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Complexation: Effect of Metal on Microbiological Activity of Sulfanilamide Derivatives

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ABSTRACT

Resistance strain which outgrows current therapeutics. As new drug development either against resistance strain or any new disease is neither easier nor cheap. Modification of the available entity generally called “sulfonamide” with metal. Sulphonamides and metal duos are promising for their antimicrobial activity. Sulfanilamide metal complexes were synthesized. Coordination complexes of sulfonamide with metal such as Copper, Barium, Zinc, Ferric, Aluminum, Cadmium, Manganese, Magnesium and Calcium were prepared by complexation reaction under stirring and control environmental conditions. The purity of complexes was checked by TLC monitoring and all synthesized complexes were characterized using I.R., Mass, Atomic Absorption Spectroscopy. The antibacterial activity of synthesized complex was tested by Agar plate method.

Keywords: Sulfonamide, Complexes, Chelates, Atomic Absorption Spectroscopy, Antibacterial, Antifungal, MIC.

INTRODUCTION

Over the past three decades, intensive efforts have been made to design novel compounds to confront new strains of resistant micro-organisms. The on-going search for novel and innovative drug delivery systems is predominantly a consequence of the well established fact that the convectional dosages are not sufficiently effective in conveying the drug compounds to its site of action and this has necessitated the need to search for more potent drugs.[1] The recognition of the potential employment of metal complexes and chelates in therapeutics application provides useful outlets for basic research in transition metal chemistry.[2]

A number of antibiotics such as bleomycin, streptonigrin and bacitracin have been reported to function properly upon coordination with metal ions.[3] Metal and antibiotics duos interact with several biomolecules such as DNA, RNA, protein receptors and lipids, making them very unique and specifically bioactive.[4,5] Also,

some metals such as iron play important roles general body metabolism. Improved efficiency have been observed upon chelation has effective replacement for novel drug development.

EXPERIMENTAL SECTION

Materials and Method

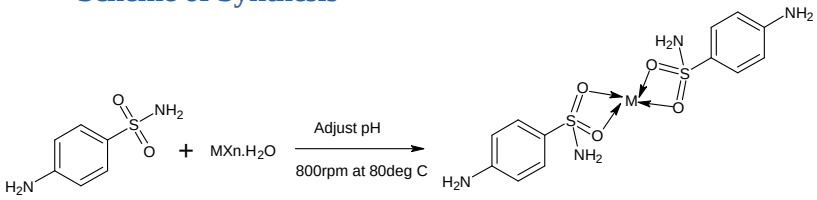
- The entire chemicals were supplied by S. D. Fine Chem. (Mumbai), Finar Chem. Ltd (Ahmedabad) and Loba Chemie. Pvt. Ltd. (Mumbai).
- Melting points were determined by open tube capillary method and were uncorrected.
- Purity of compounds was checked by thin layer chromatography (TLC) on silica gel-G.
- IR spectra of all compounds were recorded on FT-IR 8400S Shimadzu spectrophotometer using KBr.
- Mass spectra were obtained using 2010EV LCMS Shimadzu instrument.
- Atomic absorption spectroscopy was performed on AA-7000 Shimadzu instrument.

SYNTHETIC PROCEDURES

Preparation of metal complexes

- Metal salt in appropriate quantity was dissolved to get solution (0.01mol).
- Ethanolic solution of *p*-amino benzene sulfonamide was prepared (0.01mol).
- Both of above solutions were mixed at 80°C and stirred at 800rpm for 2hrs and maintained required pH.
- Cooled the reaction mixture till change in coloration occur indicating precipitation of metal complexes.
- Complexes formed were recovered by vacuum filtration from reaction mixture.
- Washed and recrystallized with appropriate solvent.

