

Absorption Spectroscopy for Antibacterial Agents Selectivity and Their Toxicity to the Host

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ABSTRACT

The use of inorganic compounds in medical practise dates back to ancient times. In spite of this, significant progress has been made in this area through the completion of species and the articulation of chelating agents. These chelating agents must, by definition, contain two or more electron donor groups that are in a position to co-ordinate with a metal ion in order to form one or more stable ring structures with five or six members each. There is compelling evidence to suggest that chelation is the fundamental mechanism behind the therapeutic effects of a large number of natural and synthesised medicinal medicines. Because it is not possible to infuse metals directly. Because they do not target specific bacteria and are hazardous to the host, they are able to enter the bloodstream and operate as antibacterial agents. Additionally, in order to carry out their function, soluble metal ions such as iron are able to pass through the membranes that surround bacterial cells. Van Bohring discovered that diphtheria may be healed by a serum that is made by injecting bacteria into a healthy animal and then collecting serum from the immunised animal after the caused sickness has passed. Biotherapy may take many forms, including vaccination and serum treatment, and its early, remarkable achievements inspired the optimism that all contagious illnesses could one day be prevented or cured using these methods. Prophylactic vaccination, also known as active immunisation, has been demonstrated to be very successful in human therapy only against smallpox, typhoid fever, and rabies. The only other disease that prophylactic vaccination has been shown to be beneficial against is scarlet fever. Serum treatment has only been shown to be effective for a select few human diseases, such as diphtheria bacillary desentry. Snake bites are one of these disorders. Biotherapy has only found limited use due to the large number of known microorganisms that may cause disease in humans.

Keywords:

Absorption , spectroscopy , antibacterial , agents

Introduction

The philosophy of bacteriology incorporates a wide variety of overarching ideas that shed light on the mysteries surrounding the beginning of life and the characteristics of contagious illnesses seen in both living and dead organisms, including humans and other animals. Many authors, including scientists, philosophers, and prices, have contributed volumes of writing to the effort to provide light on the early beginnings of the scientific field of microbiology. can explain the underlying workings of the

evolution of the animal kingdom's hidden systems. The intricacies of the processes of fermentation and outrefaction, as well as the factors that contribute to communicable illnesses.

The application of inorganic compounds discovered via experimentation in medical practise dates back to antiquity. Complexing species and particulating chelating agents, which, by definition, contain two or more electron donor groups and are able to co-ordinate to a metal ion to form one or more

stable (5-6 membered) ring structures, have made a significant contribution in this field. Their work has been hailed as one of the most important developments in the field. There is compelling evidence to suggest that chelation is the fundamental mechanism behind the therapeutic effects of a large number of natural and synthesised medicinal medicines. Since metals cannot be injected directly into the blood stream to serve as antibacterial agents due to their lack of selectivity and their toxicity to the host, metals cannot be used in the treatment of bacterial infections. Additionally, in order to carry out their function, soluble metal ions such as iron are able to pass through the membranes that surround bacterial cells.

It has been shown that both substituted and unsubstituted 6-aryl pyrimidines have the ability to combine with iron that is normally present in the host and transport the iron through the cell membranes of bacteria and fungi. However, substituted 6-aryl pyrimidines do not have this ability. Because of this, the complex has the potential to operate as an antibacterial and antifungal agent.

Prophylactic vaccination (active immunization) as Serum treatment

This important fact was first established by Robert Koch, who succeeded in isolating the microscopic rods (*Bacillus anthracis*) present in blood of cattle afflicted with anthrax (Splenic fever), growing them in a synthetic medium, and in showing that they produce the disease when inoculated into healthy animals. While Pasteur had a suspicion that contagious diseases and fermentation are caused by micro-organisms, this important fact was first established by Robert Koch (1876). In later years, Pasteur made the discovery that animals can develop immunity to anthrax by subcutaneous injection of attenuated bacilli. This discovery was made in 1881. In the year 1890, Van Bohring made the discovery that diphtheria may be treated using a serum that is created by injecting bacteria into a healthy animal and then collecting the serum from the immunised animal once the sickness that was induced has passed. Biotherapy may take many forms, including vaccination and serum treatment, and its early, remarkable achievements inspired the optimism that all

contagious illnesses could one day be prevented or cured using these methods. Prophylactic vaccination, also known as active immunisation, has been demonstrated to be very successful in human therapy only against smallpox, typhoid fever, and rabies. The only other disease that prophylactic vaccination has been shown to be beneficial against is scarlet fever. Serum treatment has only been shown to be effective for a select few human diseases, such as diphtheria bacillary desentry. Snake bites are one of these disorders. Biotherapy has only found limited use due to the large number of known microorganisms that may cause disease in humans.

