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# Synthesis of Radical Cation of Promethazine by Electrochemical Oxidation of Promethazine HCl, Free radical scavenging and Antibacterial Application

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## ABSTRACT

*Promethazine HCl was oxidized electrochemically in presence of platinum electrodes as cathode and anode. The rate constant for the formation of the oxidized compound was calculated by first order plot. The rate of formation of oxidized compound increases with increase in concentration of promethazine HCl. The reaction mechanism for the formation of oxidized compound was proposed. The oxidized product has been characterized by Ultra-violet, Infrared, Mass spectroscopy, NMR and ESR techniques. The antibacterial activity and antioxidant assay has been performed for both promethazine and oxidized compound.*

**Keywords:** Electrochemical oxidation, Promethazine HCl (PMZ), Oxidized compound (PMZ)$^{\cdot}$

## INTRODUCTION

Promethazine hydrochloride (N,N-dimethyl-1-phenothiazin-10-yl-propan-2-amine hydro chloride), which belongs to the phenothiazine group, is a pharmaceutical compound widely used for its antihistaminic, sedative, antipsychotic, analgesic and anticholinergic properties [1]. However, promethazine hydrochloride can cause adverse effects in humans, such as endocrinal, cardiac and reproductive alterations [2-3]. Most of N-substituted phenothiazines are phenothiazine drugs which are used as tranquilisers. A major industrial application of phenothiazines is as antioxidants in preventing oxidation changes in polyethylene oils [4]. They are used as high temperature antioxidants such as additives to greases for silicons clad motors and generators and as lubricants for turbines and turbojet engines. They exhibit a high order of inhibition [5-8]. They are extensively used in field of psychiatry and chemotherapy. Phenothiazine drugs such as chloropromazine, promethazine, fluphenazine and alimemazine were examined for antibacterial effect on bacterial stains [9].

## MATERIALS AND METHODS

Electrochemical oxidation of promethazine HCl (sigma chemical co.) was carried out at different concentration (0.1M, 0.15M, 0.2M). The experimental setup consists of reaction chamber and a voltage power supply, the electrode system consist of platinum wire electrodes as cathode and anode. The kinetic runs were carried out from 60 to 230 minutes with continuous stirring. A positive voltage was applied by

using battery eliminator (Neulite India) and current output of 8mA-26mA using rheostat (INSIF India). The experiment was performed with volume of 20ml solution. The rate of oxidation of promethazine was followed by using spectrophotometer (ELICO SL 171), at a wavelength of 620nm. The rate constant of the reactions were calculated which is shown in table 1. The rate of the oxidation of promethazine (PMZ) increases with increase in concentration of promethazine (PMZ).

**Table 1:** Effect of concentration of (PMZ) on rate of oxidation.

| Concentration of (PMZ) | Rate constant k |
|---|---|
| 0.1M | $0.77\times10^{-4}$ |
| 0.15M | $1.91\times10^{-4}$ |
| 0.2M | $4.98\times10^{-4}$ |

A plot of log %T versus time for various concentration of (PMZ) was plotted which is shown in fig 1.
The oxidation of promethazine (PMZ) I involve a removal of an electron from (PMZ) to give radical cation of promethazine (PMZ)$^{\cdot}$ II. Further oxidation was stopped to avoid the formation of dication III, which was confirmed from the spectral data (scheme-I).

