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Surface charge based rapid method for detection of microbial contamination in drinking water and food products

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Abstract**—**Microbial contamination, most of which are fecal born in drinking water and food industry is a serious threat to humans. *Escherichia coli* is one of the most common and prevalent among them. We have developed a sensor for rapid and an early detection of contaminants, taking *E.coli* as a threat indicator organism. The sensor is based on co-polymerizations of aniline and formaldehyde in form of thin film over glass surface using the vacuum deposition technique. The particular doping combination of thin film with Fe-Al and Fe-Cu in different concentrations changes its non conducting properties to p- type semi conductor. This property is exploited to detect the different contaminants, believed to have the different surface charge. It was found through experiments that different microbes at same OD (0.600 at 600 nm) have different conductivity in solution. Also the doping concentration is found to be specific for attracting microbes on the basis of surface charge. This is a simple, cost effective and quick detection method which not only decreases the measurement time but also gives early warnings for highly contaminated samples.

Keywords**—** Sensor, Vacuum deposition technique, thin film, E.coli detection, doping concentration**.**

I. INTRODUCTION

IN order to ensure a proper healthy lifestyle, one needs to be protected from various diseases, many of which spread through contaminated water or food. Each year millions of people are infected and thousands of them die due to water and food borne diseases. In India, more than 70% of the epidemic cases reported were either water-borne or water related [1]. In 2005 alone, 1.8 million people around the world died from diarrhoeal diseases. A great proportion of these cases can be attributed to the contamination of food and drinking water[2]. A study published in 2005 by the Centers for Disease Control and Prevention, Atlanta, estimated that E.coli O157:H7 accounts for more than 73,000 cases of food borne illness each year in the United States itself [3].

Besides the conventional fecal pathogens which are transmitted by water, several other water-borne pathogens have dangerously evolved especially in the last decade. These include Vibrio cholerae O139, Cryptosporidium parvum, shiga toxin producing E. coli especially enterohaemorrhagic E coli (EHEC), Yersinia enterocolitica, Campylobacter jejuni, Calciviruses and Microsporidia [4]. These pathogens are responsible for causing diseases like diarrhea, cramps, nausea, headaches, or other symptoms and can pose a serious health risk for infants, young children, and people with severely compromised immune systems [5]. In this regard, E.coli is widely used as an indicator organism for such microbes [6].

Various standard methods and commercial kits are available for the detection of microbes [7], especially for E.coli [8]. Though most of the kits and conventional methods like colony counting, membrane filter techniques etc., are reliable methods for the detection of even least amount of bacteria, but the time span for microbial detection is found to vary of the order of several hours or even days [9]. In order to overcome this enormous delay, rapid methods involving the use of sensors and biosensors are preferred for the same purpose of detection [10]. Of the various sensors used for these methods, many are developed on the basis of different transducing elements e.g., optical detection biosensors [11][12], Potentiometric bio-sensors [13], Amperometric [14][15], Piezoelectric [16]. etc. Moreover, according to the type of recognition unit, various enzyme sensors [17], immunosensors [18], microbial sensors, genosensors [19], proteomic sensors [20] and RNA bio-sensor[21] have also been reported for detection of E.coli. These all are rapid but they are either too expensive or are much sensitive and require special storage facilities.

Conducting polymers are the new and fast emerging materials with a growing scientific and technological interest. Although the idea of using polymers for their electrically conducting properties dates back to the 1960's [22], the field really started with the discovery by Shirakawa and his coworkers [23]. The properties and characteristics of intrinsically conducting polymers make them suitable materials for developing the sensors [Misra, Suri, Chandra, Kumar & Bhattacharya, 2000]. Apart from inorganic, there has been a considerable interest in exploiting organic substances for polymer preparations. Among those attracting and considerable interest are polyacetylene [24], polythiophene [25], polypyrrole [26] and polyaniline (PANI) [27].

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The vacuum deposited polymer thin films of aniline and formaldehyde have also been found to exhibit excellent micro detection properties [28]. These microbial detectors are inexpensive, and are operated at room temperature. Thus have the advantage of remote sensing and monitoring at hazardous places. Our effort is concentrated towards the development of a similar semi-conducting vacuum deposited polymeric thin film on glass surface which can be used as a sensor for detection of pathogenic microorganism such as E.coli in water.

