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Effect of Combinations of Mycobacteriophages and Drugs Against *Mycobacterium Spp.*

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Abstract- The emergence of multidrug-resistant tuberculosis (MDR-TB) is a matter of global concern. The use of mycobacteriophages alone or in combination with antibiotics could be used as an alternative approach to treat drug-resistant Mycobacteria. The aim of the study was the evaluation of the effect of an isolated mycobacteriophage in combination with Isoniazid, Rifampicin, Streptomycin, and Ampicillin against *Mycobacterium tuberculosis* and *Mycobacterium smegmatis.* Determination of the MIC of the above drugs for each strain was done by the standard tube dilution method. Further, the inhibitory effect of varying drug concentrations in combination with different dilutions of the mycobacteriophage was studied. The growth inhibition pattern for both the organisms was also studied in the presence of different dilutions of mycobacteriophage by turbidometric and resazurin dye reduction method. The killing pattern of drugs, at concentrations below the MIC, when combined with mycobacteriophage, was determined. The phage induced lysis of bacteria assisted in decreasing the inhibitory concentration of the drugs.

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Effect of Combinations of Mycobacteriophages and Drugs Against *Mycobacterium Spp.*

Rajitha Satish [α] & Anita Desouza [σ]

Abstract- The emergence of multidrug-resistant tuberculosis (MDR-TB) is a matter of global concern. The use of mycobacteriophages alone or in combination with antibiotics could be used as an alternative approach to treat drug-resistant Mycobacteria. The aim of the study was the evaluation of the effect of an isolated mycobacteriophage in combination with Isoniazid, Rifampicin, Streptomycin, and Ampicillin against *Mycobacterium tuberculosis* and *Mycobacterium smegmatis.* Determination of the MIC of the above drugs for each strain was done by the standard tube dilution method. Further, the inhibitory effect of varying drug concentrations in combination with different dilutions of the mycobacteriophage was studied. The growth inhibition pattern for both the organisms was also studied in the presence of different dilutions of mycobacteriophage by turbidometric and resazurin dye reduction method. The killing pattern of drugs, at concentrations below the MIC, when combined with mycobacteriophage, was determined. The phage induced lysis of bacteria assisted in decreasing the inhibitory concentration of the drugs.

Keywords: phage therapy; mycobacteriophages; combination therapies; MDR TB; Mycobacterium tuberculosis; isoniazid, rifampicin, streptomycin, ampicillin, Mycobacterium smegmatis, MIC.

I. Introduction

Organizations such as the Center for Disease Control (CDC) and the World Health Organization(WHO) have declared that bacterial antibiotic resistance is a threat to global human health. Drug-resistant TB is one of the major causes of the public health crisis. As per the Global WHO report of 2018, in the year 2017 alone, 558,000 cases of Rifampicin-resistant TB (RR-TB) were reported, and of these, 82% had multidrug-resistant TB (MDR-TB). Three countries across the world account for almost half of the world's cases of MDR/RR-TB: India (24%), China (13%), and the Russian Federation (10%). Treatment for Rifampicin-resistant TB (RR-TB) and multidrug-resistant TB (MDR-TB) is expensive (≥US$ 1000 per person). MDR-TB requires that it be treated with second-line drugs for more than 24 months. As these drugs are costly and more toxic, there is a requirement of urgent action for the improvement of the quality of diagnosis and the treatment of drug-resistant TB (Website 1).

TB awareness programs and infection control measures are crucial for preventing transmission of resistant strains. MDR-TB programs that rely on internationally recommended treatment regimens, according to WHO guidelines, must be scaled up and strengthened to prevent further second-line drug resistance and spread of XDR-TB. However, the increase in incidence of XDR-TB is an indirect indicator of the program's failure to adequately diagnose, prevent, and treat MDR-TB. The emergence of XDR-TB in 6 continents, coupled with the increased use of second-line drugs, suggests that urgent measures are needed to improve the rational use of quality-assured second-line drugs, new anti-TB drug regimens and better diagnostic tests for TB and MDR-TB. Such steps are crucial if future generations are to be protected from potentially untreatable TB(Leimane et al., 2005; Shah et al., 2007; Website 2).

The period between 1950 and 1970 was a turning point in the battle against tuberculosis during which the discovery of most of the current anti-tuberculosis drugs occurred, and the therapeutic regimens were introduced that made it possible to cure tuberculosis. However, with the exception of the fluoroquinolones, no new antituberculosis drug has been introduced in the past 45 years. The probability of successful treatment further decreases with the emergence of drug-resistant strains as MDR and XDR tuberculosis generally have a high mortality rate (Caminero et al.,2010).

The global incidence of tuberculosis is increasing, and the existing diagnostic technologies are not adequately accurate. For example, sputum smear microscopy (SSM) is the most widely used diagnostic test for TB as it is a quick test and not difficult to perform, but its sensitivity is low, and it cannot distinguish between viable and dead bacteria. Bacterial culture diagnosis, though time-consuming has better diagnostic accuracy when compared to SSM. Nucleic Acid Amplification Tests (NAATs) have high sensitivity but low specificity (Dinnes, 2007).The tuberculosis-specific ELISPOT assay (T-SPOT.TB) gives an overall measurement of the antigen load on the immune system and thus can reveal the presence of subclinical disease. Since this assay does not depend on the production of a reliable antibody response or the recovery of the pathogen, the technique can be used to detect latent tuberculosis condition(Ahmad, 2010). Due to the rise in prevalence of multidrug-resistant tuberculosis (MDR-TB) and extremely drug-resistant tuberculosis (XDR-TB),

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novel technologies for TB diagnosis, therapy, and prevention are required urgently. These growing concerns have drawn the attention of TB researchers to phages. The biological characteristics of phages enable them to be advantageous in treating drug-resistant bacterial infections (Guo and Zhi,2012).

On September 21, 2016, the United Nations General Assembly convened to discuss the problem of antibiotic resistance and deemed it "the greatest and most urgent global risk". One of the popular suggestions for an alternative strategy for prophylaxis and control of bacterial infections was phage therapy. Phage therapy, which implies the use of viruses (phages) to treat bacterial infections, has been around for almost a century. The universal decline in the effectiveness of antibiotics has generated renewed interest in phage therapy, which has several advantages over antibiotics such as host specificity, self-amplification, biofilm degradation, and low toxicity to humans (Lin et al., 2017).

Though phages have been extensively used therapeutically in the former Soviet Union countries, they have yet to find widespread use in other parts of the world. Phage preparations for use against *E.coli* and *Listeria* meat contamination have received approvals and trials are in progress for control of several infections involving skin afflictions and burns (Hatfull, 2014). Mycobacteriophages are viruses that infect mycobacterial hosts, such as *Mycobacterium tuberculosis and Mycobacterium smegmatis* (Hatfull, 2012).

A number of mycobacteriophages from different environmental samples and their sequenced genomes have been reported by the Phage Hunters Integrating Research and Education (PHIRE) program at the University of Pittsburgh, and the Science Education Alliance Phage Hunters Advancing Genomics and Evolutionary Sciences (SEA-PHAGES) program by Professor Graham Hatfull and his coworkers (Hatfull,2014). As mycobacteriophages infect *Mycobacteria*, resulting in their lysis and death, the possibility of using them as therapeutic agents against the deadly mycobacterial disease, tuberculosis is of great interest. Of the many mycobacteriophages isolated, the lytic phage TM4 and the lysogenic phage L5 have been extensively studied (Samaddar et al., 2016).

Mycobacteriophages display a remarkable genetic diversity. Till date, 10552 mycobacteriophages have been identified, of which 1795 have been sequenced (Website 3). The sequenced mycobacterio-phage genomes get grouped into clusters and subclusters based on their sequence identity. About 30 distinct types (called clusters or singletons if they have no similarities) have been identified. The clusters that span sufficient diversity warrant further division into subclusters. Host range analysis shows that phages in Cluster K, and certain subclusters(A2 and A3) of Cluster A, efficiently infect *M. tuberculosis* (Hatfull,2014).

In the year 2019, Nature Medicine reported the case of a 15-year-old cystic fibrosis patient with a *Mycobacterium abscessus* infection; treated with a cocktail of three mycobacteriophages. Hatfull and his team searched through a phage library of over 10,000 different phages to find the phages that would most effectively kill the mycobacterial strain in the abscess. One of the three phages was lysogenic and was converted by genome engineering to a lytic phage. The phages were delivered intravenously. The treatment was well tolerated and was successful in bringing about sternal wound closure, improved liver function, and substantial resolution of the infected skin nodules (Dedrick et al., 2019).

D29 mycobacteriophage was used first for TB diagnosis, and later it showed potential as a therapeutic agent against TB. It was effective in treating guinea pigs infected with both drug-sensitive and drug-resistant *M. tuberculosis* strains (Li et al., 2009). TM4 has been reported to kill fast-growing Mycobacteria such as *M.smegmatis* as well as the slow-growing *M.tuberculosis H37Rv* and *M.ulcerans(*Pope et al.,2011).Researchers have predicted that as TM4 has the most highly expressed genome and the greatest ability to kill mycobacteria in vivo, it might be the best choice for phage therapy against TB. Among the Cluster K phages, extensive research has been done to date, only with TM4, and it is anticipated that in the future more Cluster K phages will be developed for therapeutic use. For successful phage therapy, the challenge is to create an ideal transport vehicle that would allow the mycobacteriophage to be administered into the human body (Abedon et al., 2011). Although TM4 is being considered for use in TB therapy, additional novel safe phages that can treat TB effectively need to be obtained or constructed through genetic recombination. The aim would be to establish a repository of therapeutic phages and thereby provide more choices for combination therapy strategies to treat TB. In recent years, phage therapy has gained significance in light of the increasingly alarming situation of drug-resistant TB around the world(Guo and Zhi, 2012).

The efficiency with which the bacteriophages can reduce the bacterial load will depend on the number of phages present, the adsorption efficiency of the phage, its life cycle, the length of the latent phase, the burst size and the growth rate of the host. In order to put mycobacteriophages to therapeutic use, it is necessary to study the mechanism by which they bring about a reduction in the bacterial population and to determine a quantitative relationship between phage growth and decrease in bacterial numbers(Samaddar et al., 2016).

In the present study, we have attempted to study the relationship between phage and bacterial growth in order to determine the effect of the number of

mycobacteriophages on host inactivation. We also studied the combined effect of various antibiotics and mycobacteriophages which may serve as an alternative treatment for tuberculosis. We attempted to determine whether the phage induced lysis of *Mycobacterium,* would assist in decreasing, the MIC of the anti TB drug. The understanding of these mechanisms will enable the development of new strategies to combat tuberculosis.

The aim of the study was to determine the antibacterial effect of mycobacteriophage individually or in combination with varying concentrations of antibiotics so that they can eventually be used as antimycobacterial therapeutic agents either alone or in combination with antibiotics.

II. Materials and Methods

a) Cultivation of Mycobacterium spp.

Mycobacterium smegmatis MTCC 994 and *Mycobacterium tuberculosis* MTCC 300(IMTECH, Chandigarh) both non-virulent mycobacterial strains were used for the study of the mycobacteriophages previously isolated (Satish and Desouza, 2018). The strains were cultivated in Nutrient broth at 37°C for 48 hrs.

b) Phage isolation

In a previous study, ten mycobacteriophages were obtained from soil and sewage samples using *Mycobacterium smegmatis* MTCC 994 as host. Of the ten phages obtained, three were found to infect four different species of *Mycobacterium*, namely *M. fortuitum subsp. fortuitum* MTCC 993, *Mycobacterium kansasii* MTCC 3058, *Mycobacterium avium subsp. avium* MTCC 1723, and *M. tuberculosis* MTCC 300, besides the host *M. smegmatis* (Satish and Desouza, 2018). Characterization of the growth parameters of one of the mycobacteriophages which exhibited host diversity was done in an earlier study (Satish and Desouza, 2019). This mycobacteriophage was chosen in the present study to determine the combined effect of phage and antibiotics against *M. tuberculosis* and *M. smegmatis.*

c) Antimicrobial agents

Four anti-tuberculosis drugs were used in the present study. Isoniazid, Rifampicin, and Ampicillin were obtained from Hi-Media Laboratories, and Streptomycin was obtained from Ambistryn S.

d) Determination of Minimum Inhibitory Concentration of the drugs

The MIC of Isoniazid, Rifampicin, Streptomycin, and Ampicillin was determined for both the Mycobacterial strains by the standard broth dilution method. Various drug concentrations in the range, as listed in Table 1, were prepared in 5ml of Mueller Hinton medium and inoculated with 100µl of 48 hr old inoculum having a density of 0.1 OD_{530}. The tubes were incubated at 37°C for 24-48hrs. The MIC was determined as the lowest drug concentration at which there was no visible bacterial growth. Each drug concentration was tested in triplicate.

Table 1: Drug range to determine MIC

Organism	*Mycobacterium tuberculosis* MTCC 300	*Mycobacterium smegmatis* MTCC 994
Isoniazid	1.0-10.0mg/ml	0.25-1.5 mg/ml
Rifampicin	0.5-10.0 µg/ml	50.0 -500.0 µg/ml
Streptomycin	1.0 -10.0 µg/ml	0.5-5.0µg/ml
Ampicillin	50.0 -500.0 µg/ml	0.1 - 1.0 mg/ml

e) Inhibitory effect of a combination of drug and mycobacteriophage

The assay was performed in a 96well microtiter plate. The inoculum used was a saline suspension of 48 hr old culture of *Mycobacterium tuberculosis MTCC 300* or *Mycobacterium smegmatis MTCC 994* (OD_{530}–0.1). To determine the inhibitory effect of a combination of drug and mycobacteriophage; the drug concentrations chosen were the MIC value itself, and one concentration above and below the MIC for each of the drugs.

Into the wells in Row A was added 250µl of drug of the given concentration prepared in MH broth and 50µl of culture *Mycobacterium tuberculosis MTCC 300*. The drug concentrations ranged as follows – Isoniazid - 1.0-5.0mg/ml, Rifampicin - 0.25-3.0µg/ml, Streptomycin - 1.0-8.0µg/ml, Ampicillin -12.5-150.0µg/ml. Into the wells in Row B was added 125µl of each drug concentration prepared in MH medium; 125µl of mycobacteriophage (10^6pfu/ml) and 50µl of culture *Mycobacterium tuberculosis MTCC 300.* The final drug concentration achieved in Row B was the same as in Row A. Row C consisted of controls. The positive control consisted of 250µl of sterile MH broth and 50µl of culture *Mycobacterium tuberculosis* MTCC 300. The medium control consisted of 300µl of sterile MH broth. The phage control consisted of 125µl of sterile MH broth, 125µl of mycobacteriophage (10^6pfu/ml), and 50µl of culture *Mycobacterium tuberculosis* MTCC 300. The plates were incubated at 37°C for 24hrs, after which 30µl of resazurin (0.015%) were added to all the wells, further incubated at 37°C for 2-4hrs and observed for color change (blue to pink). The assay was performed similarly for *Mycobacterium smegmatis MTCC 994.* The drug concentrations used were as follows –Isoniazid - 0.25–1.5mg/ml, Rifampicin - 50.0 -500.0µg/ml, Streptomycin - 0.5-4.0µg/ml and Ampicillin - 0.1-1.0mg/ml.

f) Synergistic Inhibitory effect of drug and Mycobacteriophage

i. *Assay using resazurin dye reduction*

The assay was performed in a 96 well microtiter plate. The drug ranges used were as follows – Isoniazid - 1.0-5.0mg/ml, Rifampicin - 0.25-3.0µg/ml,

Streptomycin - 1.0-8.0µg/ml, Ampicillin - 0.0125-0.15. The additions in the five rows were as follows: Row A – In the first five wells –125µl of the various concentrations of each drug prepared in sterile MH broth,125µl of mycobacteriophage (10^6pfu/ml) and 50µl of culture suspension of *Mycobacterium tuberculosis MTCC 300*(OD_{530} – 0.1). Rows B, C, and D had the same drug concentrations as Row A, while the phage added had a count of 10^5pfu/ml, 10^4pfu/ml, 10^3pfu/ml, respectively. Row E consisted of 250µl of the various concentrations of each drug prepared in sterile MH broth and 50µl of culture suspension of *Mycobacterium tuberculosis MTCC 300.* In Row A, the last two wells were medium controls consisting of the only 300µl of sterile MH broth. In Row B, the last two wells were phage controls consisting of 125µl of sterile MH broth, 125µl of mycobacteriophage (10^6pfu/ml), and 50µl of culture suspension of *Mycobacterium tuberculosis MTCC 300.* In Row C, the last two wells were positive controls consisting of 250µl of sterile MH broth and 50µl of culture *Mycobacterium tuberculosis MTCC 300.* The plates were incubated at 37°C for 24hrs.30µl of resazurin (0.015%) were then added to all the wells, further incubated at 37°C for 2-4hrs and observed for color change. With *Mycobacterium smegmatis MTCC 994* the experiment was performed in a similar manner. The drug concentrations range used for *Mycobacterium smegmatis MTCC 994* were as follows:Isoniazid –0.25-1.5mg/ml, Rifampicin-50.0-300.0µg/ml, Streptomycin-0.5- 4.0µg/ml, Ampicillin- 0.1-1.0 mg/ml

ii. *Mycobacterial Growth Pattern in the presence of mycobacteriophage and drug*

The experimental set up was the same as in the previous experiment. The growth was determined by measuring turbidity and not by resazurin reduction. After adding the culture to the wells, the plates were incubated at 37°C for 18hrs. Culture turbidity was determined at intervals of 3 hours by measurement of the absorbance of each well at 530nm with a microplate reader with moderate shaking at intervals of 30min. A graph of Time (hrs) vs. $Absorbance_{530}$ was plotted to determine changes in growth pattern.

g) *Mycobacterial Growth Pattern in the presence and absence of mycobacteriophage*

The assay was performed in a 96well microtiter plate. For the control set, 50µl of saline suspension (OD_{530}- 0.1) of the host culture (*Mycobacterium tuberculosis MTCC 300* or *Mycobacterium smegmatis* MTCC 994) was added to wells containing 250µl of sterile Nutrient broth. For the test set 50µl of saline suspension (OD_{530}-0.1) of the host culture (*Mycobacterium tuberculosis MTCC 300* or *Mycobacterium smegmatis* MTCC 994) was added to wells containing 125µl of sterile Nutrient Broth and 125µl of phage suspension of two different counts (10^6pfu/ml, 10^5pfu/ml) respectively. The plates were then incubated at 37°C for 24hrs. Culture turbidity was determined at an interval of 3 hours for a period of 24 hours by measurement of the absorbance of each well at 530nm with a microplate reader with moderate shaking at intervals of 30min.