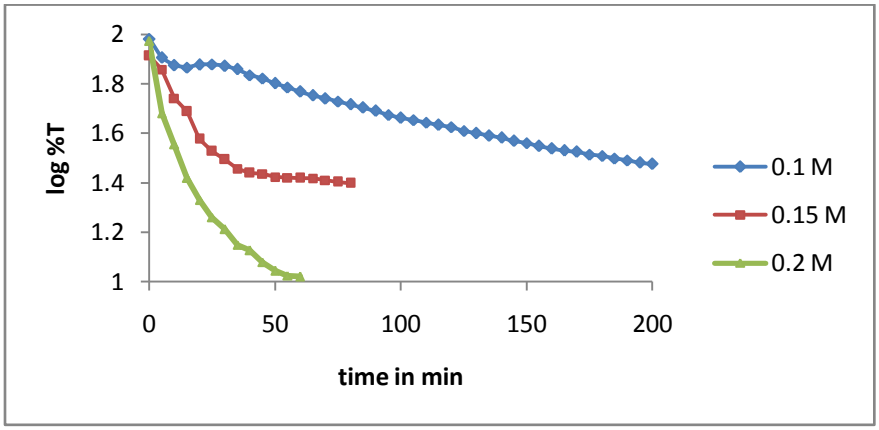
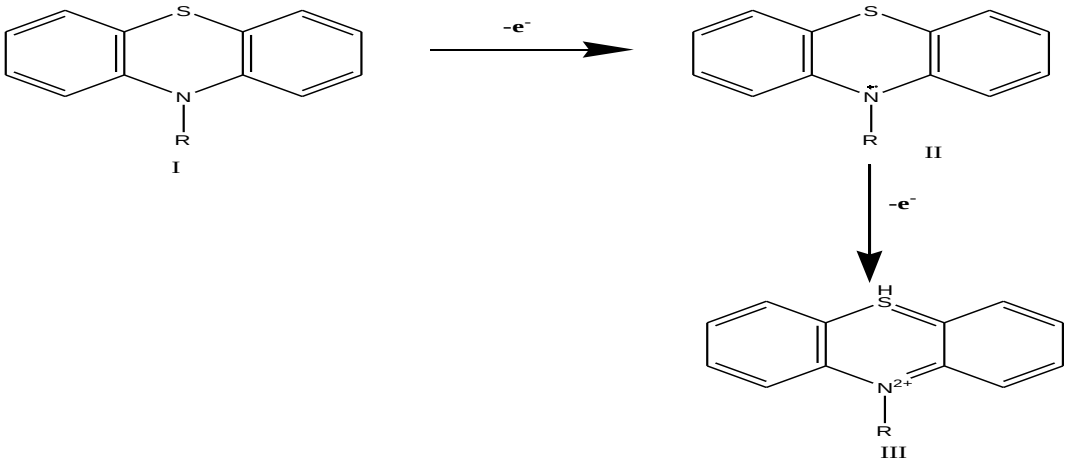



**Figure 1:** Effect of concentration of (PMZ) on the rate of oxidation.




**Scheme 1**: Probable pathway for the electrochemical oxidation of promethazine.

## RESULTS AND DISCUSSION

**Ultraviolet studies:** Maximum absorbance wavelengths for (PMZ)· was examined, which was found to be 620 nm. The UV-spectra for (PMZ)· is shown in figs 2.The observed blue colour at 620nm confirms the presence of a radical in the synthesized compound.

**Conductivity of solutions:** Solutions of (PMZ) and (PMZ)· was prepared by dissolving 15.00 mg of each compound into 20ml of distilled water, and conductivity of the solutions were measured at 20 ms using conductivity meter (Elico CM180) and results are shown in table 2.The higher value of conductance for (PMZ)· indicates more ionic than promethazine and confirms the presence of radical cation.

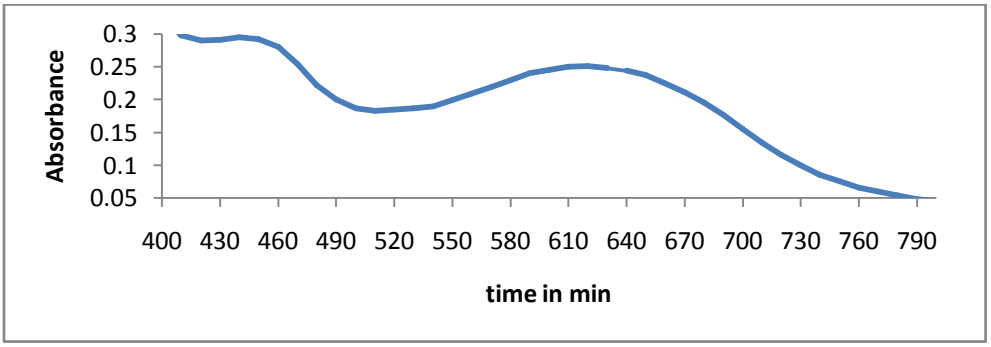



**Figure 2:** UV-Visible spectra of (PMZ)·

**Table 2:** Conductivity of (PMZ) and (PMZ)·

| Solutions | Conductivity |
|---|---|
| Promethazine HCl (PMZ) | 8.15 |
| Oxidized compound (PMZ)· | 8.50 |

**Antioxidant assay:** The DPPH radical scavenging activity of compounds was determined spectrophotometrically. DPPH reacts with an antioxidant compound that can donate hydrogen radical and thereby it is reduced. A change in colour, from deep violet to light yellow, was measured. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [10]. The intensity of the yellow colour depends on the amount and nature of the scavenger present in the sample and standard compounds.

