Synthesis, characterisation, ion chelation exchange capability and antibacterial properties of the *o-cresol-formaldehyde-catechol*-based copolymer

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Abstract – An ion exchange resin chelator was synthesized from o-cresol, catechol and formaldehyde through the condensation process in the molar ratio of 1:3:5 in the presence of 1 M hydrochloric acid as a catalyst. The resulting copolymer was characterized by IR spectral data. The cation exchange capacity was measured; the effect of metal ion concentration, pH effect, and the ratio of the cation exchange reaction were studied. The chelation ion-exchange properties were also studied with the batch equilibrium method. The formed copolymer chelation ion-exchange properties of the copolymer were analyzed for Cd^{2+}, Cr^{2+}, Fe^{2+} and Mg^{2+} ions. The resin was proved selective chelating ion- exchange copolymer for the above metals. The study was carried out over a wide pH range and in media of various ionic concentrations for the metals. The antibacterial properties of the synthesized resin were also investigated, using the well diffusion method for the gram-positive bacteria, *Staphylococcus aureus* and the gram-negative bacteria *E coli*. The results showed a significant inhibiting effect against the tested bacteria, against the formed resin copolymer.

Key words: copolymer resin, ion exchange capacity, chelation, antibacterial properties, purified resin, activated resin, inhibition zone

1. Introduction

In recent years, metal ions have a great responsible for the water pollution by anthropogenic activities. In addition, the heavy metal ions cause serious threat to environment and they affect the human health directly and by bioaccumulation. Considering their high toxicity, cadmium, arsenic, chromium, lead and mercury rank among the priority metals that are threat to human health and environment (Woloiece, 2019).

Heavy metal contamination is one of the most important threats to the human health and the environment (Woloiece, 2019). Before releasing the heavy metal ions into the environment, controlling and treating is necessary. Several treatment methods have been developed recently to avoid the greater risk of heavy metals such as are chemical precipitation, ion exchange adsorption, membrane filtration etc. (Karunakaran, 2013). There has been growing concern about the synthesis of polymer resins because of their special potential of ion exchanging capacity. Synthetic resins usually used for water treatment, but also can be used for various applications including antibacterial activity.

Resins are natural or synthetic organic compounds without consisting of crystalline or liquid viscous substance. Synthetic resins are polymers that prepared artificially by one or few monomers by copolymerization. Most of phenolic formaldehyde resins (copolymers) have been used in ion exchange applications. Ion exchange is a popular approach of synthetic resins because of their applicability to both pre-attention and separation (Arasaretnam, 2020). The uptake behavior (rate of the cation exchanging) of various metal cations such as Cd2+, Fe3+, Mg2+ and Cr3+ toward the synthetic resin studied here related with the concentration of metal cation and pH value of the sample (Arasaretnam, 2020 and Yang, 2019).

Another application of synthesized formaldehyde resin was also used to determine the antibacterial properties. Bacteria such as *Escherichia coli* and *Staphylococcus aureus* at room temperature, by measuring diameter of the inhibition area by using well diffusion method. All the types of Bacteria need water or moisture for the growth and reproduction and hence water sources can contaminate with several types of bacteria. Therefore, water systems use many water treatment methods to provide safety for the water especially for the drinking water systems. As an important method, reins can be used for the water purification from bacterial contamination. Therefore, this study described in the processes of synthesis and characterization of synthetic resin together with the systematic studies of various properties of ion exchange resin.

2. METHODOLOGY

2.1 Synthesis of resin

Three resins were synthesis by changing the mole ratios of O-cresol, formaldehyde and catechol (1:3:5, 2:2:5, 3:1:5) in the presence of 2 M HCl and the reaction was heated at 140 ˚C for 2 hours. The mixture-containing flask was shaken periodically for the homogenizing mixing then the flask was poured into crushed ice and left over night. The resin was crushed into small particles and washed several times with distilled water. Then the crystal resins were purified with rinsing 3 times with cold water, hot water and chloroform. Resin dissolving in 10 % NaOH and filtered.1:1(v/v) concentric HCl and distilled water was used for the re-precipitating. The formed resin crystals were washed with cold water and hot water. Moreover, they dried 100 % in an oven. To convert the resin sample in its activated form, the sample equilibrates with a solution of 1 M HCl for 24 hours with shaking. Then the sample was washed with deionized water until they are free from Cl^- . Then sample was filtered and dried.

