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Synthesis, Characterization and Antimicrobial Activity of Vanadium Tetracycline Metal Ion Complex

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Abstract:
With increase in antimicrobial drug resistance, metal ion complexes are playing significant roles in the development of antimicrobial drugs. Vanadium complexes of the antibiotic tetracycline was synthesized through reaction of tetracycline and vanadium sulphate ($VOSO_4$) using ethanol as reagent. The produced vanadium tetracycline complex was characterized by UV-Vis absorption spectroscopy with a peak absorption at 450nm. Furthermore, the complex was found to be soluble in water but partially soluble in ethanol, with a melting point of 240^oC. The antibacterial activity of the vanadium tetracycline complex was tested against clinical isolates of Escherichia coli using the agar well diffusion assay. The bacteria were found to be sensitive to the complex at a concentration of 6.25mg/ml, producing a zone of inhibition of 19mm. A minimum inhibitory concentration (MIC) of 12.5mg/ml was determined to inhibit growth while a minimum bactericidal concentration (MBC) of 25mg/ml was shown to effectively kill the test organism. These results were more effective than the commercial tetracycline used as control, thus indicating the potential of this complex to be a possible candidate for the treatment of bacterial infections caused by multidrug resistant microorganisms.

Keywords: *Vanadium, tetracycline, complex, spectrophotometer, antimicrobial activity, drug resistance*

1. Introduction

Tetracycline is a semi-synthetic antibiotic originally synthesized by the *Streptomyces* sp. They are broad spectrum antibiotics which have potent activity against a wide range of Gram-positive, and Gram-negative bacteria including atypical organisms such as Chlamydia, Mycoplasmas, Rickettsia, and protozoan parasites (Lan and Marilyn, 2001). The antimicrobial properties of tetracycline and the absence of major adverse side effects due to their consumption has led to their extensive use in the treatment of human and animal infections (Lan and Marilyn, 2001). However, due to its low toxicity, low cost and ease of administration, tetracycline has been abused with indiscriminate oral administration which has led to the appearance of bacterial resistance to the drug (Tumer *et al.,* 2006). The mechanism of action of tetracycline involves the inhibition of protein synthesis. Tetracycline strongly binds to the 30S ribosomal subunit of the bacteria, leading to the inhibition of protein synthesis by facilitating rupture of codon – anticodon interactions between t-RNA and m-RNA which in turn results in the interruption of the bond between the aminoacyl-t-RNA and the ribosomal acceptor site (Juan and Ibranyellis, 2004).

Metal complexes consist of a central atom or ion which is the coordination center and a surrounding array of bond molecules or ions, that are in turn known as ligands or complexing agents (Geoffrey, 2009). Metal complexes have played a significant role in the development of news drug. Historically, metal base remedies such as silver was employed for the treatment of wounds and ulcer by Greek physicians while gold was utilized to treat a variety of disease conditions in China thousands of years ago (Orvig and Abrams, 1999). An important property of metals is that they form positively charged ions in aqueous solution which bind to negatively charged biological molecules. Depending on the coordination environment, the charge can be modified, thus generating species of the metals that can be cationic, anionic or neutral. Furthermore, metal ions with high electron affinity can significantly polarize groups that are coordinated to them, fostering the generation of hydrolysis reaction (Haas *et al.,* 2009). Currently, the development of antibacterial drugs is moving away from the conventional cytotoxicity based approach and towards the rational design of selective agents that act on specific cellular targets because of the development of multidrug resistance by most microorganisms (Needle and Thurston, 2005).

This resistance of bacteria to conventional antimicrobial treatment has reduced the efficiency of current antibiotics, thus increasing the need for more efficient drugs such as synthesized metal complexes for the treatment of infections (Santos, *et al.,* 2014). Numerous derivatives of tetracyclines have been synthesized with varied success. Some have shown antimicrobial activity against bacteria species which previously was resistant to conventional tetracyclines and other antibiotics (Charest *et al.,* 2005; Sun *et al.,* 2008; Clark *et al.,* 2011). Sum *et al.,* (1994) synthesized new generations of potent antimicrobial agents through the modification of 9-aminotetracyclines which was effective against multi-drug resistant organisms. Vanadium is a metallic element commonly found in foods, water, soil, air and human. It belongs to the group of transition metals and is abundant in the environment. Like other heavy metals, vanadium although toxic, but when taken in trace amounts has health benefits and thus is referred to as an essential trace element. It forms numerous inorganic compounds (vanadyl sulfate, sodium metavanadate, sodium orthovanadate vanadium pentoxide) as well as complexes with organic compounds. On its own, vanadium compounds can exhibit antitumor and carcinogenic properties (Jan *et al.,* 2012). Other benefits of vanadium include; aiding metabolism in the body, fertility enhancement, promotion of strong bones and teeth, proper functioning of the thyroid gland and the production of hormones in the body (Hopkins and Mohr, 1974). This work is aimed at the synthesis and characterization of metal complexes of tetracycline, 2,2-bipypridine using vanadium sulphate ($VOSO_4$) and investigation of its antimicrobial activity against *E. coli* in comparison to commercially available tetracycline.

