



Potential Effect of Modified Chitosan in Environmental Treatment: Metal Uptake, Ion Selectivity, and Bioactivity

Othman A. Al-Fulaij^{1*}, Abdel-Zaher A. Elassar^{1*} and Fakharia A. Alsagheer¹

¹*Department of Chemistry, Faculty of Science, Kuwait University, P.O.B 5969-Safat-13060, Kuwait.*

Authors' contributions

This work was carried out in collaboration between all authors. Author AAE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OAA and FAA managed the analyses of the study. Author AAE managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJOCS/2018/40345

Editor(s):

(1) V. Sivamurugan, PG & Research Department of Chemistry, Pachaiyappa's College, University of Madras, Chennai, India.

Reviewers:

(1) Naoki Kano, Niigata University, Japan.

(2) Julian Cruz-Olivares, Autonomous University of State of Mexico, Mexico.

Complete Peer review History: <http://prh.sdiarticle3.com/review-history/23903>

Original Research Article

Received 11th January 2018

Accepted 18th March 2018

Published 30th March 2018

ABSTRACT

Chitosan (CS) reacted with different organic reagents to give CS-CA, CS-CN, and CS-TEOF. CS-TEOF reacted with 2-aminothiophenol, 2-aminophenol, and phenyl hydrazine to give the CSTE OF-ATP, CSTE OF-AP and SCTE OF-PH, respectively. The modified chitosan derivatives represented the N-S, N-O and N-N, as chelating centres were treated with the aqueous solution containing a mixture of different metal ions (Cu^{2+} , Cd^{2+} , Fe^{3+} , Co^{2+} , Pb^{2+} , Mn^{2+} , Hg^{2+} , Cr^{3+} , Pd^{2+}) in order to investigate their selectivity and their metal uptake capacity. The N-O chelating centre was selective Fe, Co, and Cu, while O-O was selective for Pb, Cr, Ca, Ni, Cd, and Pd. The N-S was selective to Cu(II) more than the N-N chelating centre. The removal capacity by modified chitosan ligands for Ca^{2+} , Cd^{2+} , Fe^{3+} , Co^{2+} , Pb^{2+} , Mn^{2+} exceeded 80%. The morphology of the modified polymer and their metal complexes was studied. The bioactivity of chitosan was improved through modification with sulphur-containing compounds.

Keywords: Chitosan; wastewater treatment; metal complex; bioactivity.

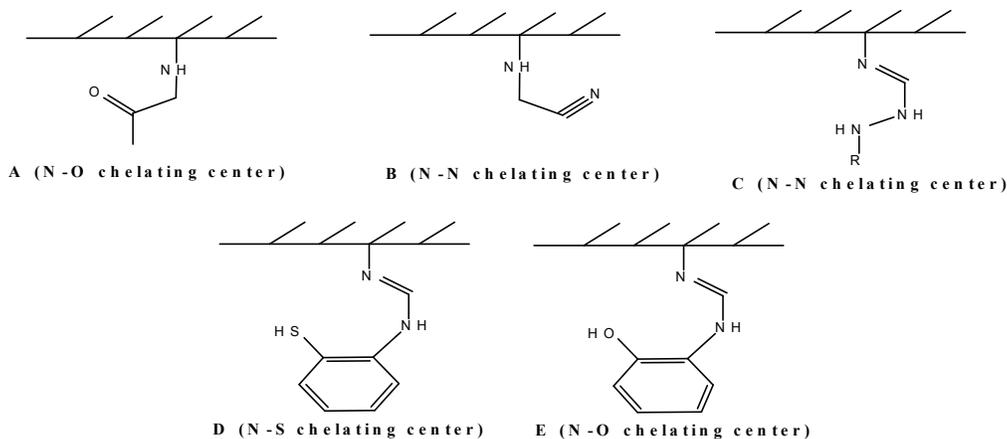
*Corresponding authors: E-mail: Othman.abdallah@ku.edu.kw, aelassar@yahoo.com;

1. INTRODUCTION

Chitosan modifications have received considerable attention in the past few decades. The metal binding ability of polyfunctional chitosan is one of the highest among natural polysaccharides. This allows chitosan to be used as a ligand not only for recovery, concentration of metal ions, and separation, but also as a matrix component of metal-containing catalysts, optical devices, chromatographic substrates, medicinal drugs, and antimicrobial materials [1]. The potential effects of chitosan in the environmental treatment for metal removal in different fields have been widely studied [2-13]. Industries are considered as one of the major sources of heavy metal pollution of water [14-16].