III. Results

The MIC was determined by the tube dilution method for four antituberculosis drugs - Isoniazid, Rifampicin, Streptomycin, and Ampicillin against two organisms-*Mycobacterium tuberculosis* MTCC 300 and *Mycobacterium smegmatis* MTCC 994.The results are presented in Table 2.

Table 2: MIC of antituberculosis drugs

Organism	*Mycobacterium tuberculosis* MTCC 300	*Mycobacterium smegmatis* MTCC 994
Drugs	MIC	MIC
Isoniazid	5.0 mg/ml	1.5 mg/ml
Rifampicin	2.0 µg/ml	200.0 µg/ml
Streptomycin	4.0 µg/ml	2.0 µg/ml
Ampicillin	0.1 mg/ml	1.0 mg/ml

The experiment for the inhibitory effect of a combination of drug and mycobacteriophage was performed using different concentrations of the drugs and mycobacteriophage in microtiter plates. Resazurin was used to determine the presence of viable cells. It is a redox indicator dye which is blue in its oxidized state and gets reduced to pink resorufin by metabolically active cells. There is a direct correlation between the reduction of resazurin in a growth medium and the proliferation of live organisms. The results interpretation was as follows – wells with no color change (blue resazurin) were reported as no growth whereas wells, where resazurin had turned pink were reported as growth.

Figures 1,2,3 and 4 show the synergistic inhibitory action of mycobacteriophages with Isoniazid, Rifampicin, Streptomycin, and Ampicillin respectively against *Mycobacterium tuberculosis* MTCC 300. In all the four figures, Row A indicates the MIC of the drug against *Mycobacterium tuberculosis* MTCC 300. The MIC so determined is as follows – Isoniazid – 5.0mg/ml (Fig.1, RowA); Rifampicin - 2.0µg/ml(Fig.2, Row A); Streptomycin - 4.0µg/ml (Fig.3, RowA) and Ampicillin - 0.1mg/ml(Fig.4, RowA). Row B, in all the four figures, contained the respective drug concentrations combined with mycobacteriophages. No growth was seen in all the wells in Row B, including the well containing the lowest concentration of the drugs. Row C, in all the figures, contained controls.

Figures 5, 6, 7, and 8 similarly show the synergistic inhibitory action of mycobacteriophages with Isoniazid, Rifampicin, Streptomycin, and Ampicillin respectively against *Mycobacterium smegmatis* MTCC

994. The MIC of the drugs against *Mycobacterium smegmatis* MTCC 994determined by the results in Rows A is as follows – Isoniazid-1.5mg/ml (Fig.5, Row A); Rifampicin-200.0µg/ml (Fig.6, Row A); Streptomycin-2.0µg/ml (Fig.7, Row A) and Ampicillin –1.0mg/ml(Fig.8, Row A). Row B, in all the four figures, contained the respective drug concentrations combined with mycobacteriophages. Similar to the experiment with *Mycobacterium tuberculosis* MTCC 300, no growth was seen in all the wells in Row B, including the well containing the lowest concentration of the drugs. Row C, in all the figures, contained controls.

Figures 9, 10, 11, and 12 represent the synergistic inhibitory action of various mycobacteriophage dilutions with varying concentrations of Isoniazid, Rifampicin, Streptomycin, and Ampicillin respectively against *Mycobacterium tuberculosis* MTCC 300. Row E, inall the four figures, contains only drug and thus indicates the minimum inhibitory concentration of the given drug. The MIC obtained are as similar to the previous experiment namely Isoniazid-5.0mg/ml (Fig.9, Row E); Rifampicin - 2.0µg/ml (Fig.10, Row E); Streptomycin - 4.0µg/ml (Fig.11, Row E) and Ampicillin –0.1mg/ml (Fig.12, Row E). In all the four figures, Row A,B,C, and D consist of combinations of drugs with different mycobacteriophage dilution. In all the four sets, Row A and B that contained mycobacteriophages at 10^6pfu/ml and 10^5pfu/ml respectively, there was no visible growth even in the wells containing the least concentration of the drugs. However, in Row C and D that contained mycobacteriophages at 10^4pfu/ml and 10^3pfu/ml respectively, there was visible growth in all the wells having drug concentrations below the MIC.

Results of the similar experiments with *Mycobacterium smegmatis* MTCC 994 are shown in figures 13, 14, 15, and 16. The MIC determined from the results in Row E is as follows – Isoniazid - 1.5mg/ml(Fig.13,Row E); Rifampicin - 200.0µg/ml (Fig.14, Row E); Streptomycin - 2.0µg/ml (Fig.15, Row E) and Ampicillin - 1.0mg/ml(Fig.16, Row E). Similar to the results obtained for *Mycobacterium tuberculosis* MTCC 300, there was no visible growth in rows A and B where the mycobacteriophage density was 10^6pfu/ml and 10^5pfu/ml respectively; while in rows C and D that contained mycobacteriophages at 10^4pfu/ml and 10^3pfu/ml respectively, there was visible growth in all the wells that had drug concentrations below the MIC. Thus the lower concentration of mycobacteriophage i.e 10^4pfu/ml and 10^3pfu/ml were not effective in killing the organisms whereas higher mycobacteriophage concentration of 10^6pfu/ml and 10^5pfu/ml were able to inhibit microbial growth.It is evident that MOI plays a role in the efficiency of the lytic activity of the phage. Thus a minimum phage count is required to be effective in inhibiting the growth of *Mycobacterium.*

Growth patterns in the presence of drugs Isoniazid, Rifampicin, Streptomycin, and Ampicillin at concentrations below the MIC of the drugs and in combination with mycobacteriophage count of 10^6pfu/ml for 18hrs are presented in figures 17, 18, 19 and 20 for *M. tuberculosis* and in figures 21, 22, 23 and 24 for *M. smegmatis*. It was observed that in all the cases, the phages were effective in killing the organisms within 3hours while in the presence of drugs alone, the growth rate was reduced to some extent, but total inhibition was not seen.

The growth patterns of *Mycobacterium tuberculosis* MTCC 300 and *Mycobacterium smegmatis* MTCC 994 were studied in the presence and absence of phage counts of 10^6pfu/ml and 10^5pfu/ml. As seen in figures 25 and 26 the mycobacteriophages having counts of10^6pfu/ml and 10^5pfu/ml were able to suppress bacterial growth of both organisms over the 24 hour period of the study.

IV. Discussions

Mycobacteriophages (10^6pfu/ml and 10^5pfu/ml) were efficient in killing both *Mycobacterium tuberculosis* MTCC 300 and *Mycobacterium smegmatis* MTCC 994. In the presence of phage, the growth of the organisms was inhibited even at the least concentration of the drug tested (Table 3).

Table 3: Inhibitory concentrations of the drugs in presence and absence of phage

Organism	*Mycobacterium tuberculosis* MTCC 300		*Mycobacterium smegmatis* MTCC 994	
Drugs	Minimum Inhibitory Concentration	Least drug concentration inhibiting growth in the presence of phage	Minimum Inhibitory Concentration	Least drug concentration inhibiting growth in the presence of phage
Isoniazid	5.0 mg/ml	1.0 mg/ml	1.5 mg/ml	0.25 mg/ml
Rifampicin,	2.0µg/ml	0.25 µg/ml	200.0 µg/ml	50.0 µg/ml
Streptomycin	4.0 µg/ml	1.0 µg/ml	2.0 µg/ml	0.5 µg/ml
Ampicillin	0.1mg/ml	0.0125 mg/ml	1.0 mg/ml	0.1 mg/ml

Bacteria on which phage has been adsorbed are ultimately destined to be lysed after about 60 min of infection, which is the latent period. In a continuous infection model, phage adsorption will take place continuously, accompanied by lysis. Simple mathematical modeling predicts that within 100 min, the number of susceptible bacteria should go down by at least 100 fold at an MOI of 0.1. Such a hundred fold reduction in cell counts should result in substantial changes in optical density (Samaddar et al.,2016). A

previous study (Pereira et al., 1983) reported the inability of phage D29 to completely lyse *M. smegmatis* host cells where it was shown that the optical density decreased to about 50% of the initial level in 2 hrs. However, they found that a mutant of D29 was able to achieve a much higher level of lysis (Pereira et al., 1983).In the present study, complete lysis of bacteria was observed at a higher mycobacteriophage concentration of 10^6pfu/ml and 10^5pfu/ml. This mycobacteriophage induced lysis of bacteria helped to reduce the MIC of the drugs against the organism. However, at lower mycobacteriophage concentrations, the extent of lysis decreased significantly.

Based on the investigations performed in the study by Samaddar et al., (2016), a model was proposed to explain lysis of bacteria in the presence of phage. As per this model, in the presence of phage, a fraction of the cells are lysed, resulting in death by lysis (DL), whereas cells present in the remaining fraction that do not come in contact with the phage undergo death through secondary mechanisms, termed death without lysis (DWL). The phenomenon of DWL probably involves superoxide radicals. However, DWL also can happen due to other reasons, such as the induction of programmed cell death (PCD) through the mediation of toxin-antitoxin (TA) systems, as observed in the case of *E. coli* cells infected with phage P4. The cycle of infection, phage release, and cell death through secondary mechanisms continue to be repeated, resulting in the lowering of viable cell counts by several orders of magnitude. However, further investigations need to be undertaken to deduce the pathways, involved in the generation of these reactive species.

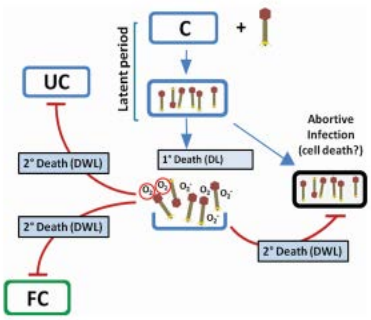

Fig. 1: Model to describe phage-induced killing of host cells (Samaddar et al, 2016)

V. Conclusion

The isolated mycobacteriophage at a concentration of 10^5pfu/ml and above was effective in killing both the organisms *Mycobacterium tuberculosis* MTCC 300and *Mycobacterium smegmatis* MTCC 994 even in the absence of drugs. This was observed by the resazurin dye reduction test. It was also confirmed by determining growth patterns by measuring turbidity. The mycobacteriophage was effective in inhibiting the growth of both the Mycobacterial spp. upto 24 hours of study. It appears that the mycobacteriophage used in this study has the potential for therapy against *Mycobacterium spp.* The phage was highly effective in reducing the MIC of the drugs against the organisms. Therefore it would be useful in reducing the concentration of drugs to be consumed and in decreasing the toxic effects of long term drug usage.

To use bacteriophages as pharmaceuticals in the future, it is necessary to obtain a quantitative and mechanistic insight into how they bring about a reduction in the bacterial population. Samaddar et al., (2016) have reported that bacteriophages can reduce bacterial counts and the efficiency with which the bacterial loads are reduced will depend on the number of phages present, their adsorption efficiency, the phagelife cycle, length of the latent period and the growth rate of the host.

Even though it is known that phages being host specific are nonpathogenic to humans, it is important to ensure that the phages are not capable of gene transduction and that they do not carry antibiotic resistance genes (Sulakvelidze et al**.,** 2001). Thus, it is necessary to make a systematic toxicological analysis along with the studies at the molecular level on the candidate phage before large scale clinical trials. Also, the phage should be of low immunogenicity, as exogenous microorganisms may trigger an immune response. A previous study showed that phages were cleared by the reticuloendothelial system when they entered the human body through the circulatory system, resulting in low phage titres at the site of infection; that were not sufficiently high to have a maximal therapeutic effect. Therefore, novel phages with low immunogenicity should be obtained by screening phage mutants, or by genetic reconstruction, or by coating them with biocompatible materials, to escape removal by the immune system(Guo and Zhi, 2012; Merril et al., 1996;Vitielloet al.,2005).

One of the most important problems in the realization of phage therapy is the emergence of resistant bacterial strains. The adsorption of phage to bacterial cells is dependent on the presence of specific receptors on the cell wall. Tanji (2004), in his study introduced a rational procedure for selecting an effective phage cocktail for controlling *E.coli*. The use of a cocktail of two phages delayed the emergence of phage resistant *E. coli* cells. Based on the results obtained in this study, a cocktail consisting of more than two phages that bind to different cell receptors will help delay and even suppress the emergence of phage resistant bacteria (Tanji, 2004). This is similar to the concept of combination antibiotic therapy. Researchers have found that phage mixtures can prevent resistant bacteria from arising during the therapeutic use of phage, and the time before emergence of resistant

bacteria could be prolonged through optimization of the phage cocktail (Tanji,2005). The question that needs to be addressed is whether administration of large doses of phage will cause any side effects or even be fatal. However, the advantage of phage therapy is that since phages are composed of protein and DNA, their toxicity is low in a different cell system(Merril et al.,1996). In addition, when phage particles get degraded, toxic metabolites are not produced, as would happen with the metabolism of antibiotics. Therefore, high dose treatment will not result in toxicity; except may be a mild allergic reaction to the phage capsid protein, though such reactions are likely to be uncommon (Abedonand Thomas-Abedon,2010).

There is a great need to explore and focus on novel treatments like phage therapy due to the fast advent of MDR TB. Phage therapy using reported or newly isolated mycobacteriophages can be considered as an adjunct to antibiotic treatment alone or in combination against drug-resistant TB. The intracellular nature of mycobacterial infection complicates the phage delivery method, which can be overcome either by using nonpathogenic Mycobacterium infected with the mycobacteriophage or employing a delivery system such as liposome-mediated phage delivery (Gondiland Chhibber, 2018).

Delivery of phages to the lungs should be relatively simple, although there is considerable doubt as to whether they would effectively reach their bacterial hosts, which may be intracellular and within granulomas. A suggestion for addressing this issue was proposed by Broxmeyar et al.,(2002), who studied the use of infected, non-virulent, surrogate mycobacterial cells for the delivery of phages.

The ability of bacteriophages to act as bactericidal agents has prompted researchers to consider them as an alternative to antibiotics as they could be used directly to destroy the pathogens. Many of the phage-encoded products, such as lysins, are highly toxic to bacteria; therefore, instead of using the phages directly, their products could also be used. Finally, it is important to note that phages have the ability to inhibit the metabolism of their hosts (host inactivation); hence, by understanding how they perform this act, it may be possible to develop new strategies that do not necessarily use the phage directly for intervention against bacterial diseases (Matsuzaki et al., 2005; Sammadar et al.,2016).

The rapid development of proteomics, genomics, bioinformatics, and other disciplines strongly supports basic phage research. Therefore, it is clear that while the study of phage therapy for treating TB is still in the in-vitro and animal experimentation stages; further research is necessary so that a novel effective method for the treatment of drug-resistant TB will be available in the future, which will potentially shorten the duration of the chemotherapy treatment.

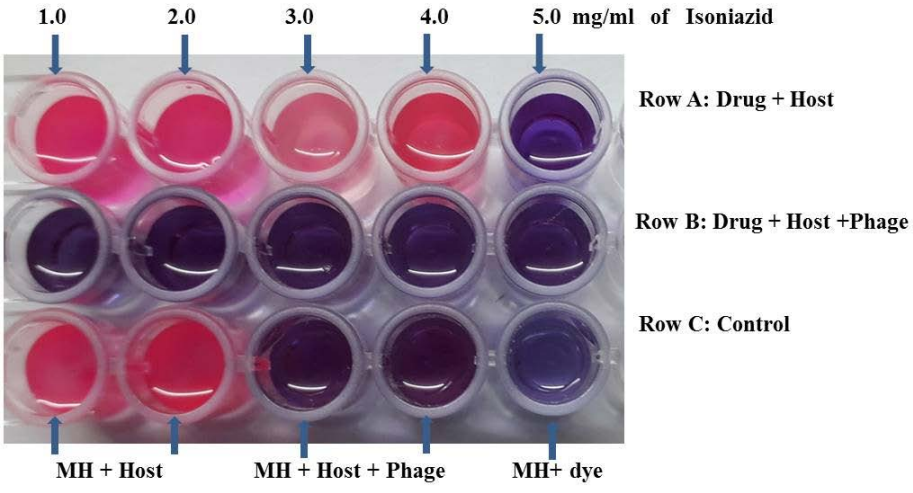

Figure 1: Synergistic action of mycobacteriophage and isoniazid on *Mycobacterium tuberculosis* MTCC 300

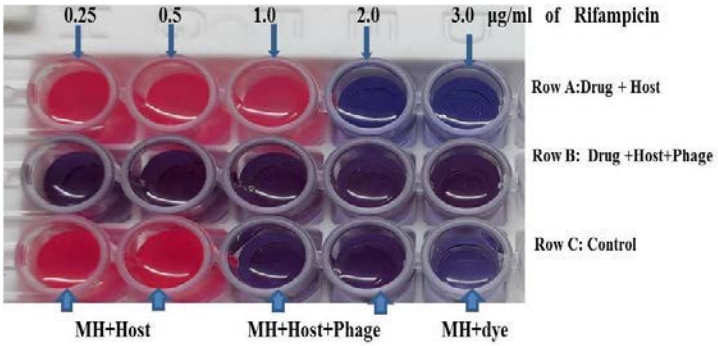

Figure 2: Synergistic action of mycobacteriophage and Rifampicin on *Mycobacterium tuberculosis* MTCC 300

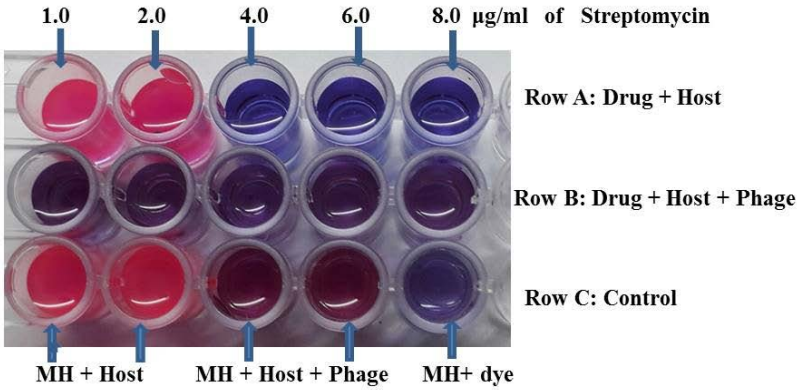

Figure 3: Synergistic action of mycobacteriophage and Streptomycin on *Mycobacterium tuberculosis* MTCC 300

