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Amination of chitosan and its application in wastewater treatment in Kitchener drain in Egypt.

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ABSTRACT

Chitosan is a non-toxic polyamino saccharide available in a variety of useful forms. The current work, the graft copolymerization of acrylonitrile (AN) onto chitosan (CS) was carried out using ceric ammonium nitrate (CAN) as an initiator. Chitosan derivatives were prepared by treatment with polyethylene imine (PEI) and 1,3-diamino propane (DAP) to produce copolymers [(CS-g-(PAN)/PEI)] and [(CS-g-(PAN)/DAP)] respectively. Thermo-gravimetric (TGA) and FT-IR spectroscopic analyses as well as electron scanning microscopy (SEM) and transmission electron microscopy (TEM) were used to characterize the obtained products. The products were tested toward their ability to uptake transition metal ions as Pb (II) and Cu (II) from their aqueous solutions. The uptake was determined by atomic adsorption. The modified chitosan copolymers showed higher capacity of adsorption than pure chitosan. [CS-g-(PAN)/PEI] showed lower capacity of adsorption compared to [CS-g-(PAN)/DAP]. The antimicrobial activity of the modified copolymers was tested against various microorganisms (*Staphylococcus aureus*, *Escherichia coli* and *Salmonella*). [CS-g-(PAN)/PEI] showed reasonable antibacterial activity against three bacterial strains.
Key words: Chitosan- Acrylonitrile- Polyethyleneimine -1,3diamino propane- Adsorption- Chemical modification- biological activity.

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INTRODUCTION

Water is one of the essential items needed for living for the survival and growth. It also maintains an ecological balance between various groups of organisms and their environment [1]. There has been an increase in effluents being disposed to natural water resources due to rapid industrialization. Major contaminants found in wastewater including biodegradable, volatile and recalcitrant organic compounds, toxic metals, suspended solids, plant nutrients, dyes, microbial pathogens and parasites [2,3].

For minimizing these hazardous pollutants a great effort has done. The effort was mainly taken to avoid the dangerous effects caused by the pollutants on plants, animals and humans. Contamination by the heavy metal has become a main critical problem recently because the metals gets persisted and accumulated in the environment [4]. Heavy metals are widely used in the industries like textiles, leather, paper, plastics, electroplating, cement, metal processing, wood preservatives, paints, pigments and steel fabricating industries [5]. The toxic metal compounds contaminate underground water in trace amounts by leaking from the soil [6]. The effluents of industrial wastewaters often contain considerable amounts of toxic and polluting heavy metals such as chromium, mercury, lead and copper [7]. A wide range of physical and chemical processes are available for the removal of these metals from waste waters, such as precipitation, ultra filtration, adsorption, ion exchange, reverse osmosis, oxidation, ozonation, coagulation, flocculation and membrane filtration processes [8].

Among these, adsorption techniques have been proved to be an excellent method to treat waste water, offering advantages over conventional processes [9]. It is now recognized that adsorption using low cost-adsorbents is an effective and economic method for the removal of heavy metals in wastewater. However, low cost adsorbents with high adsorption capacities are still under development to reduce the adsorbent dose and minimize disposal problems. Much attention has recently been focused on biopolymers as bio adsorbents. They are naturally produced by all living organisms. Polysaccharides such as chitosan a natural amino polymer plays a vital role in the treatment of waste water [10]. It is soluble in dilute acids. The solubilisation occurs by the protonation of the $-NH_2$ function on the C-2 position of the D-glucosamine repeat unit, where by the polysaccharide is converted to a poly electrolyte in acidic media. Chitosan is the only pseudo natural cationic polymer and thus it finds applications in the wastewater treatment [11]. Chitosan has more applications as biopolymers reported for their high potential of adsorption of metal ions [12]. Most adsorbents developed nowadays for the removal of heavy metal ions was based on their interactions with the functional groups on the surfaces of the adsorbents. From these interactions it was concluded that the functional groups present in the adsorbents have important effects on the effectiveness, capacity and reusability for the removal of heavy metal ions [13]. In order to improve the adsorption capacity of chitosan, chemical modification affords a wide range of novel chitosan derivatives. Among the various methods of modification graft copolymerization is an attractive technique [14]. In Chitosan, both hydroxyl and amino groups are possible sites for the reaction to incorporate new and desired Functional groups.

PEI, a highly branched aliphatic polyamine, which is characterized by the repeating chemical unit denoted as $-(CH_2-CH_2-NH)-$. PEI is manufactured by the acid-catalyzed ring opening homopolymerization of ethyleneimine monomer. The amine groups in PEI exist in primary, secondary and tertiary forms in the approximate ratio of 1:2:1 [15] with branches every 3–3.5 nitrogen atoms along any given chain segment. PEIs function as weak Lewis bases in aqueous environments. As the molecular weight of PEI increases, the polymer coils in solution and assumes a Gaussian conformation. It has been demonstrated in empirical studies that the attachment of the highly charged PEI molecule to anionic surfaces results in a near monolayer of polymer at the interface. In aqueous solution PEI exhibits a strong affinity to anionic materials such as poly anions and negatively charged organic and inorganic solids. PEIs are highly soluble in most polar materials including water, alcohols, glycols and certain organic solvents. The most prominent feature of PEI is its extremely high cationic charge density [16]. The capacity of branched PEI to bind several heavy metal ions has been evaluated [15,17]. Extensive studies have also been carried out on the use of water soluble complexing polymers including PEI in membrane filtration [18]; however, the effect of PEI on dyes removal has not been studied. No attempts have been made to optimize the efficiency of polyelectrolytes in enhancing dye removal by modifying PEI with varying functional groups [19]. PEI which contains a large number of primary, secondary and tertiary amine groups in a molecule, exhibited good sorption ability for heavy metals when they were adsorbed or cross-linked on the adsorbent surface [20].

In the present study, chitosan has been graft copolymerized with acrylonitrile with an aim to develop an intermediate which was reacted either with polyethylene imine (PEI) and 1,3 diamino propane (DAP), which has produced two copolymers [(CS-g-(PAN)/PEI] and [CS-g-(PAN)/DAP)] respectively for the sorption of heavy metal [Cu (II) and Pb (II)] ions in the industrial wastewater compared with *Kitchener* drain samples. The antimicrobial activity of the synthesized copolymers was evaluated using cut plug method and measuring the microorganisms surviving ratio under various polymer concentrations.

EXPERIMENTAL

Materials

Chitosan powder (CS) was purchased from ROTH Company, Germany. The degree of deacetylation and molecular weight were reported as 85 % and 50,000, respectively. Acrylonitrile monomer, PEI of purity 99%, was used as received from Aldrich, USA. Other chemicals, such as acetic acid was purchased from SIGMA Company. 1,3diaminopropane, ceric ammonium nitrate and glutaraldehyde of 25% solution in water used in the experiments were used as received from Merck Company, Germany without further purification.