The good behaviour of chitosan for metal binding has promoted a steady growth of theoretical and experimental studies and interest in the interactions of its derivatives with metal ions [17–19]. There has also been a growth in simple and economical methods of the preparation of the derivatives with increased selectivity and sorption [2,3,20–23]. Heavy metals accumulating in living organisms cause many health problems, such as cardiovascular disease, bladder issues, skin lesions, lung cancer, and anaemia [24]. Ions of elements such as Hg(II), Pb(II), Cr(III), Cd(II), Co(II), Cu(II) and Ni(II) are toxic to the aquatic systems. It is well-known that Cr(VI) is toxic to living systems and must be removed from wastewater before discharge. According to previous reports, the removal of Cr(VI) through the acid catalysed the chemical reduction of Cr³⁺, followed by the precipitation as hydroxide at alkaline conditions [25-27]. At certain concentration levels, Cu ions can become toxic

to humans, causing cancer and promoting oxidation. The removal of Cu by chitosan and precipitation as copper hydroxide by lime has also been reported [28,29]. Chitosan and its derivatives have also attracted considerable interest owing to their antimicrobial and antifungal activities [30]. Chitosan metal complex has showed an effect on microorganisms. Wang et al. [13] mentioned that after chitosan binding with metal ions through oxygen and nitrogen or both, the bindings are likely to leave some potential donor atoms free, which enhances the bioactivity. Chitosan is used as a pharmaceutical agent in biomedicine, a human dietary supplement for weight loss, cholesterol reducing agent, a hydrating agent in cosmetics, and an antimicrobial agent in food wrap materials [31-34]. The antimicrobial activity of chitosan has showed that low molecular weight chitosan exhibits strong bactericidal activities as compared by with high molecular weight chitosan. The antibacterial activity of chitosan–copper complexes against *Salmonella* has indicated that the complexes with molar ratio close to 1:1 exhibited an inhibition rate reaching 100% [6]. In this study, chitosan was modified to give new types of chelating centres such as N-O (structure A & E), N-N (structure B & C), N-S (structure D) and O-O (oxygen atoms of chitosan), which would increase the capacity and metal uptake selectivity (cf. Scheme 1). We aimed to increase the bioactivity of chitosan through its modification. We then investigated the bioactivity of the modified chitosan and metal complex against bacteria and fungi. The structures were established based on elemental analysis, spectral data, scanning electron microscopy (SEM), and X-ray photoelectron spectroscopy (XPS).



Scheme 1. Suggested and aimed chelating centres

2. EXPERIMENTAL

2.1 Materials

Chloroacetonitrile, triethylorthoformate (TEOF), chloroacetone, 2-aminothiophenol, and 2-aminophenol were used as received. All metal salts and other chemicals were reagent grade and used without further purification.

2.2 Characterization Methods

2.2.1 Elemental analysis

Elemental analysis was performed at Analab, on LECO CHNS – 932, Faculty of Science, Kuwait University.

2.2.2 IR spectra

IR spectra were recorded as KBr discs of monomers, polymers, and the metallo-polymer complexes on a Perkin-Elmer System 2000 FT-IR spectrophotometer (Kyoto, Japan).

2.2.3 Scanning electron microscopy

SEM was carried out for the polymers and their metallo-polymer complexes at room temperature in a model JSM 6300 JEOL scanning electron microscope (Akishima, Japan) at 20 kV.

2.2.4 X-Ray photoelectron spectroscopy

XPS spectra were recorded on a Thermo ESCALAB 250 Xi using monochromatic Al K α radiation (1486.6eV) with a spot size of 850 μ m. The spectra acquisition and processing were carried out using the Thermo Advantage software, version v5.956.

XPS analysis was performed on a sample containing C, N, O, and a metal. The sample was stuck on the sample holder using double-sided carbon tape and then introduced into the preparation chamber, which was degassed until the proper vacuum level was achieved. It was then transferred into the analysis chamber in which the vacuum was 10⁻¹⁰ mbar. The analysis was carried out using the following parameters: pass energy of 20eV, dwell time 50ms, and the step size 0.1eV. The XPS spectra, including the deconvoluted and non-deconvoluted spectra, are shown in the Figs. 1 and 2.

2.3 Synthesis

2.3.1 Preparation of CS-TEOF: reaction of chitosan with TEOF

TEOF (0.01 mol) was added to 100 mL of toluene containing a chitosan film (4x5 cm). The reaction mixture was refluxed for 12h. The obtained films were refluxed in ethanol for 1h.

2.3.2 Preparation of CS-CA and CS-CN: reaction of chitosan with chloroacetone and chloroacetonitrile

Chloroacetone or chloroacetonitrile (0.01 mol) was added to 100 mL of toluene containing a chitosan film (4x5 cm) and sodium carbonate (2g). The reaction mixture was refluxed for 10h. The obtained modified chitosan derivative films were refluxed in ethanol for 1h.

