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Synthesis, characterization and biological applications of conducting poly *o*-toludine in the absence and in the presence of ultrasonic irradiation

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ABSTRACT

The poly ortho toludine was synthesized by oxidative chemical polymerization method in the absence and in the presence of ultrasonic irradiation using potassium dichromate as an oxidant and hydrochloric acid as a dopant. The synthesized polymers were characterized using, UV, IR, TGA and DTA. The Dc conductivity of the polymers and their biological applications like antibacterial and cytotoxic effects were studied. The study revealed that the polymer prepared was conducting in nature and the value was found to be $\mu 2.x10^{-2}$ *S/cm at* 40^0 *C and they are thermally stable even up to* 840^0*C. The antibacterial study was carried by Agar well cut diffusion method and the cytotoxic effect was carried out by MTT assay .The studies indicate that the activities were found to increase with increase in concentrations. The study also revealed that the polymer prepared in the presence of ultrasonic irradiation showed better results even at lower concentrations.*

Keywords: Toludine, ultrasonic irradiation, Polymer, antibacterial, cytotoxicity.

INTRODUCTION

Conductive Polymeric Materials containing conjugated bonds have attracted much interest in scientific and technological areas in recent years. The unique optical, electrical and chemical properties offer these materials to be used in electronic displays, telecommunication, biosensors[1]anticorrosion coatings, rechargeable polymeric batteries, electromagnetic shielding, polymer photovoltaic's ,polymer actuators[2,3]. Polyaniline (PANI) is by far the most investigated conducting polymer. However, major problem related to its successful utilization lays in its poor mechanical properties and process ability due to its insoluble nature in common organic solvents [4, 5]. The common way to change the physical and chemical properties of polymer is to substitute the polymer chain with special chemical groups like $-CH_3$, $-C_2H_5$, -OH, $-OCH_3$ [6] etc**.** Poly toluidines have attracted considerable attention as they exhibit better solubility in many solvents and better processability than PANI [5]. MacDiarmid and co-workers [7] suggested that introduction of $-CH_3$ substituent on the phenyl rings increases the torsion of neighboring benzene rings on the polymer chain thereby resulting in decrease in the extent of conjugation of Poly Ortho Toluidine (POT). It has been demonstrated that the conductivity of POT at room temperature is one or two orders of magnitude (10^{-1}-10^{-2} S/cm) lower than that of PANI(10^0-10^1 S/cm) [9]. Basically the stages of polymerization of aniline and its derivatives (including o-toluidine) can be described by a sequence of reactions involving oxidation and coupling reactions.

Electrochemically active groups are either built in the polymer structure inside the chain or as a pendant group or incorporated into the polymer phase in the course of the polymerization [10]. Properties of polymers are originated from their highly regulated molecular and morphological structures. The electrical conductivity, appearance,

toughness and morphology are markedly affected by the conditions of polymerization. Recently sonochemistry has attracted considerable interest since irradiation of reaction mixtures with ultrasound produces a variety of effects on reactions.

Conducting polymer having good biocompatibility combined with antibacterial and cytotoxicity property can lead to tremendous development in the field of biomedicine and controlled drug release, the latter of which can be tremendously useful in cancer treatment . A range of biomedical applications for conducting polymers are considered which includes development of artificial muscles, controlled drug release and stimulation of nerve regeneration. Low cytotoxicity and good biocompatibility are evident from the growth of cells on conducting polymers and from the low degree of inflammation seen in test animals over a period of several weeks [12]. Given that conducting polymers are redox-active, and can shuttle between reduced and oxidized forms, potential interactions of the polymers with biological media need to be considered [13].

The imine group present in various natural, natural-derived and non-natural compounds like Schiff bases has been shown to be critical to their biological activities including antibacterial, antifungal and antipyretic properties [14-18]. Since Polytoluidines also, possess amine and imine units in their structure [6], they could also exhibit biological activity like antibacterial property. Besides appropriate mechanical and physical chemistry properties, the most important requirement for a material to be used in biomedical fields is its biocompatibility in a specific environment. Cytotoxicity testing of a material by MTT colorimetry is the initial step to evaluate its biocompatibility and the fast effective method to choose the right material.

The literature survey shows that exhaustive work has been carried out in conducting polymers and their applications in different fields, but no literature is available on synthesis of Poly ortho toluidine in the absence and presence of ultrasonic irradiation. Hence an attempt has been made to synthesize poly ortho toludine and to study their biological applications.