Preparation of Adsorbent

Preparation of chitosan-g-poly acrylonitrile copolymer (CS-g-PAN)

A weight of 5 g of chitosan was dissolved in100 mL of 2% aqueous acetic acid. 15 ML of glutaraldehyde (GA) solution was added in chitosan solution and stirred for 20 minutes using a magnetic stirrer to crosslink chitosan solution. A solution of 0.1 M CAN in 10 ml of 1 N nitric acid was added followed by 7 ml of acrylonitrile drop wise with continuous stirring at 60 ^{o}C for 2 hrs under nitrogen atmosphere. The product was precipitated by pouring into (2N) sodium hydroxide solution with vigorous stirring. The precipitate was washed several times with distilled water, filtered and dried in vacuum oven at 50 ^{o}C [21, 22].

Amination of CS-g-PAN with PEI (CS-g-(PAN)/PEI)

The amination procedure of CS-g-PAN with PEI was a modification of previously described amination of PAN with PEI [23]. A weight of 5 g of CS-g-PAN was reacted with 30 ml of PEI and 20 ml of distilled water was added to the reaction mixture, the temperature was maintained at 50 ^{o}C for 4 hrs with continuous stirring using mechanical stirrer, then the reaction was left at room temperature with continuous stirring for 12 hrs. The obtained precipitate was then washed several times with distilled water to remove homopolymer formed and it was dried in vacuum oven at 40 ^{o}C overnight.

Amination of CS-g-PAN with DAP (CS-g-(PAN)/DAP)

The amination procedure of CS-g-PAN with DAP was a modification of previously described amination of PAN with DAP [24]. A weight of 5 g of CS-g-PAN was reacted with 60 ml of DAP at 90 ^{o}C for 12 hrs with continuous stirring using mechanical stirrer. The aminated product was washed several times with distilled water several times and it was dried under vacuum at 40 ^{o}C.

Characterization of polymers

Fourier transform infrared (FTIR) spectra were obtained in the transmission mode using Mattson 1000, Unicam infrared spectrophotometer Cambridge, England. The spectra were obtained in the infrared region 4000 to 400 cm^{-1} using KBr pellets. Thermo gravimetric analysis was carried out using Shimadzu thermo gravimetric analyzer TGA-50. Measurements were carried out from ambient temperature to 600 ^{o}C at a heating rate of 10 ^{o}C /min under a nitrogen atmosphere at flow rate of 20 ml/min. The morphology of the samples was examined using transmission electron microscope (TEM) and Scanning Electron Microscopy (SEM) The surface morphology of the copolymers by (JEOL- JEM-100cx) and (JEOL- JSM-6510LV) respectively.

Experimental process of removal of lead and copper

Batch studies were performed with concentrations of lead nitrate Pb (II) and copper nitrate Cu (II) to investigate the extent of adsorption of the modified polymers. The procedure for metal ion uptake by chitosan and modified chitosan copolymers was carried out as follows: 25 ml of aqueous solutions containing 4mL of the metal ion solutions (1000ppm) were treated with 50 mg of the polymer in flasks. The extent of the removal of the two metals was investigated separately changing the time of stirring the adsorbent metal solution mixture. The pH of each solution was adjusted to fixed value. The flasks were stirred at room temperature in fixed speed 150 rpm for various time intervals. The adsorbates were separated using filter paper and supernatant liquid was analyzed for residual concentration of the metals by atomic absorption spectrophotometer (VARIAN-Model: AAS240FS).

In all batch sorption studies, the percentage of metal ion removal was evaluated from the following equation:

$$\% \text{ Removal} = (C^0 - C_e) \backslash C^0 \times 100$$

Where C^0 is the initial metal ion concentration and C_e is the metal ion concentration at equilibrium.

Antibacterial activity of chitosan and modified chitosan copolymers using well diffusion method.

Microorganisms

The microorganisms included the gram-negative bacteria *Escherichia coli* and *Salmonella typhae* and the gram-positive bacteria *Staphylococcus aureus.* Bacteria were maintained on nutrient agar. These bacteria strains used to evaluate the antibacterial activity of the synthesized polymers. The antibacterial activities of the samples were determined by well diffusion method [38]. Bacteria were cultured in nutrient agar medium for 24 hr at 35 ºC. A 100 mg of suspension of test microorganisms, containing 1×10^{-6} CFU/mL of bacteria spread on Nutrient agar medium.

Antimicrobial activity test

Cut plug method

The tested polymers were not soluble in water, so the cut plug method provided by Kenawy was suitable to determine the antimicrobial activity of the synthesized polymers [25]. A 0.5 mL spores or cell suspension were prepared and counted, then mixed with 9.5 mL of the corresponding sterilized melted media and left to be solidified at room temperature. Wells are made in seeded agar plate with different organisms under investigation by cork borer and each one was filled with 50 mg of the tested polymers. All the plates were incubated at proper temperature for 36 hr and then the inhibition zone diameters were measured after 24 hr.

Minim inhibitory concentration (MIC)

MIC determination for the most efficient antimicrobial compounds against test microorganisms were determined previously described MIC [26]. Each standard organism suspension (0.5 mL) was mixed with 9.5 mL of 10-fold diluted corresponding media in sterile test tube contained the tested polymers to give 12.5, 25, 50, 75, and 100 mg . Then the seeded tubes were shaken at 250 rpm overnight,

The three-folds dilution were carried out and 100 mL of each dilution was spread into agar plate of corresponding media. Controls without the polymers were run and the plates were incubated for 24 hr, and then the colony forming units was recorded. The surviving ratio was calculated for each chosen organism at different polymers concentrations and compared to the control.

RESULTS AND DISCUSSION

Synthesis of modified chitosan copolymers (Chemically Induced Graft Copolymerization)