2.3.3 Preparation of CSTEOP-ATP, CSTEOP-AP, and CSTEOP-PH: reaction of CS-TEOF with amines

2-aminothiophenol, 2-aminophenol, or phenyl hydrazine (0.01 mol) was added to 100 mL of a toluene/ethanol mixture (3:1) containing a CS-TEOF film (4x5 cm), and then heated under reflux for 10h. The produced modified chitosan derivative films were refluxed in ethanol for 1h.

2.3.4 Preparation of metallo-polymer complex

CS-CA film (4 x 5 cm) was submerged in a hot solution of metal nitrate salts (0.01 mol) in DMF, and then heated under reflux for 5h. The metallo-polymer complex films obtained were purified by heat in ethanol for 1h.

2.3.5 Ion selectivity

A modified chitosan film was added to an aqueous solution containing (0.01 mol) of metal nitrate mixture (M = Cu²⁺, Cd²⁺, Fe³⁺, Co²⁺, Pb²⁺, Mn²⁺, Hg²⁺, Cr³⁺, Pd²⁺). The mixture was left for 24h with stirring. The reaction product was filtered, and ICP measurements of the metal contents for the original solution and the obtained filtrate were obtained.

2.3.6 Concentration of metal ions in the Gulf water samples

Real Gulf water sample (5 L) was pre-concentrated to a final volume of 150 mL. The metal ions concentration was determined by ICP-

OES. 150 mL of the pre-concentrated sample was divided into three portions of 50 mL and then treated with a modified chitosan film under a shaking rate of 350 rpm for 24h. The mixture was filtered and the polymer film was washed several times with doubly distilled water. The results of ICP-OES measurements before and after treatment and the percentage of metal uptake onto the chelating polymer were estimated according to the following equation:

$$\% \text{ sorption} = [C_i - C_f / C_i] \times 100 \quad (1)$$

Where C_i and C_f are the initial and final concentrations of the metal ion (mg/L or in $\mu\text{g/L}$) before and after treatment, respectively. The results are the average of three measurements, and the precision in most cases is very close to $\pm 1.5\%$.

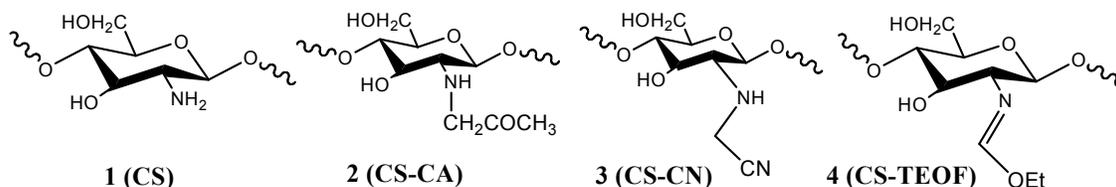
2.3.7 Procedure of antimicrobial tests

A solution or suspension of the tested modified polymers and polymer metal complexes prepared by dissolving 400 $\mu\text{g/mL}$ (w/v) in sterile DMSO was poured aseptically in a well of 6 mm diameter made by a borer in the seeded agar medium. After transferring *via* pipette the same volume in wells of all tested microorganisms, bacteria test plates were incubated at 37°C for 24 h, and fungal test plates were incubated at 25°C for 48 h. The activities were expressed as inhibition zones (mm, diameter, as clear areas). The least concentration, which showed inhibitory effect on any specific microorganism, was considered as the minimum inhibitory concentration (MIC) and was determined using streptomycin and mycostatin (50 $\mu\text{g/mL}$) as the references.

3. RESULTS AND DISCUSSION

3.1 Modification of Chitosan and Characterization

Chitosan (**1**) alkylated with chloroacetone and chloroacetonitrile in the presence of sodium



Scheme 2. Modification of chitosan with different organic reagents

carbonate to give the *N*-alkylated products **2** and **3**, respectively (Scheme 2). The reaction product was established based on the IR spectrum, for example, the modified polymer, CS-CA (**2**), displayed two carbonyl bands at 1678 and 1649 cm^{-1} , while CS-CN (**3**) showed the cyano and amide carbonyl groups at 2244 and 1649 cm^{-1} , respectively (Table 1). Moreover, chitosan reacted readily with triethyl orthoformate to give, CS-TEOF, **4**. The structure of derivative **4** was established based on IR, which reveals the presence of a new band at 1659 cm^{-1} for C=N, in addition to the presence of a new band at 3098 for imine-H and NH band at 3343 cm^{-1} for amide NH_2 , and the disappearance of the amino group of chitosan, which exhibited two bands at 3393 and 3168 cm^{-1} (Table 1).