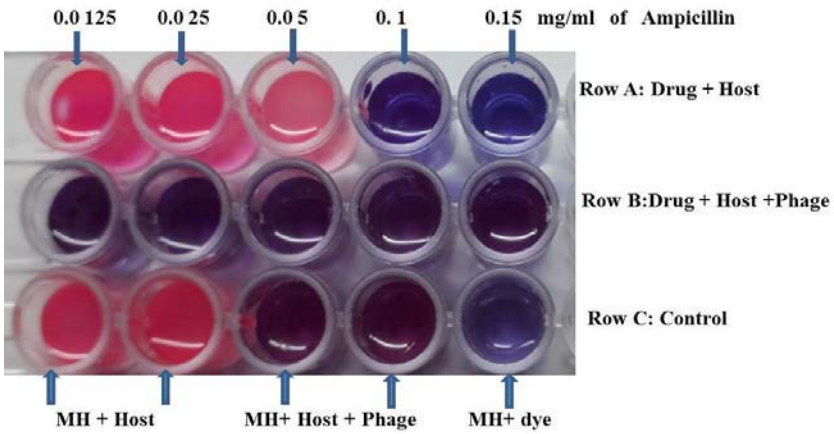

Figure 4: Synergistic action of mycobacteriophage and Ampicillin on *Mycobacterium tuberculosis* MTCC 300

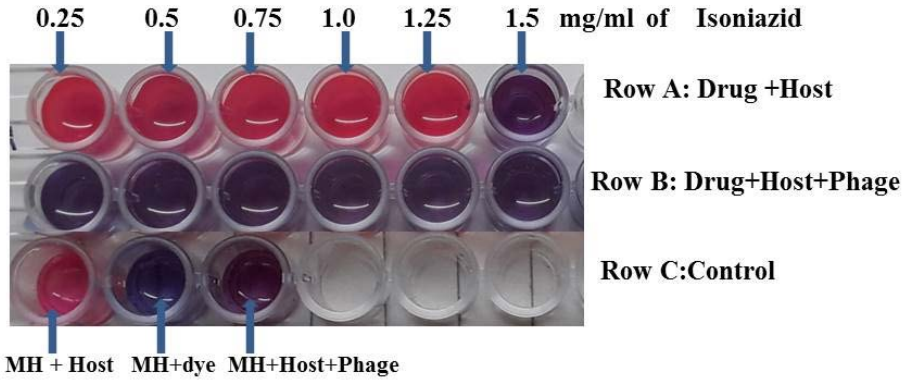

Figure 5: Synergistic action of mycobacteriophage and Isoniazid on *Mycobacterium smegmatis* MTCC 994

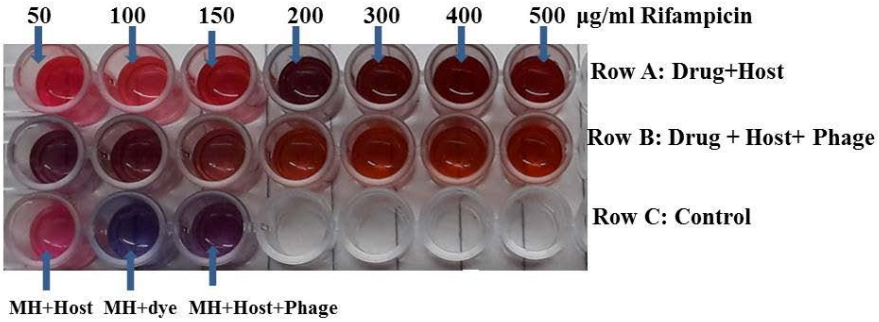

Figure 6: Synergistic action of mycobacteriophage and Rifampicin on *Mycobacterium smegmatis* MTCC 994

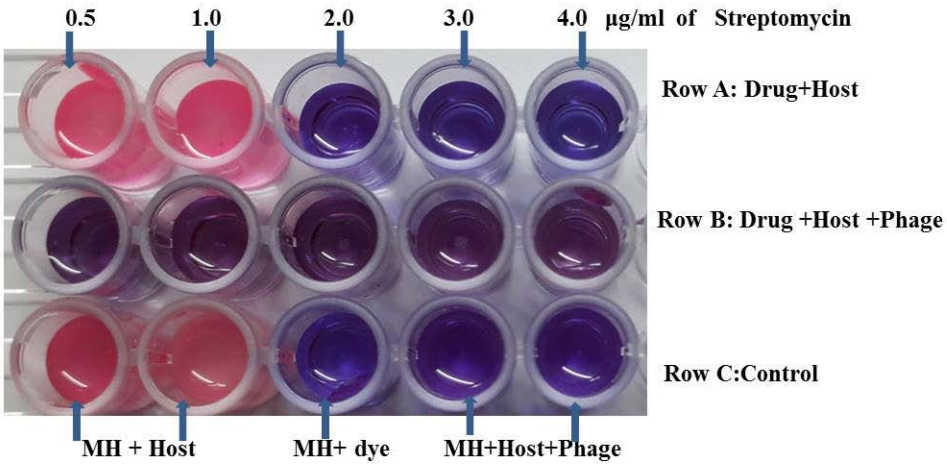

Figure 7: Synergistic action of mycobacteriophage and Streptomycin on *Mycobacterium smegmatis* MTCC 994

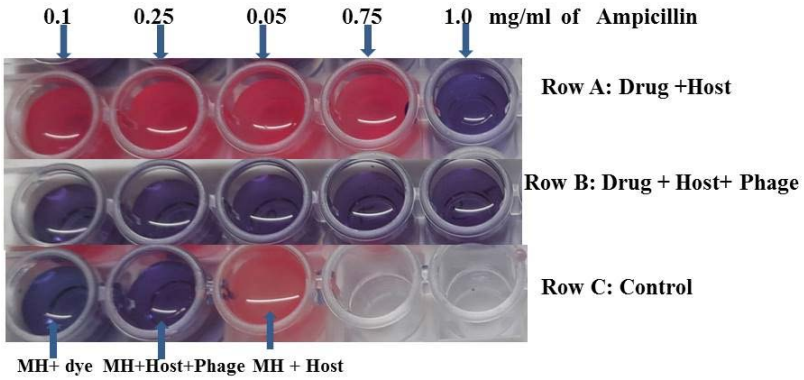

Figure 8: Synergistic action of mycobacteriophage and Ampicillin on *Mycobacterium smegmatis* MTCC 994

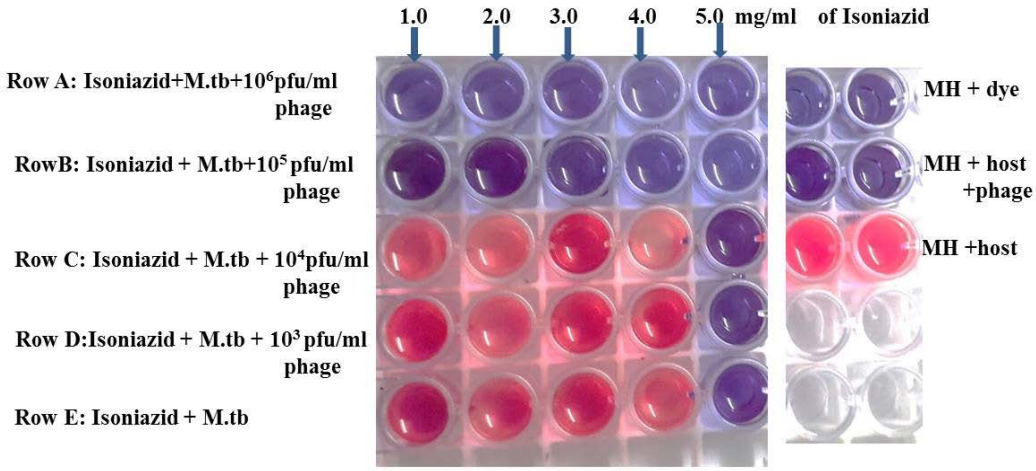

Figure 9: Synergistic action of combinations of mycobacteriophage and Isoniazid on *Mycobacterium tuberculosis* MTCC 300

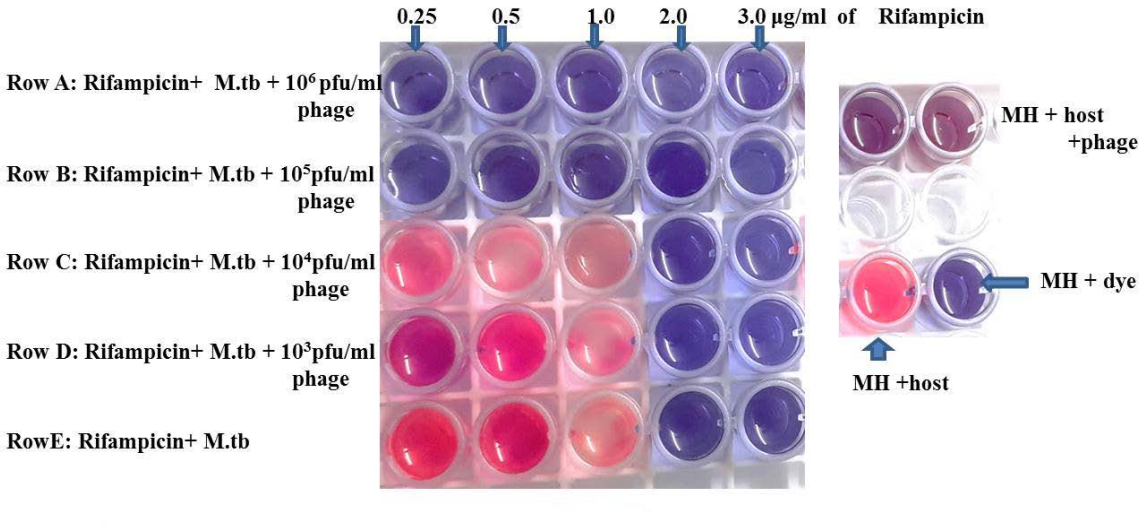

Figure 10: Synergistic action of combinations of mycobacteriophage and Rifampicin on *Mycobacterium tuberculosis* MTCC 300

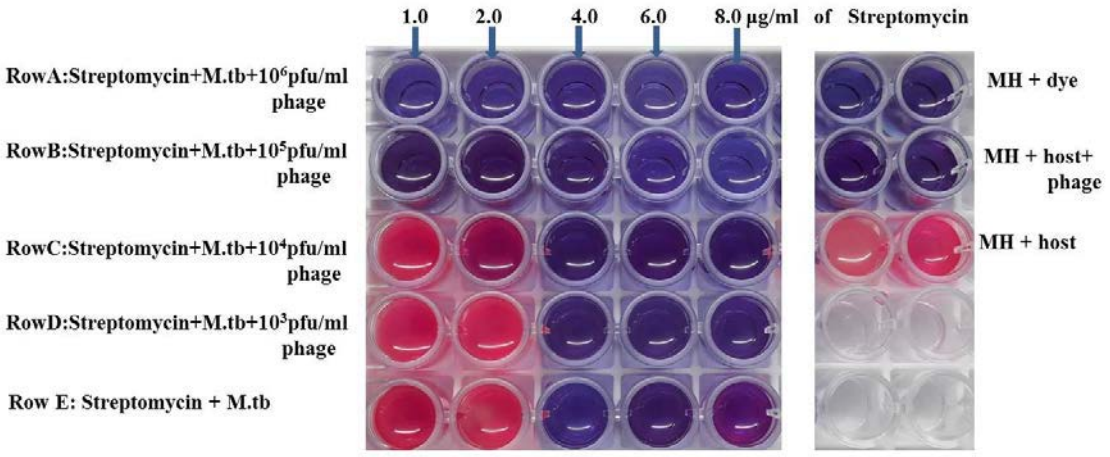

Figure 11: Synergistic action of combinations of mycobacteriophage and Streptomycin on *Mycobacterium tuberculosis* MTCC 300

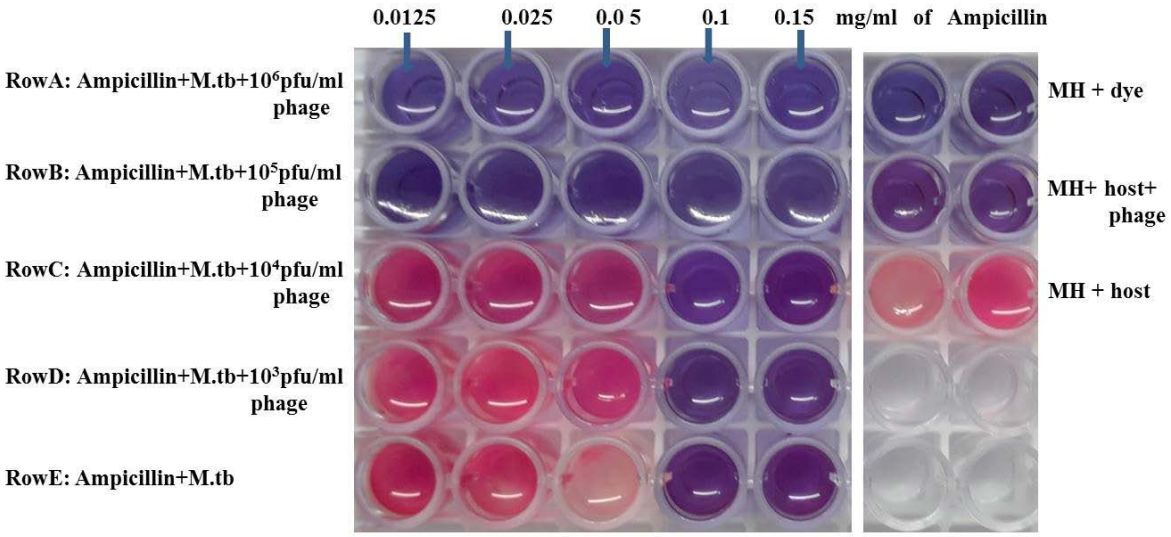

Figure 12: Synergistic action of combinations of mycobacteriophage and Ampicillin on *Mycobacterium tuberculosis* MTCC 300

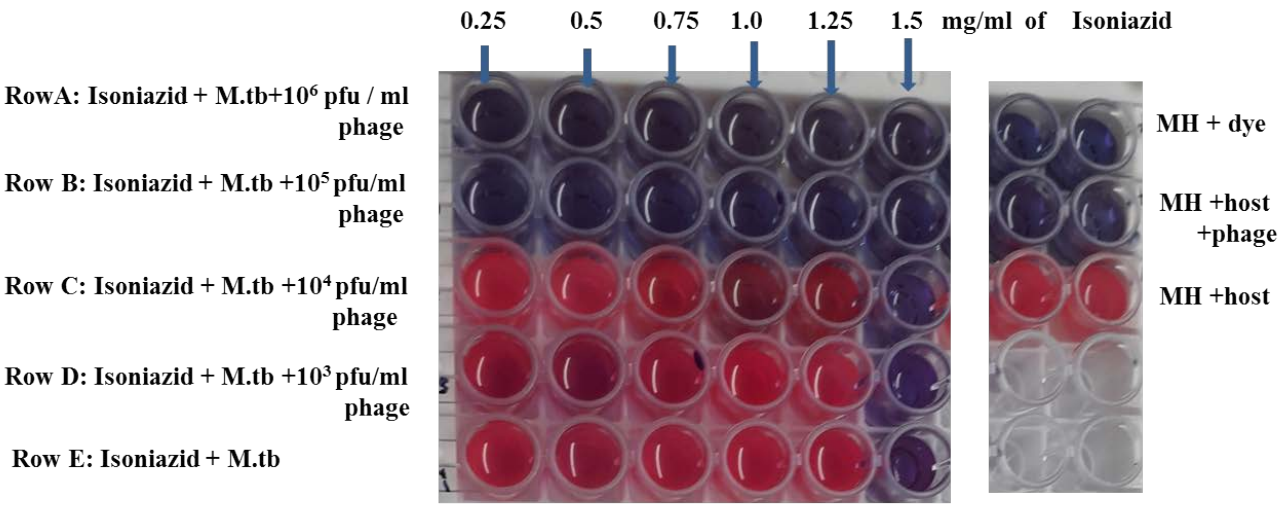

Figure 13: Synergistic action of combinations of mycobacteriophage and Isoniazid on *Mycobacterium smegmatics* MTCC 994

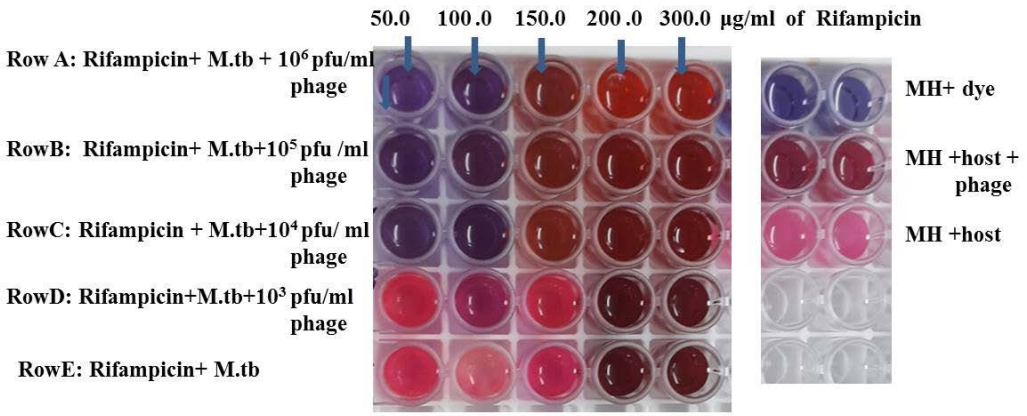

Figure 14: Synergistic action of combinations of mycobacteriophage and Rifampicin on *Mycobacterium smegmatics* MTCC 994

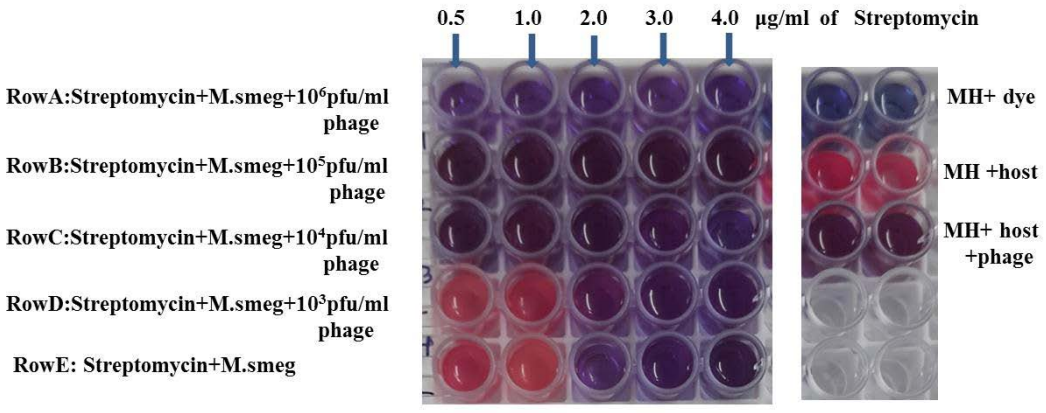

Figure 15: Synergistic action of combinations of mycobacteriophage and Streptomycin on *Mycobacterium smegmatics* MTCC 994