There is nothing particularly novel about antibiotic action. According to reports by Pasteur and others, pollutants found in air and soil have an effect similar to that of an inhibitor, preventing the growth of germs that cause disease. However, these early insights did not result in any significant advancements.

In the year 1929, Alexander Fleming was employed at St. Mary's Hospital in London, England. He had put away some laboratory cultures of the staphylococcus aureus bacteria that caused sickness.

At the year 1952, W. M. Mela more and his colleagues in the new York Research Laboratories discovered the structure (-) 2 (5-corboxypentyl - 4 - thiazolidone)(I) for a Streptomyces Antibiotic that shown in vitro anti tubercular action.

Bacteriological activities of substituted and unsubstituted 6-aryl pyrimidine-metal chelates:

The cultivation of bacteria in test tubes that already include a fluid medium and to which the medication has been added is one of the most common and commonly used ways for determining the level of antibacterial activity that a given medicine possesses. There are also other ways that have been utilised, such as the utilisation of the slide cell "as well as a number of other adaptations of the Agar Cupplate. Washman and More specialised procedures that include the use of Reilly were the ones that published the Agar streck method for assessing antibacterial activity. The difficulty of determining how effective chemotherapies are

has also been tackled using chick embryos, bone cultures, marrow, and several other types of tissue.

However, in this study, the anti-bacterial characteristics of the complexes were investigated using the standard cup plate Agar diffusion technique.

"In order to determine whether or not the compounds have any antibacterial activity, they were tested against the following two microorganisms:

- 1) Gram-Positive staphylococcus aureus
- 2) Gram-negative Escherichia coli

The 'Sensitivity Way Test' for cup plate Agar diffusion consists of the following steps:

- 1) Preparation of medium, sterilisation, and tubing
- 2) The treatment of the glass instruments and their subsequent sterilisation
- 3) Transferring the seeded media to petri dishes that have been previously sterile and cutting the cups
- 4) The dilution of the compounds in cups and their pouring in the respective cups
- 5) Incubation at a given temperature.
- 6) Establishing the Boundaries of the Inhibition Zones.

Objectives of the Study

1. To study on Bacteriological activities of substituted and unsubstituted 6-aryl pyrimidine-metal chelates:
2. To Study on Absorption Spectroscopy

Research Method

Test Media

Among the many factors that might have an effect on findings obtained in vitro. It is widely agreed upon that the component makeup of the test medium is the factor that frequently has the largest influence on the activity of the medication being evaluated. Numerous species, including *S. aureus* and *E. coli*, may be cultured in straightforward media with well-defined chemical constituents."

Sesler and Schmidt found that sulfa thiazole and sulfa diazine were from ten to one thousand times more active in a synthetic medium than in a beef heart in fusion broth against strain briedlander's bacillus. These findings were reported

by Sesler and Schmidt. The influence of test media upon activity has also been reported by these two researchers. In addition to the composition of the test, it has been established that there is a thousand fold variation in the activity of each of many in various media against strains of *E. coli*. "In the case of media, the pH of the medium is a factor that can either directly or indirectly impact the action of a medicine. The ideal pH range for the development of many different bacterial species is a rather limited one.

The composition of the basal media used in the experiment

- Peptone 10 g
- Beef Extract 3g
- Sodium chloride 5g
- Yeast extract 1.5g
- Sucrose 1.0g
- Distilled water 1000ml
- Agar 3%

Preparation of the medium:

The numerous components that were discussed before were accurately weighed into two separate small flasks, and then they were dissolved in approximately 400 millilitres of distilled water using a gentle heating method. After the ingredients had been fully dissolved, the volume of the solution was brought up to 1000 times its original size using purified water. Bromothymol blue was used as an indication to help bring the pH of the medium down to 7.6 once it had been adjusted to that level. This medium autoclave had about all agar-agar added to it, and the mixture was steamed for one hour. A transparent solution was obtained by passing the hot medium through cotton wool pads and filtering it (when difficulty was experienced in getting perfectly clear medium, a little agar albumin was added, the agar reheated in an autoclave and refiltered through cotton until the medium was completely clear) After filling test tubes measuring 6 inches by one inch with 20 millilitre portions of this agar medium, the tubes were sealed with cotton wool, placed in an autoclave for 30 minutes at a pressure of 15 pounds, and then allowed to cool to room temperature.