2. Experimental

2.1. Materials and Instrumentation

All chemical reagents used was of analytical standard and purchased from Aldrich. Agar media was purchased from Biotech. All glassware used for the experiment was sterilized in a hot air oven maintained at 160^{O}C for one hour and all microbiological media was autoclaved at 121^{O}C for 15 minutes at 15psi.

2.2. Synthesis of the Vanadium Tetracycline Complex

0.222g (0.5mmol) of tetracycline (tc) was dissolved in 10ml of ethanol to give a clear yellow solution. Similarly, 0.0515g (0.5mmol) of vanadium sulphate ($VOSO_4$) was dissolved in 10ml of ethanol to give a clear blue solution. The two aqueous solutions were mixed dropwise into a single beaker and the resulting mixture was stirred magnetically for two hours until a colour change to brown was noted. The reaction mixture was allowed to stand overnight, protected from direct light by wrapping in aluminum foil. The mixture was then subjected to gentle heating to reduce volume prior to centrifugation. The resultant precipitate formed was washed with ethanol, filtered and dried under vacuum.

2.3. Evaluation of Physico-Chemical Parameters

Solubility test of the synthesized metal complex was carried out in water and ethanol as described by USEPA (1996). Its solubility was compared to $VOSO_4$ and tetracycline. Similarly, the melting point was determined using a Thiele tube as described by Blank (1933). The UV-Visible absorption spectra of the vanadium tetracycline complex were recorded using a Shimadzu (model 160IPC) UV-Visible spectrophotometer.

2.4. Antimicrobial Assay

Serial dilution of the vanadium tetracycline complex and control was undertaken to obtain concentrations of 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml. The antimicrobial assay of the complex was tested on the bacterium *Escherichia coli.* The test organism was first grown overnight in nutrient broth, then the concentration of the test bacteria was adjusted to the MacFarlands standard by diluting with sterile distilled water and measuring to an optical density (OD) value of 0.1 at 600nm wavelength using a UV/Vis spectrophotometer.

The well-in-agar method was used to determine the antimicrobial activity of the complexes as described by Cheesbrough, (2006), followed by incubation at 37°C for 24 hrs in an upright position. At the end of incubation, inhibition zones formed around the wells are measured. Antimicrobial activity was expressed as the mean diameter of the clear zone (mm) of growth inhibition produced by the complexes.

The Minimum Inhibitory Concentration (MIC) test is carried out to determine the lowest concentration of the complex that is able to inhibit the growth of the test organism. This test was performed using nutrient broth by mixing different concentrations of the complex with the test bacterium inoculated into various tubes containing nutrient broth. After overnight incubation, the broths were examined for turbidity/cloudiness using a spectrophotometer. Turbidity resulting from microbial growth indicates that the complex/tetracycline concentration present in the test tube had no antimicrobial effect on the test organism while the clear tubes indicate no microbial growth (thus showing growth inhibition by the complex/tetracycline). The MIC is the broth containing the lowest concentration of the extract/tetracycline, which was clear (able to inhibit microbial growth).

The minimum bactericidal concentration test (MBC) was performed to determine if the growth inhibition observed from the MIC test was bactericidal (causing cell death) or simply bacteriostatic (growth inhibition) and to determine the lowest concentration of the crude extract which is bactericidal. Briefly, 0.1ml of nutrient broth is collected from the MIC test tubes showing growth inhibition and inoculated onto sterile nutrient agar. These were incubated for 24hrs at 37°C. After

incubation, the plates are checked for colony formation. If the concentration of the complex/tetracycline in the nutrient broth tube was able to only inhibit the growth of the microbes, without effecting cell death, there would be growth on the Petri-dishes. However, if the concentrations were able to kill the cells, colonies would not be formed on the agar plates. The minimum bactericidal concentration is the lowest concentration of the extract/tetracycline that caused cell death.