Scheme of Synthesis



Spectral Characteristics of Synthesized Complexes

1. Cadmium sulfanilamide complex: I.R. spectra shows absorptions bands at 1000.99 (-S=O bend), 1299.93 (-SONH bend), 3473.56 (-N-H str.), mass spectra shows characteristic M^+ peaks at 458.1 (m/z) and AAS indicates metal level in complexes 10ppm.
2. Barium sulfanilamide complex: I.R. spectra shows absorptions bands at 1072.35 (-S=O bend), 1311.5 (-SONH bend), 3461.99 (-N-H str.), mass spectra shows characteristic M^+ peaks at 481.7 (m/z) and AAS indicates metal level in complexes 12ppm.
3. Calcium sulfanilamide complex: I.R. spectra shows absorptions bands at 1083.92 (-S=O bend), 1288.36 (-SONH bend), 3363.62 (-N-H str.), mass spectra shows characteristic M^+ peaks at 385.2 (m/z) and AAS indicates metal level in complexes 20ppm.
4. Zinc sulfanilamide complex: I.R. spectra shows absorptions bands at 1047.27 (-S=O bend), 1294.15 (-SONH bend), 3359.77 (-N-H str.), mass spectra shows characteristic M^+ peaks at 411.0 (m/z) and AAS indicates metal level in complexes 10ppm.
5. Magnesium sulfanilamide complex: I.R. spectra shows absorptions bands at 1078.13 (-S=O bend), 1301 (-SONH bend), 3492.85 (-N-H str.), mass spectra shows characteristic M^+ peaks at 369.1 (m/z) and AAS indicates metal level in complexes 08ppm.

6. Manganese sulfanilamide complex: I.R. spectra shows absorptions bands at 1000 (-S=O bend), 1296.08 (-SONH bend), 3434.98 (-N-H str.), mass spectra shows characteristic M^+ peaks at 399.0 (m/z) and AAS indicates metal level in complexes 20ppm.
7. Copper sulfanilamide complex: I.R. spectra shows absorptions bands at 1022.2 (-S=O bend), 1246.64 (-SONH bend), 3411.84 (-N-H str.), mass spectra shows characteristic M^+ peaks at 408.0 (m/z) and AAS indicates metal level in complexes 10ppm.
8. Aluminium sulfanilamide complex: I.R. spectra shows absorptions bands at 1072.75 (-S=O bend), 1311.5 (-SONH bend), 3456.20 (-N-H str.), mass spectra shows characteristic M^+ peaks at 543.9 (m/z) and AAS indicates metal level in complexes 14ppm.
9. Ferric sulfanilamide complex: I.R. spectra shows absorptions bands at 1043.42 (-S=O bend), 1290.29 (-SONH bend), 3438.84 (-N-H str.), mass spectra shows characteristic M^+ peaks at 573.2 (m/z) and AAS indicates metal level in complexes 25ppm.

BIOLOGICAL EVALUATION

Microbiological Screening

All the synthesized complexes were screened for determining antibacterial activities *in-vitro* by using cup plate method at concentrations 200-700µg/ml against Gram-negative bacteria viz. *E.coli* and Gram-positive bacteria viz. *B.subtilis* and *S.aureus* using nutrient agar media. The results have been compared with sulphanilamide. The activity against bacteria was evaluated by cup plate method.[6]

20g of Agar, 10g of peptone, 10g of beef extract, 5g of sodium chloride were dissolved in 1000ml of distilled water by heating. This mixture was adjusted to pH 7.00 by NaOH. The content was sterilized by autoclaving.

All the synthesized compounds were dissolved separately to prepare a stock solution containing 1000µg/ml and 800µg/ml in water. 1ml of this solution was aseptically transferred to the sterile nutrient broth medium and made up to 10ml with sterile nutrient media, thus 1ml of the resulted solution produced 1000µg/ml. 7ml of the above solution has been transferred to 3ml of water to give 700µg/ml concentration of first. Thus, from 1000µg/ml stock solution, a successive concentration like 700, 500, 300, 200 and so have been prepared in a similar manner up to 6 dilutions from sixth 5ml of the solution has been discarded. Thus, from 800µg/ml stock solution, a successive concentration like 700, 500, 300, 200 and so has been prepared in a similar manner up to 6 dilutions from sixth 5ml of the solution has been discarded. The tubes have been mixed well after each addition. All the tubes have been inoculated with 0.5ml of the test organism culture. The process has been repeated with different test organism. A positive control and a negative control have been also prepared to confirm the nutritive property and sterility, respectively of the prepared to confirm nutritive property and sterility, respectively of the prepared medium. The tubes have been incubated at 37°C for 48hours. The presence or absence of growth of organisms have been observed after incubation.[6-8]

DISCUSSION

- Prominent peaks at **3300-3500cm^{-1}** for primary amine in IR for all complexes and starting material revealed that sulfone moiety.
- Mass spectra of all complexes indicate presence of molecular ion [M^+] peaks as well various fragment ion peaks. Base peaks in mass spectra around **171.00 to 172.00** m/e are of sulfonamide moiety.