2.2 Evaluation of the rate of metal ion Uptake

Samples were accurately weighted (0.250 ± 0.001g) in the activated form of dry resin for the experiment. Each sample was taken into different stoppered glass bottles, equilibrated with desired pH values (3.6, 4.0, 4.4, 4.8, 5.2 and 5.6) with buffer solutions (50 ml), and kept for 24 hours. The buffer solution was decanted and resin samples equilibrated with metal ion solutions (Cd^{2+}, Fe^{3+}, Cr^{3+} and Mg^{2+}) with the same pH values and kept for 24 hours again. Then metal solutions were decanted and GBC AA dual determined the non-chelated metal ion atomic absorption spectrometer fitted with cadmium, iron, chromium and magnesium hollow cathode lamps were used to analyze the concentration of metal ions under study. -e wavelength was set at 228.8 nm resonance line, the spectral band pass at 0.5 nm, and the measurements were carried out in an air/acetylene flame at fixed time intervals (1 h, 2 h, 3 h, 4 h and 5 h) (Gharbi, 2014).

2.3 Evaluating the distribution of metal ions at different pH

To study the pH effect for the metal ion exchange capacity to the formed resin, resin samples were equilibrated with the desired pH values. pH values (3.6, 4.0, 4.4, 4.8, 5.2 and 5.6) have been prepared from 0.1 M acetic acid and 0.1 M sodium acetate. (0.250±0.001 g) dry resins were equilibrated for 24 hours with buffer solutions with different stoppered bottles. After 24 hours' buffer solution have been decanted 0.1 M metal ion solutions 25 ml was added and equilibrated with resin samples for 24 hours with shaking at room temperature. After 24 h metal solutions were decanted and metal ion concentration of the supernatant was measured by atomic absorption spectrometer to study the distribution ratio (D) of the each of metal ions, defined by the following relationship.

$$D = \frac{Weight(in\ mg)\ of\ the\ metal\ ion\ uptake\ by\ 1g\ of\ polymer}{Weight\ (in\ mg) of\ metal\ ion\ present\ in\ 1ml\ of\ solution}$$

2.4 Study of pH effect for the metal ion exchange capacity

To the formed resin, resin samples were equilibrated with the desired pH values (3.6, 4.0, 4.4, 4.8, 5.2 and 5.6). The pH values have been prepared from 0.1 M acetic acid and 0.1 M sodium acetate. (0.250±0.001 g) dry resins were equilibrated for 25 hours with buffer solutions with different stoppered bottles. After 24 h, buffer solution has been decanted. 0.1 M metal ion solutions 25 ml was added and equilibrated with resin samples for 24hours with shaking at room temperature. After 24 h metal solutions was decanted and metal ion concentration of the supernatant was measured by the atomic absorption spectrometer. (Gharbi, 2014)

The ion-exchange capacity was calculated by using the following equation:

$$Exchange\ capacity\ (mmolg^{-1}) = \frac{I - R}{M \times W}$$

Where, *I*, *R*, *M* and *W* respectively represent the Initial molarity of the metal ion, Remaining molarity of the metal ion, Atomic molar mass of the metal and the weight of the resin.

2.5 Evaluation of the effect of metal ion concentration exchange capacity

To study the metal ion concentration effect on uptake of different metal ions by formed resin, the resin has been equilibrated with buffer solution of desired pH for 24 hours and buffer solution was decanted. Accurately weighted (0.25±0.001 g) dry resin has been equilibrated with metal ion solutions (25 ml) with different concentrations at the same pH value at room temperature with intermitted agitation for 24 hours. After 24 hours, the metal ion solutions were decanted and non-chelated metal ion was estimated using Atomic absorption spectrometer (Gharbi, 2014).