The sensing element is a thin film of aniline-formaldehyde polymer doped with certain metals, Fe-Al or Fe-Cu. Doping makes the sensor suitable for attracting microbes to the surface. Later on, gases produced by these microbes change the conductivity at the polymeric surface which can be recorded and treated as an indication of microbial presence.

II MATERIALS AND METHODS

A. *Reagents*

Aniline, Formaldehyde, Sodium hydroxide, Aluminum chloride, Ferric chloride used was of analytical grade and were obtained from E. Merck (I) Ltd., Mumbai, India. All other chemical and media used for microbial culture were purchased from Hi-Media Laboratories, India.

B. *Organisms and their cultivation*

The bacteria *E. coli*, *Lactobacillus sporlac* , *Bacillus subtilis* and *Lactobacillus casie* (Collected from IMTECH, Chandigarh, India) were grown in bacterial nutrient media. The cells were grown in 250 ml Erlenmeyer flasks containing 50 ml of the above sterile medium at 30°C for 24 hr on an orbital shaker (n=250 m^{-1}) cells were harvested at late exponential phase, by centrifugation (at 10,000 g for 15 min) washed with distilled water. Cells were suspended in phosphate buffer at pH-7.0 in order to achieve optical density 0.604 at 600 nm. Cell suspensions were used to measure the conductivity using conductivity meter of Elico Limited, India. All measurements were performed in duplicate.

C. *Preparation of polymeric film*

The process for the formation of aniline formaldehyde condensate (polymer) was based on the reaction of acidified aniline and formaldehyde. It was performed as described by Misra et al . A copolymer of aniline and formaldehyde was prepared by dissolving 0.5gm of aniline in 12.5 ml of 10M hydrochloric acid, diluted with 11 ml distilled water. 12 ml of 25% formaldehyde solution was added to this solution along with simultaneous addition of doping solution. The doping solution of Fe-Al and Fe-Cu was separately prepared in a predetermined quantity.

The resultant mixture was stirred for 60 min and poured into 200 ml of 5–10% NaOH solution. The precipitate obtained was filtered, washed and dried. The polymer was then converted into thin pellets each having a diameter of 10 mm and a thickness of 1 mm using Polylab Molding Machine. These powdered and pellets form were then used for characterization of polymer and also for the preparation of thin polymeric films by evaporation and vacuum deposition on glass surface under a vacuum of 10^{-6} mmHg. The deposition was done using vacuum deposited Unit, Hind vacuum India Pvt. Ltd. Before deposition, the glass surface was cleaned using Hielscher's UP50H sonicator.

D. *Polymer characterization*

The FTIR analysis of the polymer formed was performed to check the composition of the polymer with and with out doping. Solid samples were milled with potassium bromide (KBr) to form a very fine powder. This powder was compressed into a thin pellet which was then analyzed for FTIR. The melting point of doped and undoped powdered polymer was found out using Toshniwal Melting Point Apparatus. The conductivity of various polymers were done in pellet form using Keithley 617 Programmable Electrometer.

E. *Fabrication of sensing element*

After the polymer had been deposited on the glass surface by vacuum deposition technique, silver electrodes were prepared over the polymeric film using the same vacuum deposition technique. Later ohmic contacts were made on the surface using silver paste and copper wire. An original picture of sensing device was shown in Fig 1.

F. *Testing of various microbial samples*

To detect *E.coli* and other microorganisms, experiments were performed in a measuring cell made of glass. The test samples were taken from the stock cultures and were maintained at the same optical density of 0.604 at 600 nm in phosphate buffer. The I-V characteristics of the polymeric film were performed using different concentrations of *E.coli* cells and other microorganisms.