Graft copolymer based on chitosan was synthesized by grafting acrylonitrile onto a polysaccharide molecule using ceric ammonium nitrate (CAN) as initiator in H_2O under nitrogen atmosphere. This technique enables the production of new polymeric materials with the desired properties such as high functionality. Further modifications were carried out on the CS-g-PAN to introduce repeat units with amino or imino groups along the polymer chain. For example the CS-g-PAN was modified with PEI and DAP to produce [CS-g-(PAN)/PEI] and [CS-g-(PAN)/DAP].This reaction was confirmed by following the –CN group attached to PAN with FTIR indicated that cyanide groups of PAN on the surface of [CS-g-(PAN)] copolymer may be reacted with amino groups of both PEI and DAP respectively showed in **Scheme (1)**.
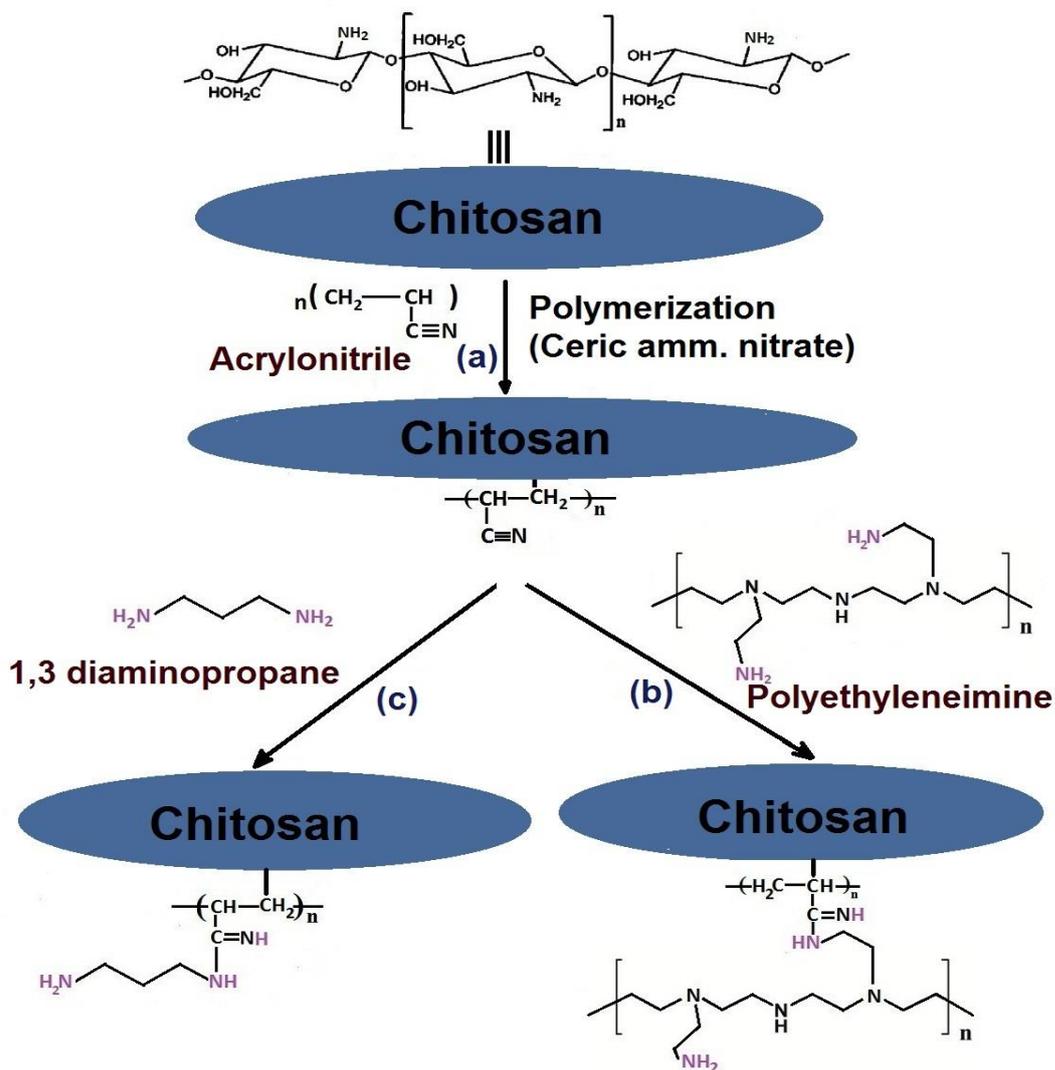
Scheme 1: Preparation of modified chitosan polymers (a) CS-g-(PAN), (b) CS-g-(PAN)/PEI and (c) CS-g-(PAN)/DAP

Characterization of the grafted copolymer

 FTIR spectrum

Figure (1) shows the FTIR spectrum of the modified chitosan copolymer. The spectrum of CS displays a strong absorption band at 3437 cm^{-1} due to OH and amine N-H symmetrical stretching vibration. A peak at

2921 cm^{-1} was due to symmetrical C-H stretching vibration attributed to pyranose ring. The sharp peak at 1383 cm^{-1} was assigned to CH$_3$ in amide group. The broad peak at 1095 cm^{-1} was indicated C-O –C stretching vibration in CS [27], peaks at 1649 and 1425 cm^{-1} were due to C=O stretching (amide I) and N-H stretching (amide II). The absorption band at 1153 cm^{-1} was assigned to the anti-symmetric stretching of C-O-C bridge and 1095 cm^{-1}, 1010 cm^{-1} were assigned to the skeletal vibration involving the C-O stretching. The FTIR spectrum of the modified chitosan (CS-g-PAN) was showed strong peak at around 2244 cm^{-1} spectrum was assigned to the stretching absorption of CN, which proved the successful graft copolymerization of chitosan and Poly acrylonitrile (PAN) [28]. The FTIR spectrum of CS-g-(PAN)/PEI shows the strong peaks at around 2244cm^{-1} partially disappeared in spectrum (c) was assigned to the stretching absorption of CN, which proved the PEI macromolecules have been introduced to the structure of PAN [29]. The FTIR spectrum of CS-g-(PAN)/DAP shows the strong peaks at around 2244cm^{-1} partially disappeared in spectrum (d), which proved the 1,3 diaminopropane molecules have been introduced to the structure of PAN.
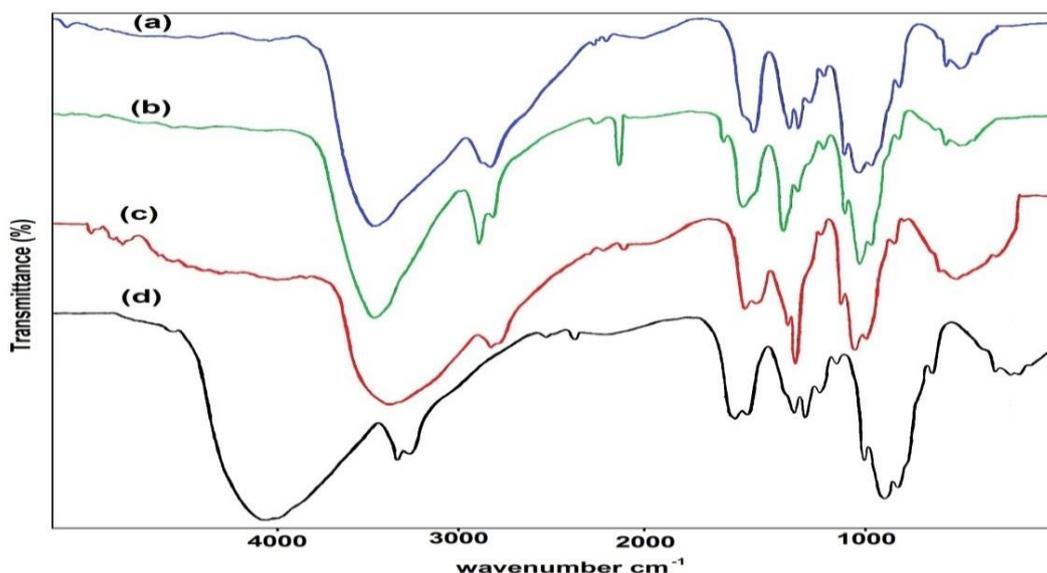

Figure (1): FTIR analysis for (a) CS, (b) CS-g-PAN, (c) CS-g-(PAN)/PEI and (d) CS-g-(PAN)/DAP

Thermo gravimetric analysis (TGA)