CS-TEOF reacted with 2-aminothiophenol, 2-aminophenol, and phenylhydrazine in a mixture of toluene/ethanol (1:1) to afford the modified chitosan **5-7** (scheme 3). The structure of compounds CSTEOF-ATP (**5**) was assigned as the reaction product depending on the elemental analysis, which showed a sulphur analysis of 6.72%. From the analysis, a rough percentage of functionalization is about 65%, which is close to the values reported in the literature [35,36]. It is worth noting that the sulphur percentage increases with the duration of heating. Moreover, IR reveals the presence of a new aromatic C-H band at 3099 cm^{-1} . Derivative **6** was established based on IR, which showed the hydroxyl and NH groups at 3459, 3406, and 3335–3210 cm^{-1} . In addition, a new band at 3135 cm^{-1} was assigned to the aromatic C-H. Similarly, compound **7** was established as the reaction product.

3.2 Complex Formation

The modified chitosan CS-CA was refluxed with the metal salt solution in a suitable solvent to give the corresponding complexes. The structures of the obtained complexes were established based on elemental analysis, FT-IR

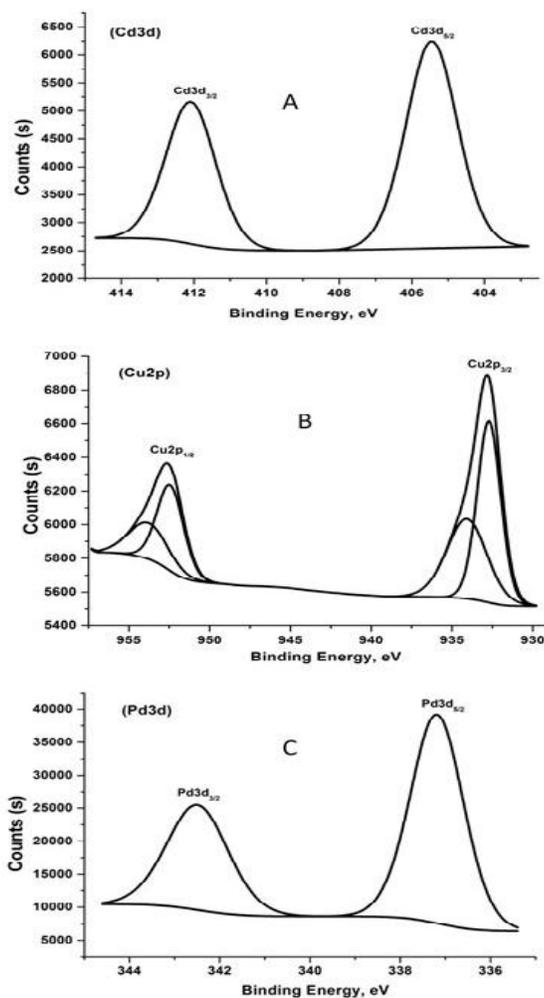
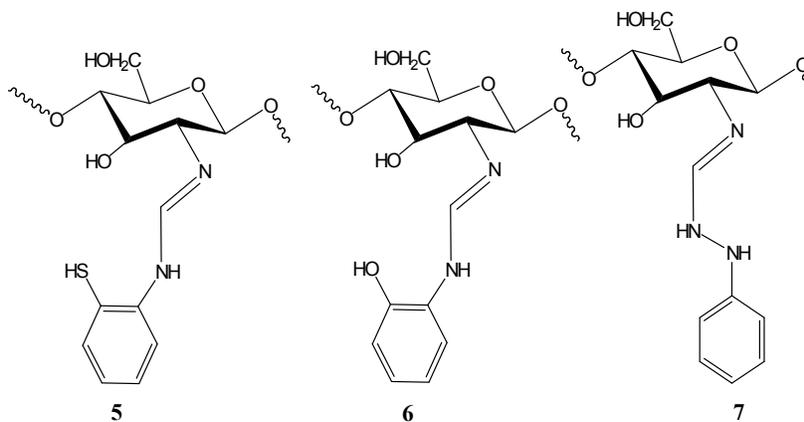


Fig. 1. XPS data of modified CS complex: (A) CSCA-Cd complex, (B) CSCA-Cu complex, and (C) CSCA-Pb complex



Scheme 3. Synthesis of N-N, N-O, and N-N chelating centres

(cf. Table 1), and XPS data. The XPS of metallo-polymer complex structure confirmed the presence of metal chelation, and it refers to the centre of chelation through study the binding energy.