2.6 Antibacterial activity Screening Methodology

The *in vitro* screening of antibacterial activity of the synthetic resins was carried out using the gram-negative bacteria, *Escherichia coli* and a gram-positive bacteria, *Staphylococcus aureus*. The cultures of these two bacteria were obtained from Faculty of Health-Care Sciences, Eastern University, Sri Lanka, Chenkalady. The Bacterial cultures were sub cultured in nutrient agar (NA) medium and incubated for 24 hours at 37 ˚C before using for the Antibacterial activity studies. The antibacterial activity of the synthetic resins was studied in NA medium, using well diffusion method. The concentration of 2000 ppm, 3000 ppm, and 4000 ppm solutions of the synthetic resins were used in triplicate form. As the reference Antibacterial agent, Amoxicillin (Amino penicillin) was used with same concentrations of synthetic resins (2000 ppm, 3000 ppm and 4000 ppm). The samples containing Nutrient Agar plates were incubated for 24 hours at 37 ˚C. The antibacterial activity was determined by measuring the zone of inhibition and the mean value of the diameter of the inhibition zone was recorded.

2.7 Statistical Analysis

The diameter of zone of inhibition was expressed using one way Anova (analysis of variance) by using software Minitab 17.

3. RESULTS AND DISCUSSION

3.1 Physical properties

The synthesized resin from o-cresol, formaldehyde and catechol with the presence of hydrochloric acid catalyst resin observed as a solid, hard crystal like appearance. Moreover, the appearance is black colour in nature.

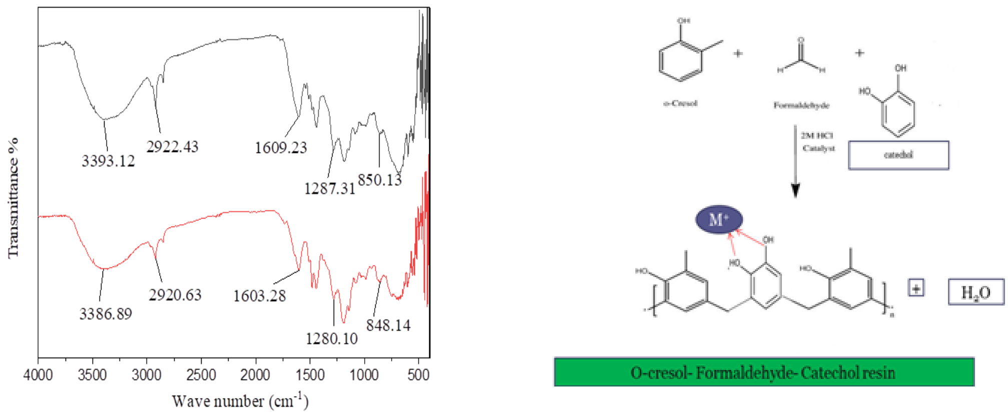

Figure 1: FTIR spectrum of synthesized resin and proposed structure.

3.2 Spectral characterization of resin

The FTIR spectrum of the purified resin (A) and activated resin (B) are shown in (Figure 1). In the purified resin, broad band in the region 3393.12 cm^{-1} is assigned to the stretching vibrations of phenolic hydroxyl groups (-OH) which exhibit intermolecular hydrogen bonding. A medium peak was observed at 2922.43 cm^{-1} due to stretching vibration of aliphatic (-CH) in the resin polymer. The band at 1609.23 cm^{-1} can be designed to (-C=C) aromatic ring stretching. A band at 1287.31 cm^{-1} is due to aromatic (-CO) stretching. A peak at 850.13 cm^{-1}, can be assigned to the presence of 1, 2, 3, 5 tetra substituted benzene ring in the formed resin. (Lazar, L, 2014). Activated resin all the peaks showed lower values than purified resin because of the activation of the resin.

3.3. Ion exchanging properties

The results of the equilibrium study carried out with the polymer resin presented from figures 1 and 2. From the study with the influence of experimental conditions, certain generalization may be made about the behavior of the polymer resin.