EXPERIMENT SECTION

Materials:
All chemicals used in the present investigation are of Analytical Reagent (AR) grade and used as received.

Preparation of Poly Ortho Toluidine:
The polymerization of the monomer was initiated by the drop wise addition of oxidizing agent Potassium Dichromate and Conc. Hydrochloric acid as dopant under constant stirring for 5 hours at room temperature in 1:1 ratio [6]. After 24 hours the product Poly ortho toluidine (POT) was filtered, washed with distilled water until the filtrate was colorless. Finally the polymer was dried and powdered. The same procedure with slight modification was adopted for the preparation of poly ortho toludine in the presence of ultrasonic irradiation (POTS).

Characterization:
The UV- Visible spectra was recorded using systronics double beam UV-Visible spectrophotometer in the range 300-900nm. FT-IR spectra of POT were recorded in the mid IR region between 4000^{-1}cm to 400^{-1}cm using Thermo Nicolet Model 6700. The samples were prepared in the pellet form using spectroscopic grade KBr powder. The thermo gravimetric analysis (TGA-DTA) measurements were made using a Schimadzu TGA-50 Japan system under a nitrogen atmosphere with a heating rate of 10^{o}C / min from room temperature to 900˚ C. The electrical conductivity was measured using Systronics Four probe instrument at 40^{o}C.The samples were measured in pellet form of 13mm diameter.

Antibacterial Activity:
Agar Well Diffusion Method was employed for this study. Gram positive bacteria *Staphylococcus auereus* and gram negative bacteria *Escherichia coli* were utilized [19]. Sterile Nutrient broth was inoculated with freshly isolated bacterial culture and incubated for 24 hrs at 37^{o}C.The bacterial suspension was found to be approximately 10^{7}-10^{8} cells/mL after the incubation period, they were used as inoculums. About 0.1mL of suspension containing 10 Colony Forming Unit (CFU/mL) of bacterial strain was used to study by Agar well cut diffusion method [20]. The polymer (both in presence and absence of ultrasonic irradiation) were taken at different concentrations viz., 50, 75,100 µg/mL and their zone of inhibition were monitored after 24 hrs and the inhibition zone was compared with the standard Gentamycin whose zone of inhibition is 29mm.

Cytotoxic Activity - (*3-(4, 5-Dimethylthiazol – 2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay*

Cytotoxicity was determined using MTT assay. It determines the inhibitory effect of the crude extracts on cell growth using MTT assay. To determine cell viability, cell number was quantified using the standard Colorimetric MTT assay. This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to the purple formazan derivatives by mitochondrial succinate dehydrogenase in viable cells. The cells were grown in a 96-well plate in Delbucco's Minimum essential medium (DMEM) (HiMedia) supplemented with 10% fetal bovine serum (Gibco Laboratories) and antibiotics (streptomycin, penicillin-G, kanamycin, amphotericin B). About 1 ml cell suspension (10^5cells/mL) was seeded in each well and incubated at 37^0 C for 48 hour in 5% CO_2 for the formation of confluent monolayer. The monolayer of cells in the plate was exposed to various dilutions (1000, 500, 250, 125µg)**.** The cell viability was measured using MTT assay with MTT (5 mg/mL) and DMSO. This tetrazolium salt is metabolically reduced by viable cells to yield a blue insoluble Formosan product measured at 570nm spectrophotometerically. Controls were maintained throughout the experiment (untreated wells) as cell control. The assay was performed in triplicate for each. The mean of the cell viability values was compared to the control to determine the effect of the polymer on cells and % cell viability was plotted against concentration of the polymer. Viability was defined as the ratio (expressed as percentage) of absorbance of treated cells to untreated cells [21]. Controls were maintained throughout the experiment. Untreated wells were used as cell control and diluents treated wells as diluents control.