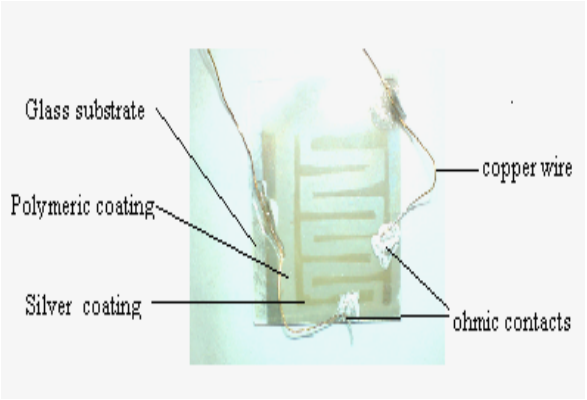

Fig 1. Sensing chip made by depositing thin layer of aniline-formaldehyde polymer on thin glass surface on which Silver electrodes were deposited in a comb shaped manner. Both deposition were done by vacuum deposition techniques. Ohmic contacts to the electrodes were provided using copper wire and silver paste

III. RESULTS AND DISCUSSION

The conductivity of different microbial suspensions was measured for all the different experimental strains. It was observed that different bacterial strains have different conductivities (Fig 2). These differences in the conductivities are mainly due to the fact that each strain have different surface charge due to different surface composition of cell membrane. A thermal property of the doped and undoped polymer was found out using Toshniwal melting point apparatus. The results showed that the boiling points were different for doped and undoped polymer. Boiling point for the undoped was found to be 142°C and for doped it varied from 149°C to 199°C (Fig 3). Doping of metal ions seems to affect the melting point of polymer.

Two probe measuring technique was used for conductivity measurements of various polymers doped with different concentration of dopants. For better ohmic contact silver paste was applied on the surface of the films. This increases the sensitivity and generates a specified path for electron transfer. Doping resulted in a clear increase of conductivity from non conducting range for undoped (5×10^{-8} S/cm) to semi conducting region (8×10^{-3} S/cm) for doped polymers.

Moreover, FTIR analysis showed the shifts of peaks with the nanocomposite copolymer when doped with different dopants.

This observation indicates that doping the copolymer has a remarkable effect on optical properties and hence on electronic properties of the copolymer which is directly related to charge carriers and the polymer characteristics. FTIR studies of the polymer formed was done using Varion 3100 FTIR instrument. The various major peaks for doped and undoped were within the range (Fig 4,5) implying that all the functional groups were well preserved during the doping process. Peak height and area was reduced in doped case that shows low concentration of organic compound and less transmittance or more absorbance by the doped polymer. A common observed property was shifting of peaks on doping which increase with concentration.

The sensor was tested for *E.coli*, *Lactobacillus*, *Bacillus subtilis*, air and water for the I-V characteristics. In case of air the output current was in the range of Pico ampere but in aqueous environment appreciable change was observed in the form of 100 times increase in the output which was almost similar for *Lactobacillus* and *Bacillus sp*. Furthermore, there was intense increase in the response for the *E.coli* solution. The voltage was applied both in forward bias and reverse bias and both results were found to be satisfactory (Fig 6). These results were quite similar to those obtained by Dixit et al . Thus using silver instead of gold does not have much effect on the net output.

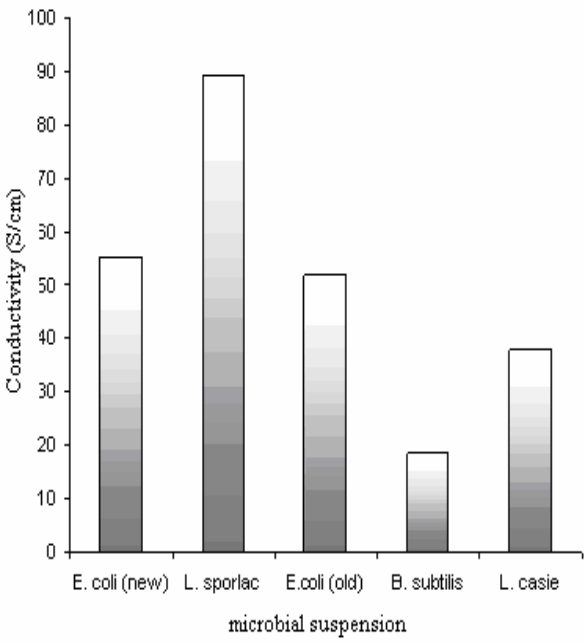

Fig. 2. Effect of conductivity of different microbial suspensions at 600nm. The surface charge of microbes may be different due to different polar ions in the cell membrane which resulted in different conductivity. The OD for all the suspensions were 0.604.