The antioxidant assay was carried out for ascorbic acid, promethazine HCl (PMZ) and oxidized compound (PMZ)· and the rate of reaction was followed spectrophotometrically at 517nm and compared. 1mg/ml solutions of ascorbic acid, promethazine(PMZ) and oxidized compound (PMZ)· were prepared in methanol and DPPH solution of 1mg/25ml in methanol. 0.1ml of standard, promethazine (PMZ) and oxidized compound (PMZ)· were added to three separate testubes and made up to 3 ml with methanol, followed by 1ml of DPPH solution. The solutions were placed in dark, and absorbance was measured at 517nm at different interval of time. The percentage radical scavenging activity was calculated by equation.

$$\% \text{ Inhibition} = \left[\frac{A0-A1}{A0}\right] \times 100 \qquad \rightarrow (1)$$

Where $A_0$ was the absorbance of the blank and $A_1$ was the absorbance in the presence of test solutions. A graph of % inhibition verses time has been plotted for ascorbic acid, (PMZ) and (PMZ)· Which is shown in fig 3 and the rate constant has been calculated in table 3. The % inhibition is in the order of Ascorbic acid > (PMZ)· > (PMZ), which indicates the $IC_{50}$ values for (PMZ)· is higher than (PMZ) . Further the rate of inhibition for (PMZ)· is higher than (PMZ) . This shows that the synthesized compound behaves as strong

antioxidant than (PMZ), because of the presence of free radical in (PMZ)·, which conforms the oxidation of (PMZ) to (PMZ)·.The reaction mechanism of DPPH with (PMZ)·is given in scheme-II.

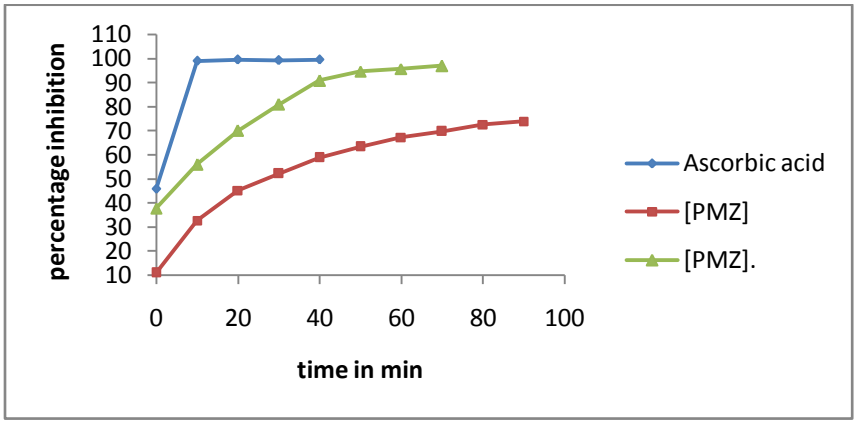
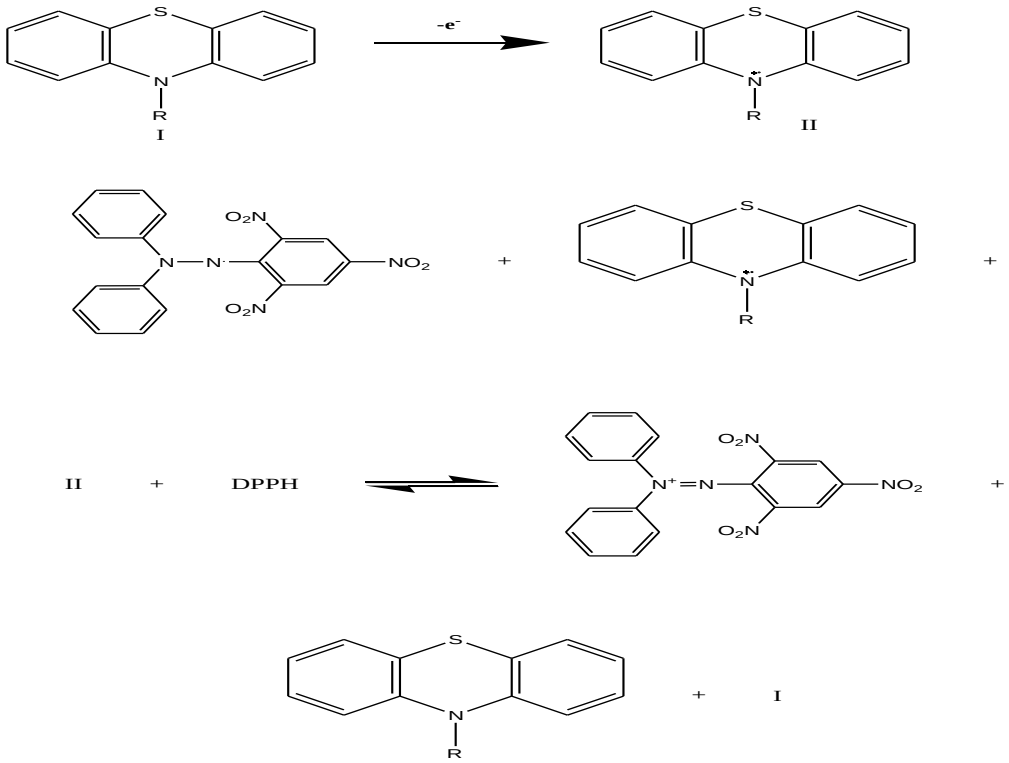