3.3 X-Ray Photoelectrons Spectroscopy

Modified chitosan CS-CA was heated with different metal ions, e.g., Cd(II), Cu(II), and Pd(II), to give the corresponding complexes. Fig. (1A) shows the core level spectra of Cd (3d) consisting of the Cd3d_{5/2} and Cd3d_{5/3} components located at 405.45 and 412.08 eV, respectively. The structure A or B was suggested as a reaction product. The results suggest that the binding energy of Cd is attributed to the Cd²⁺ bonding state [35] and is assigned to Cd-O bond. Thus, structure B was ruled out while structure A (scheme 4) was assigned as a reaction product.

The peak at 934.07eV in the Cu2p XPS spectrum in Fig. (1B) suggested the coordination bonding between Cu and the nitrogen of the polymer (Cu-N). The Cu2p XPS spectrum evidenced Cu⁺ (932.7eV) and Cu²⁺ (934.07eV) in a polymer-Cu complex, which confirmed that metals may be simultaneously redox-transformed when complexed with the polymer. The peak at a binding energy of 953.8 eV is assigned for the Cu-O bond. Structure B (scheme 4) was assigned as a reaction product. Fig. (1C) shows the XPS spectra of PdCl₂ with CSCA-PdCl₂. The XPS data show that the Pd3d centred at 337.1 and 342.5 eV were assigned to the Pd(II) species and mostly for the Pd-O bond. Structure a (scheme 4) was assigned as a reaction product. Fig. 2 shows the XPS spectra of Pd(II) with the CSCA-PdSO₄ complex. Similarly, Structure A was assigned for the CSCA-PdSO₄ complex. In addition, the elemental analysis of CSCA-PdSO₄ complex showed a sulphur analysis of 5.97%. All binding energy values for the metal-polymer complex

are slightly different than that mentioned in the literature. The difference could be attributed to the coordination action of the metal and the modified chitosan polymer. The Cu(II) complex (structure B) is different than that of Cd(II) and Pd(II) (structure A), possibly due to the difference in the ionic radius. The ionic radius of Cu(II) fit better with the bidentate chelating centre N-O.

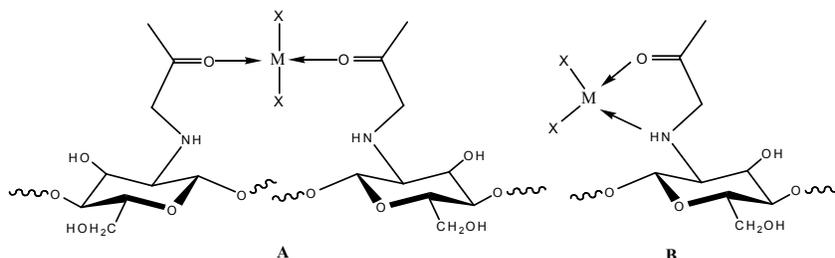
3.4 Ion Selectivity

A mixture of different metal salts was treated with different polymers, and ICP measurements for the blank solution and the solution after treatment for 24h with polymer were carried out. The differences refer to the metal uptake value.

The ICP-OES measurements before and after treatment and the percentage of metal uptake onto the chelating polymer are reported in Tables 2-6 and were computed according to equation (1).

Atomic absorption measurements of a mixture of the modified polymers CSTEOP-PH with a mixture of metal nitrate, M = Ca²⁺, Cd²⁺, Co²⁺, Cr³⁺, Cu²⁺, Fe³⁺, Mg²⁺, Mn²⁺, Ni²⁺ or Pb²⁺ before and after treating with the polymer are presented in Table 2. The polymer selectivity order for the modified polymer CSTEOP-PH could be arranged as follows: Pb > Co > Ca > Cd > Mn > Fe > Cu > Cr > Mg.

It is clear from the data that CSTEOP-PH was selected for six ions (Pb, Co, Ca, Cd, Mn, and Fe) and less selective to Cu, Cr, and Mg. The study of metal uptake of Hg(II) from an aqueous solution by CSTEOP-PH is reported in Table 3. The sorption of Hg decreased with increasing the duration to 48h instead of 24h and also decreased upon heating for 2 h. Furthermore, the polymer selectivity order for modified polymer



Scheme 4. Suggested complex structure depending on XPS data

CSTEOF-AP is reported in Table 4 and can be arranged as follows: $Pb > Co > Fe > Mn > Cd > Ca > Cu > Cr > Mg$. The data reflected that CSTEOF-AP was selected for Pb, Co, Fe, Mn, Cd, and Ca and less selective to Cu, Cr, and Mg. The ICP data onto modified chitosan CSTEOF-ATP is reported in Table 5. The CSTEOF-ATP selectivity could be arranged as follows: $Pb > Co > Ca > Fe > Mn > Cd > Cr > Mg > Cu$. The data showed that CSTEOF-ATP was selected for Pb, Co, Ca, Fe, Mn, and Cd. The CSTEOF-ATP was less selective for Cr, Mg and Cu. The data generally showed that the modified chitosan was selective for some metal ions depending on the type of chelating centre furnished with the modification process, e.g, N-N, N-O, O-O and N-S. The metal ions Fe, Co, and Cu preferred N-O, while Pb, Cr, Ca, Ni, Cd, and Pd preferred O-O chelating centre. In contrast, copper ions preferred the N-S chelating centre more than the N-N in the modified chitosan.