To evaluate the thermal properties of the grafted copolymer, the samples were characterized by TGA data in Fig.(2 a - d) shows the weight loss curves of grafted chitosan (CS), grafted chitosan with acrylonitrile (CS-g-PAN), PEI [CS-g-(PAN)/PEI] and DAP [CS-g-(PAN)/DAP]. It is seen that weight loss is directly related to increasing the heating temperature. Three steps weight loss are observed from TGA of CS. First step from 50-110 can be assigned to the release of hydroscopic water molecules; this stage shows 10% weight loss. The second weight loss observed from 200-350 is typical to degradation of saccharide ring while the third weight loss between 575-798 $^{\circ}$C can be assigned to further degradation and decomposition of acetylated unit of CS [30]. TGA of (CS-g-PAN) in (Fig.2- b) show the same characteristic weight losses of CS. Second weight loss observed between 380-560 $^{\circ}$C in TGA of (CS-g-PAN). This may be assigned to interaction of CS with acrylonitrile and decomposition derivatives. These stage show that 50 % of weight loss is due to the bulk decomposition of the polymer residue. Also, the TGA of the [CS-g-(PAN)/PEI] observed the same behavior with some changes in the 3 rd. stage. It started decomposing from 280 $^{\circ}$C until 371 $^{\circ}$C .The weight loss reached 55.6% at 600 $^{\circ}$C. This indicates that the [CS-g-(PAN)/PEI] more stable than (CS-g-PAN) . TGA of the [CS-g-(PAN)/DAP] decomposing at 420 $^{\circ}$C with weight loss reached to 50 %.

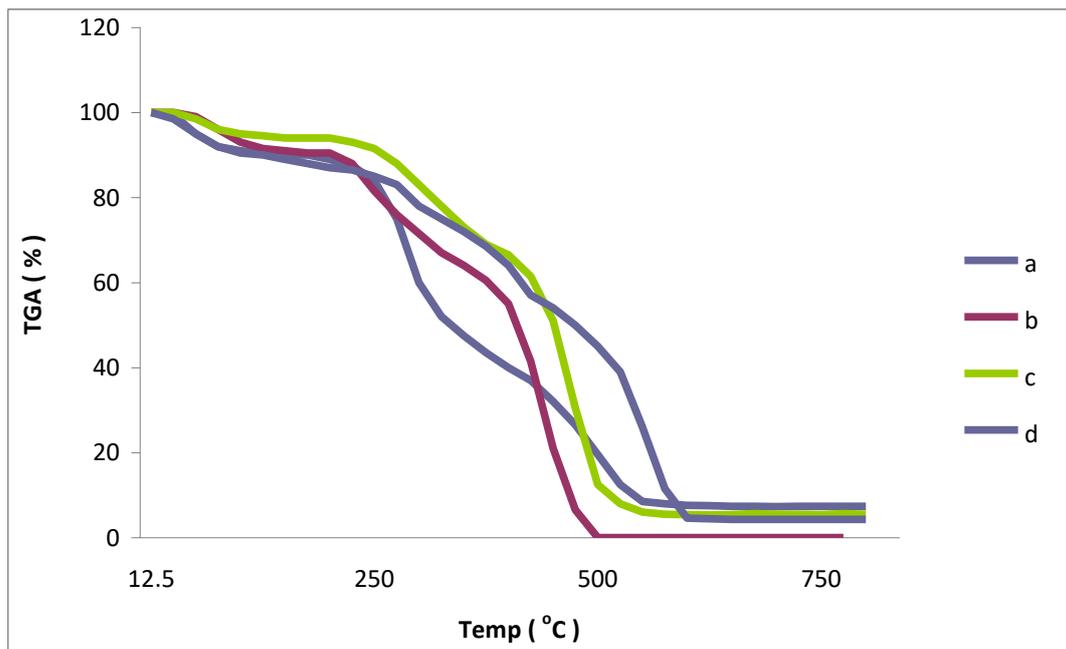**Figure (2): TGA analysis for (a) CS, (b) CS-g-PAN, (c) CS-g-(PAN)/PEI and (d) CS-g-(PAN)/DAP**

Scanning electron microscopy (SEM)

SEM images of CS, CS-g-(PAN)/PEI and CS-g-(PAN)/DAP were as shown in **Figure (3)**. The morphology was observed that CS showed chitosan shows smooth surface with very few pores, because there are stronger interaction between the chitosan molecules. CS-g-(PAN)/PEI showed fibrous and less homogeneous structure with a semi pores and appearance of cavities covered with small nano molecules as scales. This less homogeneous due to PEI branches. CS-g-(PAN)/DAP showed fibrous and layered sheets less homogeneous structure with a semi pores and disappearance of spherical morphology covered with small nano molecules as scales.
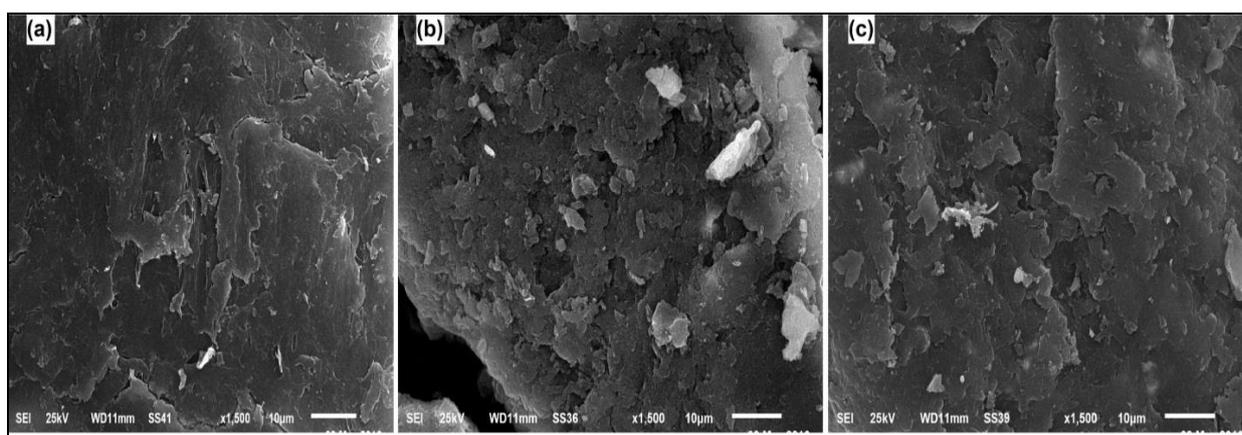

Figure (3): SEM analysis for (a) CS, (b) CS-g-(PAN)/PEI and (c) CS-g-(PAN)/DAP

Transmission electron microscopy (TEM)

In TEM analysis, the morphology of CS nanoparticles was characterized by TEM observations. The TEM image (**Fig.4**) shows a relatively narrow size distribution of mono dispersed nanoparticles with spheroid morphology with size average of (33–45) nm and standard deviation (S.D) was ± 5 nm. The size of these nanoparticles is smaller than that determined (>200 nm) previously [31, 32]. It has been reported that [CS-g-(PAN)/PEI] and [CS-g-(PAN)/DAP] nanoparticles are rough in shape [32]. TEM image in Fig.4 shows that the

average sizes of these nanoparticles are varied according to the type of amines derivatives from (75 – 83) nm and (80-98) with S.D were ±7, ±8 nm for both polyethyleneimine [CS-g-(PAN)/PEI] and 1,3-diaminopropane [CS-g-(PAN)/DAP] respectively.