Before they were used, each piece of glass equipment, such as test tubes, pipettes, and

petridishes, was cleaned and disinfected. After being filled with a combination of chromic acid and left for 18 to 24 hours, these glass goods were cleaned completely with distilled water and then interrinsed many times with tap water. In the end, these various pieces of equipment were disinfected in an oven with hot air at a temperature of 160 degrees Celsius for one hour.

Procedure for The Assay

After melting a sufficient number of agar medium tubes in a steam bath, the measured quantity of the culture of the test organism (0.2 ml) was added to each tube after allowing the tubes to cool down to 45 degrees Celsius. Once the agar medium had melted, the tubes were allowed to cool down to 45 degrees Celsius. The inoculation medium was to be put over the petridishes that had been sterilised earlier, and

then testing was to be permitted to take place. Just before starting the experiment, these petridishes were moved into a refrigerator where the temperature was maintained at 4.8 degrees Celsius. After removing the dishes, holes were made into the culture media using a needle. The holes had a diameter of around 5 millimetres and were cut out using a glass tube cutter that had been sanitised. The test solutions of the compounds, 1% (w/v), were prepared in a mixture (20%) containing equal volumes of isopropyl alcohol and dioxane. In the case of some compounds, which were insoluble or less soluble in the previously mentioned solvent, the activities were tested under fine suspension in the previously mentioned solvent, while maintaining a strengths of the compounds 1% (w/v).

Table 1 The antibacterial activity of the compound 2 chloro, 4 phenyl thio uracil, and 6 phenyl pyrimidine (CpTUpP) and its metal cheletes

Sl No.	Compound	Composition	Antibacterial activities against	
			S. aureus	E. Coli
1.	CpTUpP	$C_{17}H_{13}N_4SCl$	(-)	(-)
2.	Ag(I)-CpTUpP	$Ag(C_{17}H_{12}N_4SCl)$	(-)	(±)
3.	Cu(I)-CpTUpP	$Cu(C_{17}H_{12}N_4SCl)$	(-)	(+)
4.	Hg(I)-CpTUpP	$Hg(C_{17}H_{12}N_4SCl)$	(±)	(-)
5.	Cu(II)-CpTUpP	$Cu(C_{17}H_{12}N_4SCl)_2$	(+)	(-)
6.	Cd(II)-CpTUpP	$Cd(C_{17}H_{12}N_4SCl)_2$	(-)	(±)
7.	Ni(II)-CpTUpP	$Ni(C_{17}H_{12}N_4SCl)_2$	(-)	(-)
8.	Co(II)-CpTUpP	$Co(C_{17}H_{12}N_4SCl)_2$	(+)	(-)
9.	Mn(II)-CpTUpP	$Mn(C_{17}H_{12}N_4SCl)_2$	(-)	(±)
10.	Fe(III)-CpTUpP	$Fe(C_{17}H_{12}N_4SCl)_3$	(+)	(-)
11.	Bi(III)-CpTUpP	$Bi(C_{17}H_{12}N_4SCl)_3$	(-)	(+)
12.	Cr(III)-CpTUpP	$Cr(C_{17}H_{12}N_4SCl)_3$	(+)	(±)
13.	Al(III)-CpTUpP	$Al(C_{17}H_{12}N_4SCl)_3$	185 (±)	(-)

Data Analysis

Absorption Spectroscopy: -

Absorption Spectroscopy is one of the scientific subfields that dates back to the beginning of the discipline. In the early years of the eighteenth century and a century later, Newton presented the optical principles required for spectroscopic investigations. A few years later, Bouguer published his observations on the change in intensity of a beam as it passes through various thicknesses of absorbing material.