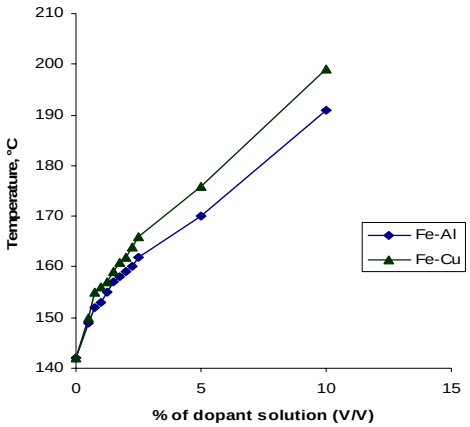

Fig 3. Effect of different dopants and doping concentration on melting point of aniline –formaldehyde polymer. Higher melting point is due to the presence of metal ions in the polymer

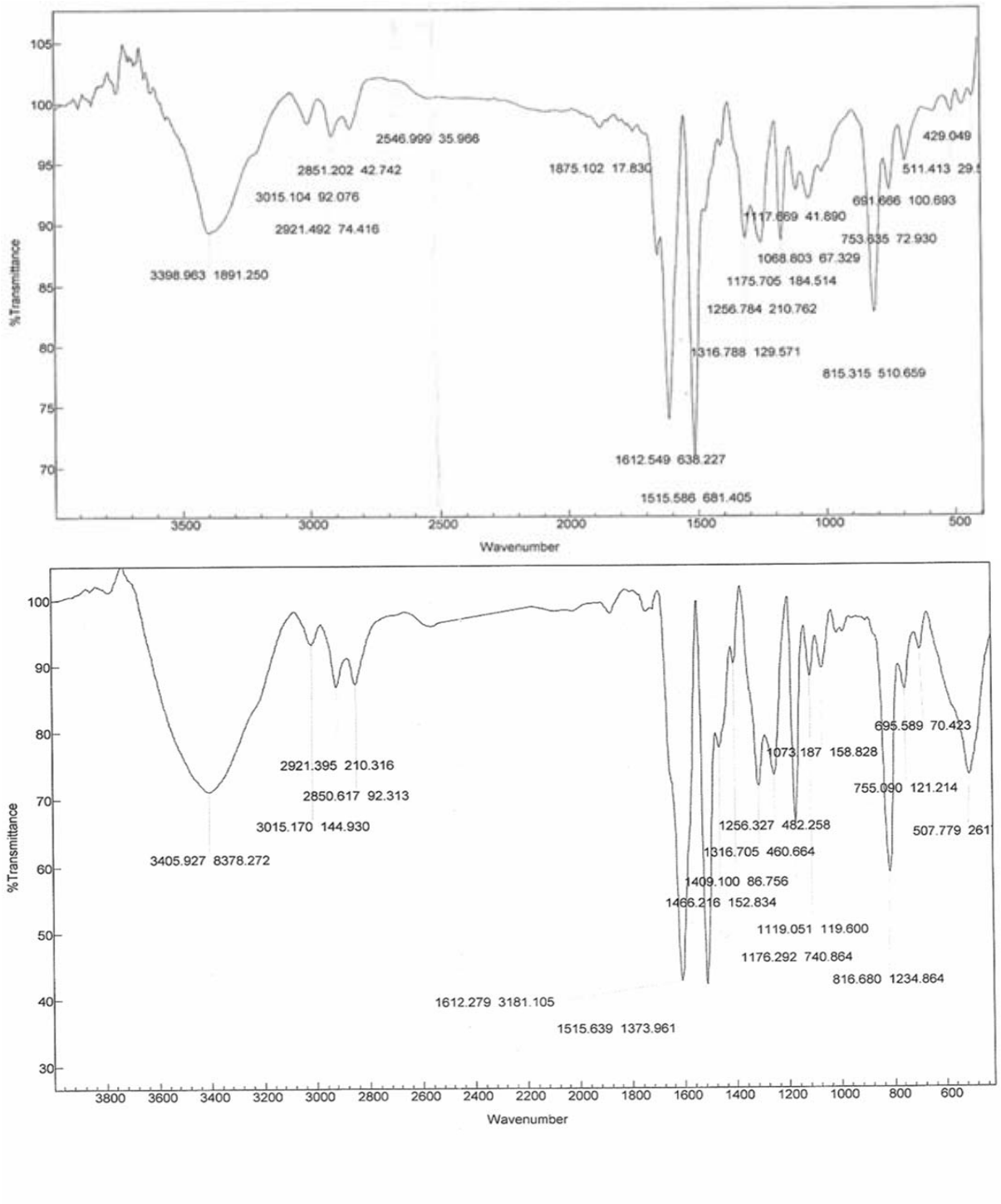

Fig. 4 FTIR spectroscopy studies for doped (top) and undoped (bottom) aniline formaldehyde co-polymer