RESULTS AND DISCUSSION

Characterization:

The FT-IR spectrums of the synthesized polymer in the absence and in the presence of ultrasonic irradiation are given in figure 1&2. The peak at 3398cm^{-1} and 3430 cm^{-1} correspond to N-H stretching vibration for POT and POTS [22]. The higher frequency vibration at 1598 cm^{-1} and 1614 cm^{-1}has a major contribution from the C-N stretching of quininoid ring for POT and for POTS. The lower frequency mode around 1497 cm^{-1} depicts the presence of bezenoid rings [23]. The presence of these bands clearly shows that the polymers are composed of the amine and imine units .The peaks at 826cm^{-1} and 800cm^{-1} corresponds to para dissubstituted aromatic rings indicating polymer formation [24] which proceeded in a head to tail fashion [11].The new band which is not found in Polyaniline appears at 1155cm^{-1} which could be attributed to the $-CH_3$ rocking mode. Moreover the bands at 1155cm^{-1} seem to be very intense and broad in the spectra of the sample prepared in presence of ultrasonic irradiation compared with its absence. This band is vibrational mode of B – N^+H = Q or B–N^+H–B (B: Benzenoid, Q: Quininoid unit) and may be attributed to the existence of positive charge and the distribution of the dihedral angle between the benzenoid and quinioid rings [19]. Therefore this could be considered that the polytoluidine prepared in the presence of ultrasonic irradiation has higher doping level than its absence.

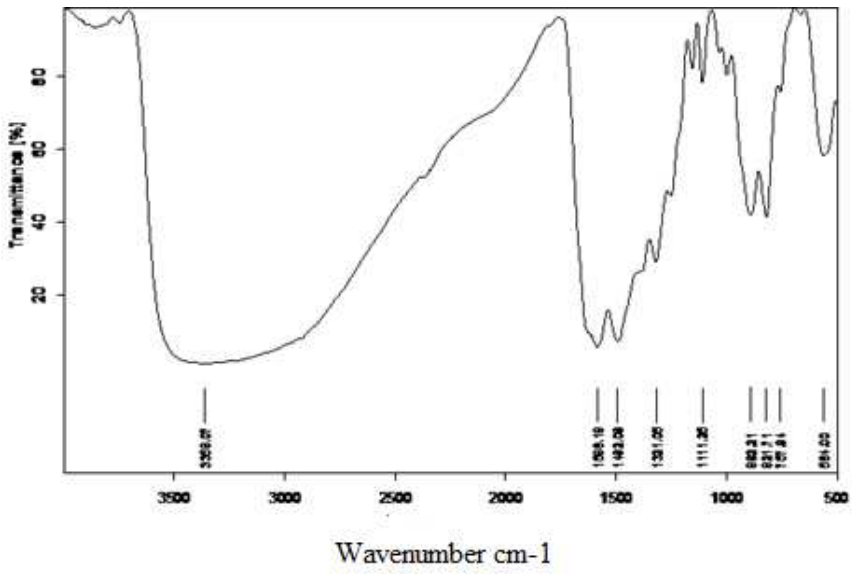

Figure 1- FT- IR Spectra of POT

Therefore this could be considered that the polytoluidine prepared in the presence of ultrasonic irradiation has higher doping level than its absence. All the other peaks found to be almost same for the polymer prepared in the absence and in the presence of ultrasonic irradiation.

The absence of the characteristic strong absorptions of carbonyl group (1680 cm^{-1}) indicates the absence of any significant quantity of *o*-quinone structure which may result from over oxidation of the POT [33]. Based on the above results, the POT is expected to have orthotoluidine unit and such units are linked to each other via the C–NH–C and C - N - C bonds. Some of the polymeric units may have a ladder-like planar structure with phenazine rings

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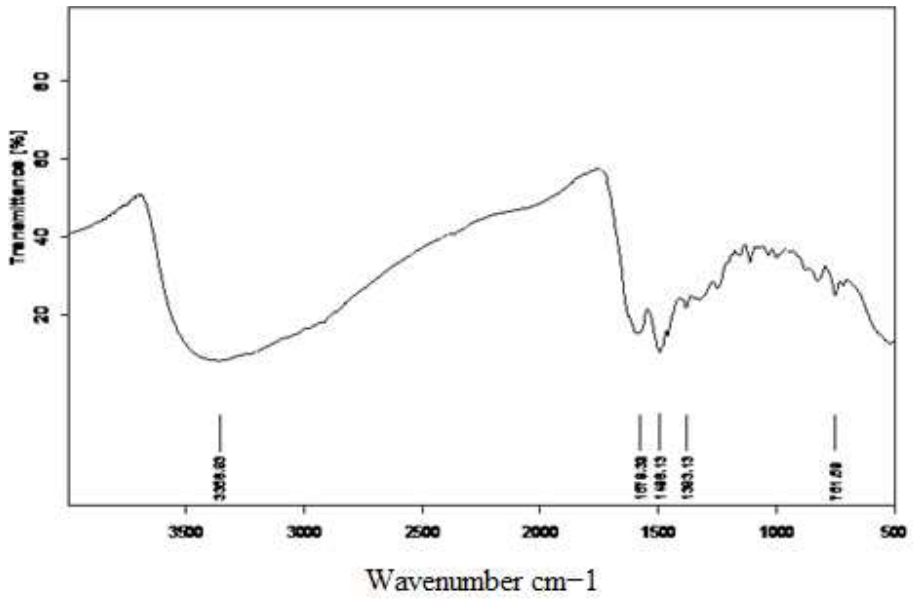

