SYNTHESIS, PHYSICOCHEMICAL ANALYSES, AND ANTIBACTERIAL ACTIVITY OF THE Fe(III) SALT OF 4-AMINE-N-2-THIAZOLYL-BENZENE SULFONAMIDE

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ABSTRACT

The synthesis and characterization of the Fe(III) complex of sulfathiazole, is presented herein. The compound was prepared by using a conventional precipitation technique, and was characterized by thermogravimetric analysis (TGA), differential thermal (DTA), differential scanning calorimetry (DSC) under oxygen atmosphere, chemical analysis, infrared spectroscopy, powder ray-X diffraction (PXRD), and scanning electron microscopy (SEM). The antibacterial activity of the complex was screened against *S. aureus*, showing an appreciable activity, compared with the free ligand.

Keywords: Sulfonamide - iron(III) complex - sulfathiazole ligand.

1. INTRODUCTION

Sulfathiazole (ST) or *p*-amino-benzenesulfonamide is a synthetic substance derived from sulfanilamide by replacing one amidic hydrogen by a thiazole group^{1,2}. Sulfathiazole was the first effective chemotherapeutic agent systematically employed for the prevention and cure of bacterial infections in humans³. In addition, the sulfa drugs have been used in food-producing animals, to promote their growth, and for therapeutic and prophylactic purposes^{4,5}. The sulfanilamides carry out their antibacterial action by the competitive inhibition of the enzyme dihydropterae synthetase towards the *p*-aminobenzoate substrate⁶.

ST is clinically one of the most used drugs. Casanova *et al.* have reported its use in the inhibition of carbonic anhydrase and superoxide dismutase activity^{7,8}.

Besides, ST and their metal complexes have many applications, such as diuretic, antiglaucoma or antiepileptic drugs, among others,^{9,10} and in many cases, the activity of the complexes is much better than the free ligand^{11,12}.

In the last years, we have synthesized and studied the structural and physicochemical properties of metallic complexes which have interesting applications, such as their use as precursors to obtain oxides by thermal decomposition¹³⁻¹⁶.

In this work, we report the preparation, physicochemical analysis and antibacterial activity of iron(III) sulfathiazole.

The interest for studying the behavior of Fe(III) against sulfathiazole is due to the fact that most of the researches relate to divalent ions. Moreover, the synthesized sulfa could possibly have topical therapeutic applications^{8-12,17,18}.

2. EXPERIMENTAL

2.1. Synthesis of the iron complex

The preparation of this compound was carried out at room temperature, using an ordinary precipitation method¹⁹. A 0.1 M solution of iron(III) chloride was added dropwise and with stirring to a 0.3 M solution of sodium sulfathiazole. An abundant yellow precipitate was obtained, isolated by filtration with Whatman N° 42 filter paper, appropriate for fine crystals, washed with distilled water until absence of iron (III) in the filtrate, and dried in an oven at 100 °C. The weight of the solid obtained corresponded to 97 % of the theoretical yield.

Aging of the precipitate: the compound, prepared as described above, was left in contact with bidistilled water, at room temperature for 210 days. At certain time intervals, samples were taken to analyze Fe(III)^{16,19}.

2.2. Chemical analysis and physicochemical properties

Fe(III) was determined using two different techniques whose results were compared: a) A dry chemical technique for measuring reflectance in a Vitros Analyzer 250 equipment, and b) the classic technique of *o*-phenanthroline²⁰.

To determine sulfathiazole, 0.1 g the precipitate was dissolved in 1 mL

of concentrated HCl and then diluted with 10 mL of distilled water. ST was confirmed through a process of diazotation^{21,22}. To avoid color interference it was necessary to mask Fe(III) cation with H₃PO₄. Density, solubility, specific surface area and Kps of the compound were calculated, and the used methodology was reported in a previous work^{16,23}.

2.3. Characterization

The thermal decomposition process of the complex was studied by thermogravimetric, differential thermal analysis (TGA/DTA) and differential scanning calorimetry (DSC). TGA and DTA were performed on a Thermogravimetric and Differential Thermal Analyzer Shimadzu TGA/DTA-50 at 5 °C / min in a gas flow of 20 mL / min. Al₂O₃ was used as DTA standard. The DSC was performed in a Perkin Elmer Thermal Analysis with a heating rate of 10 °C / min.

IR spectra were recorded with a FTIR Perkin Elmer 1600 in the transmission mode from 4000-400 cm⁻¹, using KBr pellets.

The size and morphology of the particles were determined by scanning electron microscopy (SEM) with a JEOL JSM 35 CF.

PXRD pattern was performed in a Rigaku Miniflex Diffractometer with CuK α radiation (λ =1.5418 Å) between 20-90° in 2 θ , steps of 0.02° and counting time of 4 s.