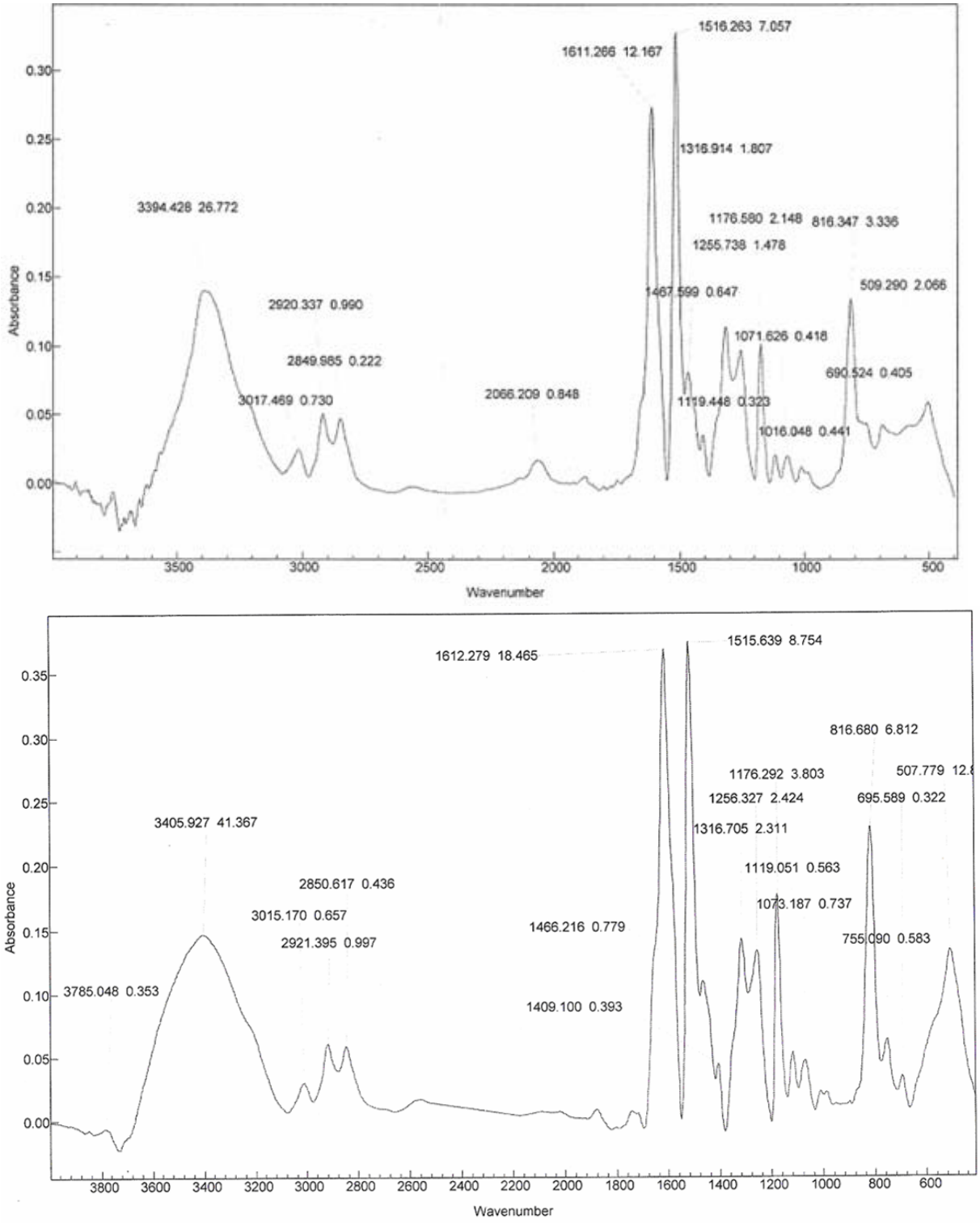

Fig. 5 FTIR spectroscopy studies for different doped concentration (top .5% , bottom 1.5 %). All the characteristics peaks are present and the common observed property is shifting of peaks for different doping concentration.