Figure 1 FT- IR Spectra of POTS

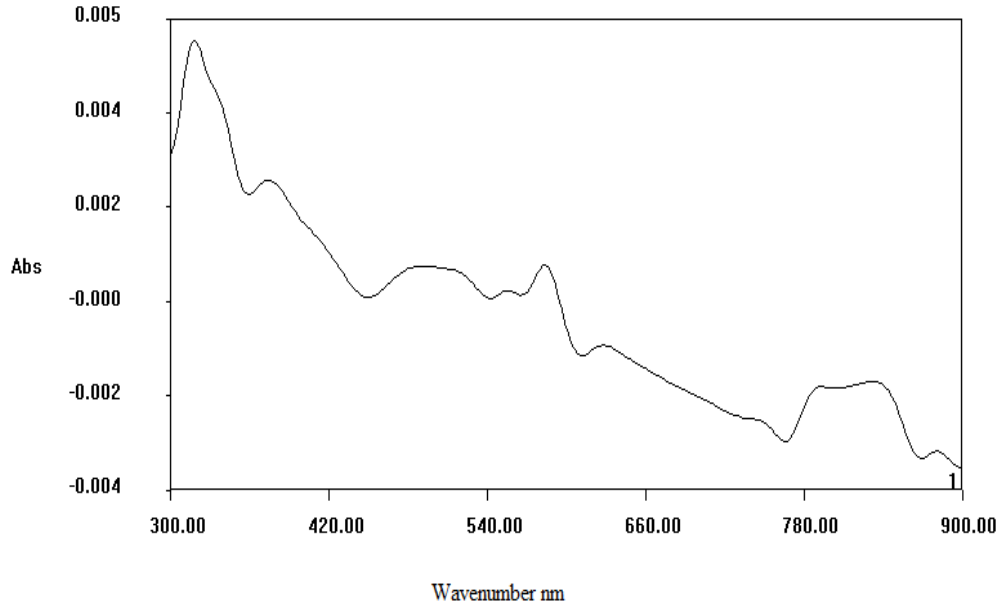

Fig 3:UV-Vis spectra of POT

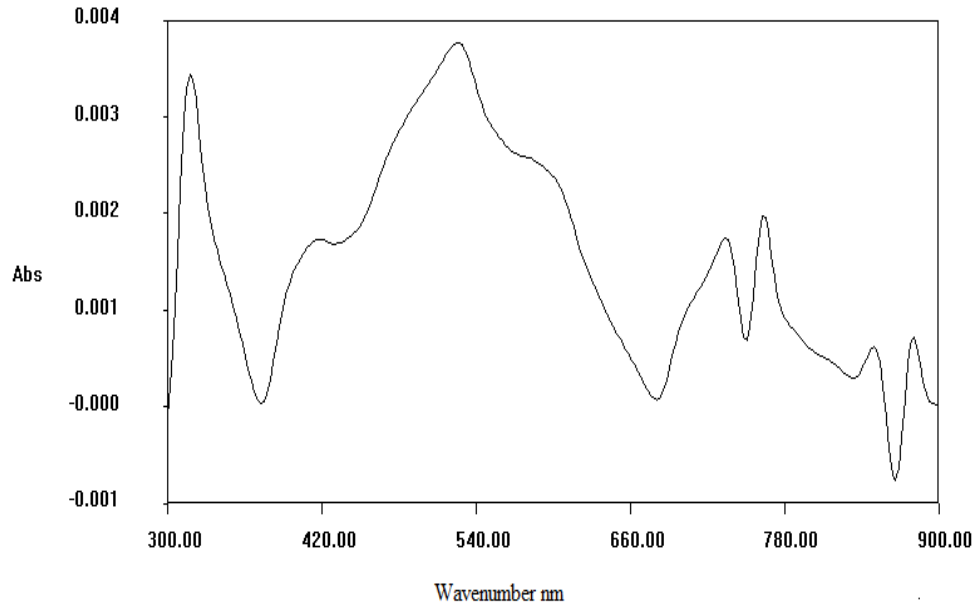

Fig 4: UV-Vis spectra of POT S

The UV-Vis spectra of POT and POTS were recorded in DMSO and are shown in Figs 3 and 4. In the polymer prepared in the presence and absence of ultrasonic irradiation, the first absorption band appears in the region of 312-376.8nm is assigned to the π-π^* transition of the benzenoid ring. It is related to the extent of conjugation between the phenyl rings along the polymeric chain. The absorption band at 582nm and 632nm for POT and at 528nm and 600nm corresponds to n-π* transitions and insulating pernigraniline phase of the polymer. The peaks at 806nm for POT and 844nm correspond to conducting emeraldine phase of the polymer.

It can be observed that absorption for the polymer prepared in the presence of ultrasonic irradiation is higher at longer wavelength than that for the polymer prepared in its absence which can be attributed to dissimilar electrical properties and redox behavior [25]. The other broad band appearing beyond 800nm is attributed to the bipolaronic transitions arising from the two step oxidation of reduced POT and POTS [32].