3. Results and Discussion

3.1. Physico-Chemical Characteristics

The vanadium tetracycline complex occurred as a dark red crystalline powder which is very soluble in water but partially soluble in ethanol. The complex has a melting point of 240^{0}C at 18^{0}C/min (Rate). Upon subjecting vanadium tetracycline complex and tetracycline to UV-Vis spectrophotometer analysis, the following absorption spectra was observed.

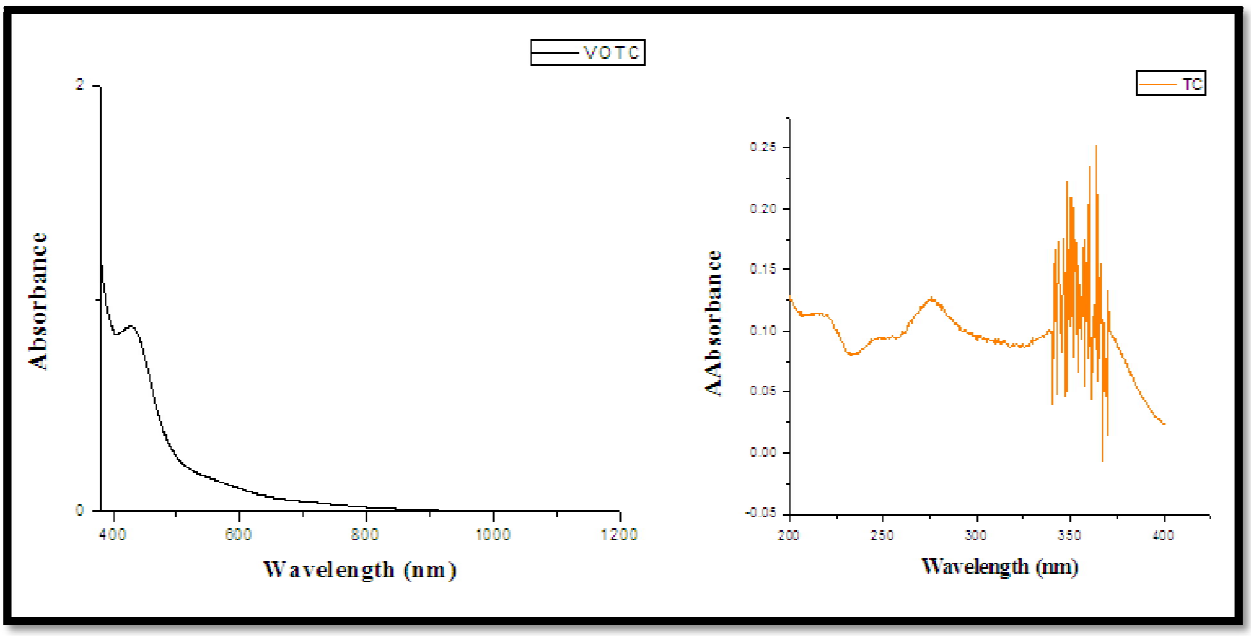

Figure 1: Absorption Spectra of Tetracycline (TC) and [VOTC] Complex

The vanadium tetracycline complex shows absorption in the visible region, with a peak absorbance at 450nm which clearly indicates a transition metal. While tetracycline shows absorption in the ultraviolet region, with a peak absorbance at 355nm thus indicating the presence of a ligand only which is tetracycline. This is similar to the absorption spectrum obtained for doxycycline and its complexes by Obaleye *et al.* (2016), thus suggesting a distorted octahedral geometry typical of the Jahn-Teller effect in hexa-coordinated d^9 metal ion as described by Lever (1984).

3.2. Well-in-Agar Diffusion Assay

In the disk diffusion assay, the tetracycline and VOTC (Vanadium tetracycline complex) samples gave varied zones of inhibitions as shown in table 1 below.