2.4. Bacteriostatic assays

In order to evaluate the bacteriostatic activity, the minimum inhibitory concentration (MIC), of iron(III) complex and sodium sulfathiazole salt was determined against *S. aureus* ATCC 25923 (American Type Culture Collection) and *S. aureus* obtained from a patient of the private microbiology laboratory. It was used the microdilution method following the specifications of the National Committee for Clinical Laboratory Standard (NCCLS)²⁴. Briefly: 1 mL of bacterial suspension in the last phase of growth was inoculated in 1 mL of Mueller Hinton Broth containing the compounds in the final concentration ranging from 128 to 0.25 µg/mL. The final inoculum was approximately 1x10⁵ viable bacteria/mL and final volume 2 mL. Inoculated tubes were incubated at 35 °C for 24 h. The readings were made visually by turbidity. The MIC was defined as the lowest concentration of antimicrobial agent showing complete inhibition of growth.

3. RESULTS AND DISCUSSION

3.1. Composition of precipitate. Physicochemical properties

From the chemical analysis it is determined that the precipitate contains 91.00 % of sulfathiazole and 8.80 % of Fe(III). The results of the solubility for the original and aged precipitate were consistent in the used methods. The results of density, solubility, specific surface area and Kps of the aged and recent compound are shown in Table 1, and the values of pKps and ln C for different aging times in the Table 2, and Fig. 1.

Table 1: Physical and chemical properties of iron(III) sulfathiazole.

Properties		Recent	Aging
Density (g/mL)		1.478	1.516
Solubility(g/L)	<i>o</i> -phenanthroline	1.04×10^{-3}	1.31×10^{-8}
	Dry chemical	1.07×10^{-3}	1.38×10^{-8}
surface area (m ² g ⁻¹)		0.2744	0.2132
Kps (mol ⁴ /L ⁴)		3.45×10^{-18}	9.55×10^{-24}

Table 2: Values of pKps and ln C for different aging times.

Time (days)	pKps	ln C
3	17.49	-13.88
15	17.89	-14.19
30	18.66	-14.78
40	18.92	-14.99
70	19.87	-15.71
120	21.70	-17.12
150	22.79	-17.96
180	23.00	-18.11
210	23.04	-18.15

Fig. 1: pKps as a function of aging time.

3.2. Structure of Fe(ST)₃

In the structure of the complex, the central atom is Fe(III) which forms d^2sp^3 hybrid orbitals, allowing us to assign a coordination number six²⁵. These coordination sites can be occupied by three bidentate ligands of the sulfathiazole anion. The binding of the ligand to the central atom occurs through the oxygen of the sulfonyl group and thiazolic nitrogen, forming six-membered rings of the type chelate. The most likely spatial disposition would be octahedral.

From PXRD data using the Le Bail Method and the Fullprof Program is determined that the complex crystallizes in the monoclinic crystal system, *P2₁/m* space group^{26,27}. The results of refinement are shown Fig. 2, and Table 3.

Fig. 2: PXRD pattern of iron(III) sulfathiazole refined in the space group *P2₁/m***Table 3:** Crystal data and structure refinement for Fe(ST)₃

Empirical formula	C ₂₇ H ₂₄ FeN ₉ O ₆ S ₆
Formula weight	818.789
Wavelength, Å	1.5418
Crystal system	Monoclinic
Space group	<i>P2₁/m</i>
Unit cell dimensions, Å	a = 18.70219 (5)
	b = 7.48602 (5)
	c = 9.73557 (6)
Angle, °	α = γ = 90
	β = 95.27918 (9)
Volume, Å ³	1357.25 (6)
Z	2
Refinement method	Le Bail
Program	FULLPROF
Parameters discrepancy	R _{wp} = 25.8; χ ² = 1.78

3.3. Thermogravimetric analysis (TGA), differential thermal analysis (DTA) and differential scanning calorimetry (DSC)

TGA curve (Fig. 3) shows that the synthesized iron(III) sulfathiazole is anhydrous because the weight remains constant up to 250 °C. The decomposition is carried out in three stages with a total weight loss of 98.50 %. At about 440 °C starts the most significant weight loss and ends at about 600 °C. The final product is iron(III) oxide, which was identified by IR spectroscopy.

DTA curve (Fig.3) shows two endothermic peaks located at 165 °C and 198 °C. The first peak corresponds to a crystallographic phase change, and the second to the sample melting. This was confirmed by studies of DSC (Fig. 4), which indicated that the first transition begins at 162.38 °C and ends at 170.46 °C and the second onsets at 198.31 °C and ends at 202.19 °C. The crystallographic phase change involves a ΔH = 26.609 J/g and the sample fusion involves a ΔH = 111.520 J/g. These findings agree with previous studies on polymorphism of sulfonamides^{28,29}. After that, in the DTA there are three exothermic peaks at 247.5 °C, 455.2 °C and 477.5 °C that correspond to the decomposition of iron(III) sulfathiazole, as shown in the TGA curve.