Century During the second half of the nineteenth century, spectroscopists working

with emission and absorption techniques made discoveries that laid the groundwork for the early twentieth century theories of atomic and molecular structure. Recent refinements of these theories are based primarily upon modern spectroscopic measurements, and these discoveries have been made. The infrared zone, which was discovered for the first time by Herschel and Coblentz in the early years of this century, was investigated in great detail by both of these astronomers. As a result of these investigations, the existence of "characteristic frequencies" for the absorption of

electromagnetic radiation by functional groups in organic molecules was established. These radiations are measured only in wave length (expressed in micron 10⁻⁴ cm or 10⁻⁴) but also considered in frequency, which is expressed not in cycles per second but in wave numbers cm⁻¹, which are also sometimes referred to as reciprocal centimetres. The ultraviolet radiation ranges from 2000-4000 Å, and the visible radiation ranges from 4000-7500 Å. The infrared radiation ranges from 1-33.

A. Electronic excitation:

In which the electrons are excited to higher energy levels, which results in either absorption or emission in the visible or ultraviolet portions of the spectrum.

B. Increase in vibrational and rotational energy:

Because of the enhanced vibrational and rotational movements of the atoms or groups included inside the molecules, it puts out either an absorption or an emission in the infrared area of the spectrum, depending on which you look at. A figure that shows the quantity of electromagnetic radiation absorbed or transmitted at each frequency is referred to as a compound's spectrum. It is a feature of the make-up of the compound that this property possesses. There is no one type of spectroscopy that is superior to the others; rather, each one is utilised to complement the others. The following equation serves as a place of departure for calculating the light absorption spectra of pure substances:

$$\log (I_0/I) = A = ECL$$

Table 2

Groups	λ max. (m μ)	E. max.
C = C	175	5,000
C = O	279	15,000
>C = C - C = C <	217	21,000
>C = C - C = O	217	16,000
C = C - C = C - C = C	258	35,000
C = C - C = O	220	16,000
>C = C - COOH	206	13,000
>C = C _H (Cis)	264	9,500
^H >C = C _H (trans)	273	21,800
C = N	190	5,000
C \equiv C	175	6,000
C ₆ H ₆	255	230

UV spectra below 200 μ spectrophotometric vacuum metres are used to measure volumes in millilitres..

Infra red absorption spectro-photometry:

The analysis of infrared spectra can yield a significant amount of information, such as the existence of a variety of functional groups and the strength of hydrogen bonds (intra molecular and inter molecular). The determination of the

conformational orientations of cis and trans isomers, the orientation of aromatic compounds, and so on.

In order for a substance to be able to absorb in the infrared region, it is necessary for the vibrations in the molecule to give birth to an asymmetrical charge distribution. This is the key need. Because of this, it is not required that the molecules have a dipole moment that is stable throughout time. The portion of the form

2.5 that is of interest for the purposes of analysis

μ L through 25, or wave numbers between 4000-400 (waves per centimetre, cm). Glass and quartz, which are the two most common types of optical materials, both have a high absorption rate in the infrared spectrum. Because of this, the apparatus used to measure spectra in the infrared range is noticeably distinct from the apparatus used to measure spectra in the visible and ultra violet areas. The many patterns of vibration and rotation that occur within a

molecule are what give rise to its infrared spectrum. Below 25 microns in wave length

μ , Changes in the vibrational and rotational levels of the molecule can be brought about as a result of the rotation since it possesses enough energy to do so. Pure rotating spectra of molecules can only be observed at wave lengths that are significantly longer than the long wave length limit (25. μ) in the case of the majority of infrared spectrophotometers

Table No.2 Absorption Frequencies of 2 Chloro-4-Nitro-Phenyl-Thio-Uracil-6-Phenyl-Pyrimidine and Metal Chelates of Metals