Compound	Conc. (mg/ml)	Zones of Inhibition (mm)	Compound	Conc. (mg/ml)	Zones of Inhibition (mm)
Tetracycline	50	40	VOTC	50	39
	25	37		25	38
	12.5	27		12.5	32
	6.25	18		6.25	19
	3.125	17		3.125	19

Table 1: Zones of Inhibition Obtained from the Agar Diffusion Assay

From the table above, the highest zones of inhibition was recorded for tetracycline compound at 40mm for the 50mg/ml concentration. Similarly, a comparable zone of inhibition of 39mm was recorded by the 50mg/ml concentration of the Vanadium Tetracycline Complex. The lowest zone of inhibition was recorded by tetracycline (17mm) at a concentration of 3.125mg/ml, followed by the vanadium tetracycline complex (19mm) at the concentration of 3.125mg/ml.

3.3. Minimum Inhibition Concentration (MIC) Test

On analysis of the inhibitory properties of the tetracycline and VOTC compound, the result shows that 12.5, 25, and 50mg/ml concentrations of the VOTC compound was able to inhibit the growth of the test organism (*E. coli*). While only the 25mg/ml and 50mg/ml of tetracycline was able to inhibit the test organism. As shown in table 2 below, a minimum inhibitory concentration (MIC) of 12.5mg/ml was recorded for the Vanadium tetracycline complex while that of tetracycline was recorded as 25mg/ml. Thus, the activity of the VOTC complex was greater than that of the parent ligand (tetracycline) in the inhibition of bacterial growth. This is in line with the findings of Tella and Obaleye (2010) who synthesized metal complexes of sulphadimidine.

Concentration	[VOTC]	Tetracycline
3.125mg/ml	Turbid	Turbid
6.25mg/ml	Turbid	Turbid
12.5mg/ml	Clear	Turbid
25mg/ml	Clear	Clear
50mg/ml	Clear	Clear
MIC	12.5mg/ml	25mg/ml

Table 2: Minimum Inhibition Concentration Assay of the Complexes

3.4. Minimum Bactericidal Concentration (MBC) Test

On sub-culturing the broth cultures from the MIC test that showed growth inhibition onto solid nutrient agar plate, only the 50mg/ml concentration of the tetracycline showed bactericidal activity, completing killing the test organism and preventing growth. While the 25mg/ml and 50mg/ml of the Vanadium tetracycline complex (VOTC) killed the test organism (bactericidal) and prevented its growth, as shown in the table below. Thus, the MBC of tetracycline is 50mg/ml while MBC for Vanadium tetracycline complex is 25mg/ml indicating that the VOTC complex was more effective than the parent tetracycline in eliminating bacteria at low concentrations.

Concentration	VOTC Compound	Tetracycline
12.5mg/ml	Growth	-
25mg/ml	No growth	Growth
50mg/ml	No growth	No growth
MBC	25mg/ml	50mg/ml

Table 3: Minimum Bactericidal Concentration (MBC) Test

From the results obtained above, both the vanadium complex and tetracycline were able to cause growth inhibition of the test organism as shown by the comparable zones of inhibition (40mm and 39mm) obtained for the tetracycline and vanadium complex respectively in the agar diffusion assay. On measurement of the minimum inhibitory concentration (MIC), the VOTC compound performed relatively better at inhibiting the test organism (*E. coli*) in comparison to tetracycline with an MIC of 12.5mg/ml against that of 25mg/ml recorded for tetracycline.

Furthermore, from the MBC test, a lower concentration of the VOTC compound (25mg/ml) completely killed the test organism in comparison to tetracycline (50mg/ml). Since it is desirable that as low a dose as possible of a compound is effective in the elimination of a microbial pathogen, the VOTC compound promises to serve as a good antimicrobial agent with the potential of replacing tetracycline in light of the development of bacterial resistance to tetracycline antibiotics, subject to purification, characterization and evaluation of its toxicity.

4. Conclusion

The interaction of tetracycline with metal salts of vanadium resulted in the formation of tetracycline vanadium complex. The complex was characterized by solubility, melting point determination and UV-Vis absorbance. The complex was shown to be stable, hygroscopic and soluble in water but only partially soluble in ethanol. On assessing the bactericidal activity of the complex on *E.coli*, the complex was able to inhibit and kill the test bacteria at concentrations lower than the commercial tetracycline antibiotics, thus proving to have potential as an antimicrobial drug for the treatment of infections caused by bacteria which has developed resistance to tetracycline and other antimicrobials.

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