Fig. 3: TGA and DTA data of iron(III) sulfathiazole.

Fig. 4: DSC data of iron(III) sulfathiazole.

3.4 IR spectra

The IR spectrum of the compound (Fig. 5 a) displays the typical bands (stretching and deformations) of the thiazole, sulfonyl, amine and benzene groups³⁰⁻³².

The stretching vibrations in free sulfathiazole, attributed to the SO₂ group, appear at 1320, 1150 and 560 cm⁻¹. However, when the SO₂ group is bound to metal through the O atom, the anti-symmetric and symmetric stretchings become apparent at 1325 cm⁻¹ and 1140 cm⁻¹, respectively³¹.

As a result of the deprotonation of the sulfathiazole ligand and its coordination through the N thiazole atom, significant changes in the bands assigned to the thiazole ring at 1540 cm⁻¹ in ST, and at 1531.6 cm⁻¹ in Fe(ST)₃, and to the S-N stretching at 920 cm⁻¹ in ST, and at 945 cm⁻¹ in the complex, are observed.

In order to isolate the intermediate products and obtain their infrared spectra, the pyrolysis of the compound was performed in furnace. In Figures 5b and 5c, the spectra of the wastes obtained to 350 °C and 600 °C, respectively, are shown. In the IR spectrum it is confirmed the elimination of thiazole ring at 350 °C by the disappearance of the band located at 1531.6 cm⁻¹. Furthermore, the presence of the typical bands of the -SO₂ group suggests that iron benzene-sulfonate was formed as an intermediate product (Fig.5 b). We noted that the final residue at 600 °C corresponds to reddish brown Fe₂O₃, which was checked against an infrared spectrum of a true sample (Fig. 5 c). From the results of IR spectroscopy, the chemical reactions during thermal decomposition could be represented as:

Fig. 5: a) IR spectrum of original compound. b) IR spectrum of residue at 350 °C and c) IR spectrum of residue at 600 °C.

3.5. Scanning Electron Microscopy (SEM)

Fig. 6 a and b show the SEM photographs of recent and aged Fe(ST)₃ precipitate, respectively. It is noted that the aging of the compound in the presence of the mother liquor leads to the formation of crystals about a hundred times larger than the original.

Fig. 6: SEM of iron (III) sulfathiazole: a) recent, b) after aging.

3.6. Antimicrobial Activity

The bacteriostatic activity of the complex and of the sodium sulfathiazole salt was evaluated against *S. aureus* using the microdilution method. Table 4 shows minimum inhibitory concentration (MIC) of the drugs for *S. aureus* ATCC 25923 and *S. aureus* obtained from a patient of the private microbiology laboratory.

MIC values of Fe(ST)₃ were lower than the corresponding free ligand in the same conditions. The activity of complex was better against the *S. aureus* from the laboratory private patient than against the ATCC 25923 one. Bellú *et al.* have obtained similar results in the determination of the antibacterial activity of Hg(II) sulfathiazole against *E. Coli*¹².

Recently, it has been reported the antibacterial activity of benzohydrazone derivatives against *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumonia* and the results indicate that the compounds show moderate activities against the growth of the tested strains³³.

Table 4: Minimum inhibitory concentration (MIC, µg/mL) of the drugs for *S. aureus* ATCC 25923 and *S. aureus* obtained from a patient of the private microbiology laboratory.

Sulfadrag	MIC, µg/mL <i>S. aureus</i> ATCC 25923	MIC, µg/mL <i>S. aureus</i> from patient
Fe(ST) ₃	47	15
Sulfathiazole (sodium salt)	>95	>95

4. CONCLUSIONS

The preparation of iron(III) sulfathiazole was carried out at room temperature using an ordinary precipitation method.

Through chemical and spectroscopic analysis we found that sulfathiazole forms an anhydrous complex with Fe(III) in a 3:1 molar ratio, which is slightly soluble in water, according to the Kps values.

The aging of the synthesized product leads to significant growth of crystals. This explains why the solubility and specific surface area decrease with time.

The MIC values indicate that Fe(III) sulfathiazole presents better antibacterial activity against *S. aureus* than the corresponding ligand.