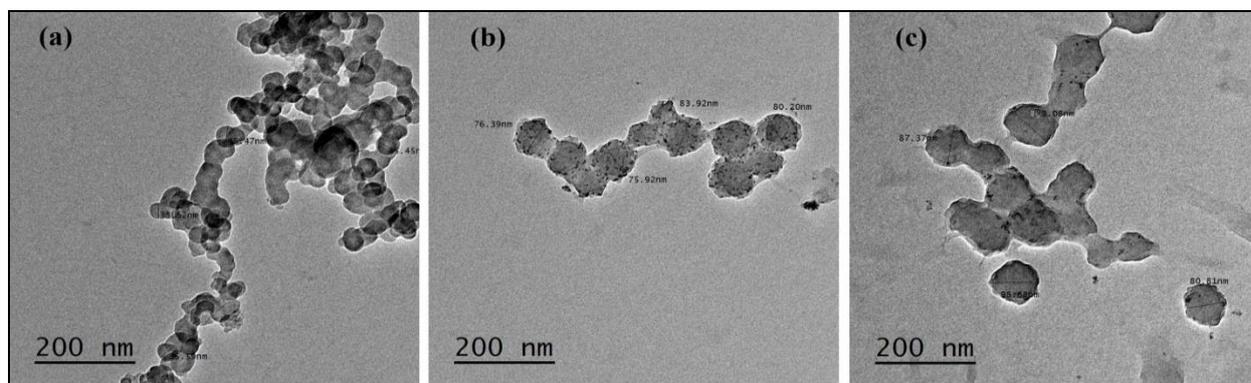**Figure (4):TEM analysis for (a) CS, (b) CS-g-(PAN)/PEI and (c) CS-g-(PAN)/DAP**

Metal ions solution uptake studies

Amino groups (-NH_2) in polymers uptake metal ions by interaction bonds in aqueous solution. The pair of electrons on nitrogen atom of amino group share with metal ions which forms bonds. **Scheme 2** is example for the interaction between amino groups of [CS-g-(PAN)/PEI] polymer with lead ions Pb (II).
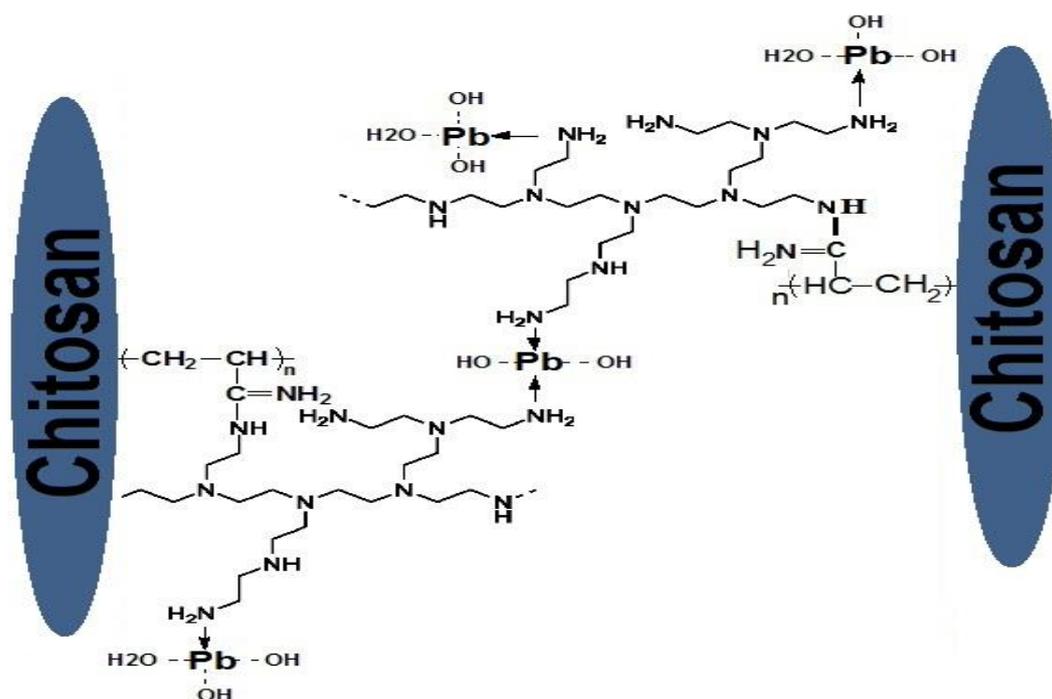
Scheme 2: Lead ions uptake by CS-g-(PAN)/PEI

Adsorption studies of Pb (II) and Cu (II) by chitosan and modified chitosan polymers.

The amount of Pb (II) and Cu (II) ions adsorbed by chitosan and modified chitosan as a function of time are listed in **Tables (1, 2)** and plotted in **Figures (5, 6)**. The maximum lead ions Pb (II) uptake for chitosan, [CS-g-(PAN)/PEI] and [CS-g-(PAN)/DAP] are 59.2%, 72.3% and 81.4% respectively from the aqueous solution. Also the maximum copper ions Cu (II) uptake for chitosan, [CS-g-(PAN)/PEI] and [CS-g-(PAN)/DAP] are 52.4%, 70.2% and 82.2% respectively. The modified chitosan copolymers showed higher capacity of adsorption than

pure chitosan. This means that the modification of CS improved its metal uptake properties due to increasing the affinity of the polymer to the metals by introducing more free amino groups due to reaction the latter with PEI and DAP. [CS-g-(PAN)/PEI] is lower capacity of adsorption than [CS-g-(PAN)/DAP] due to steric hindrance of PEI branches and the metal ions cannot penetrate the polymer body. High adsorption rates were observed at the beginning, and then plateau values (i.e. adsorption equilibrium) are gradually reached within 300 min.

Table (1): Lead ions uptake ratio from aqueous solution at different contact time

Metal ion Pb (II) Uptake ratio (%)	50 min	100 min	150 min	200 min	250 min	300 min
CS	19.4	34.4	46.7	54.9	58.6	59.2
CS-g-(PAN)/PEI	41.7	58.3	67.4	70.9	72.3	72.1
CS-g-(PAN)/DAP	58.7	74.8	78.6	80.2	80.9	81.4

Table(2): Copper ions uptake ratio form aqueous solution at different contact time