The Thermo gravimetric analysis of both polymers POT and POTS are shown in figures 5 and 6. It is generally known that three weight loss steps are observed in the TGA measurements for Polyaniline and their derivatives. The thermo gravimetric analysis exhibits a three step loss in the range of 72^{o}C-840^{o}C for POT. The first weight loss (13.43wt. %) starts from room temperature to 300^{o}C corresponds to the loss of water molecules/moisture and dopant HCl present in the polymer matrix. The second weight loss from 300 to 600^{o}C is associated decomposition of the oligomers from the polymeric matrix. The weight loss after 600^{o}C is due to the complete degradation and decomposition of the polymer backbone [25]. The four step weight loss was resulted for POTS similar to POT associated to the loss of water, dopant, decomposition of oligomers and the polymer.

At the end of the analysis 25.81% of the POT sample remained as residue and for POT S it was 27.27% .From the comparison of the remaining residue it can be confirmed that POT S is thermally more stable than POT. In DTA a weak exotherm around 220^{o}C was found for Poly-o-toluidine and strong exotherm at 420^{o}C and for POTS a weak exothermic peak around 140^{o}C and strong exotherm at 410^{o}C and it can be concluded that polymer decomposition takes place exothermally.

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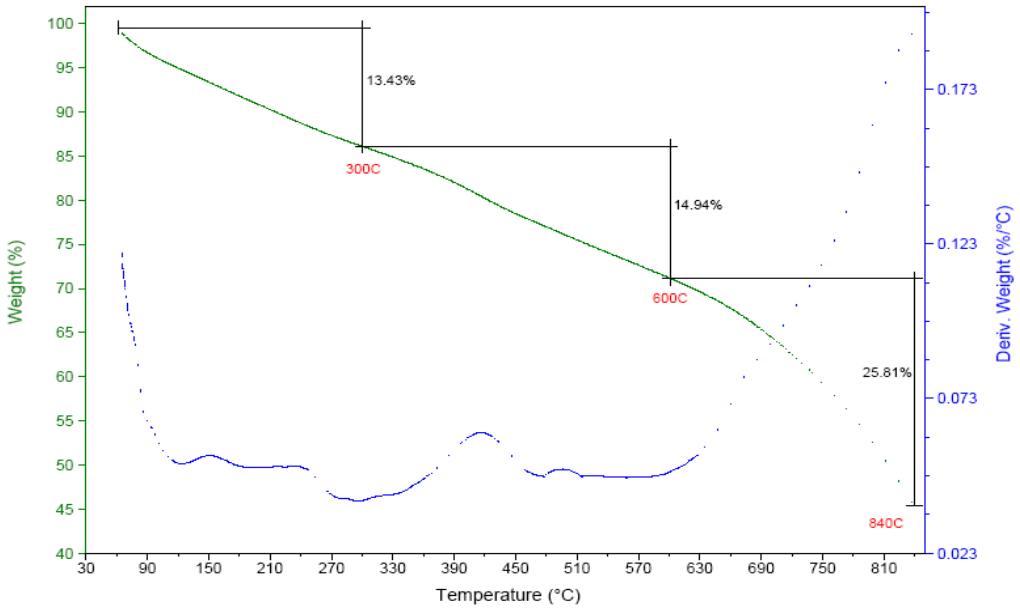