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5. REFERENCES

- M. Litter. Las sulfonamidas y otros quimioterápicos: las diaminopirimidinas. Compendio de Farmacología, El ateneo, Buenos Aires, Argentina, 749 (1992).
- A. Velasco, P. Lorenzo, J. Serrano. Farmacología Velásquez, 16th Ed., Interamericana McGraw-Hill, Madrid, España, 894, (1993).
- F. Blasco, L. Perelló, J. Latorre, J. Borrás, S. García Granda, J. Inorg. Biochem. **61**, 143, (1996).
- D. E. Dixon Holland, S. E. Katz, J. Assoc. Off. Anal. Chem. **74**, 784, (1991).
- Y. Wen, M. Zhang, Q. Zhao, Y. Feng, J. Agric. Food Chem. **53**, 8468, (2005).
- A. García-Raso, J. J. Fiol, S. Rigo, A. López-López, E. Molins, E. Espinosa, E. Borrás, G. Alzueta, J. Borrás, A. Castiñeiras, Polyhedron **19**, 991, (2000).
- D. Alzueta, J. Inorg. Biochem. **75**, 189, (1999).
- J. Casanova, G. Alzueta, S. Ferrer, J. Latorre, J. Ramírez, J. Borrás, Inorg. Chim. Acta **304**, 170, (2000).
- A. Scozzafava, L. Menabuoni, F. Mincione, F. Briganti, G. Mincione, C. Supuran, J. Med. Chem. **42**, 2641, (1999).
- C. Supuran, A. Scozzafava, J. Enzyme Inhib. **13**, 37, (1997).
- G. M. Golzar Hossain, J. Saudi Chem. Soc. (2011) doi 10.1016/j.jscs.2011.04.002.
- S. Bellú, E. Hure, M. Trapé, M. Rizzotto, E. Sutich, M. Sigrist, V. Moreno, Química Nova **26**, 188, (2003).
- D. Gil, R. Carbonio, M. I. Gómez, J. Chil. Chem. Soc. **55**, 189, (2010).
- D. Gil, M. Avila, E. Reguera, S. Pagola, M. I. Gómez, R. Carbonio, Polyhedron **33**, 450 (2012). Doi: 10.1016/j.poly.2011.12.006.
- D. Gil, J. Guimpel, A. Paesano Jr., R. Carbonio, M.I. Gómez, J. Mol. Struct. **1015**, 112, (2012).
- M. Bordier, J. de Morán, E. Navarro y M. I. Gómez, Inf. Tecnol. **18**, 77, (2007).
- J. R. Anaconda, J. Diaz, J. Chil. Chem. Soc. **53**, 1702, (2008).
- S. Bellú, E. Hure, M. Trapé, C. Trossero, G. Molina, C. Drogo, P.A.M. Williams, A. Atria, J.C. Muñoz Acevedo, S. Zacchino, M. Sortino, D. Campagnoli, M. Rizzotto, Polyhedron **24**, 501, (2005).
- V. Acevedo, M. Rodríguez, J. Giménez, Inf. Tecnol. **12**, 81, (2001).
- D. Skoog, D. West, F. Holler, S. Crouch. Química Analítica, 7th Ed., McGraw-Hill, Guadalajara, México, (2003).
- Argentina National Formulary, 7th Ed., Codex, Buenos Aires-Argentina, (2000).
- US Pharmacopeia, 29th Ed., The United States Pharmacopeial Convention, Rockville, MD, (2006).
- C. H. Giles, A. S. Trivedi, Chem. and Industry **40**, 1426, (1969).
- National Committee for Clinical Laboratory Standard Performance Standards Antimicrobial Susceptibility Testing, NCCLS Document M 100- S10, NCCLS 771 East Lancaster Avenue, Villanova, Pennsylvania 19085, USA, (2000).
- J. Huheey, E. Keiter, R. Keiter. Química Inorgánica. Principios de estructura y reactividad, 4th Ed., Ed. Alfaomega. D.F.México, (2005).
- A. Le Bail, H. Duroy, J. L. Fourquet, Mat. Res. Bull. **23**, 447 (1985).
- J. Rodríguez-Carbajal, Physica B **192**, 55 (1993).
- D. Wirth, G. Stephenson, Org. Process Res. Dev. **1**, 55, (1997).
- G. Z. Zhang, C. Gu, M.T. Zell, R.T. Burkhardt, E.J. Munson, D.J.W. Grant, J Pharm. Sci. **91**, 1089, (2002).
- K. Nakamoto. Infrared and Raman Spectra of Inorganic and Coordination Compounds, 4th Ed., A Wiley. Interscience Publication, (1986).
- B. Smith. Infrared spectral interpretation, 1st Ed., CRC Press, New York, United States of America, (1998).
- R. Silverstein, F. Webster. Spectrometric identification of organic compounds, 6th Ed., John Wiley & Sons, Inc. New York, United States of America, (1996).
- Z. Wang, F. Zhi, R. Wang, L. Xue, Y. Zhang, Q. Wang, Y-Lin Yang, J. Chil. Chem. Soc. **58**, 2, (2013).