Metal ion Cu (II) Uptake ratio (%)	50 min	100 min	150 min	200 min	250 min	300 min
CS	21.1	36.7	47.3	50.8	52.4	51.8
CS-g-(PAN)/PEI	33.5	51.3	62.1	68.6	69.4	70.2
CS-g-(PAN)/DAP	41.7	62.6	77.5	81.8	82.2	81.6
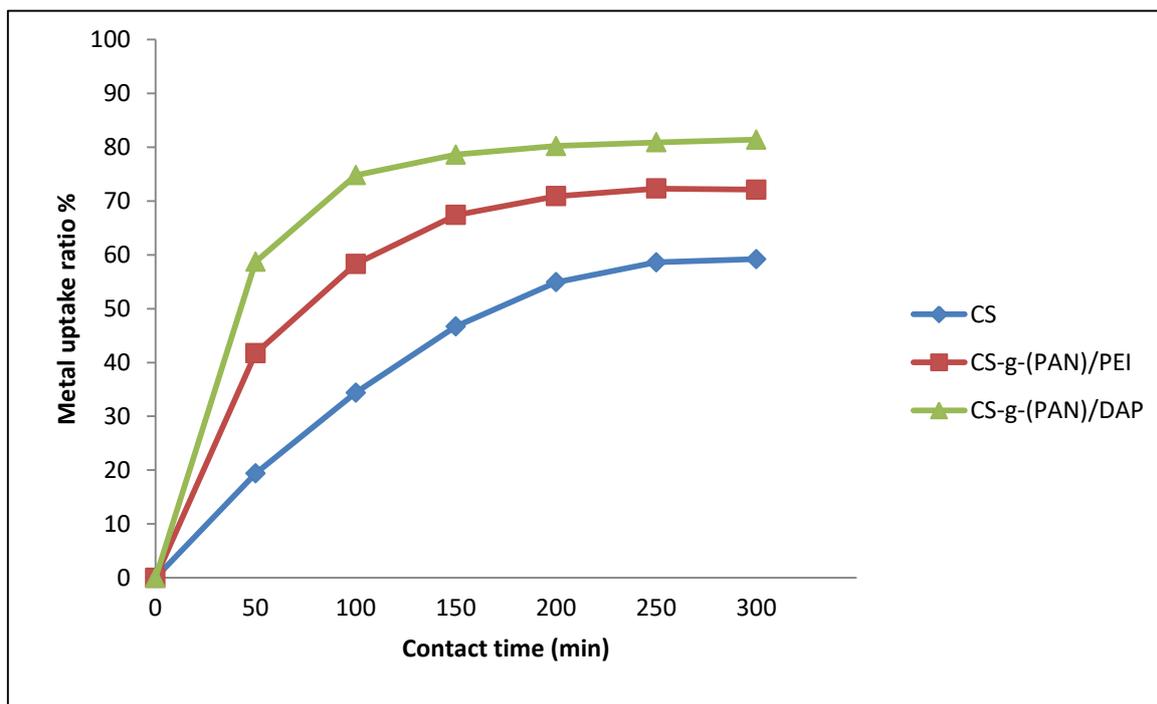
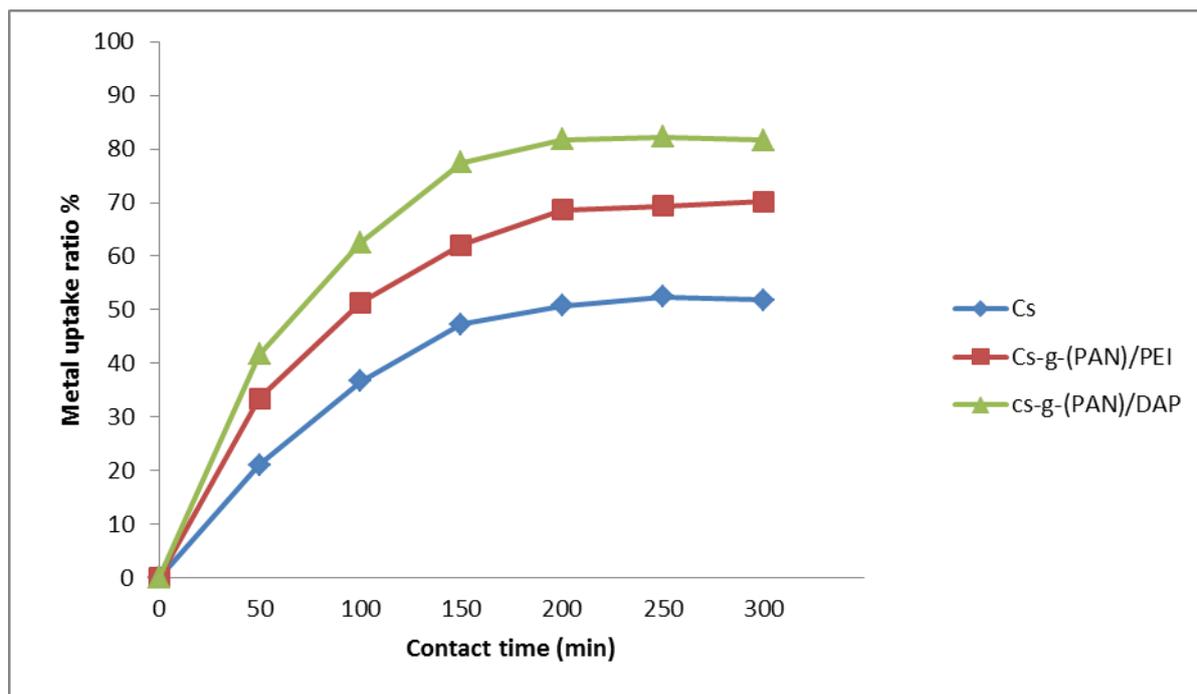**Figure 5: Lead ions uptake using CS, [CS (PAN)/PEI] and [CS (PAN)/DAP]**

Figure (6): Copper ions uptake using CS, [CS-g-(PAN)/PEI] and [CS-g-(PAN)/DAP]

Adsorption studies of heavy metal ions from *Kitchener* drain water using chitosan and its modification

The Kitchener drain was came from *El- Mahalla El- Kubra* city in Egypt and was ended in the Mediterranean Sea. The Kitchener drain is an agricultural drain which includes industrial wastewater. Moreover, the use of chemical fertilizers and pesticides has increased the pollution, particularly by heavy metals. Heavy metals are severe contaminants in the environment. Unfortunately, drainage water of *Kitchener* canal is reused for irrigation. A sample from The *Kitchener* drain was taken in October 2016 from *Kafer-dokhmees* village. Before treatment, the heavy metals ions (Pb, Cu, Mn, Zn, Cd, Fe and Cr) were measured in the sample of *Kitchener* drain and other chemical parameters. Chemical analysis for Kitchener drain sample was showed in the **Table (3)**.

Table 3: Chemical and metals analysis of Kitchener's drain sample

Chemical parameter	T.D.S	pH	Cu	Fe	Mn	Zn	Pb	Cd	Cr
Unit	ppm	-----	ppb	ppb	ppb	ppb	ppb	ppb	ppb
Kitchener drain sample	805	7.2	160	340	480	19.6	8.7	6.8	5.4

The procedure for metal ions uptake by chitosan and modified chitosan copolymers was carried out as follows: 1 liter of Kitchener drain sample were treated with 100 mg of the polymer at room temperature in flasks with continuous stirring at 150 rpm for 1 hour. The concentrations of the metals ions in the sample, after treatment were determined in (**Table 4**) and the desired treatment period was measured by using an atomic absorption spectrophotometer "AAS" at suitable wavelength. As shown in **Figure7,** Chitosan has high adsorption capacity for cadmium ions (49%) in the sample while low adsorption capacity for chromium ions (23%). [CS-g-(PAN)/PEI] has high metal ions uptake for copper ions (69%) while it has low metal ions uptake for chromium ions (39%).[CS-g-(PAN)/DAP] has high adsorption for Lead ions (78%) while it has low adsorption for chromium ions (44%). [CS-g-(PAN)/DAP] copolymer has the highest metal ions uptake for all the metals ions in this study except manganese ions. CS polymer has the lowest metal ions uptake for all the metals ions in this study except cadmium ions.