3.5 Determination of Metal Ions in Gulf Water Samples

The use of the modified polymer for the Gulf ocean water analysis showed that the CS-CA could uptake many metal ions (such as Na, K, Mg, Ca, Co, Ni, Cu, Cd, Pb, Fe, and Hg) from the Gulf ocean water. The ions Cu, Ni, Fe, Hg, and Co was measured in $\mu\text{g/L}$, but Na, K, Mg, and Ca was measured in mg/L . The ICP-OES measurements before and after treatment and the sorption percentage of the chelating polymer

are reported in Table 6 and calculated according to equation (1).

3.6 The Structures and Morphology

The change in the surface morphology may be due to the modification of the polymer or/and the introduction of the metal ions in the polymer matrix. SEM was carried out to confirm the chelation of the metal ions with the polymeric chain and to study how the change in the surface occurred. The SEM of CS-CA and its metallo-polymer complexes with different metal ions are shown in Fig.3, with 10,000x magnification. The crystallinity of the metal complexes of CSCA-Cu, CSCA-Cd, and CSCA-Pd is more than that of CS and its modified derivatives CS-CA. The SEM for both CS and CS-CA show a smooth surface. This means that the chelation mostly occurred on the surface of the polymer. The SEM micrograph of CS-CA in Fig. 3A seems to be a smoothed surface, which is somewhat similar to that of chitosan. Figs. 3B and 3C show the CSCA-Cu complex and CSCA-Cd complex, respectively. The micrograms of the CSCA-Cu and CSCA-Cd complexes show similar surfaces, which are tube-shaped. In contrast, from the SEM micrograph of CSCA-Pd complex shown. According to the figure, Pd-complex showed a different change in the surface as compared to the Cu-complex and Cd-complex.

3.7 Biological Activity

The well-known diverse biological activities of compounds containing sulphur and nitrogen as