- Atomic Absorption spectroscopy data indicates presence of metals in complexes as well safety mark of metal concentration to human in **08 to 20ppm**.
- Antimicrobial screening studies data shows diverse mode of metal influence. On one hand complexes of zinc of compound code **4** had MIC of **100µg/ml** and of copper having compound code **7** had MIC of **200µg/ml** for bacterial strain. So it reduces dose **1/2 to 1/3rd** compare to drug having MIC **300µg/ml** without complexation with metal, other hand metal complexes of calcium, barium, ferric metal compound code **3, 2, 9** respectively had no influence on activity. Metal complexes of cadmium, aluminium, manganese, magnesium had in between influence.

CONCLUSION

From the I.R., Mass and Atomic Absorption spectroscopy it has been abstracted that complexes were formed according to planned task. Promising results of complexes antimicrobial screening against bacteria observed. These attributed to high activity in bacteria.

Improved activity of complexes better explained by Chelation Theory: **tailoring and tuning of hydrophilicity and lipophilicity of drug by coordinating metal ion by lead to bring down the solubility and permeability barriers to get into cells, this in turn increase bioavailability and increase activity**. Other reason for improved activity of complexes is the presence of metal in enzymes such as zinc in carbonic anhydrase presence in bacterial species.

From result it has been abstracted that metal complexes can be divided into three categories according to significance of metal on activity.

1. Metal highly significant such as Copper and Zinc metal complex.
2. Moderate significant such as Manganese, Magnesium, Cadmium, Aluminium complex.
3. Metal not significant such as Calcium, Barium, Ferric metal complex.

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REFERENCES

1. Rybicki EP, "The classification of organisms at the edge of life, or problems with virus systematic." *S. Afr. J. Sci*. **1990**, *86*, 182-186.
2. LWOFF, "The concept of virus." *J. Gen. Microbial*. **1957**, *17(2)*, 239-253.
3. Christner BC, Morrise CE, Foreman CM, Cai R and Sands DC, "Ubiquity of biological ice nucleators in snowfall." *S. Afr. J. Sci*. **2008**, *31*, 5867.
4. Schlegel HG, Kaltwasser H and Gottschalk G, "Ein Submersverfahren zur Kultur wasserstoffoxidierender Bakterien: Wachstums physiologische Untersuchungen." *Arch. Microbial*., **1961**, *38*, 209–222.
5. Jain NK., Pharmaceutical Microbiology; 1st edition; Vallabh Prakashan, Delhi, **2001**, pp 46-58.
6. Ahluwalia V., and Aggaraval R., Comprehensive Practical Organic Chemistry Preparation and quantitative, 1st edition, University press, New Delhi, **2000**, pp. 120-128.
7. Pelczar MJ, Chan ES, Pelczar JR and Krieg NR. Microbiology McGraw-Hill Book company; 5th edition, **1997**, pp 73-98.

8. Ashutosh Kar, Pharmaceutical Microbiology; 1st edition, New Age International Limited Publishers, New Delhi, **2008**, pp 268-278.