***Rate of metal ion uptake*:** The rate of the metal ion adsorption was determined to find out the shortest period of time for which equilibrium could be carried out while operating as close to equilibrium conditions as possible. Figure 1 shows the dependence of the rate of metal ion uptake on the nature of the metal. The rate refers to the change in the concentration of the metal ions in the aqueous solution, which is in contact with the given polymer. The results show that the time taken for the uptake of the different metal ions at desired pH conditions. It is found that Cr^{3+} ion required 5 hours for the establishment of equilibrium while Cd^{2+}, Fe^{2+} Mg^{2+} required more than 5 hours for the equilibrium. The rate of the metal ion uptake follows the order $Cr^{3+} > Mg^{2+} > Cd^{2+} > Fe^{3+}$ (Figure 2**).**

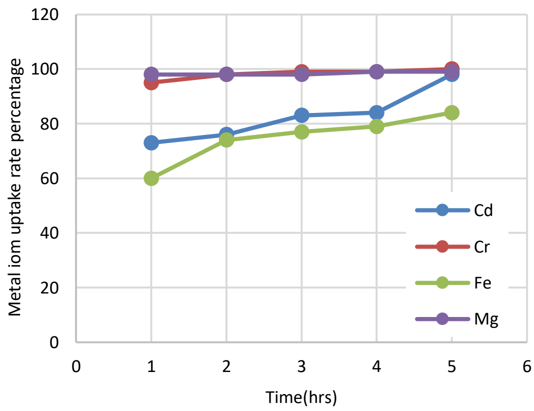

Figure 2: Comparison of the rates of metal ion uptake by synthesized resin at desired pH.

Distribution ratios of the metal ions at Different pH: The effect of pH on the amount of the metal ions distributed between two phases can be explained by the results shown in (Table 1 & Figure 3). The data on the distribution ratio as a function of pH indicates that the relative

Table 1: Distribution ratio (D) of different metal ions as a function of the pH

Metal ion	Percentage of metal ion uptake at different periods of time (h)				
	1h	2h	3h	4h	5h
Cd^{2+}	13.475	11.961	11.961	11.961	1.820
Cr^{3+}	5.348	1.823	1.187	0.651	0.00
Fe^{3+}	30.171	19.661	17.358	15.212	12.305
Mg^{2+}	0.363	0.357	0.356	0.196	0.165

amount of metal ions taken up by the polymer increases with increasing pH of the medium. The study was carried out up to a definite pH value for the particular metal ion to higher pH values. The increase in magnitude, however, is different for the different metal cations.

The resin takes up of Cd^{2+} ion is more selective than any other metal ions under study. The lowest distribution ratio of Cd^{2+} may be attributed to steric hindrance. Amongst the other metal ions, Cr^{3+}, Fe^{3+} and Mg^{2+} ions are taken up by the resin more selectively. The order of the four metal ions distribution ratio (D) is $Mg^{2+} > Cr^{3+} > Fe^{3+} > Cd^{2+}$ .Thus the results of such type of study are helpful to selecting the optimum pH for a selective uptake of a particular ions of metal from a mixture of different metal ions.

For example, using the resin as ion exchanger, at pH 4.8, 5.2 and 5.6 the distribution ratio D for Mg^{2+}, Cr^{3+} and Cd^{2+}, Fe^{3+} have huge difference. It will be useful for active separation of these ions from any mixture. As the pH of medium increase to 4.8 and above, the amount of adsorbed ions by the resin also increase. From the results of distribution ratio, it can be observed that the polymer shows highest affinity towards Cd^{2+} whereas least affinity for Mg^{2+}. Because of the considerable difference in the adsorption capacity at different pH, resin may be utilized for the separation of particular metal ion from any mixture.

Effect of pH on ion exchange capacity: Metal ion removal from an aqueous solution by sorption is strongly dependent on the pH value of the metal ion solution, which influences the surface charge of the sorbent. (Gharbi, 2014) Chelating ligands form complexes with various metal ions in specific pH values (Gharbi, 2014). Therefore, synthesized resin has been used to study the effect of the pH on metal ion exchange capacity. When varying the pH value of the metal ion chelating ability is different. The results of the exchange capacity depending on the pH for different metal ions are presented in (Figure 3). The results show that Cr^{3+} has the highest ion exchange capacity and the Cd^{2+} has the lowest ion exchange capacity. Fe^{3+} has higher ion exchange capacity than Mg^{2+}.