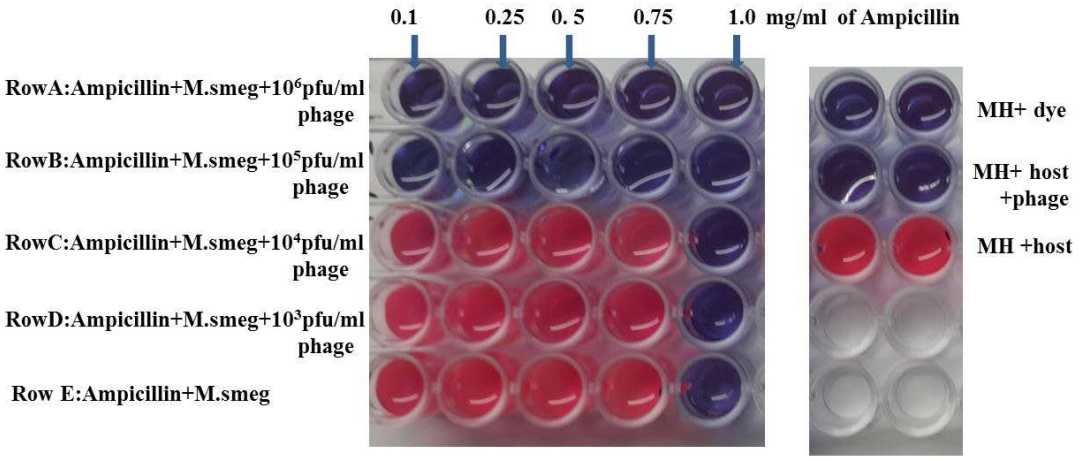

Figure 16: Synergistic action of combinations of mycobacteriophage and Ampicillin on *Mycobacterium smegmatics* MTCC 994