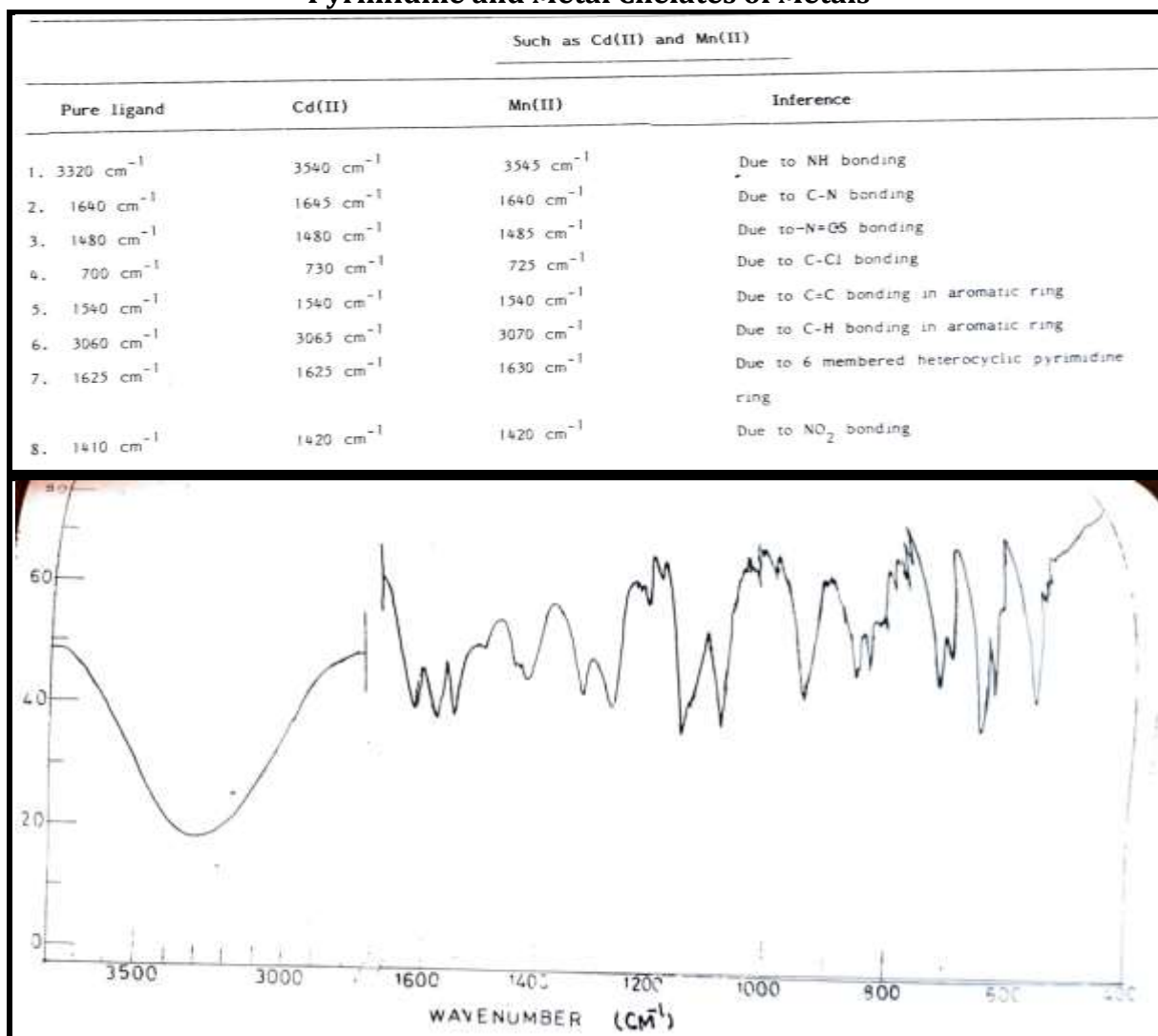


Figure 1 IR SPECTRUM OF CpTUpP

Conclusion

Micro organisms may gain resistance to in vivo and in vitro. There have been reports of such resistant infections occurring spontaneously in

diverse illnesses, in especially in cases when therapy was continued with an insufficient dose to sustain bacterio static at the site of the infection. Once an organism has developed

resistance to one of the powerful sulfa medications, it is frequently discovered that it has also developed resistance to all of the routinely used sulfa drugs, the sulphadruugs, and strains with a variety of other drugs. This was discovered to be the case strains of shigella sonnei and shigella paradysentaria cocci, both of which are resistant to pneumococcal strains. In contrast to this, it was discovered that the strain Flexner, which was resistant to sulfathiazole and sulfa diazine, had the same amount of resistance to sulfapyrazine as the parent strain.

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