TABLE 4: Metals ions uptake ratio after polymer treatment (CS, CS-g-(PAN)/PEI and CS-g-(PAN)/DAP).

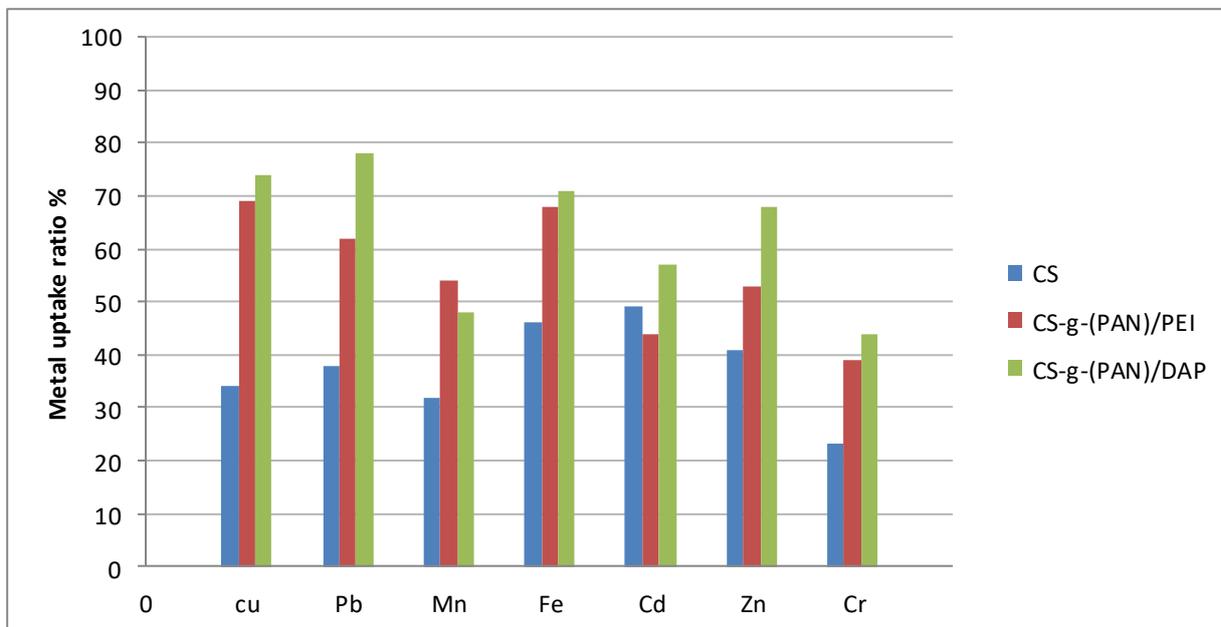| Metal removal ratio (%)	Cu	Pb	Mn	Fe	Cd	Zn	Cr
CS	34	38	32	46	49	41	23
CS-g-(PAN)/PEI	69	62	54	68	44	53	39
CS-g-(PAN)/DAP	74	78	48	71	57	68	44

FIGURE 7: Metal ions uptake ratio from the Kitchener drain sample by different polymers (CS, CS-g-(PAN)/PEI and CS-g-(PAN)/DAP)

Antibacterial activity

Chitosan inhibits the growth of a wide variety of fungi, yeasts, and bacteria [33].Chitosan is readily soluble in various acidic solvents and has high antimicrobial activity against many pathogenic including Gram-positive and Gram-negative bacteria [34]. The antimicrobial effects of chitosan were occurred without migration of active agents [35]. As chitosan is in a solid form, therefore, only organisms in direct contact with the active sites of chitosan is inhibited. Chitosan is incapable to diffuse through the adjacent agar media [36]. The agar diffusion test is a method commonly used to examine antimicrobial activity regarding the diffusion of the compound tested through water-containing agar plate. The diffusion itself is dependent on the size, shape and polarity of the diffusion material [37]. To compare the antibacterial activity of chitosan and its modification with (PEI) polyethyleneimine and (DAP) 1,3-propane diamine loaded on chitosan nanoparticles the samples were tested against gram positive (*Staphylococcus aureus*) and gram- negative *(Escherichia coli* and *salmonella)* bacteria. It is seen (**Fig.8**) that [CS-g-(PAN)/PEI] have a significant antibacterial activity against the tested microorganisms with variable inhibition zones expressed as mm. For *E.coli, S.aureas and salmonella* this holds true regardless of the kind of bacterial used than [Cs-g-(PAN)/DAP] due to small particle size nanoparticles [38].There is no clear zone surrounding, [Cs-g-(PAN)/DAP] we assumed that there is no inhibitory zone and furthermore, the diameter was valued as zero against all tested microorganisms.

In comparison with [CS-g-(PAN)/PEI] have small particle sizes derivatives nanoparticles were more active and good antibacterial activity against the Gram positive bacteria (*Staphylococcus aureus*) than Gram negative bacteria (*Escherichia coli* and *salmonella*) respectively as shown in Fig.9. The inhibition zones of [CS-g-(PAN)/PEI] were determined to change in the following order: *Staphylococcus aureus* (14 mm) **>** *Escherichia coli* (9 mm) **>** *salmonella* (8 mm). The antibacterial activity of chitosan in acidic environment may result from its poly cationic structure due to the protonation of –NH$_2$ on the C-2 position of the D-glucosamine. Positively charged chitosan can bind to and protonated with bacterial cell surface which is negatively charged and

disrupt the normal functions of the membrane microorganisms, e.g. by promoting the leakage of intracellular components or by inhibiting the transport of nutrients into cells and damages its intracellular components [39].
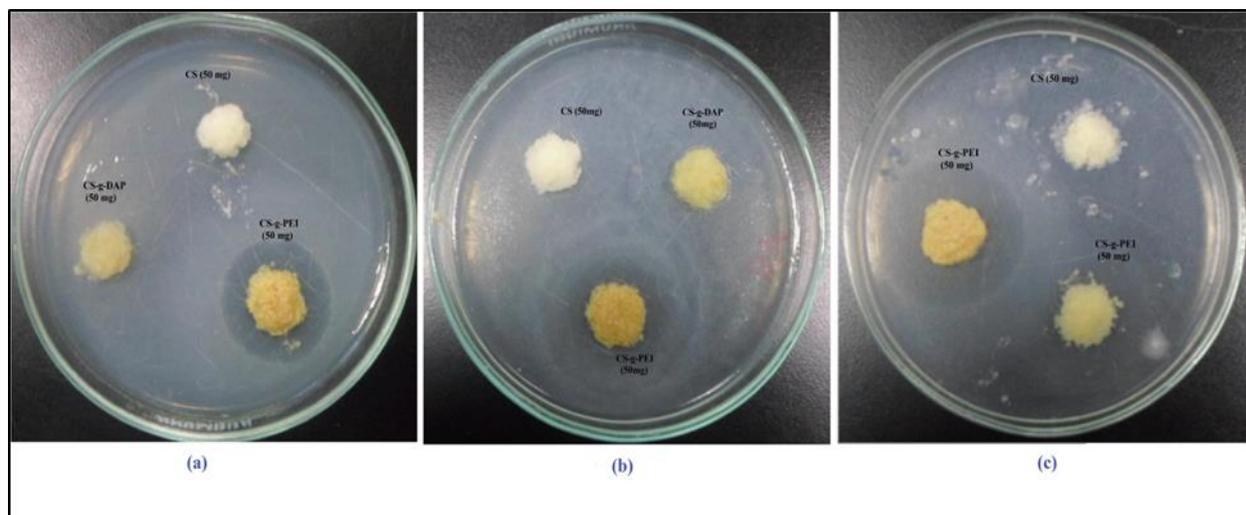