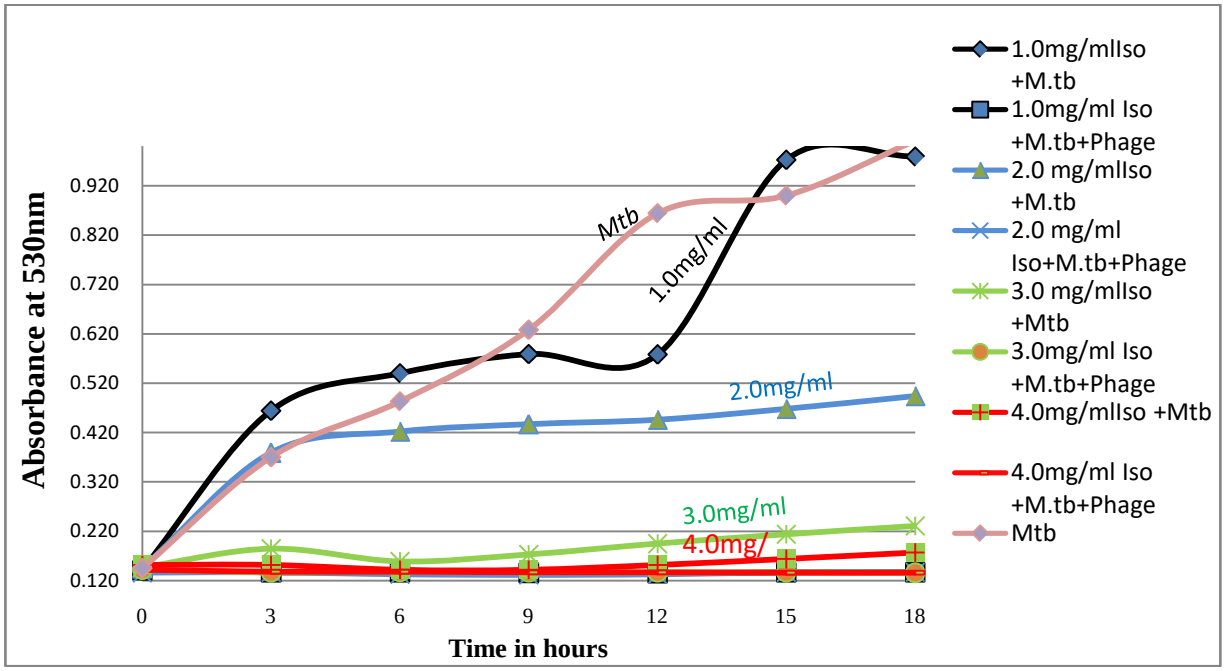

Figure 17: Growth curve of *M.tb* in presence of phage and Isoniazid

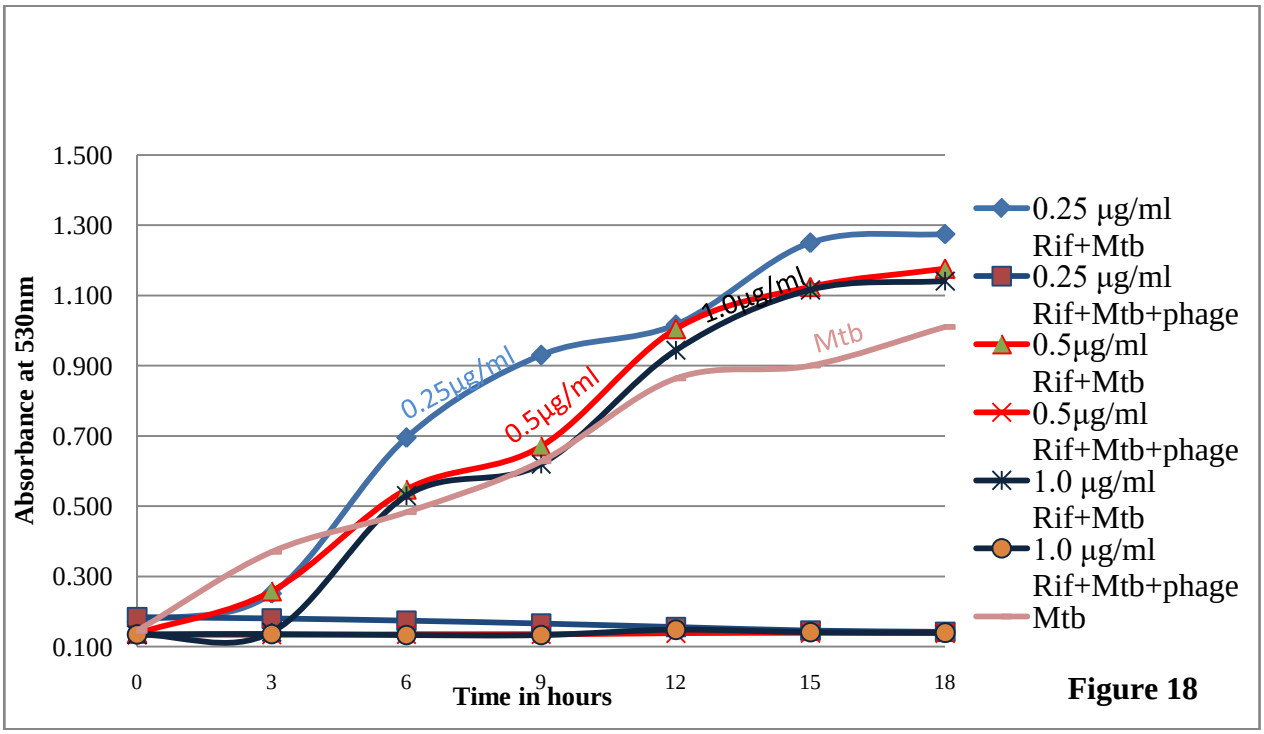

Figure 18: Growth curve of *M.tb* in presence of phage and Rifampicin

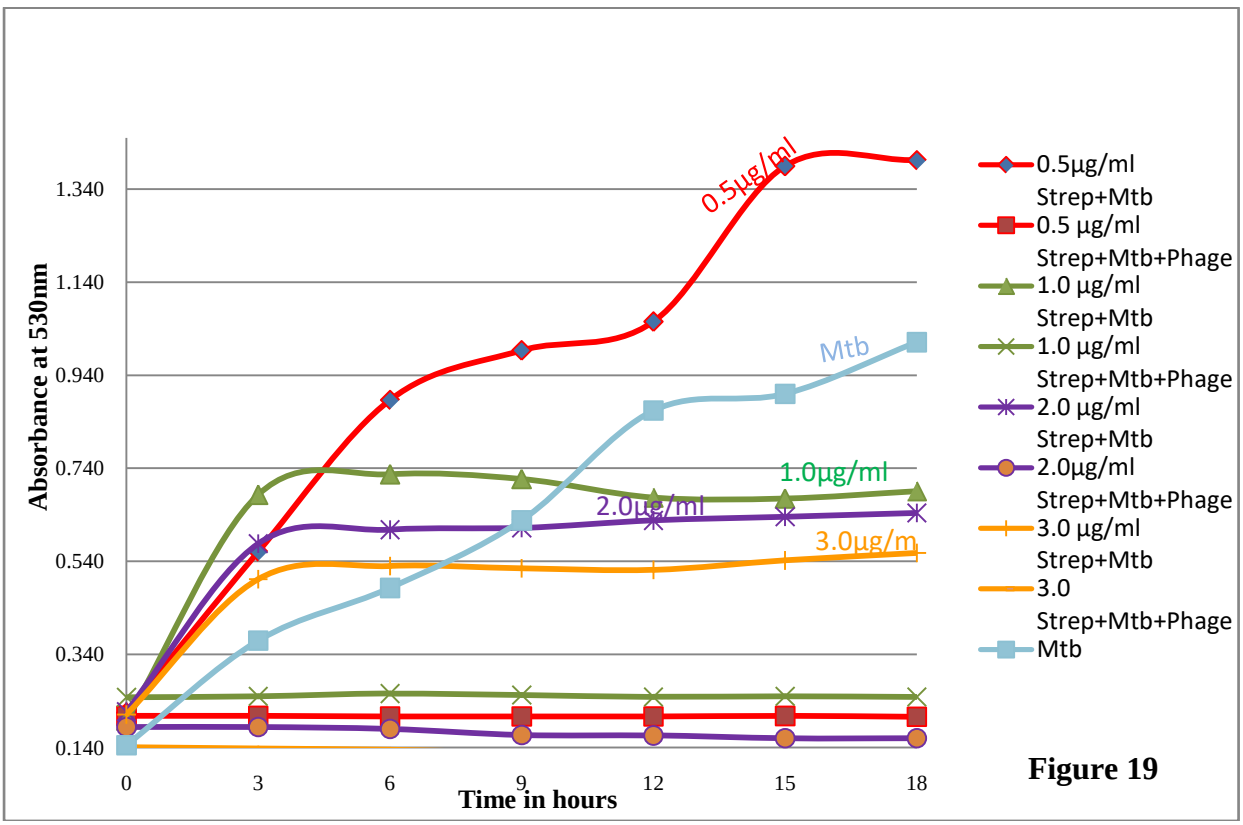

Figure 19: Growth curve of *M.tb* in the presence of phage and Streptomycin

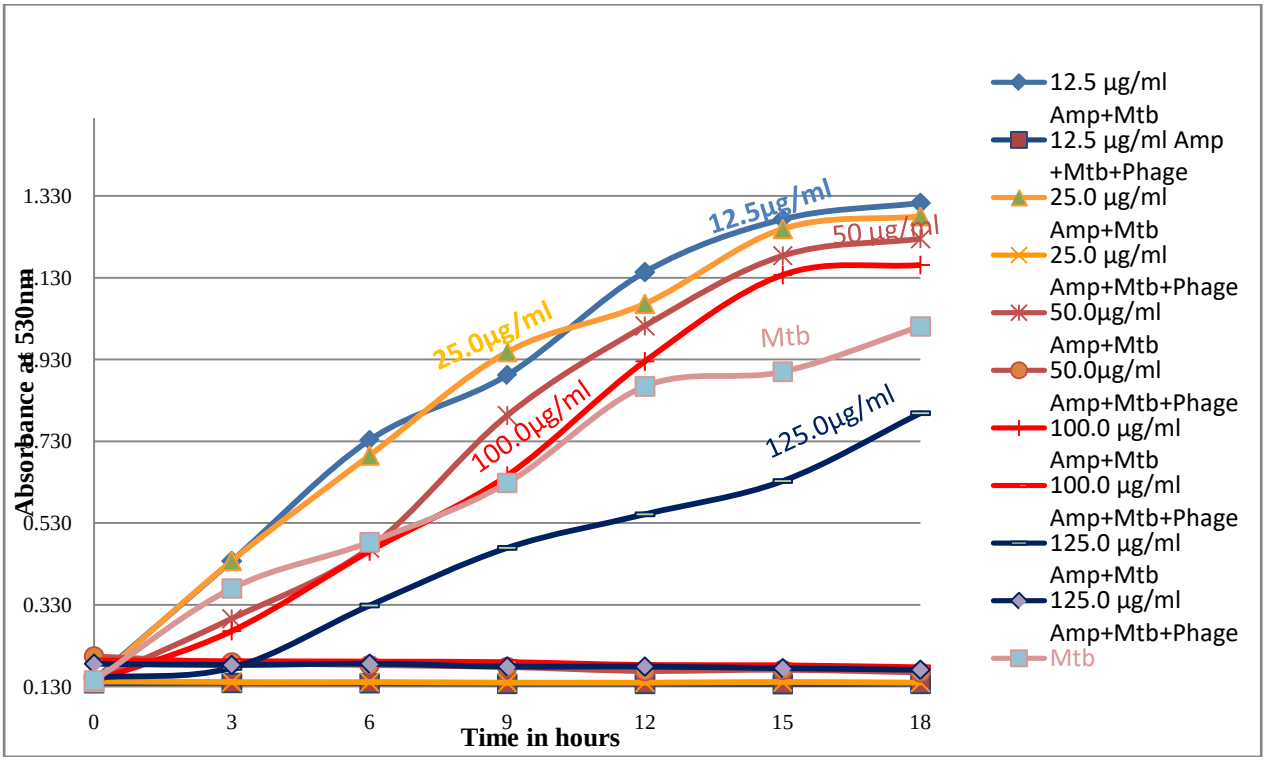

Figure 20: Growth curve of *M.tb* in the presence of phage and Ampicillin

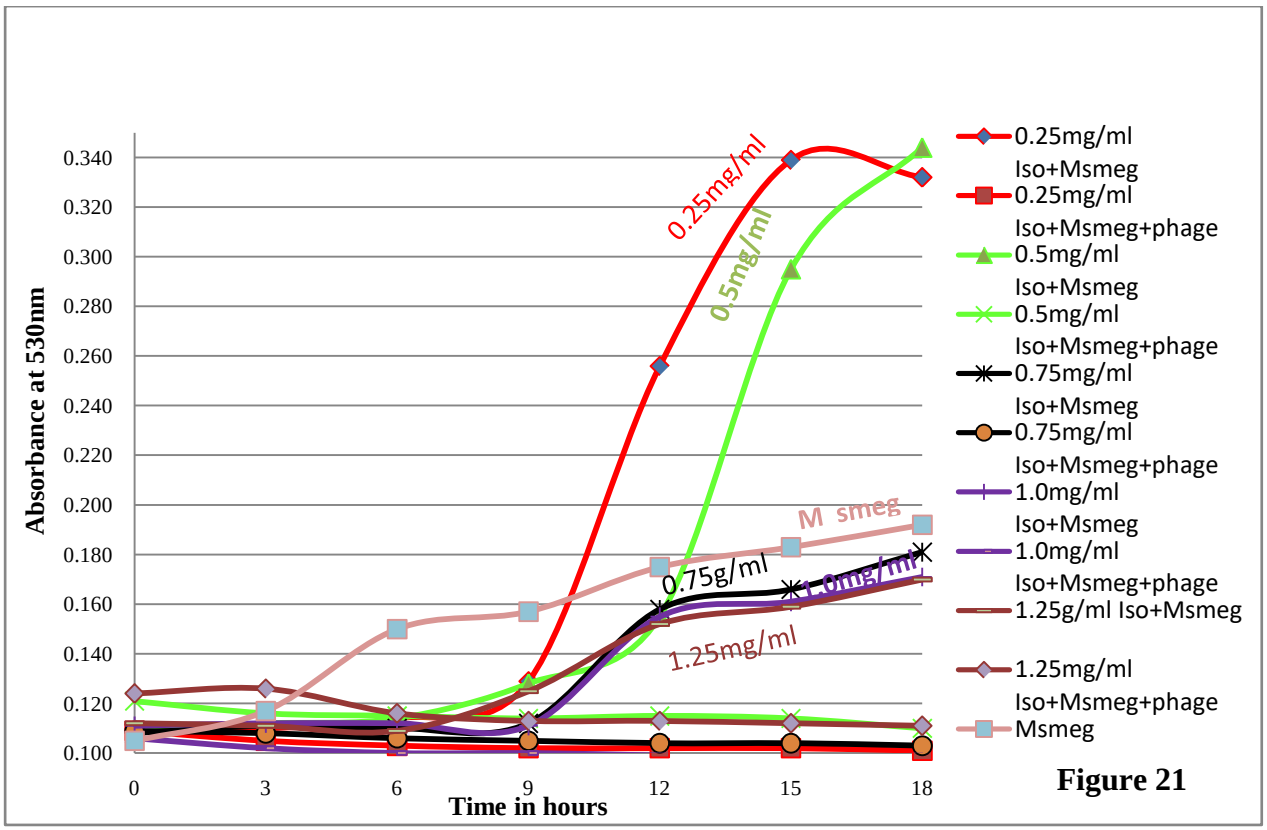

Figure 21: Growth of *M.smeg* in the presence of phage and Isoniazid

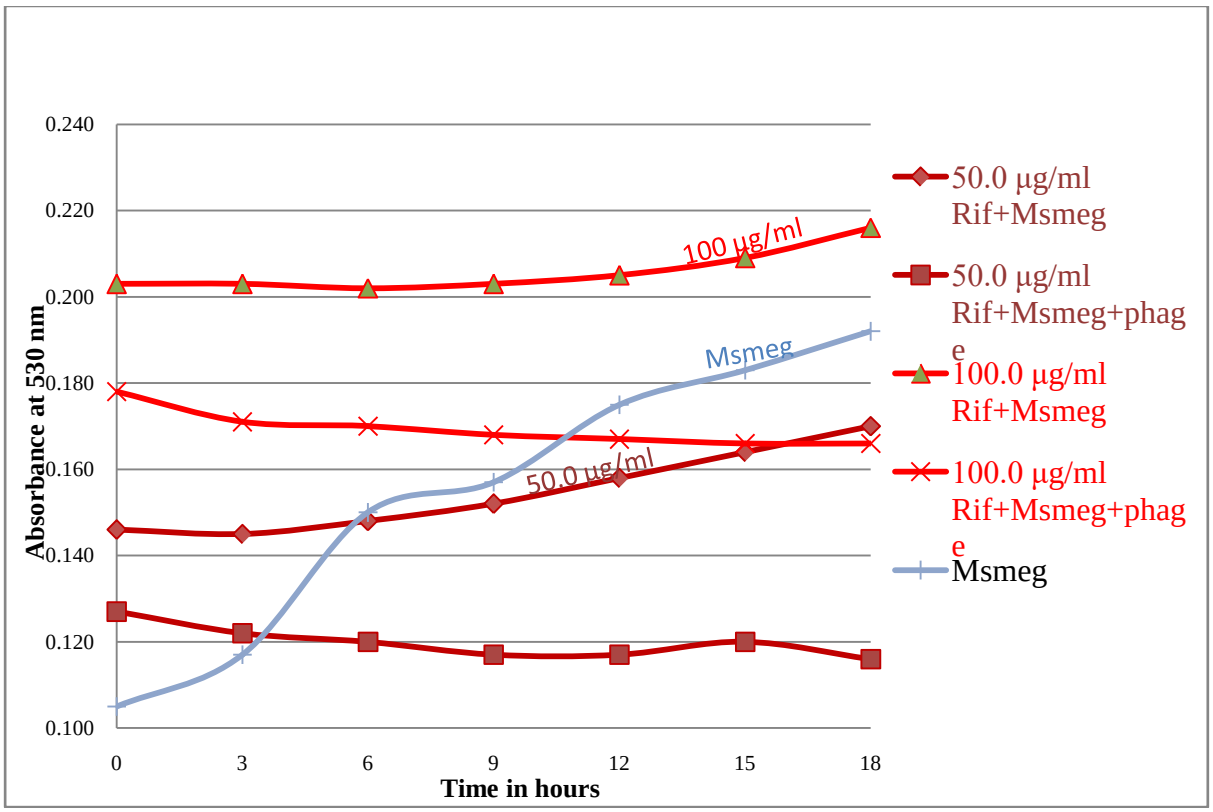

Figure 22: Growth curve of *M.smeg* in the presence of phage and Rifampicin

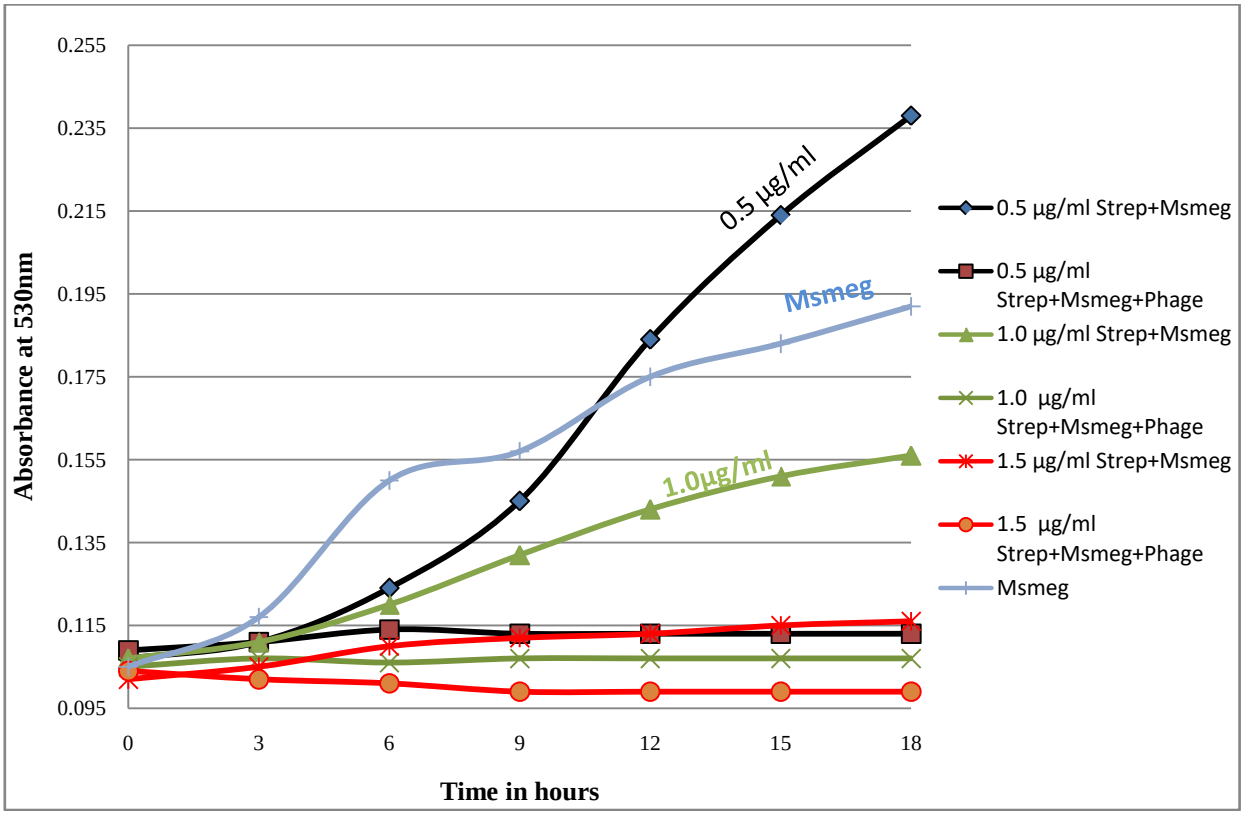

Figure 23: Growth curve of *M.smeg* in the presence of phage and Streptomycin

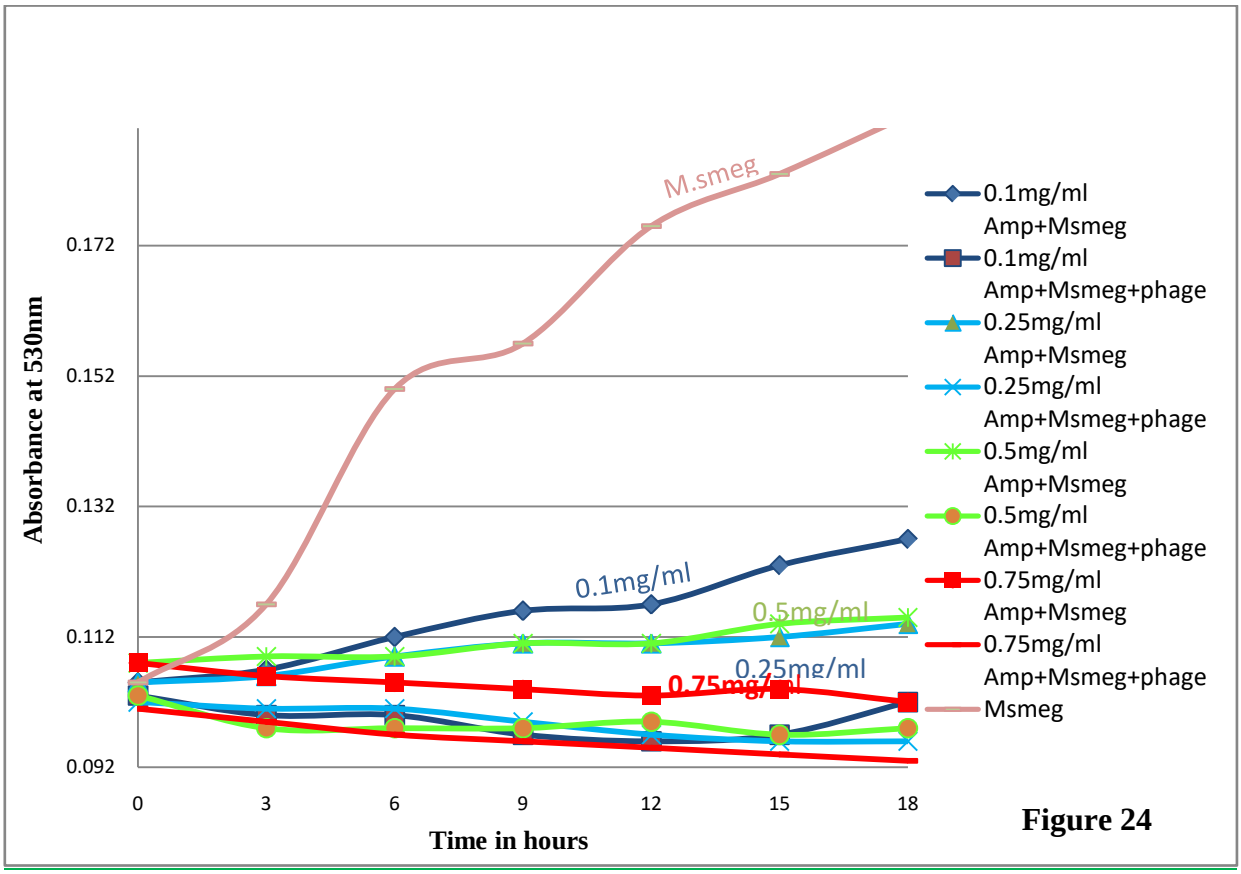

Figure 24: Growth curve of M.smeg in the presence of phage and Ampicillin

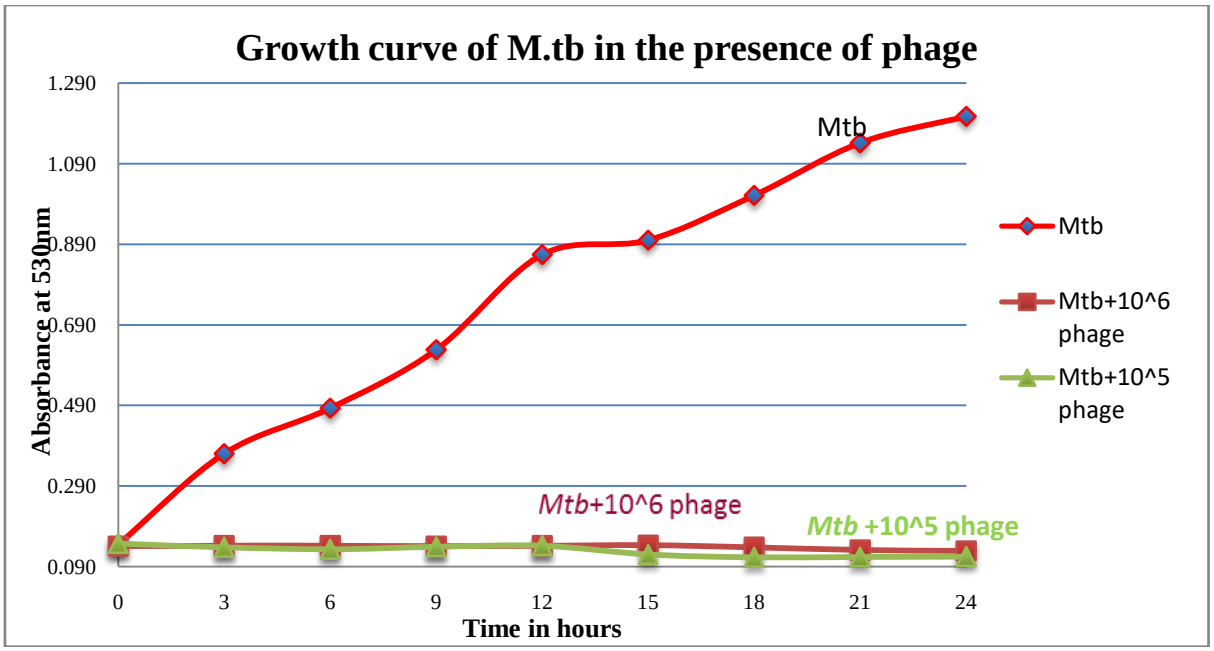

Figure 25: Growth curve of M.tb in the presence of phage

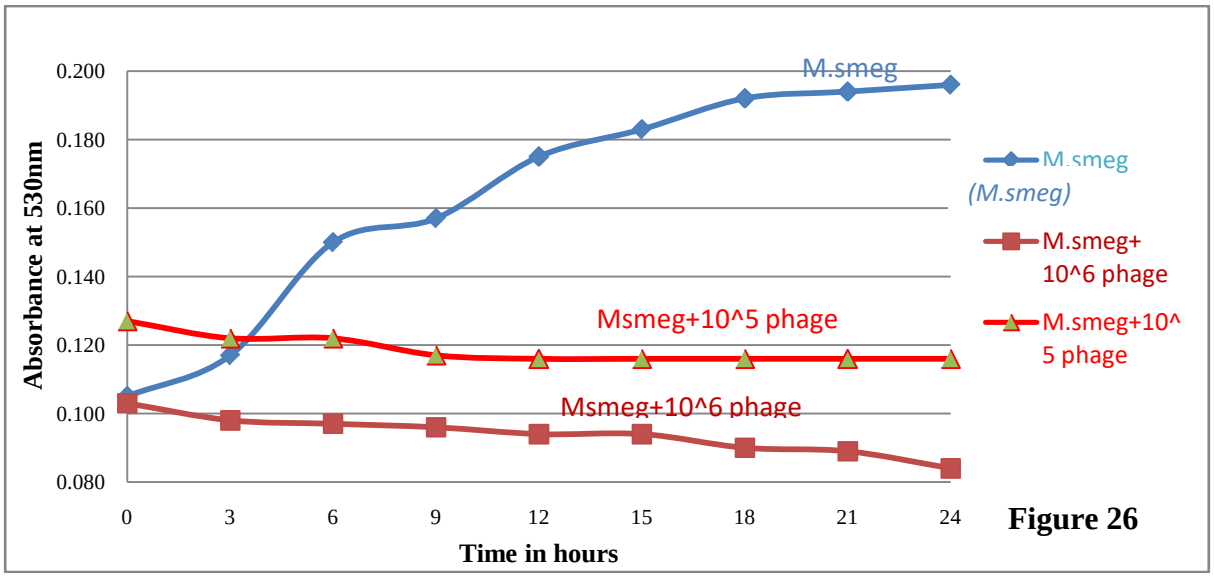

Figure 26: Growth curve of M.smeg in the presence of phage

Website

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Structural, Raman and Electrical Characterization of Nanocrystalline CdSe Thin Films Deposited by Pulse Laser Deposition Technique

By Pawan Kumar, Aravind Kumar, Rajesh K. katiyar, Alvaro Instan & Ram S. Katiyar

Gurukul Kangri Vishwavidyalaya Haridwar

Abstract- Cadmium Selenide (CdSe) thin films were deposited by Pulse Laser Deposition(PLD) technique onto Indium Tin Oxide (ITO) coated glass substrates at different substrate temperatures. The structure of CdSe confirmed by using the X-ray diffraction pattern. The surface element analysis and morphology of thin films were done by X-ray photoelectron spectroscopy and Atomic force microscopy, respectively. Raman spectroscopy is used for atomic bond behavior at room temperature and lower than room temperature. The band gap of the thin films was estimated (1.75 eV to 2.3 eV) using the UV-Visible absorption spectra. Electrical behavior (I-V characteristic) of thin films also studied at different temperatures. These films have possible applications in thin films based on solar cells, and sensors.

Keywords: *CdSe thin films, PLD, XRD, raman and I-V characterization.*

GJSFR-B Classification: *FOR Code: 250403*

Strictly as per the compliance and regulations of:

Structural, Raman and Electrical Characterization of Nanocrystalline CdSe Thin Films Deposited by Pulse Laser Deposition Technique

Pawan Kumar [α], Aravind Kumar [σ], Rajesh K. katiyar [ρ], Alvaro Instan [ω] & Ram S. Katiyar [¥]

Abstract- Cadmium Selenide (CdSe) thin films were deposited by Pulse Laser Deposition(PLD) technique onto Indium Tin Oxide (ITO) coated glass substrates at different substrate temperatures. The structure of CdSe confirmed by using the X-ray diffraction pattern. The surface element analysis and morphology of thin films were done by X-ray photoelectron spectroscopy and Atomic force microscopy, respectively. Raman spectroscopy is used for atomic bond behavior at room temperature and lower than room temperature. The band gap of the thin films was estimated (1.75 eV to 2.3 eV) using the UV-Visible absorption spectra. Electrical behavior I-V characteristic) of thin films also studied at different temperatures. These films have possible applications in thin films based on solar cells, and sensors.

Keywords: CdSe thin films, PLD, XRD, raman and I-V characterization.

I. Introduction

Cadmium selenide is one of the II-VI materials which have been extensively considered by researchers due to their various applications such as photovoltaic applications, solar cells, thin-film transistors, gamma-ray detectors, lasers, sensors [1-3], and photo electrochemical cells [4] because of having high efficiency of radiative recombination, high absorption coefficient, high photosensitivity, direct band gap matching to a wide spectrum of wavelengths from ultraviolet to infrared regions[5]. Semiconducting nanocrystalline thin films such as CdSe, CdS, and CdTehave attracted considerable attention owing to their remarkable optical and electrical properties, which depend on crystallite size[6-9]. The optical properties, including broadband absorption and high luminescence, make these materials attractive in photovoltaic applications[10, 11]. CdSe has shown to be the most promising candidate owing to its direct band gap, high absorption coefficient, n-type conductivity, and crystallite size- tunable band gap that can vary its optical response over the entire visible range of the electromagnetic spectrum[12-14].Although there are many reports available on the optical and transport properties of CdSe thin films, the optical and sub-band gap absorption parameters are much less explored [15-19]. The performance of the devices based on CdSe thin films depends on the structural and electronic properties of the layers obtained under various experimental conditions [20]. The reported thin film deposition methods for CdSe are mainly based on vacuum and non- vacuum film deposition, spray pyrolysis[21], electrochemical deposition [22], vacuum evaporation, co evaporation[23-25], chemical deposition[26], laser ablation[27], hot wall deposition technique[28], and molecular beam epitaxy[29].In recent years, many efforts have been devoted to the research of doped metal chalcogenide nanostructured materials [30 - 34]. The electronic and optical properties of semiconductors are strongly influenced by the methods of preparation and doping process, which provides the basis for tailoring the desired carrier concentration and conscquently the absorption, emission, and transport properties. Nanocrystalline thin films with varying concentrations of dopants are a matter of interest from the view point of basic physics and applications. The Raman spectroscopy has been widely used to characterize the phonon properties of thin films.Due to the dependence of Raman shift, intensity, and linewidth on temperature, recently, Raman spectroscopy has also been actively used for thermometry.