Fig 5: Thermal Analysis of POT

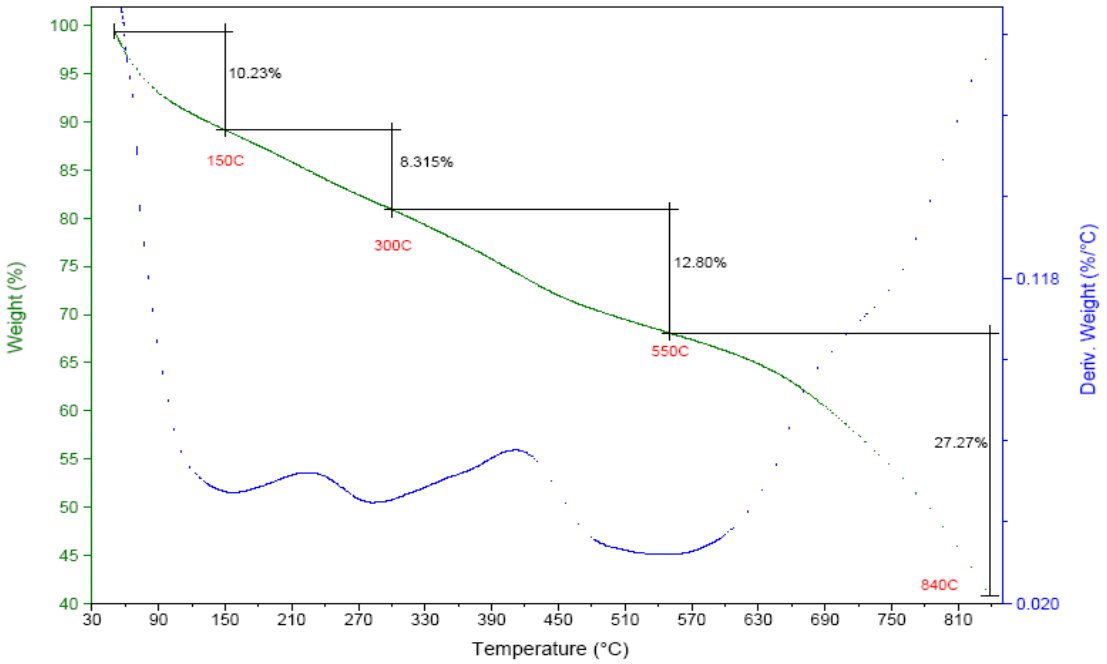


Fig 6: Thermal Analysis of POTS

Electrical Conductivity

The electrical conductivity of POT and POTS were found to be 2 $X10^{-2}$ S/cm and 2.087 X 10^{-2} S/cm respectively at 40^0C. Electrical conductivity of POTS was found to be slightly higher than POT .The electrical conductivity was calculated using the formula [26].

$$\sigma_{dc} = \frac{d}{RA}$$

Where d is inter electrode distance, R is resistance and A is cross sectional area

Biological Applications:
Biological applications like antibacterial activities against gram positive and gram negative bacteria and cytotoxicity using MTT assay were studied.

The antibacterial activities of the polymers prepared in the presence and absence of ultrasonic irradiation were investigated against *S.aureus* (gram positive) and *E.Coli* (gram Negative) and their zone of inhibition are given in table 1 at the concentration of 50, 75 and 100µg/mL.[27,28,29]

Table1: Antibacterial Activity of POT and POTS

Concentration (µg/mL)	*S.auereus* gram (+ve) mm		*E.coli* gram(–ve) mm	
	POT	POT-S	POT	POT-S
100	20	20	7	8
75	15	15	6	7
50	12	13	6	6

Depending on the measured values with the zone of inhibition including the well in millimeter, the antibacterial activity can be classified into the following types: > 12mm zone of inhibition high sensitive, 9-12mm zone of inhibition – moderate sensitive, 6-9mm zone of inhibition – less sensitive and < 6mm zone of inhibition – bacterial resistant. It was found that zone of inhibition was highest for polymer prepared in presence of ultrasonic irradiation than its absence. The zone of inhibition increased as concentration of polymer increased and inhibition was highest for *S.aureus* (20mm).