Physical characteristics of sulfanilamide metal complexes

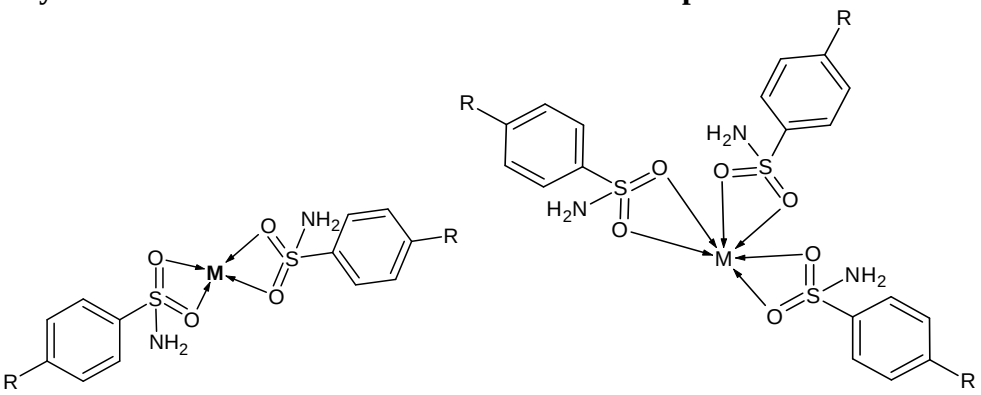

Table 1. Physicochemical parameters

Compound code	Metal (M)	R	Molecular formula	Molecular weight (g/mol)	Melting point (°C)	% yield (%w/w)
1	Cd	NH_2	$C_{12}H_{16}N_4O_4S_2Cd$	456.40	178-180	60.22
2	Ba	NH_2	$C_{12}H_{16}N_4O_4S_2Ba$	481.32	214-216	75.00
3	Ca	NH_2	$C_{12}H_{16}N_4O_4S_2Ca$	384.40	190-192	71.69
4	Zn	NH_2	$C_{12}H_{16}N_4O_4S_2Zn$	409.40	240-242	81.25
5	Mg	NH_2	$C_{12}H_{16}N_4O_4S_2Mg$	368.30	208-210	69.00
6	Mn	NH_2	$C_{12}H_{16}N_4O_4S_2Mn$	398.93	181-183	76.00
7	Cu	NH_2	$C_{12}H_{16}N_4O_4S_2Cu$	407.5	>300	85.07
8	Al	NH_2	$C_{18}H_{24}N_6O_6S_3Al$	543.0	>300	68.88
9	Fe	NH_2	$C_{18}H_{24}N_6O_6S_3Fe$	572.00	222-224	65.00

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Table 2. Antibacterial screening

Compound Code	Concentration (µg/ml)	Zone of inhibition (mm)		
		Gram +ve		Gram-ve
		S.aureus	*B.subtilis*	*E.coli*
1	200	00	00	00
	300	06	07	09
	500	05	09	11
	700	08	10	13
2	200	00	00	00
	300	00	00	00
	500	07	08	10
	700	09	09	12
3	200	00	00	00
	300	00	00	00
	500	05	06	09
	700	07	07	11
4	200	11	11	13
	300	12	14	16
	500	15	15	17
	700	16	15	19
5	200	05	06	09
	300	07	07	12
	500	10	09	14
	700	11	09	15
6	200	00	00	00
	300	05	08	11
	500	06	10	13
	700	11	12	15
7	200	07	09	11
	300	08	11	14
	500	11	13	14
	700	13	14	17
8	200	04	05	06
	300	05	08	07
	500	07	08	09
	700	08	09	11
9	200	05	04	07
	300	06	06	08
	500	07	08	10
	700	08	08	12
Sulfanilamide	300	05	04	04
	500	07	06	08
	700	10	08	09

Table 3. MIC for Antibacterial screening

Compound code	Minimum Inhibitory Concentrations (µg/ml)		
	Gram +ve		Gram –ve
	S.aureus	*B.subtilis*	*E.coli*
	MTCC 96	MTCC 121	MTCC 521
1	300	300	250
2	400	400	400
3	500	500	400
4	100	100	100
5	200	200	150
6	300	300	250
7	200	150	100
8	200	200	200
9	200	200	200
Sulfanilamide	300	300	300