In the present work, we present a systematic study on the investigation of structural, Raman, and electrical properties CdSe thin films deposited by PLD with varying different substrates temperaturewhich performance a significant impact on the CdSe layer. The substrate temperatures were fixed at 150^{0}C (S1), 200^{0}C (S2), 250^{0}C (S3), and 300^{0}C (S4), respectively, during the growth of samples.

II. Experimental

CdSe Powder(99.999%, Sigma Aldrich Chem. Co.) was used to make the target of the PLD technique.

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The target was sintered at 850^{0}C in the Ar gas atmosphere in vacuum condition for 5 hours. The laser beam was focused onto the target material surface using a lens of focal length 5 cm. The target was maintained in continuous horizontal and vertical displacement to refresh the ablated zone. The incident laser pulse energy and repetition rate for the film deposited on ITO substrates was obtained as 90 mJ, and 10 Hz, respectively. The target and the glass substrate holder were rotating at 5 rpm and -5 rpm, respectively. The distance between the substrate holder and the target inside the vacuum chamber was ~5 cm. A resistive type heater was used to heat the substrate temperature to reach the set value. The substrates were clean up sequentially, using isopropyl alcohol, detergent solution, methanol followed by deionized water in the ultrasonic bath cleaner and subsequently,CdSe thin films were deposited by PLD on the highly cleaned Indium Tin Oxide (ITO) coated glass substrates. To obtain free-risking pinhole thin film each parameter of the PLD was optimized (25 milli torr, 250 milli J) at high vacuum condition using a KrF Laser(248nm, 10number of shots per sec,30Hz) to ablate a sintered CdSe target in nitrogen atmosphere. Laser ablation was carried out by scanning the sample inside the vacuum chamber which is kept at 10^{-6} Torr, by using a molecular pump together with a mechanical pump [26]. Thin film of thickness around 200nm were prepared using pulsed laser deposition technique. No reactive gas was introduced to the chamber only N_2 gas pass in the chamber for deposition with the 25ml torr pressure.

Phase purity of synthesized CdSe thin films were characterized using X-ray diffraction method (Rigaku, Smartlab) using Cu(K_α) 1.54Åoperated in $\theta/2\theta$ configuration. Long scanning times were carried out to obtain good quality of diffraction patterns. A PHI 5000 Versaprobe system was used to examine the surface chemistry with X-ray photoelectron spectroscopy (XPS) analysis using Al K_α X-rays of energy 1486.6 eV. Raman spectroscopic measurements were carried out using a Horiba–Yobin (T-64000) *micro*-Raman system using a laser excitation line 514.5 nm from an argon ion laser.The surface morphology of the films were investigated employing atomic force microscopy (AFM) (Digital Instruments, Veeco Metrology Group) in the contact mode.The transmittance of thin films was performed using a Lambda 950 UV–vis spectrophotometer from PerkinElmer equipped with a spectralon integrating sphere. The I-V measurements were carried out by using aprecision impedance Analyzer (4294A) in the frequency 40 Hz –110 MHz in the temperature 273 – 373K in the interval of 10 ^{0}C.

III. Results and Discussion

a) Structural characterization

Fig. 1 shows the typical XRD pattern of CdS thin films. Diffraction patterns were recorded in the 2θ range of 10^0 to 80^0 for each layer. These patterns were used to identify the crystal structures, phases and lattice planes for the observed diffraction peaks from atomic planes of deposited thin films.The XRD pattern shows the highest intensity peak at $2\theta = 24.3^0$ along (111) with another small intensity peak at $2\theta = 44.6^0$ along (311).These peak are indexed using standard results JCPDS card no.00-019-0191 having cubic structure with lattice constant a = b = c = 6.077.Some distortion also obtained due to ITO substrate and most distortion are marked as (*). As the substrate temperature increased from 150°C to 300°C the crystallinity of thin films also changed. The effect of grain size, peak broadening may be due to other reasons, such as inhomogeneous strains, lattice bending, twinned structure or other point defect that may be present in the CdSe nanomaterials; hence Scherrer's method may produce results which are different from the actual size[35].The crystallite size varied from 50 nm to 120 nm at different substrate temperature. The cubic structure is obtained corresponding to the peak (111) with a=b=c =5.984.

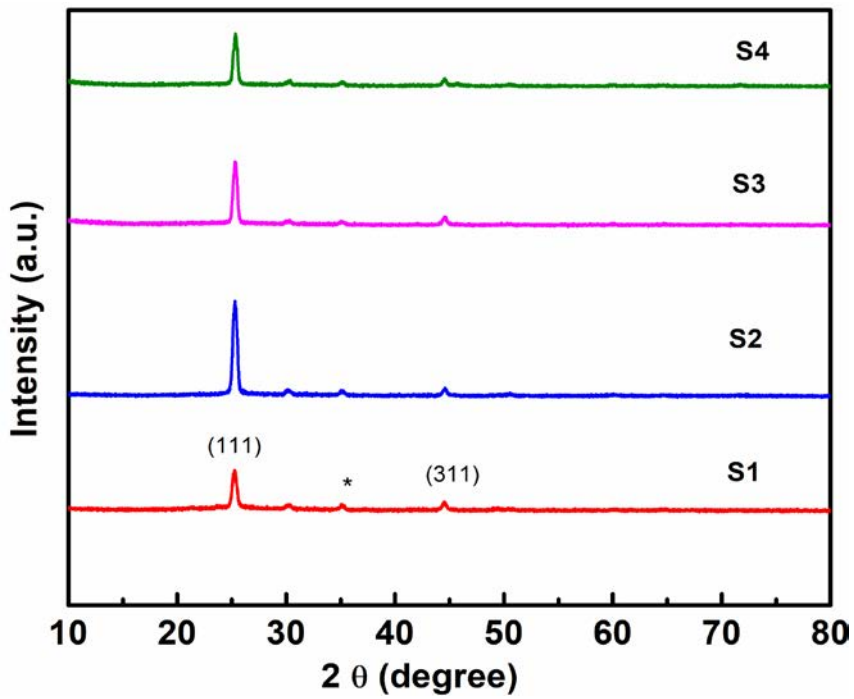

Fig.1: XRD diffraction Pattern CdSe thin films

b) X-ray Photoelectron spectroscopy (XPS)

The XPS spectra of the CdSe thin films were analyzed to confirm their compositions as shown in Fig.2. The different survey spectra obtained using Al K_α X-rays with energy 1486.6 eV. The survey diagram (Fig. 2(a)) of CdSe thin film indicates the presence of cadmium (Cd), selenium (Se), carbon (C), and oxygen (O) elements. The detected carbon is related to the carbon adsorbed on the surface during the exposure of the sample to the ambient atmosphere. All binding energies were corrected for the charge shift using the C 1s peak of graphitic carbon (284.6 eV) as a reference [36].High resolution core level spectrum for Cd 3d is shown in Fig.2 (b). The two peaks in Cd 3d core level spectrum arise with Cd3d5/2 peak position at 405.03 eV and Cd3d3/2 at 412.18 eV binding energies respectively. The binding energy of Cd $3d_{5/2}$ indicates the Cd^{2+} states; this is in good agreement with the literature [37, 38]. Both sample S1 and S3 shows the same peak position for Cd 3d. High-resolution core level spectrum for Se 3d depictedin Fig.2(c). Core level spectrum for Se 3d was fitted using singlepeak for binding energy at 54.10 eV [39, 40].The area of the peak remain same but a little shift obtained in Se 3d peak that may be due to lattice mismatching as some distortion obtained in the XRD results. The values of binding energies for Cd and Se are at their respective positions for Cd^{2+} and Se^{2-} states. It means that Cd^{2+} and Se^{2-} exist in as-deposited CdSe thin films with stoichiometric formula CdSe, confirm the presence of the Cd and Se materials.

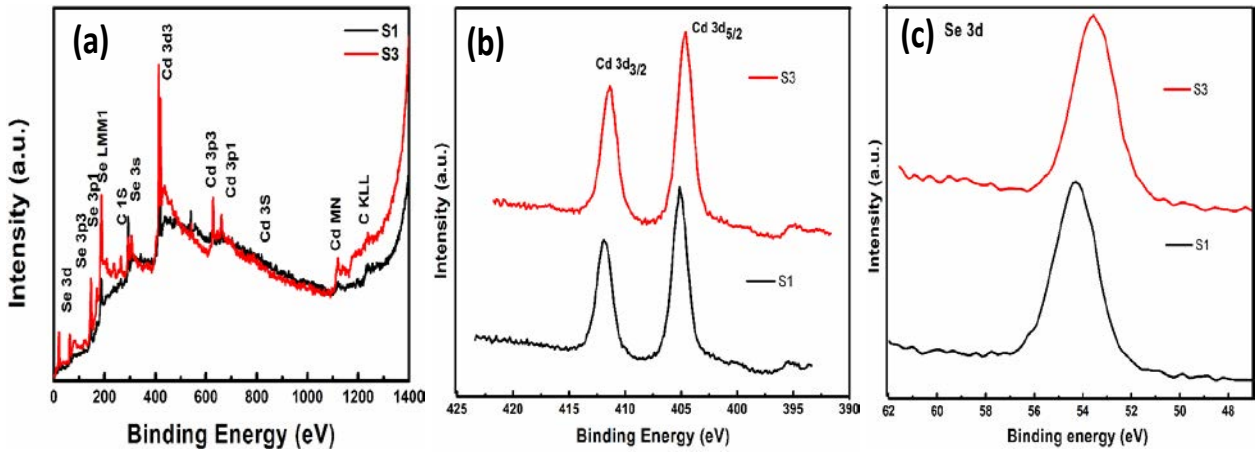

Fig. 2: XPS studies of CdSe thin films

c) Optical absorption and transmission

Trasmission studies are carried out in the wavelength range280-800nmto investigate the optical absorption properties of CdSe thin films. Fig.3 shows the variation of transmittance T with wavelength (λ). The transmission spectra exhibit interference fringes for photon energies below the absorption edge, which shifts towards longer wavelengths with increase in temperature. The shift in absorption edge can be assigned to thermally induced defects, quantum confinement, or the formation of additional phases and change in the structural properties [41]. Moreover, a lower grain size, resulting in an increase in scattering of photons at grain boundaries, could contribute to the decrease in transmittance.

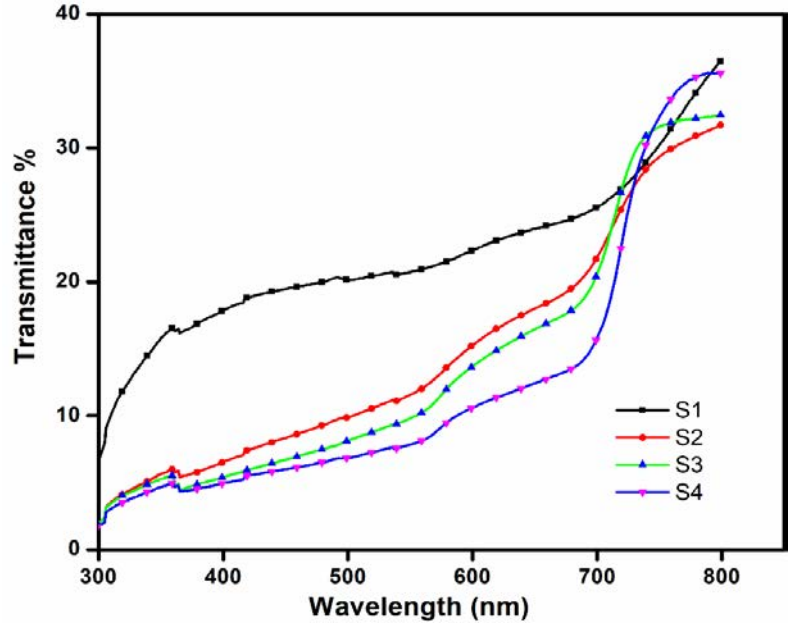

Fig. 3: Transmittance spectra of CdSe thin films

The absorbance spectra obtained from transmittance data shown in Fig. 4 (a). The optical band gap of the thin films has been determined from the absorption coefficient using the equation [42];

$$(\alpha h\upsilon)^n = B(h\upsilon - E_g)$$

Where α is the absorption coefficient, hυ is the energy of incident photon, B is a constant which is characteristic of the material, $E_g$is the optical band gap and n = 2 for direct band gap transitions or n =1/2 for indirect band gap transitions. The intercept on the abscissa of the plot of $(\alpha h\upsilon)^2$versus hυ·given in fig.4 (b), provides the direct band gap energy. The value of Eg varies from 2.3 eV to 1.75 eV for CdSe thin films owing to size quantization effect as reported earlier [43].

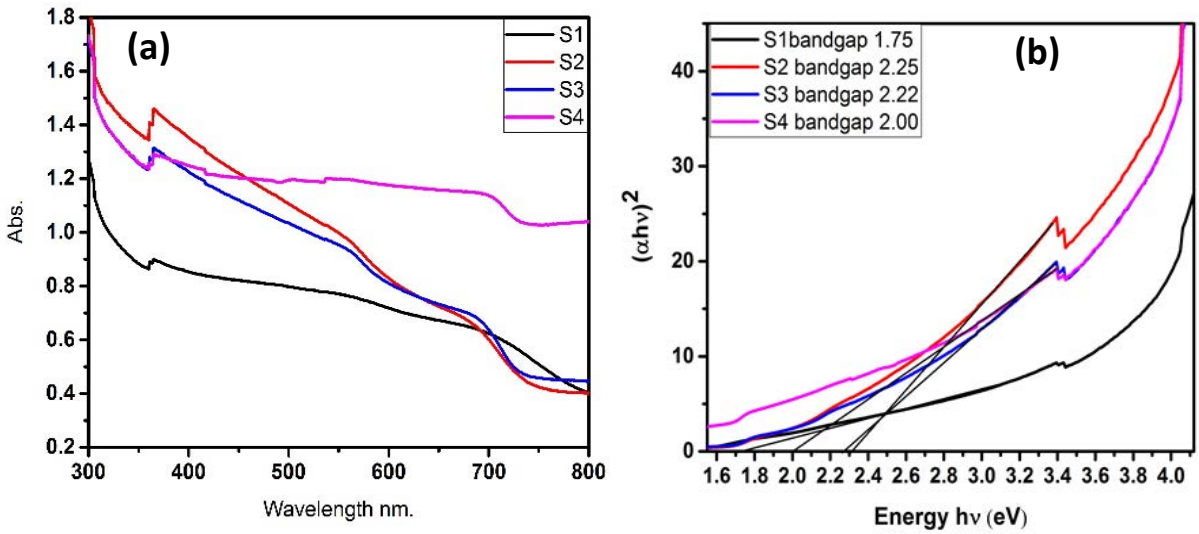

Fig. 4: Absorbance spectra and bandgap diagram of CdSe thin films

d) Low temperature Raman Spectroscopy

Raman spectroscopy is used to identify the various vibrational modes of the molecules in the material by shining the laser beam of light onto the CdSe thin films. Raman spectroscopy is an alternative method for quality control of materials grown in aproduction line. Raman spectra are generally applied as fingerprints for identifying material phases. The optimal conditions (power and wavelength of excitation, accumulation time) for Raman scattering measurements chosen in such a way to provide the good quality spectra. In present work micro-Raman scattering was performed in the backscattering configuration using the 514.5 nm line of an argon ion laser to a spot size of ~2 μm, and with an incident power of ~3 mW.Fig. 5 shows the Raman spectra of the samples: S3 (CdSe thin films at substrate temperature 250^{0}C) at low to room temperature 83K to 300K. In the Raman Spectra it has been found that the peak at 208 cm^{-1} corresponds to the basic vibrational (longitudinal) LO mode of CdSe structure vibrations [44]. The peak in ~ 415 cm^{-1} region corresponds to the second order 2LO mode which is observed at low temperature. Some distortion also obtained marked as (*) in the spectra. The Raman peak at ~517 cm^{-1}originates from substrate property and get wider with decreasing temperature. It should be noted that scanning of the surface of CdSe thin films by micro-Raman did not reveal any changes in values of Raman shift for the related modes across the surface. It means that surface distribution of compound elements in samples is uniform.

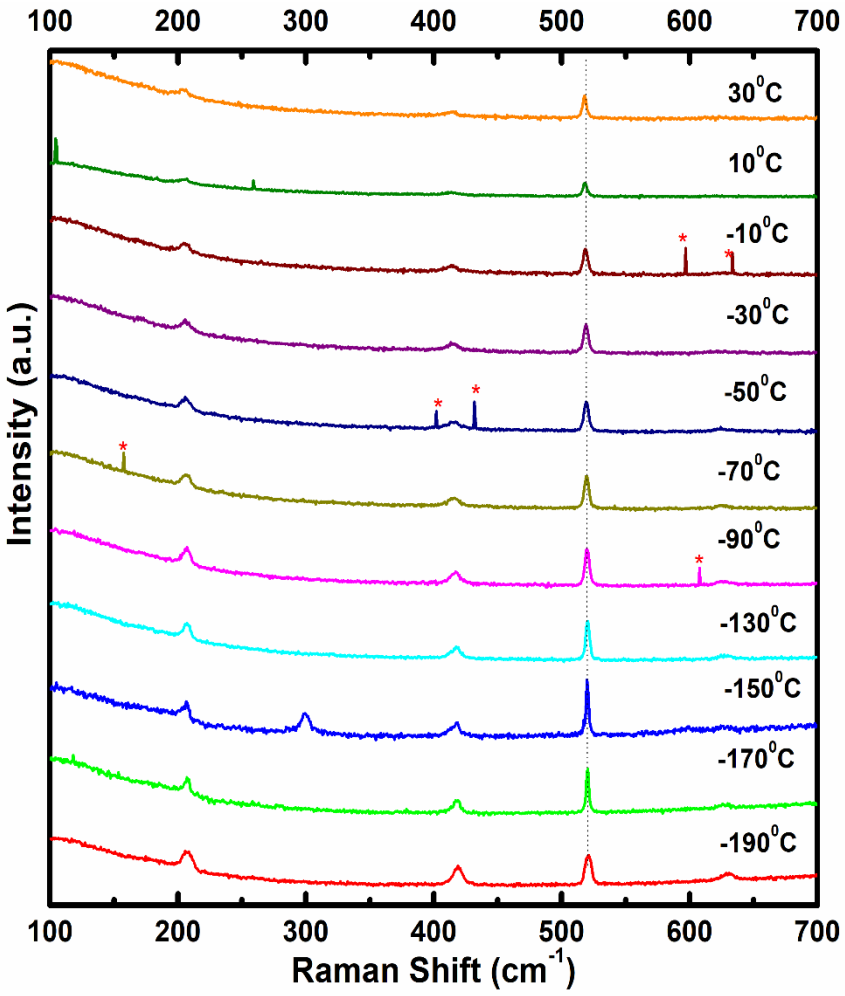

Fig. 5: Raman spectra of CdSe thin films at different temperature

e) Surface Analysis

Figure 6 shows the surface morphology of the grown films analyzed by atomic force microscopy. It is observed from the surface image that the particles are uniformly distributed on the surface of the thin film and the grains of CdSe particles were found to exist in spindle shape. Highly elliptical shape of the grains has a narrow size distribution on the surface of the film. The roughness of the film is found to be relatively low with a RMS value of 20 nm.