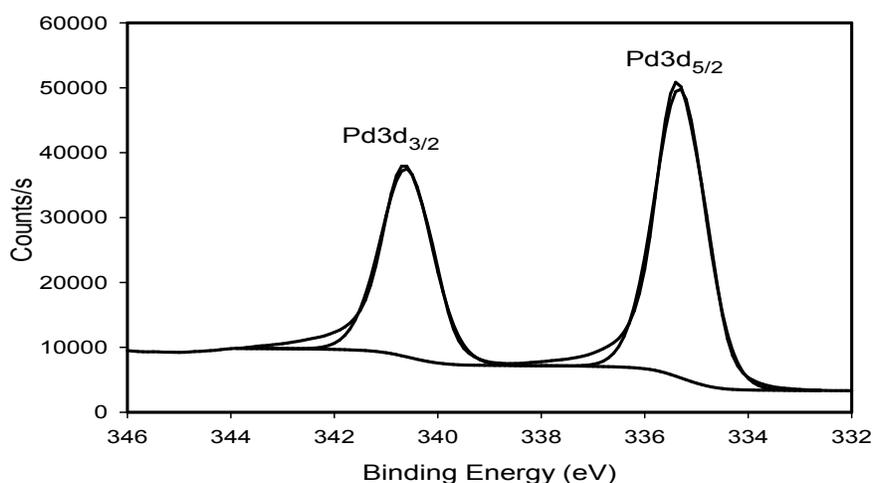


Fig. 2. XPS data of the CSCA-PdSO₄ complex

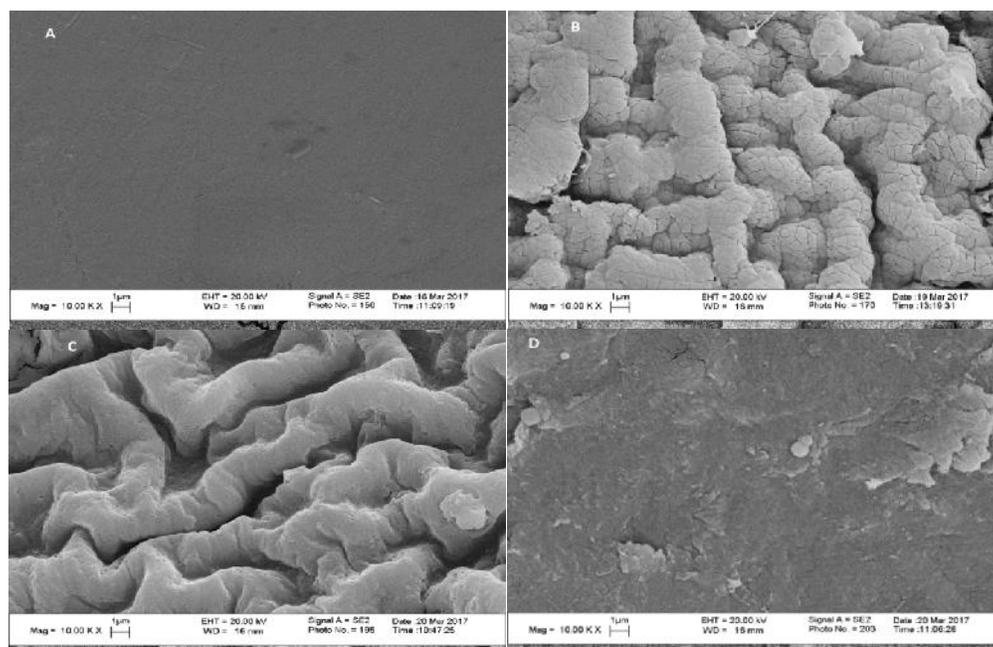


Fig. 3. SEM image with 10,000x magnification: (A) CS-CA, (B) CSCA-Cu complex, (C) CSCA-Cd complex, (D) CSCA-Pd complex

Table 1. Elemental analysis and IR measurements of chitosan, modified chitosan and metal complexes

Compound	C%	H%	N%	S%	IR (cm ⁻¹)
CS	39.04	7.53	5.88		3393, 3168 (NH, NH ₂); 2924, 2880 (CH), 1642 (C=O), 1554, 1408, 1321
CS-CA	41.12	7.02	5.62		3348, 3294, 3235, 3199, 2881, 1678, 1649, 1559, 1380, 1317, 1257, 1150, 1104, 1009, 896, 614
CS-CN	39.07	7.39	6.76		3358, 3216, 2886, 2244, 1649, 1551, 1377, 1316, 1150, 1112, 1025, 896, 612
CSTEOF-PH	48.74	6.90	7.10		3343, 3098, 2993, 2882, 1652, 1647, 1552, 1384
CSTEOF -ATP	55.25	7.22	9.51	6.72	3459, 3342, 3285, 3200, 3135, 2884, 1650, 1561, 1381
CSTEOF -AP	41.80	6.52	6.97		3469, 3406, 3335, 3283, 3248, 3210, 3099, 2926, 2884, 1705, 1653, 1559, 1406, 1324
CSTEOF S-AP-Cu	39.21	6.36	7.99		3469, 3406, 3335, 3283, 3248, 3210, 3099, 2926, 2884, 1653, 1559, 1406, 1324
CSCA-PdSO ₄	33.53	6.43	5.32	5.97	3596, 2648, 1630, 1590, 1513, 1421, 1350
CSCA-Cu	33.56	6.10	8.72		3426, 3345, 3254, 3150, 3107, 3008, 2977, 2838, 2080, 1680, 1632, 1535
CSCA-Cd	32.17	5.80	8.54		3636, 2680, 2076, 1727, 1671, 900
CSCA-PdCl ₂	39.79	6.75	6.09		3624, 2487, 2129, 1686

Table 2. Ion selectivity (ICP) measurements of different metal ions from aqueous solution by CSTEOP-PH

Metal ion	(C _i) mg/L	(C _f) mg/L	(C _i - C _f) mg/L	Sorption %
Ca ²⁺	1642.5	184.5	1458	88.8
Cd ²⁺	2366.8		336.8 2030	85.8
Co ²⁺	1836	161.3	1674.7	91.2
Cr ³⁺	2382.6	912.5	1470.1	61.7
Cu ²⁺	1877.5	703.8	1173.7	62.5
Fe ³⁺	2420.56	461.7	1958.86	80.9
Mg ²⁺	1485.5	686.1	799.4	53.8
Mn ²⁺	1795.5	265.1	1530.4	85.2
Pb ²⁺	3315.1	281.7	3033.4	91.5

Table 3. Ion selectivity of Hg(II) ions from aqueous solution by CSTEOP-PH

Reaction time (h)	C _i mg/L	C _f mg/L	(C _i -C _f) mg/L	Sorption %
After 24h	165398	24236	141162	85.3
After 48h	165398	87873	77525	46.9
After heating 2h	165398	99123	66275	40.1

Table 4. Ion selectivity (ICP) measurements of different metal ions from aqueous solution by CSTEOP-AP