**Figure 3:** DPPH radical scavenging activity of (PMZ) and (PMZ)·

**Table 3:** Rate constant for (PMZ)·and (PMZ) in DPPH radical scavenging assay.

| Test solutions | Rate constant |
|---|---|
| Ascorbic acid | $1.53\times10^{-4}$ |
| (PMZ)· | $1.05\times10^{-4}$ |
| (PMZ) | $0.77\times10^{-4}$ |




**Scheme 2:** Probable pathway of the mechanism of DPPH radical scavenging with (PMZ)·

The mass spectrum of (PMZ) and (PMZ)$^{\cdot}$ is shown in fig 4a and 4b. The spectra shows the mass peak at 285.1 and 285.0 respectively, which indicates there is no change in mass of synthesized compound as only an electron is removed from (PMZ) to form (PMZ)$^{\cdot}$. The IR spectra of (PMZ) and (PMZ)$^{\cdot}$ is shown in fig 5a and 5b the spectra shows similar spectral bands indicating no changes in functional groups. Further the NMR spectra of (PMZ) and (PMZ)$^{\cdot}$ in fig 6a and 6b shows similar structural data.

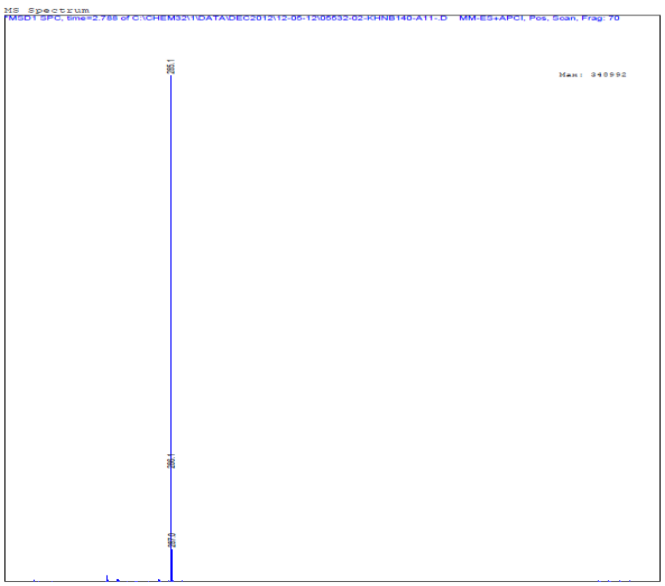


**Figure 4a:** Mass spectra of (PMZ)

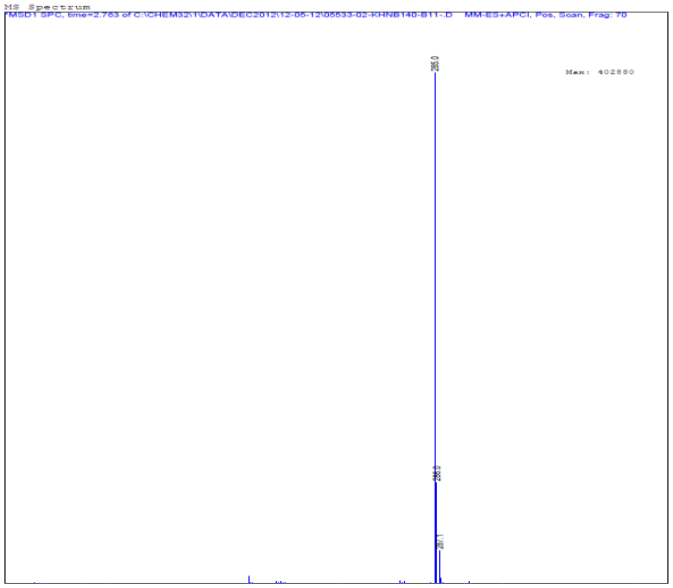


**Figure 4b:** Mass spectra of (PMZ)$^{\cdot}$

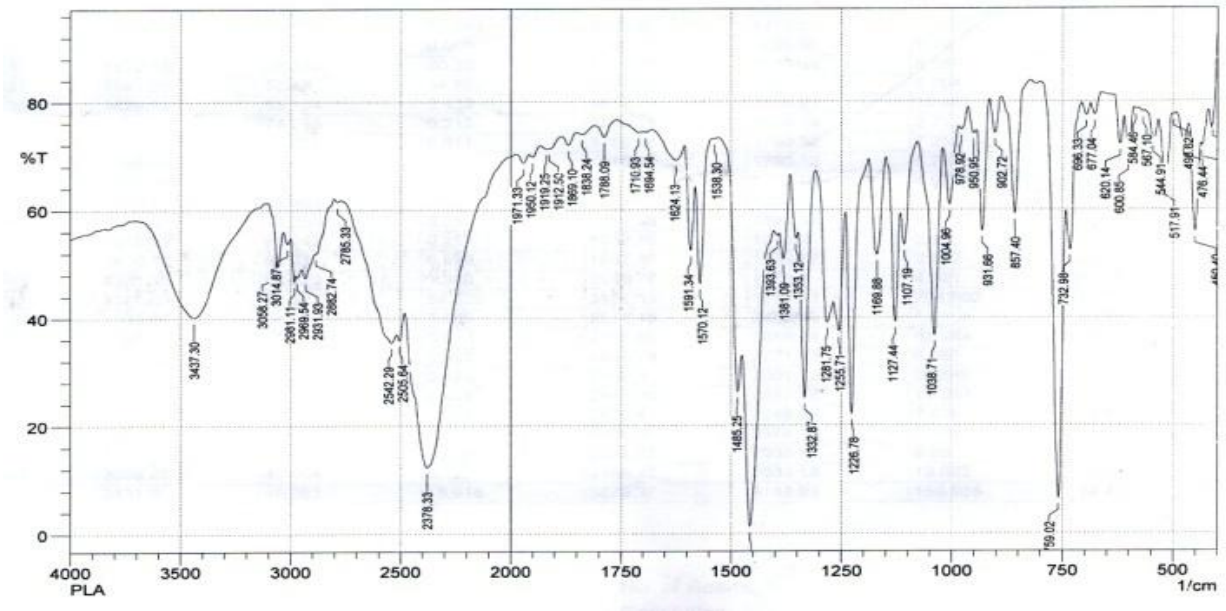



**Figure 5a:** FT-IR spectra of (PMZ)

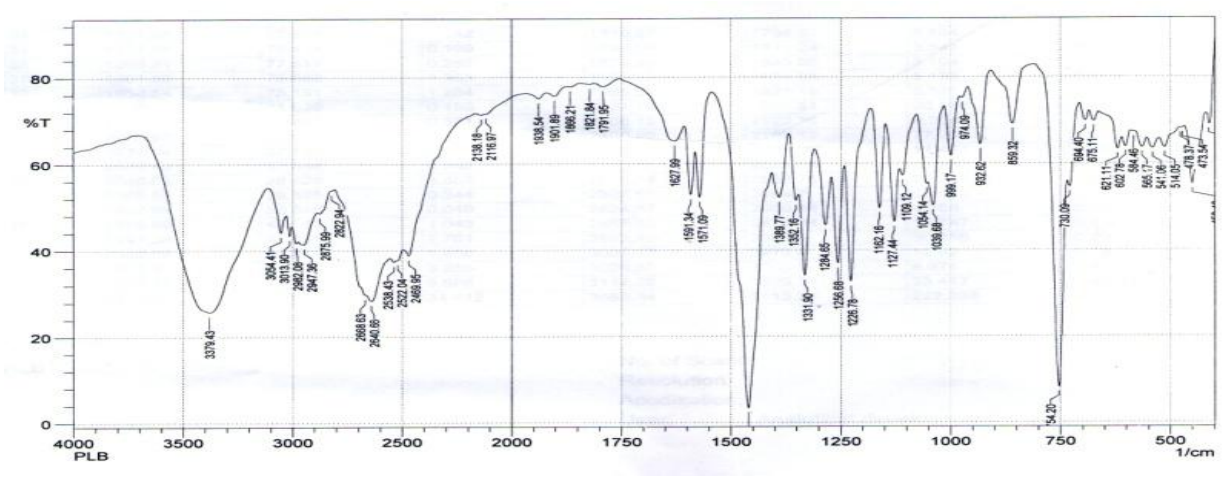



**Figure 5b:** FT-IR spectra of $(PMZ)^{\cdot}$

The ESR spectra for both (PMZ) and $(PMZ)^{\cdot}$ is shown in fig 7a and 7b. Oxidation of (PMZ) gives structure II and III shown in scheme I. However in the present case since structure III is not ESR active and structure II is only ESR active, Hence the formation of dication III does not takes place. The existence of radical cation II was conformed from the ESR spectra 7b.

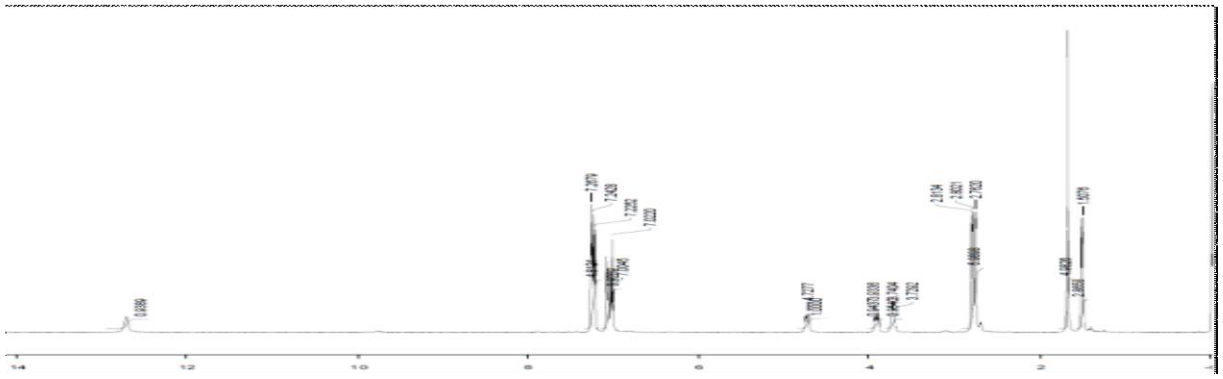


Figure 6(a): NMR spectra of (PMZ)