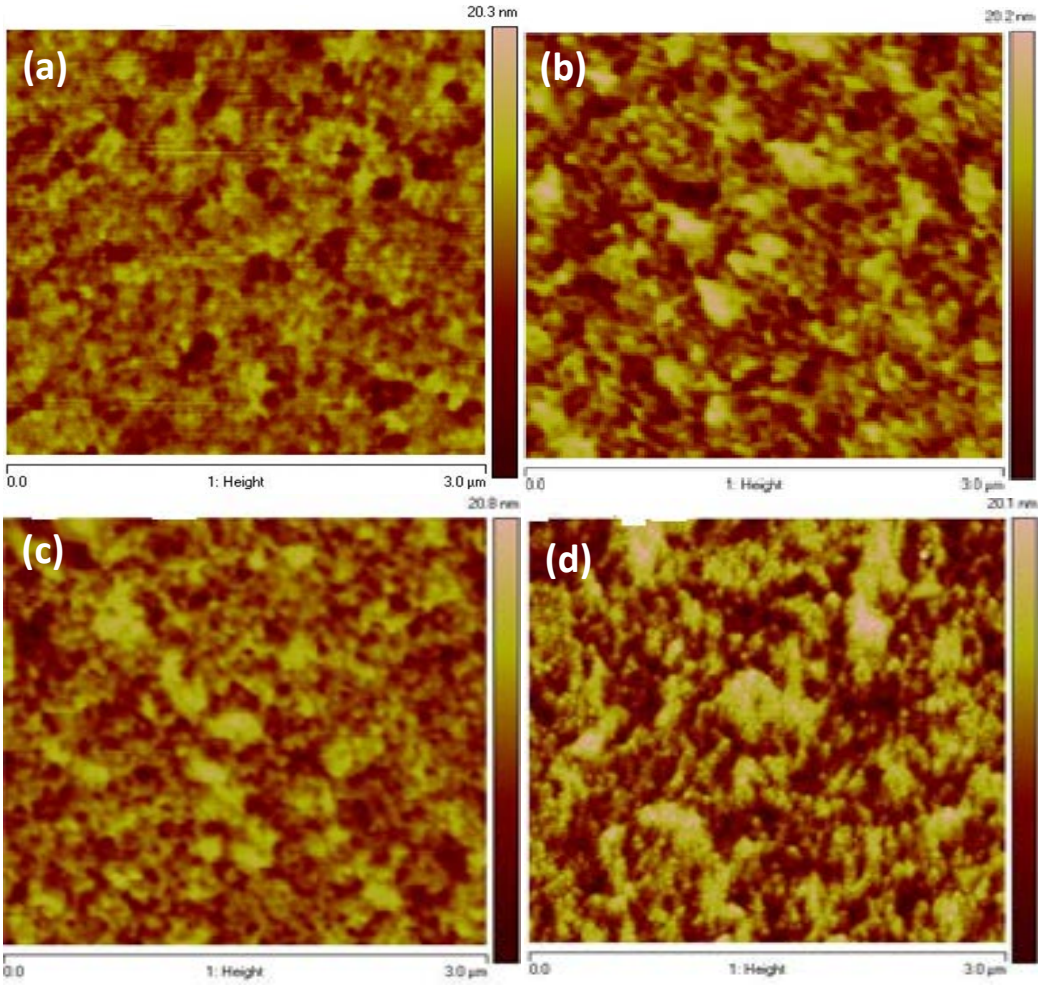

Fig. 6: AFM image of CdSe thin films at different substrate temperature

f) Electrical Measurements

The electrical property of the materials is an important factor which reveals the important and reliable information about the transport phenomenon and other physical properties of the material. The electrical properties are dependent on various film or growth parameters such as composition, thickness, and substrates temperature and deposition rate.The study of I-V characterization is done by varying the temperature from 273K to 373K of CdSe thin films. Measurement of electrical conductivity of CdSe/ITO (S1 and S3) was made by using d.c. two probe method at different temperatures and the corresponding characteristic curves are shown in Fig-7. At room temperature the current flow for 1 volt potential difference is 0.005µA while at low temperature 273K the current increases to 0.024µA which is approximately 5 times to the room temperature value. Similar work is done on the CdSe thin films (S3) and obtained that when the substrate temperature increased the conductivity also increased.S. Mahato et al. [45] also observed the variation of the electrical conductivity with temperature during heating and cooling cycles was found to be different. The low value of conductivity may be attributed to the nanocrystalline nature of thin film, discontinuities at crystallite boundary, presence of surface states and small thickness of the film. It is observed that the electrical conductivity is increased with the increase of temperature and the curves show typical semiconducting nature of thin films. [46, 47].

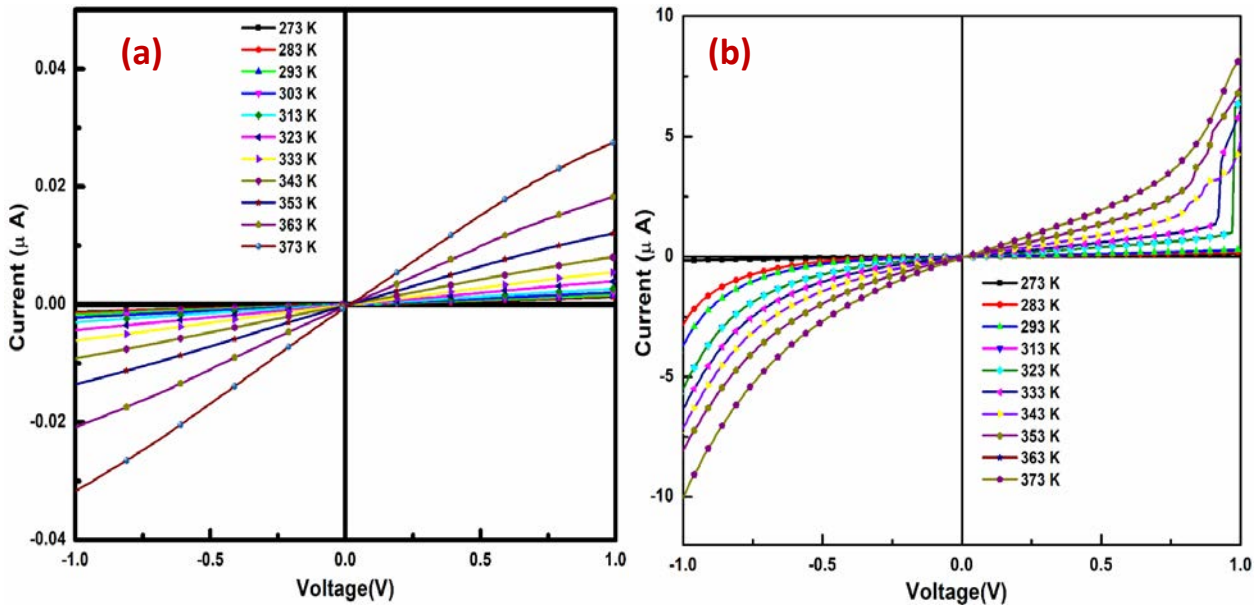

Fig. 7: I-V measurement of CdSe thin films at different substrate temperature

IV. Conclusion

The CdSe thin films successfully synthesized by pulse laser deposition method at different substrate temperature. The recorded diffraction pattern matched with the standard data and have cubic structure. The XPS results of CdSe thin film indicates the presence of cadmium (Cd), selenium (Se), carbon (C), and oxygen (O) elements. The Cd 3d (Cd^{2+}) and Se 3d (Se^{2-}) exist in as-deposited CdSe thin films. The bandgap calculated by the absorption coefficient varies from 2.3 eV to 1.75 eV for CdSe thin films. Raman Spectra shows that basic vibrational LO mode observed at 208 cm^{-1} and it's double corresponds to at 415 cm^{-1}. The AFM image shows spindle shape morphology with lest roughness. The I-V results shows that at room temperature, the current flow for 1-volt potential difference is 0.005μA while at low temperature 273K the current increases to 0.024μA which is approximately five times to the room temperature value.

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Kinetics of Transition Metal Ion Catalyzed Oxidation of Some Industrially Important Alcohols using Ammonium Metavanadate in Acidic Medium

By Harichandra A Parbat, D V Prabhu & Anna Pratima Nikalje
Wilson College

Abstract- Oxidation of alcohols has industrial importance as it yields several useful products.

Toxic and costly metal ions like Os(VIII), Cr(VI), and Ru in different oxidation states are widely used for the oxidation of a variety of organic compounds. We are reporting herein the oxidation of the industrially useful primary alcohols, 2-Chloroethanol, 2-Butoxyethanol and 2-Phenoxyethanol using Ammonium metavanadate in acidic medium. Relatively less toxic and cheaper transition metal ions of the first series are effectively used as homogeneous catalysts for the oxidation of the alcohols to the corresponding aldehydes.

2-Chloroethanol is used as a precursor for ethylene oxide and is useful in the manufacture of crop protection chemicals, and pharmaceuticals.2-Butoxyethanol finds use in the making of paints, varnishes and industrial and household cleaners.

Keywords: *alcohols, ammonium metavanadate, kinetics, ionic strength, thermodynamic activation parameters, the entropy of activation, transition metal ion catalysts, reaction mechanisms.*

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Kinetics of Transition Metal Ion Catalyzed Oxidation of Some Industrially Important Alcohols using Ammonium Metavanadate in Acidic Medium

Harichandra A Parbat [α], D V Prabhu [σ] & Anna Pratima Nikalje [ρ]

Abstract- Oxidation of alcohols has industrial importance as it yields several useful products.

Toxic and costly metal ions like Os(VIII), Cr(VI), and Ru in different oxidation states are widely used for the oxidation of a variety of organic compounds. We are reporting herein the oxidation of the industrially useful primary alcohols, 2-Chloroethanol, 2-Butoxyethanol and 2-Phenoxyethanol using Ammonium metavanadate in acidic medium. Relatively less toxic and cheaper transition metal ions of the first series are effectively used as homogeneous catalysts for the oxidation of the alcohols to the corresponding aldehydes.

2-Chloroethanol is used as a precursor for ethylene oxide and is useful in the manufacture of crop protection chemicals, and pharmaceuticals.2-Butoxyethanol finds use in the making of paints, varnishes and industrial and household cleaners.

2-Phenoxyethanol serves as a perfume fixative. The oxidation is studied in the presence and absence of transition metal ions under first- order kinetic conditions concerning the inorganic oxidant. The dependence of the oxidation rates of alcohols on concentrations of substrate and oxidant, ionic strength, and temperature is investigated. From the variation of the oxidation rate with temperature, the energy of activation and other thermodynamic activation parameters are evaluated and interpreted in terms of the molecular dynamics of the oxidation process.

The oxidation rates of the alcohols follow the sequence: 2-Chloroethanol > 2-Butoxyethanol > 2-Phenoxyethanol, which is explained based on their structural features.

The catalytic effect of the transition metal ions, Mn(II), Co(II) and Ni(II) on the oxidation rates of alcohols is studied in the range [M(II)]= 2.5 to 4.5 x 10^{-4} mol dm^{-3}, and the sequence of catalytic efficiencies of the metal ions determined. A suitable reaction mechanism is suggested for the oxidation of alcohols using Ammonium metavanadate in acidic medium.

Keywords: alcohols, ammonium metavanadate, kinetics, ionic strength, thermodynamic activation parameters, the entropy of activation, transition metal ion catalysts, reaction mechanisms.

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I. Introduction

The oxidation of alcohols to carbonyl compounds is extensively reported [1-5]. We have studied the kinetics of oxidation of a variety of alcohols and phenols using organic and inorganic oxidants [6-14]. This paper reports the oxidation of the primary alcohols, 2-Chloroethanol, 2-Butoxyethanol and 2-Phenoxyethanol by Ammonium metavanadate in acidic medium, using transition metal ions as catalysts.

The dependence of oxidation rate on alcohol and oxidant concentrations, ionic strength, and temperature is studied. The thermodynamic activation parameters of the oxidation reaction are evaluated from the variation of oxidation rate with temperature .The sequence of oxidation rates of the alcohols is explained based on their structural features.

Transition metal ions, Mn(II), Co(II), and Ni(II) are successfully used to catalyze the oxidation of alcohols, and the sequence of their catalytic efficiencies is determined for each substrate. A suitable reaction mechanism is suggested for the oxidation of the primary alcohols under study.

II. Materials and Methods

The alcohols were obtained from S H Kelkar & Co., Mumbai, and used after distillation. All other chemicals, solvents, and reagents were of Analytical Grade.

The oxidation of alcohol is studied under first-order kinetic conditions concerning the inorganic oxidant, ie [oxidant] << [alc.]. The progress of the reaction is monitored by arresting the reaction using ice and titrating the unreacted oxidant at regular time intervals against standard $Na_2S_2O_3$ using starch as indicator. The first-order rate constants (k) are obtained from the slopes of the plots of log (unreacted oxidant) versus time. K_2SO_4 was used in the range μ = 0.05-0.25 mol dm^{-3} to find out the influence of ionic strength on oxidation rates of alcohol. The thermodynamic parameters are evaluated from the Arrhenius plots of log k versus T^{-1}.

A similar procedure is adopted to study the catalytic impact of transition metal ions, Mn(II), Co(II) and Ni(II) on the oxidation of alcohols.

III. Results and Discussion

The primary alcohols, 2-Chloroethanol, 2-Butoxyethanol and 2-Phenoxyethanol are oxidized to the corresponding aldehydes in acidic medium. Their oxidation rates increased with [alc.] but decreased with increasing oxidant concentration (Table1, Figure1).

a) Reaction mechanism of oxidation of alcohols

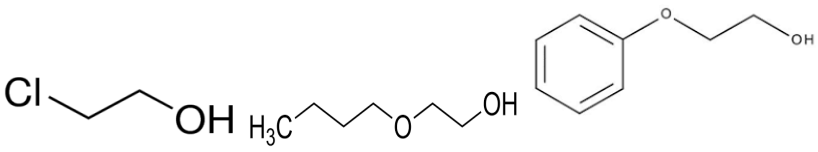
In acidic medium, Ammonium metavanadate forms Vanadic acid, which is a strong acid and strong oxidant. For primary alcohol, the oxidation reaction is shown as

$$RCH_2OH + (VO_3)^{-1} + 2H^{+} \text{--------} \rightarrow RCHO + (VO)^{+1} + 2H_2O$$

The oxidation product, i.e. aldehyde is identified by 2,4-dinitrophenyl hydrazone test and confirmed by TLC.

The oxidation rates follow the sequence:

2-Chloroethanol>2-Butoxyethanol > 2-Phenoxyethanol (Table 1, Figure 1)



The Chlorine atom in 2-Chloroethanol facilitates the removal of hydrogen atoms making it most susceptible to oxidation.

The influence of ionic strength (μ) on the oxidation rates of alcohol is studied using K_2SO_4 in the range μ = 0.05 -0.25 mol dm^{-3} at 313K (Table 2). The graphs of log k versus $\sqrt{\mu}$ are found to be straight lines parallel to the $\sqrt{\mu}$ axis, indicating that the rate of oxidation is independent of ionic strength due to the involvement of a nonionic species in the oxidation reaction (Figure 2).

The oxidation of alcohol is carried out in the temperature range 303-313K, and the thermodynamic activation parameters are determined from the Arrhenius plots of log k versus T^{-1}. The negative values of ΔS^* indicate a decrease in entropy due to the formation of a short- lived activated complex, followed by the reorientation of solvent molecules around the activated complex[15, 16].

Transition metal ions are used to catalyze the oxidation of alcohol to aldehyde using Ammonium metavanadate in acidic medium. Mn(II), Co(II) and Ni(II) ions are used in the concentration range [M(II)] =2.5 - 4.5 x 10^{-4} mol dm^{-3} at 303K. The rate constants of oxidation of alcohols are determined from the linear plots of log (unreacted oxidant) versus time. In each case, the rate (k) increased with [M(II)](Tables 4a, 4b, and 4c, Figures 3a. 3b. and 3c).

The stability order for the complexes of the transition metal ions under study is expected to be Ni(II) > Co(II) > Mn(II) and hence the sequence of their catalytic efficiencies is expected to be Mn(II) > Co(II) > Ni(II) [17,18]. However, several discrepancies are observed and reported in literature [19.20]. In our study the sequences of catalytic efficiencies of transition metal ions are as follows:

2-Chloroethanol Mn (II)>Ni(II)>Co(II) (Table 4a, Figure 3a)

2-Butoxyethanol Co(II) > Ni(II)>Mn(II) (Table 4b, Figure 3b)

2-Phenoxyethanol Co(II) > Ni(II)>Mn(II) (Table 4c, Figure 3b)

IV. Conclusions

1) The alcohols are oxidized as per the sequence, 2-Chloroethanol>2-Botoxyethanol>2-Phenoxy-ethanol.
2) The oxidation of alcohols is independent of ionic strength in dilute solution by the Bronsted –Bjerrum equation, $\log k = \log k_0 + 1.02 Z_A Z_B \sqrt{\mu}$.
3) There is a decrease in entropy of activation ΔS^* during the oxidation of alcohols.
4) Transition metal ions, Mn(II), Co(II) and Ni(II) are effectively used to catalyze the oxidation of alcohols.