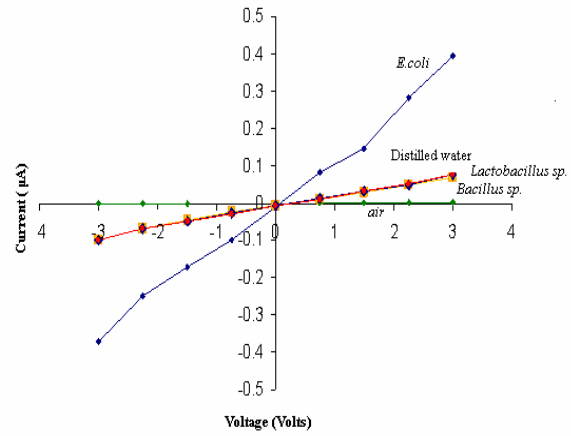

Fig. 6. V-I characteristic results in both reversed and forward bias for different microbial concentration (all having same concentration of 100 ppm), air and distilled water, using doped polymeric thin film based sensor

The original polymer was almost non-conducting in nature but due to doping of metal ions it started acting as a p-type semiconductor; conduction in thin film is because of transport of polarons and bipolarons. Moreover, the conduction in thin film occurs by crossing-over of the charge carriers through the inter crystallites boundaries, which offer a charge barrier [29][30] and when the thin film comes in contact with the gases produced by microbes, they result in a reduction of the barrier height at the inter-crystallite grain boundary, thus lowering the inter-crystallite barrier .This results in an increase in current flow through the sensor, which can be recorded as an output. In our experiments change was seen only in the case of *E.coli* thus it can be said that the doping concentration we used was attracting only *E.coli* and may attract the microbes with similar surface charge. Thus it may be possible that some other doping concentration can be used for other microbes. Furthermore, the output current is directly proportion to the amount of gas absorbed at the surface which in turn is proportional to the microbial concentration in the sample. To check this property different microbial concentration were taken at 3.0 V. The respective results were shown in the (Fig.7).

It was found that the sensor starts giving stable response after 10 seconds of its immersion in the sample. Thus the response time was quiet low making it suitable for using in rapid detection. Moreover, the sensor does not require specific storage precautions as in case of enzymatic or the biosensors. Thus the developed sensors are robust, cheap and give an rapid indication in advance of the presence of fatal microbes in the sample.

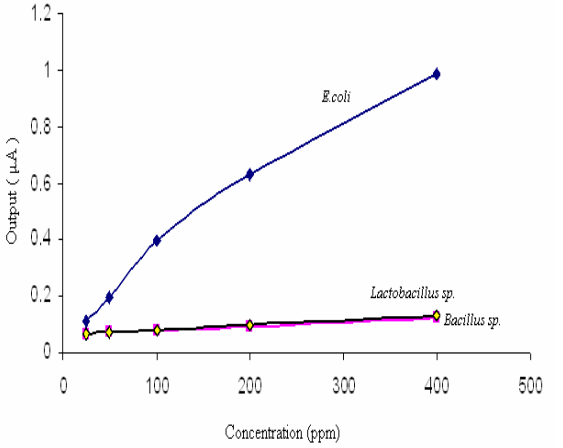

Fig. 7. V-I characteristic results for different microbial suspension at different concentrations. E.*coli* showed a significant response in comparison to other species

IV CONCLUSION

The non conducting polymer was converted to a conducting one through the process of doping. This makes it specific to attract different microbes. It also increases its thermal stability. However no structural change was seen in the chemical structure as all the groups were well preserved. The output was found to depend on the following four factors- microbial strain, microbial concentration, type of dopant and dopant's concentration. It was not affected by the type of material used for electrode deposition. However it was also found that silver electrodes starts dissociating if the sample contains salts. Thus to improve the device, gold should be incorporated as the electrode material, as it has high conductivity and is highly resistant to corrosion. . A more deep research is required to test the sensor with more numbers of different strains and different dopants.

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