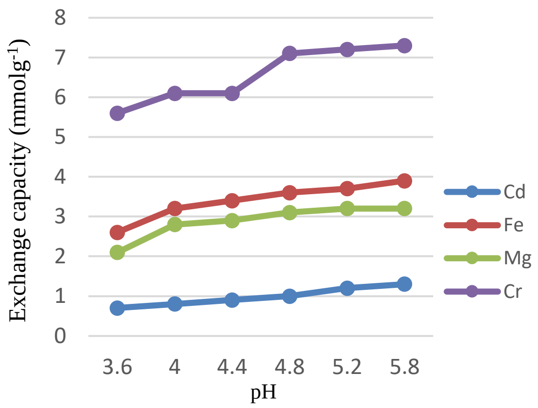

Figure 3: Effect of pH Ion exchange capacity

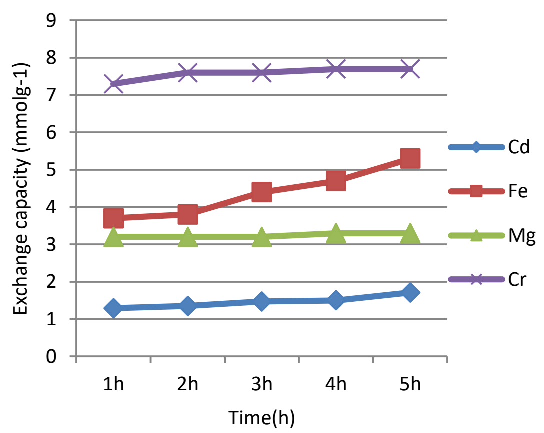

Figure 4: Metal ion exchange capacity on 5.2 and 5.6 pH.