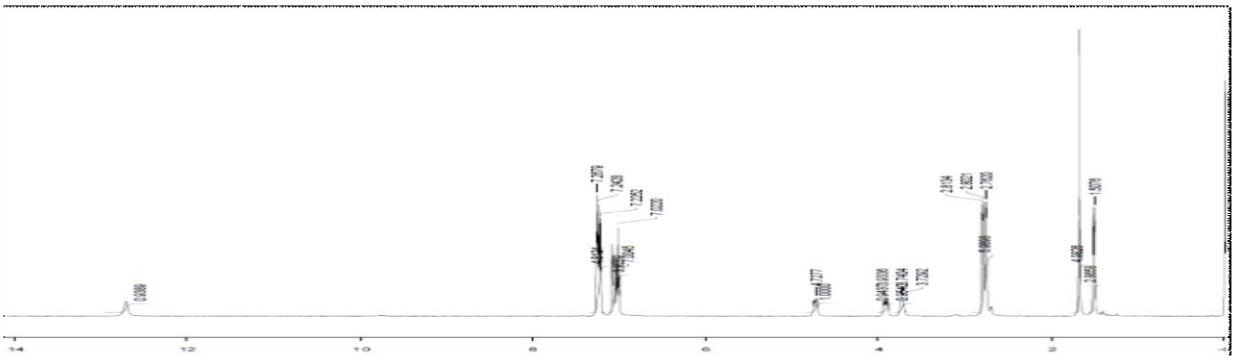


Figure 6(b): NMR spectra of $(PMZ)^{\cdot}$

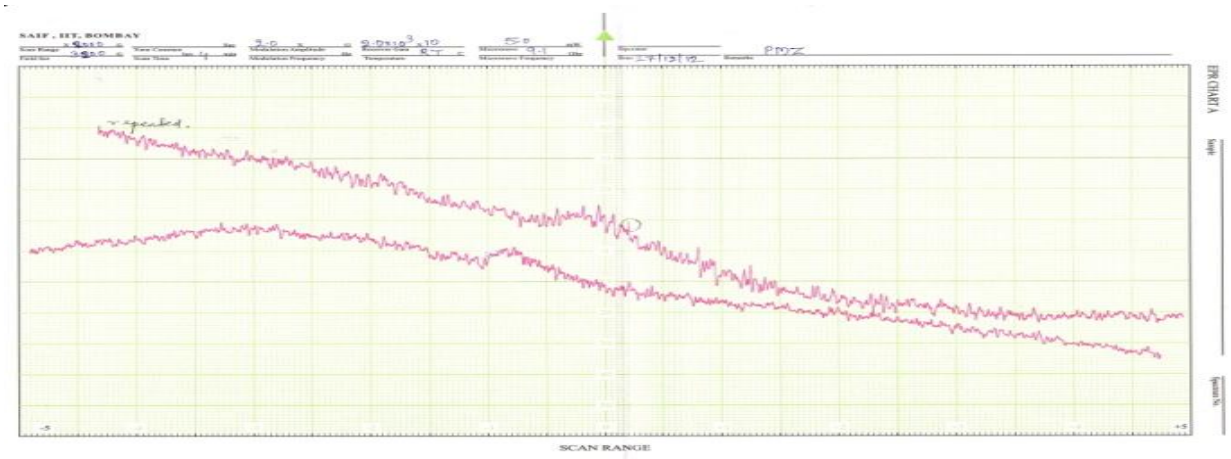



**Figure 7a:** ESR spectra of (PMZ)

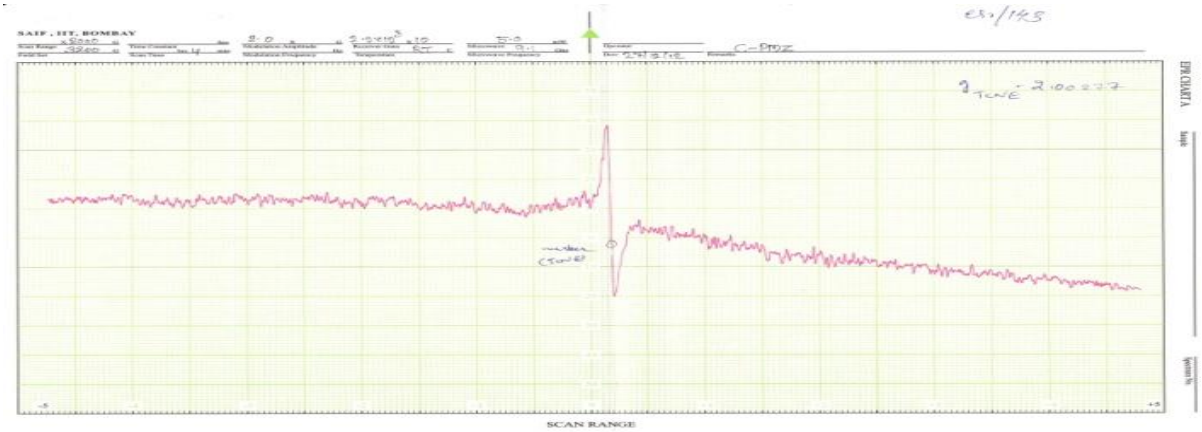


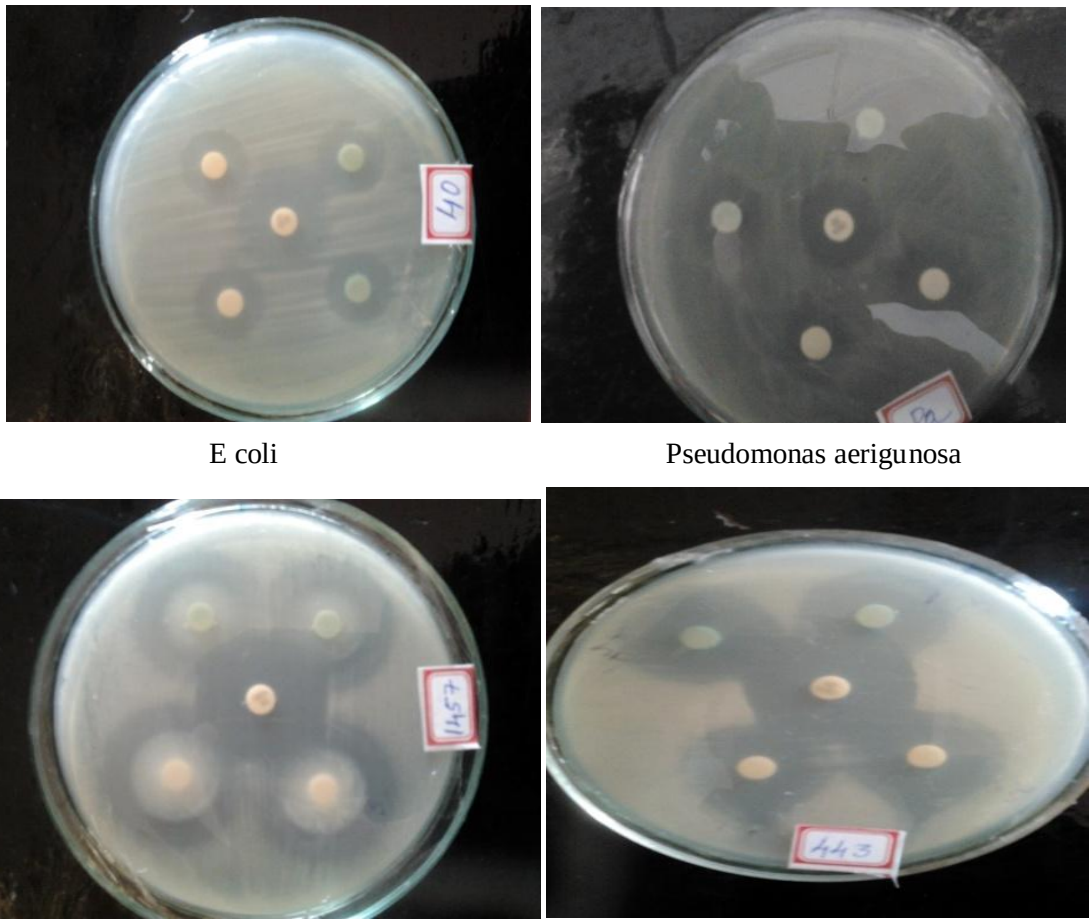

**Figure 7b:** ESR spectra of (PMZ)$^{\cdot}$

## APPLICATIONS

**Anti-Bacterial Activity:** Antibacterial activity study was carried out in duplicate for (PMZ), (PMZ)$^{\cdot}$ and a positive control as Gentamicin against E. coli, Pseudomonas aerigunosa, Shigella flexneri and Salmonella typhi by disc diffusion method, and the zone of inhibition of both the compounds were measured in