74	(9.22) 9.44	(5.27) 5.68	21.9