Cytotoxicity was determined using MTT assay[30]. In this experiments the cytotoxicity of the polymers were assessed by inhibition of proliferation of $HepG_2$ cells. It determines the inhibitory effect of the crude extracts on cell growth using MTT assay. Different concentrations of polymer were used for this study. The mean of cell viability values was compared with a positive control cyclophosphamide whose concentration is 90µg/mL. Untreated cells were used as positive and negative controls respectively. The minimum concentration of sample that was toxic to liver cancer cells was recorded as the effective drug concentration when compared to positive control (PC-cyclophosphamide). Ec_{50} was derived at a concentration lesser than 100µg/mL of the polymer. Toxicity increased as the time and concentration increased.

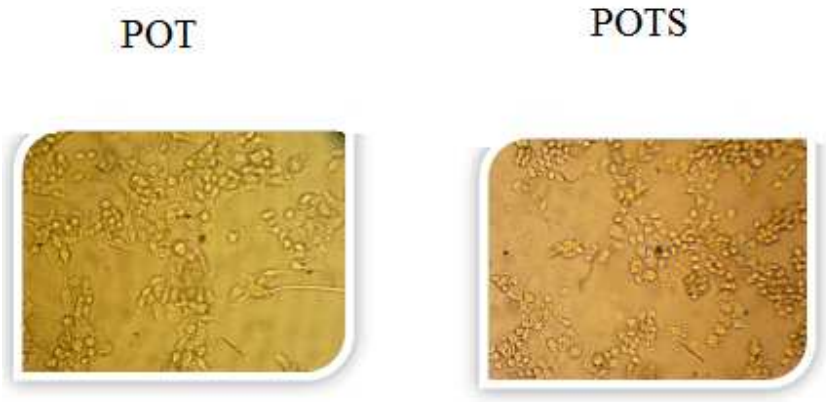


Fig 7: Cytotoxicity of POT and POT S (24 hours)

At 24 hours the EC_{50} values of the POT is 240µg/mL whereas for the polymer in presence of ultrasonic radiation it is lesser than 125µg/mL. Hence it is evident that cytotoxic activity was exhibited by the POTS at lower concentration than in their absence. (Fig7). This could be attributed due to increased oxidation at the polymeric surface and enhanced decomposition of oxidant [31].

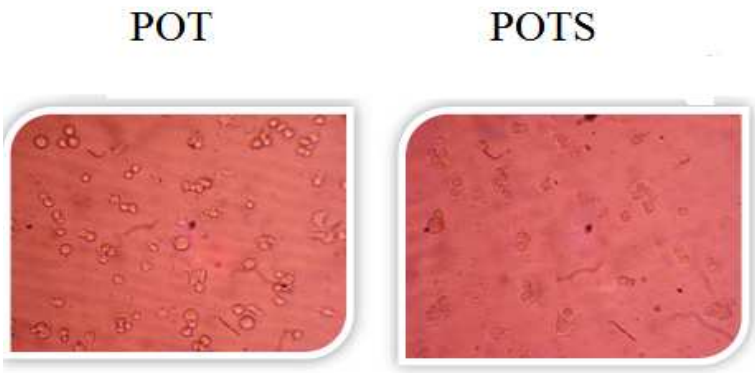
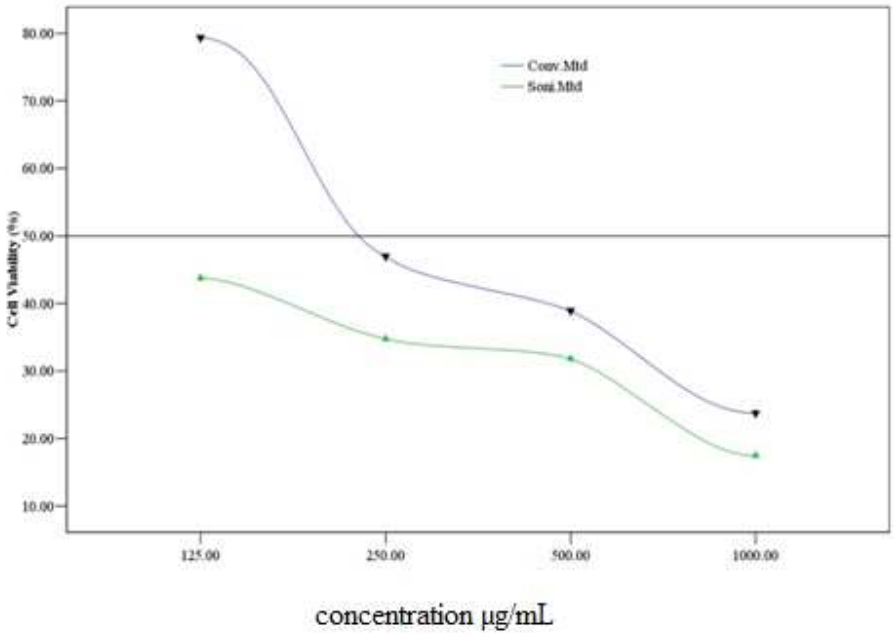