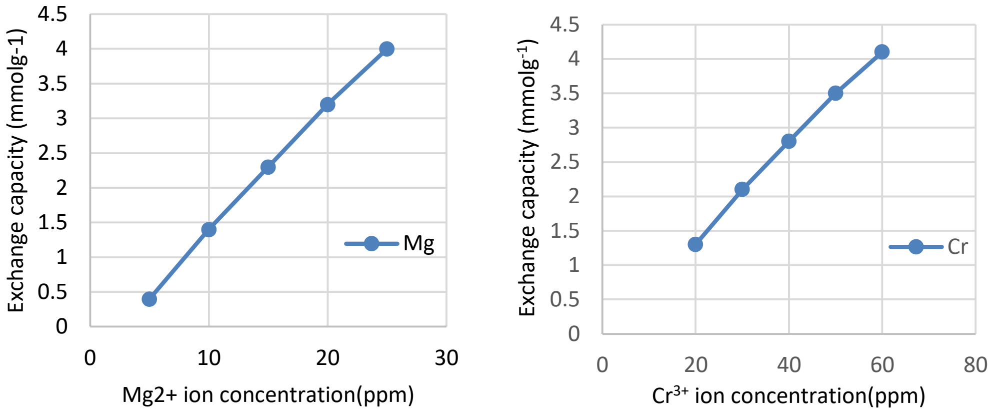

Figure 7: Effect of cation concentration on exchange capacity for Cr^{3+}

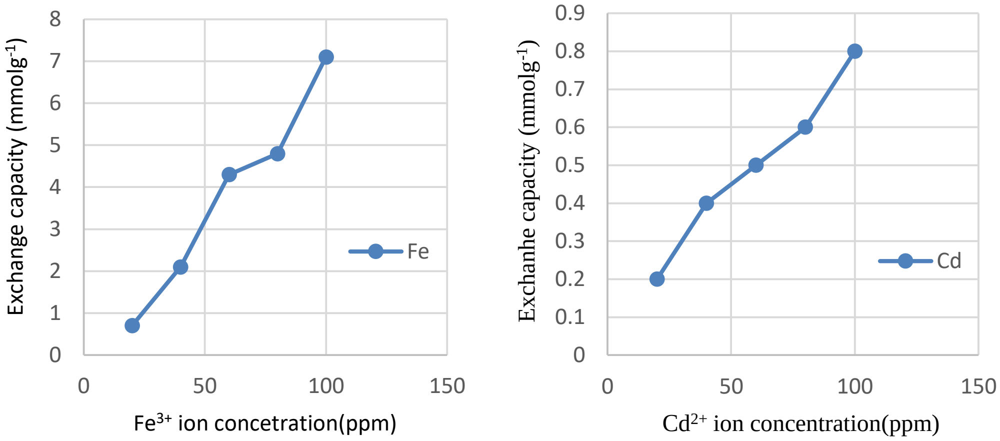

Figure 8: Effect of cation concentration on exchange capacity for Cd^{2+}

Metal ion exchange capacity in desired pH: Figure 4 shows that the metal ion exchange capacity in desired pH values for the different metal ions. The evaluated pH values are 3.6, 4.0, 4.4, 5.2 and 5.6. For the Cd^{2+}, Mg and Cr^{3+} metal ion, best-exchanged pH value is 5.6. In addition, Fe^{3+} shows high exchange capacity at pH 5.2. Within 5 hours their metal ion exchange capacities were evaluated. Cr^{3+} has the highest capacity among the other used metals. In addition, Cd^{2+} has the lowest ion exchange capacity. Fe^{3+} ion exchange capacity is higher than the Mg^{2+} ion exchange capacity.

Effect of metal ion concentration on exchange capacity: Examination data presented in (figures 5, 6, 7 and 8) shows that the amount of adsorbed metal ions increases with increasing concentration of metal ions in the solution. A low concentration of metal ions, the available number of metal ions in the solution is low relative to the arrangement sites remaining on the sorbent. (Gharbi, 2014) Fe^{3+} has the high ion exchange capacity. When increase the concentration of the metal ion exchange capacity also increasing. Cd^{2+} has low ion exchange capacity than the Fe^{3+}. Moreover, (figures 5, 6) shows the ion exchange capacities of the Mg^{2+} and Cr^{3+} ions. Mg^{2+} has high ion exchange capacity than Cr^{3+}. However, at higher concentration, the sorption available sites remain same as more metal ions are available for sorption and subsequently sorption becomes almost constant then after.

Table 2: Diameter of inhibition zone of purified resin at different concentrations

Concentrations (ppm)	Diameter (cm) of Inhibition Zone		
	2000 ppm	3000 ppm	4000 ppm
1	0.9	1.1	2.1
2	0.9	1.0	2.2
3	1.1	1.2	2.2
Mean value	0.9	1.1	2.2
Amoxicillin	3.4	3.7	4.0

Table 3: Diameter of inhibition zone of activated resin at different concentrations (H^+ form)

Concentration (ppm)	Diameter (cm) of Inhibition Zone		
	2000 ppm	3000 ppm	4000 ppm
1	0.9	1.2	2.2
2	1.1	1.1	2.3
3	1.1	1.3	2.4
Mean value	1.0	1.2	1.3
Amoxicillin	3.4	3.7	4.0

Table 4: Diameter of Inhibition zone of purified resins, against *S. aureus.*

Concentration (ppm)	Diameter (cm) of Inhibition Zone		
	2000 ppm	3000 ppm	4000 ppm
1	0.7	0.9	1.9
2	0.8	0.9	1.8
3	1.0	1.1	1.9
Mean value	0.8	0.9	0.9
Amoxicillin	3.2	3.7	4.1

Table 5: Diameter of Inhibition zone of activated (H^+ form) resin against *S. aureus.*

Concentration (ppm)	Diameter (cm) of Inhibition Zone		
	2000 ppm	3000 ppm	4000 ppm
1	0.8	0.9	1.1
2	0.9	0.9	1.0
3	0.8	1.0	1.1
Mean value	0.9	1.0	1.0
Amoxicillin	3.2	3.7	4.1

3.4. Antibacterial properties of resin

The antibacterial effect of the synthetic resin was evaluated by measuring the zone of the inhibitions (diameter) in the nutrient agar medium.

According to the statistical analysis from one-way ANOVA, the antibacterial activity of purified resin was significant against *E Coli* at all concentrations, tested. That is there is a significant ($p= 0.00$) effect of inhibition at 95 % confidential level among the diameter of inhibition zone of each samples of activated resin (Table 2).

According to the statistical analysis from one-way ANOVA test for antibacterial activity of activated resin was significant against *staphylococcus aureus* at all the concentrations, tested. There is a significant ($p= 0.00$) effect of inhibition at 95 % confidential level among the diameter of inhibition zone of each sample of activated resin (Tables 3-5).