FIGURE 8: Inhibition zone (mm) of Polymers (CS, CS-g-(PAN)/PEI and CS-g-(PAN)/DAP) against *salmonella* (a), *Escherichia coli* (b) and *Staphylococcus aureus* (c) bacterial strains.

Minimum inhibitory concentration (MIC)

The effect of polymer (CS-g-(PAN)/PEI) on the surviving ratio of different tested microorganisms was ranged from 0.00 surviving ratio to 0.75 (Fig. 9). Polymer (CS-g-(PAN)/PEI) killed 23% of at concentration of 12.5 mg/mL, and by increasing the concentration to 75 mg/mL, 66% was killed. *E. coli* is the second sensitive organism to polymer (CS-g-(PAN)/PEI) after *S. aureus*, where 75 mg/mL of polymer (CS-g-(PAN)/PEI) killed 74% of *S. aureus* cells. Furthermore, increasing polymer (CS-g-(PAN)/PEI) concentration from 75 to 100 mg/mL did not increase the killing efficiency of the polymer in case of *S. aureus* and *E. coli*. *Salmonella* were shown the less affected by polymer (CS-g-(PAN)/PEI), here 12% of *Salmonella were* killed at 12.5 mg/mL of that polymer. Additionally, 100 mg/mL of polymer (CS-g-(PAN)/PEI) exhibited 69% of cells killing for *salmonella*.

The growth inhibiting effect was quantitatively determined by the ratio (*M/C*) of the surviving cell number (**Fig. 9**). The antimicrobial activity for polymer (CS-g-(PAN)/PEI) is distinctive in case of *S. aureus* where it reached to only 25% surviving ratios at concentration 75 and 100mg/mL. However, *Salmonella* is the least affected organism with this polymer and gave 41% of organism as a surviving ratio at 100 mg/mL. As shown in **Fig. 9**, the growth inhibitory effect of polymer (CS-g-(PAN)/PEI) differed among the bacteria species. The inhibition becomes stronger in the order: *Salmonella < E. coli < S. aureus*. The results show also that the inhibitory effect increased by increasing the concentration of the polymer. Generally, the potency of inhibition varied according to the polymer and the examined species.
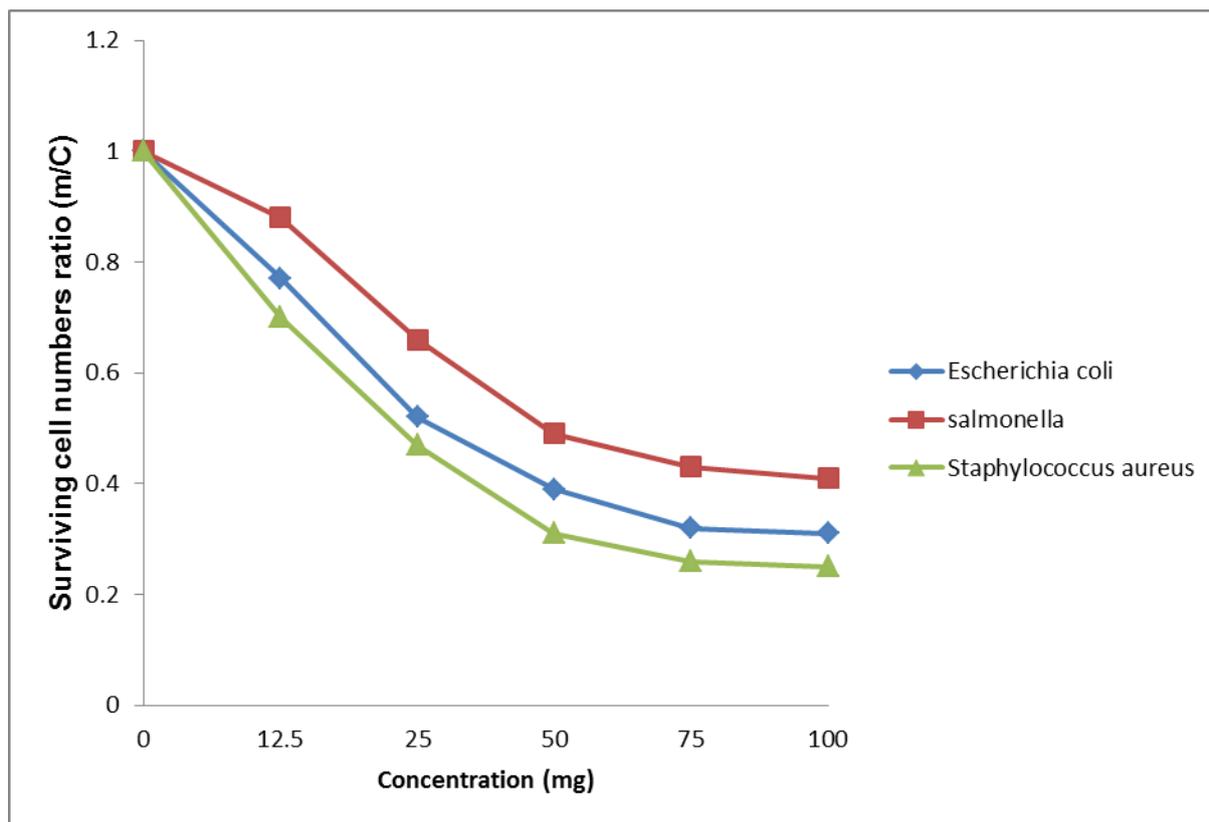
FIGURE 9: The effect of CS-g-(PAN)/PEI concentration on the tested bacteria, *Escherichia coli, Staphylococcus aureus* and *salmonella*.

CONCLUSION

In the present study, chitosan was successfully modified to CS-g-PAN which was modified to both CS-g-(PAN)/PEI and CS-g-(PAN)/DAP. These modifications have been proved by several tools such as FTIR, TGA, SEM, and TEM. Both chitosan and modified chitosan copolymers were tested for adsorption of heavy metals such as Pb(II), Cu(II).These results indicate that, the modifications of chitosan was much better in adsorption of metal ions than chitosan itself. CS-g-(PAN)/PEI has higher antibacterial activity against three bacterial strains *salmonella*, *Escherichia coli* and *Staphylococcus aureus.*

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