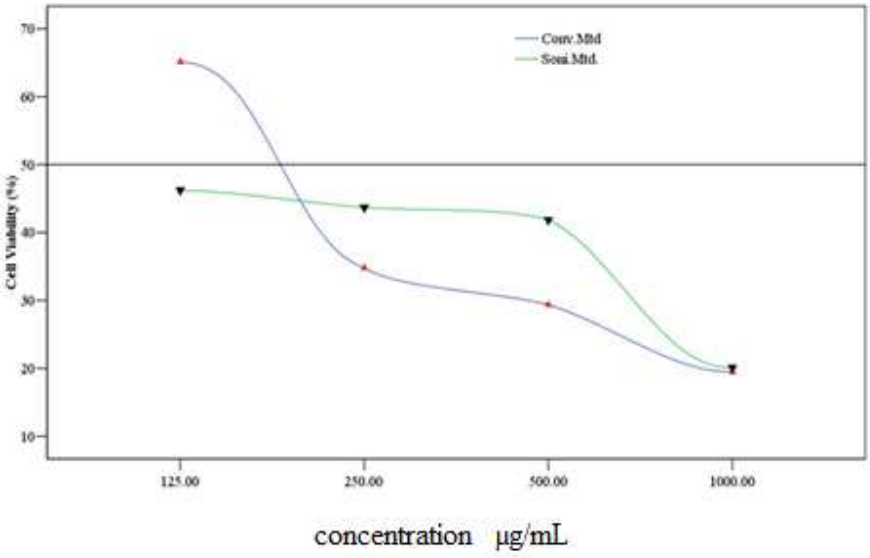
Fig: 8 Cytotoxicity of POT and POT S (48 hours)

At 48 hours toxicity was almost equal to 80% for polymer in absence and in the presence of ultrasonic radiation at 1000µg/mL. (fig 8)



Cytotoxic activity POT &POT S (24 Hrs)

The Ec_{50} values for POTS is <125µg/mL and for POT 240 µg/mL at 24 hours.



Cytotoxic activity POT &POT S (48 Hrs)

The Ec_{50} values for POTS are 125µg/mL and for POT 220µg/mL at 48 hours.

But, at lower concentration polymer prepared in presence of ultrasonic radiation was found to have good toxicity against liver cancer cells.

CONCLUSION

The conducting polymer poly ortho toludine was prepared by chemical oxidation method in the presence and in the absence of ultrasonic irradiation and they were characterized using UV, IR, TGA and DTA. The Electrical conductivity was measured and POTS showed a slightly higher conductivity than POT. The biological applications like antibacterial and cytotoxic activities were carried out. The results confirm that they can be effective against *S. aureus* and *E.Coli* and can act as good bactericidal agents and they can be also used against liver cancer cells. The results show that the polymer prepared in the presence of ultrasonic irradiation is more efficient than its absence. So the polymers prepared in the presence of ultrasonic irradiation can pave way for their application in various fields. Although the present investigations reveal that poly ortho toludine have excellent antibacterial and cytotoxicity activities, further in vivo studies for their compatibility in biological media are very essential before exploiting the fabulous scope of these synthetic polymers in biomedical applications. Further research could pave way for the development of novel drugs to control diseases and infections.

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