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Table 1: Rate constant data for the oxidation of alcohols by Ammonium metavanadate In 0.1 M H_2SO_4 Temp.=303 K

			$k \times 10^4$ s^{-1}	
[alc.] x 10^1	[NH_4VO_3] x 10^3	2-Chloroethanol	2-Butoxyethanol	2-Phenoxyethanol
mol dm^{-3}	mol dm^{-3}			
0.25	5.00	14.80	12.43	11.80
0.50	5.00	17.00	14.11	13.80
0.63	5.00	18.42	16.39	15.00
0.75	5.00	20.72	17.04	16.60
0.88	5.00	27.63	18.42	17.45
1.00	5.00	32.24	21.41	21.25
1.00	2.50	34.54	29.90	20.95
1.00	5.00	25.33	23.03	16.58
1.00	10.00	21.18	20.72	9.21
1.00	15.00	18.42	16.12	8.24
1.00	20.00	11.52	13.81	7.44
1.00	25.00	9.90	12.60	5.76

Table 2: Effect of Ionic strength on the oxidation rates of alcohols by Ammonium metavanadate in 0.1M H_2SO_4. [alc.] = 0.1M, [NH_4VO_3] = 2.5 x 10^{-3}M, Temp.=313 K

			$k \times 10^4$ s^{-1}	
μ mol dm^{-3}	$\sqrt{\mu}$	2-Chloroethanol	2-Butoxyethanol	2-Phenoxyethanol
0.00	0.00	6.82	4.54	2.24
0.05	0.22	6.72	4.27	2.28
0.10	0.32	6.48	4.64	2.31
0.15	0.39	6.53	4.50	2.21
0.20	0.45	6.50	4.07	2.39
0.25	0.50	6.58	5.00	2.35

Table 3: Thermodynamic activation parameters of the oxidation of alcohols by Ammonium metavanadate in 0.1M H_2SO_4

Temp.(K)	k x 10^4 s^{-1}	E kJ mol^{-1}	K* x 10^{16}	ΔG* kJ mol^{-1}	ΔH* kJ mol^{-1}	ΔS* kJ K^{-1} mol^{-1}
2-Chloroethanol						
303	18.42	14.24	2.92	90.13	11.72	-0.2588
308	20.58	14.24	3.21	91.37	11.68	-0.2587
313	27.95	14.24	4.29	92.10	11.64	-0.2571
2-Butoxyethanol						
303	16.12	6.23	2.55	90.46	3.71	-0.2863
308	17.27	6.23	2.69	91.82	3.67	-0.2862
313	19.34	6.23	2.97	93.06	3.62	-0.2857
2-Phenoxyethanol						
303	8.24	18.08	1.31	92.15	15.56	-0.2528
308	12.34	18.08	1.92	92.68	15.52	-0.2505
313	13.95	18.08	2.14	93.91	15.48	-0.2506

Table 4: Catalytic effect of transition metal ions on the oxidation of alcohols by Ammonium metavanadate in 0.1M H_2SO_4 [alc.]=0.1M, $[NH_4VO_3]$= 2.5 x 10^{-3}M, Temp.=303K

[M(II)] x 10^4 mol dm^{-3}		k x 10^4 s^{-1}	
	Mn(II)	Co(II)	Ni(II)
Table 4a. 2-Chloroethanol			
0.00	4.84	4.84	4.84
2.50	16.09	7.84	9.95
3.50	22.22	8.24	11.75
4.50	27.18	9.21	14.11
Table 4b. 2-Butoxyethanol			
0.00	3.93	3.93	3.93
2.50	6.63	14.96	9.90
3.50	7.46	16.74	13.35
4.50	11.67	18.42	16.35
Table 4c. 2-Phenoxyethanol			
0.00	4.34	4.34	4.34
2.50	5.76	8.70	6.91
3.50	8.61	14.73	9.30
4.50	9.21	16.42	18.65

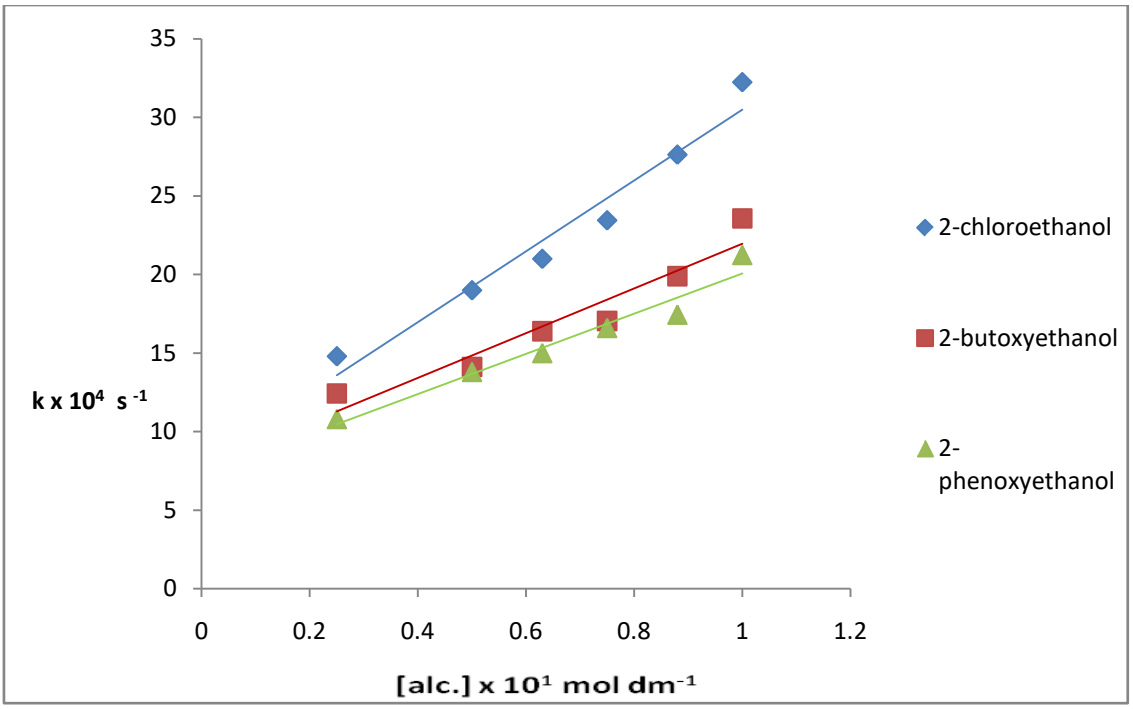

Fig. 1: Variation of rate constant of oxidation of alcohols by Ammonium metavanadate in acidic medium with [alc.]

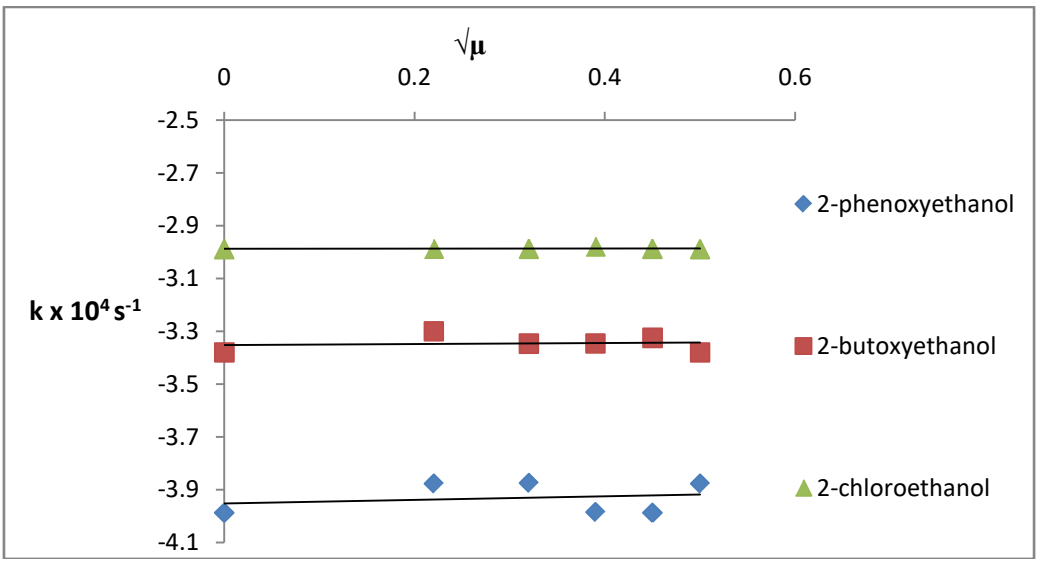

Fig. 2: Effect of ionic strength on the rate constant of oxidation of alcohols by Ammonium metavanadate in acidic medium

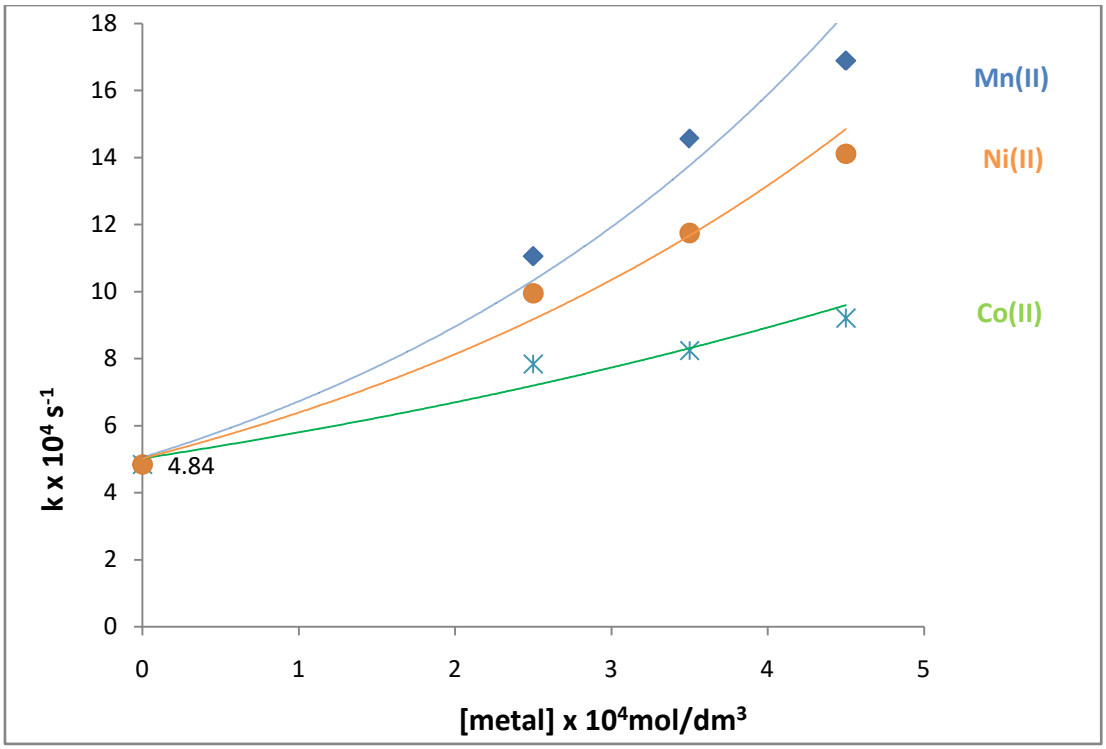

Fig. 3a: Variation of rate constant of transition metal ion catalyzed oxidation of 2-Chloroethanol with [M(II)] Sequence of catalytic efficiencies : Mn(II) >Ni(II) > Co(II)

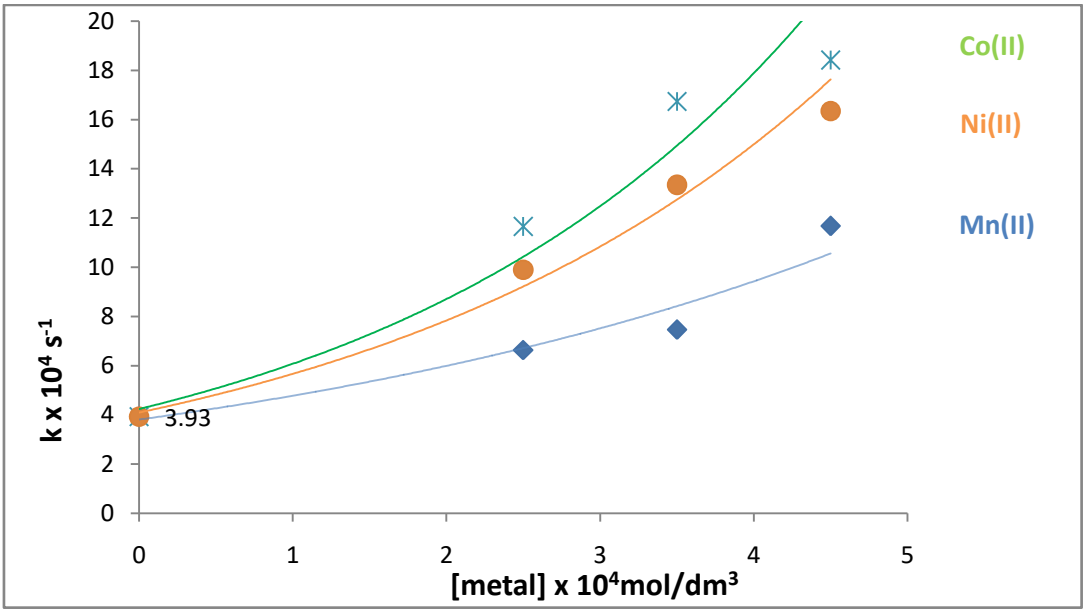

Fig. 3b: Variation of rate constant of transition metal ion catalyzed oxidation of 2-Butoxyethanol with [M(II)] Sequence of catalytic efficiencies: Co(II) > Ni (II) > Mn(II)

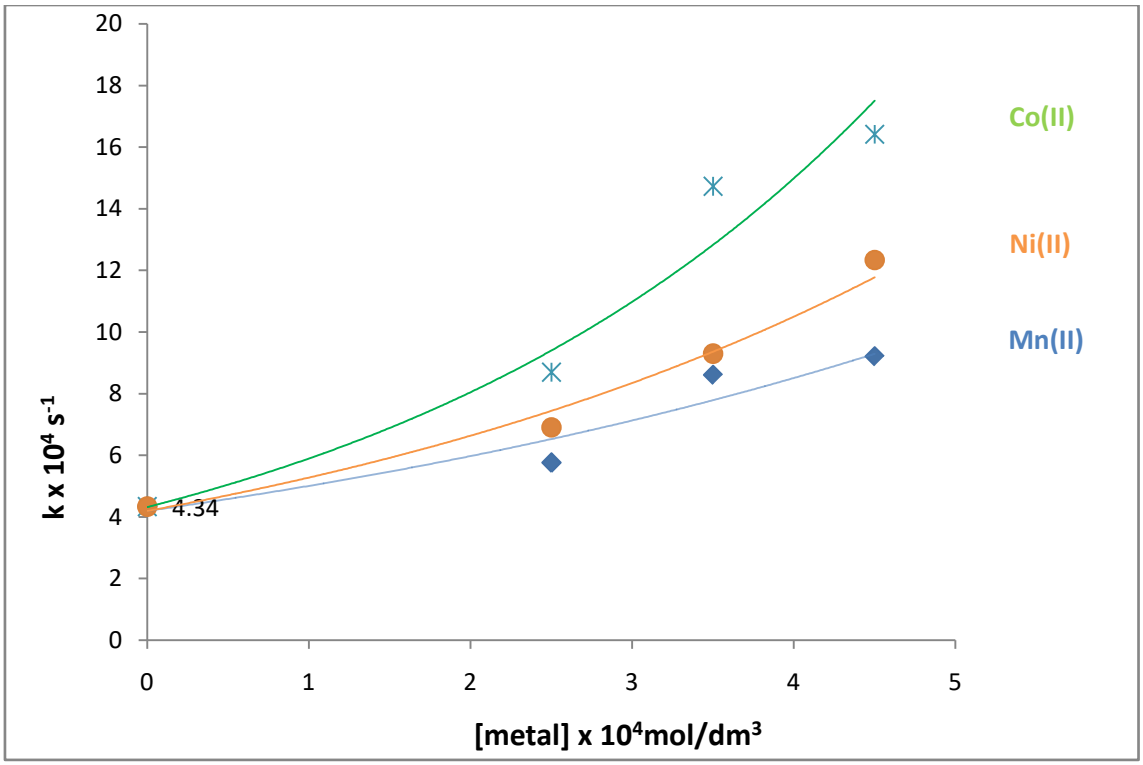

Fig. 3c: Variation of rate constant of transition metal ion catalyzed oxidation of 2-Phenoxyethanol with [M(II)]
Sequence of catalytic efficiencies: Co(II) > Ni(II) >Mn(II)

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- • This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in–depth understanding of the application of suitable techniques to a particular area of research practice.

Note :

"

- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone.

"

Preferred Author Guidelines

We accept the manuscript submissions in any standard (generic) format.

We typeset manuscripts using advanced typesetting tools like Adobe In Design, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

Alternatively, you can download our basic template from https://globaljournals.org/Template.zip

Authors should submit their complete paper/article, including text illustrations, graphics, conclusions, artwork, and tables. Authors who are not able to submit manuscript using the form above can email the manuscript department at submit@globaljournals.org or get in touch with chiefeditor@globaljournals.org if they wish to send the abstract before submission.

Before and during Submission

Authors must ensure the information provided during the submission of a paper is authentic. Please go through the following checklist before submitting:

1. Authors must go through the complete author guideline and understand and *agree to Global Journals' ethics and code of conduct,* along with author responsibilities.
2. Authors must accept the privacy policy, terms, and conditions of Global Journals.
3. Ensure corresponding author's email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s') names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
5. Authors should submit paper in a ZIP archive if any supplementary files are required along with the paper.
6. Proper permissions must be acquired for the use of any copyrighted material.
7. Manuscript submitted *must not have been submitted or published elsewhere* and all authors must be aware of the submission.

Declaration of Conflicts of Interest

It is required for authors to declare all financial, institutional, and personal relationships with other individuals and organizations that could influence (bias) their research.

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Plagiarism is not acceptable in Global Journals submissions at all.

Plagiarized content will not be considered for publication. We reserve the right to inform authors' institutions about plagiarism detected either before or after publication. If plagiarism is identified, we will follow COPE guidelines:

Authors are solely responsible for all the plagiarism that is found. The author must not fabricate, falsify or plagiarize existing research data. The following, if copied, will be considered plagiarism:

- Words (language)
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- Findings
- Writings
- Diagrams
- Graphs
- Illustrations
- Lectures

- Printed material
- Graphic representations
- Computer programs
- Electronic material
- Any other original work

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1. Substantial contributions to the conception and acquisition of data, analysis, and interpretation of findings.
2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

Changes in Authorship

The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

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Appealing Decisions

Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

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Preparing your Manuscript

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.

Format Structure

It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

Title

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

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Tips for Writing a Good Quality Science Frontier Research Paper

Techniques for writing a good quality Science Frontier Research paper:

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of science frontier then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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