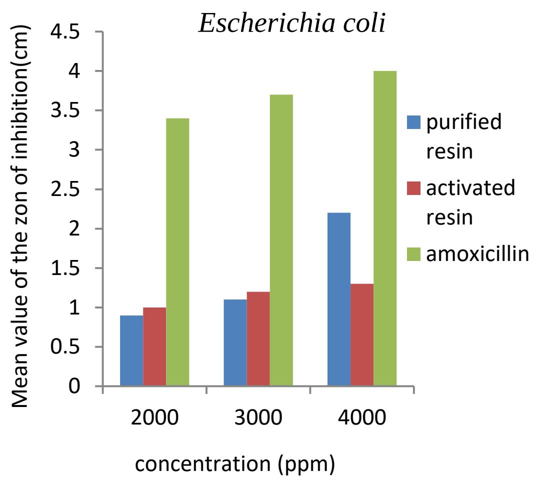

Figure 9: Antibacterial activity of purified resins, activated resins and Amoxicillin against *Escherichia coli*.

As per the results, the inhibition zone was increased with the increase of the concentration of resins. In addition, in the activated resin the antibacterial activity against *E coli* was greater than that of purified resin at three concentrations, tested except 4000ppm (Figure 9).

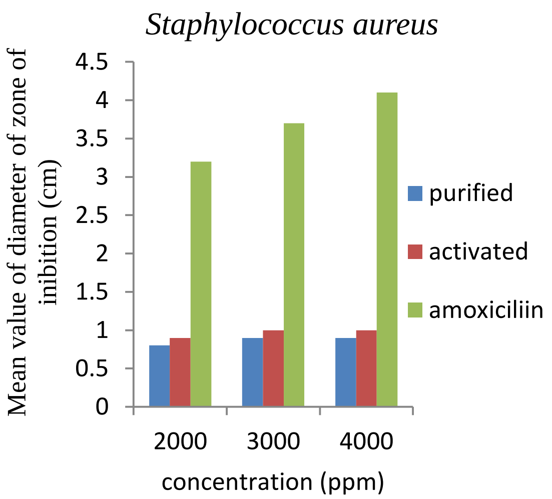

Figure 10: Antibacterial activity of purified resins, activated resins and Amoxicillin against *Staphylococcus aureus*.

The results also revealed that, for the activated resin, the antibacterial activity against *S. aureus* was greater than that of purified resin at all concentrations, tested (Figure 10).

According to the above results, the diameter of inhibition zones of standard Amoxicillin was higher than that of purified and activated resins, at all three concentrations (2000 ppm, 3000 ppm and 4000 ppm) (Li, R, 2021). Moreover, both the purified resin and activated resin showed greater inhibition activity against *E coli*, the gram-negative bacteria and it was higher than that of the gram-positive bacteria, *S. aureus*.

4. CONCLUSION

A novel polymer has been synthesized by condensing o-cresol, catechol and formaldehyde in the presence of hydrochloric acid catalyst. The polymer has a good crystal appearance with black color. Chelating properties of the copolymer with various metal cations are found to be pH dependent. For each metal ion, the optimum chelating property was observed in a particular pH. Therefore, the order of the chelation of various metal ions with the polymer is a subject of interest for employing the polymer to separate a metal ion from a solution. Though the polymer was able to absorb a host of metal ions, the study shows that it was also selective and preferential to a few metal ions like Cr^{3+}, Cd^{2+}, Mg^{2+} and Fe^{3+}. Most preferential pH for the Cd^{2+}, Mg^{2+} and Cr^{3+} is 5.6. In addition, for the Fe^{3+} is 5.2. For the metal ion, concentration effect of the metal ion exchange capacity of Fe^{3+} has the higher exchange capacity than the Cd^{2+} and Mg^{2+} has capacity than Cr^{3+}. When increase the metal ion concentration, the ion exchange capacity also increased. According to the results, purified resin and activated resin can inhibit the growth of bacteria. The diameter of the zone of inhibitions were higher in *Escherichia coli* in Nutrient agar than the *Staphylococcus aureus.* In addition, the diameter of inhibition zone was increased when the concentration of the resin samples was increased. Activated resin sample can inhibit the growth of *Escherichia coli* than purified resin sample. The synthetic resin can be used as an antibacterial agent and can inhibit the growth of *Escherichia coli* than *Staphylococcus aureus.*

5. REFERENCES

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