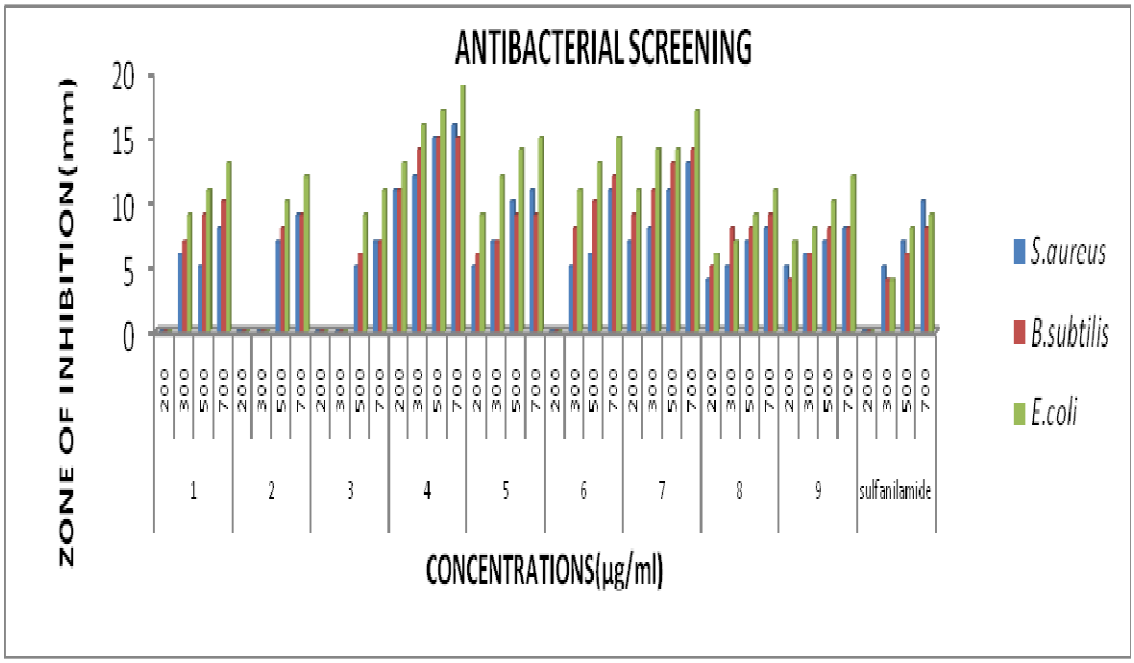

Figure.1. Histogram for antibacterial screening

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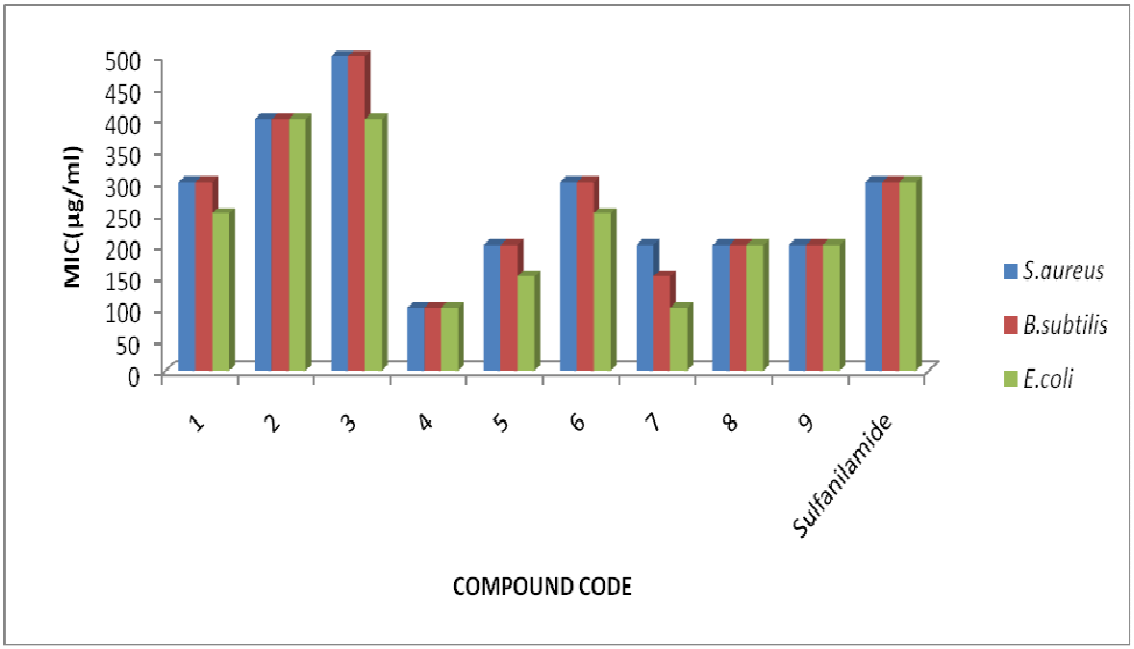

Figure.2. Histogram for MIC of antibacterial screening