centimeters. Table (4) and fig (8) shows higher anti bacterial activity for synthesized (PMZ)$^{\cdot}$compared to (PMZ). The higher activity of (PMZ)$^{\cdot}$ indicates stronger antioxidant than (PMZ).This confirms that electrochemical oxidation of (PMZ) leads to the formation of a radical cation (PMZ)$^{\cdot}$ .which is stable because of its highly conjugated structure.

**Table 4:** Inhibition of growth of microorganisms by (PMZ) and (PMZ)$^{\cdot}$

| **Test organisms** | **(PMZ) Trial I** | **(PMZ) Trail II** | **(PMZ)$^{\cdot}$ Trial I** | **(PMZ)$^{\cdot}$ Trail II** |
|---|---|---|---|---|
| Ecoli | 0.8 | 0.8 | 0.95 | 0.95 |
| Pseudomonas aerigunosa | 0.9 | 0.9 | 1.1 | 1.1 |
| Shigella flexneri | 1.6 | 1.6 | 1.8 | 1.8 |
| Salmonella typhi | 1.3 | 1.3 | 1.4 | 1.4 |



E coli Pseudomonas aerigunosa

Shigella flexneri Salmonella typhi

**Figure 8:** Inhibition of growth of microorganisms by (PMZ) and (PMZ)·

## CONCLUSIONS

Radical cation of promethazine was synthesized by electrochemical oxidation of promethazine, which shows higher antioxidant an antibacterial activity. The synthesized free radical cation substance is quite stable because of resonance involved in its structure.

## ACKNOWLEDGEMENTS

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