Metal ion	C _i mg/L	C _f mg/L	(C _i - C _f) mg/L	Sorption %
Ca ²⁺	1642.5	248.6	1393.9	84.9
Cd ²⁺	2366.8	351.2	2015.6	85.2
Co ²⁺	1836	222.6	1613.4	87.9
Cr ³⁺	2382.6	876.5	1506.1	63.2
Cu ²⁺	1877.5	612.5	1265	67.4
Fe ³⁺	2420.56	326.1	2094.46	86.5
Mg ²⁺	1485.5	650.3	835.2	56.2
Mn ²⁺	1795.5	244.9	1550.6	86.4
Pb ²⁺	3315.1	349.5	2965.6	89.5

Table 5. Ion selectivity of different metal ions from aqueous solution by CSTEOP-ATP

Metal ions	C _i mg/L	C _f mg/L	(C _i - C _f) mg/L	Sorption %
Ca ²⁺	1642.5	199.2	1443.3	87.9
Cd ²⁺	2366.8	463.8	1903	80.4
Co ²⁺	1836	215.7	1620.3	88.3
Cr ³⁺	2382.6	789.5	1593.1	66.9
Cu ²⁺	1877.5	555.8	1321.7	70.4
Fe ³⁺	2420.56	358.4	2062.16	85.2
Mg ²⁺	1485.5	487.3	998.2	67.2
Mn ²⁺	1795.5	296.7	1498.8	83.5
Pb ²⁺	3315.1	358.8	2956.3	89.2

Table 6. ICP-OES measurements of a gulf ocean water sample before and after treatment with the chelating polymer CS-CA

Metal ion	C _i mg/L	C _f mg/L	(C _i - C _f) mg/L	% of metal uptake
^a Na ⁺	16427.55	2451.6	13975.95	85.1
^a K ⁺	763.9	164.9	599	78.4
^a Mg ²⁺	1611.4	123.3	1488.1	92.3
^a Ca ²⁺	509.5	31.3	478.2	93.9
^b Co ²⁺	2.54	0.156	2.384	93.9
^b Ni ²⁺	13.75	0.177	13.573	98.7
^b Cu ²⁺	13.7	2.3	11.4	83.2
^b Cd ²⁺	BDL	ND		
^b Pb ²⁺	74.2	15.6	58.6	79.0
^b Fe ³⁺	1.07	0.11	0.96	89.7
^b Hg ²⁺	15.1	1.2	13.9	92.1

a: C_i in mg/L; b: C_i in µg/L; ND: not detected; BDL: below detection limit.

Table 7. In vitro bactericidal and fungicidal activity of some newly synthesised compounds

Compound	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>Salmonella</i>	<i>Candida albicans</i>
CS-CA	++	++	+	Nil	Nil
CS-CN	+++	+++	+++	++	++
CS-TEOF	++	++	+++	+	+++
CSTEOF-PH	++	++	+++	++	+++
CSTEOF-ATP	+++	+++	+++	++	+++
CSTEOF-AP	+++	++	+++	++	+
CSCA-Cu complex	+++	++	+++	+++	++

Slight effect = + (10–12mm); Moderate effect = ++ (13–15mm); Severe effect = +++ (16–20 mm)

components of organic compounds [36-40] in addition to chitosan, prompted us to test and study the antibacterial and antifungal activities of some of the modified chitosan and metallopolymer complexes. As shown in Table 7, most of the tested compounds had high to low activity against the tested microorganisms. The data reported in Table 7 expressed in terms of (+) for slight effect from 10 to 12 mm diameter, (++) for moderate effect from 13 to 15 mm diameter, and (+++) severe effect from 16 to 20 mm diameter. It has been reported that chitosan derivatives killed bacteria through disrupting the inner and outer membranes [41].

From Table 7, most of the tested compounds showed high to low effect on bacteria and fungi. Thus, CS-CN, CSTEOF-AP, and CSTEOF-ATP exhibit a severe effect on *S. aureus*, while CS-CN and CSTEOF-ATP showed a severe effect on *B. subtilis*. In addition, CS-CN, CS-TEOF, CSTEOF-ATP, and CSTEOF-ATP exhibit severe effect on *E. coli*. The tested compound showed medium to low effect on *Salmonella*, while CS-TEOF, CSTEOF-PH, and CSTEOF-ATP showed a high effect on *Candida albicans*. CSCA-Cu complex showed a severe effect on *S. aureus*, *E. coli*, and *Salmonella*. Investigations of CS-CA

showed a moderate to slight effect in the case of bacteria, and a non effect was reported on both tested fungi.

4. CONCLUSION

In this article, chitosan was updated through modifications under normal condition. The modifications carried out by the reaction of different organic reagents with the amino group as an active site in chitosan. Further reactions of the modified chitosan were also successful and produced different chelating active centres and biologically active derivatives. The use of the modified chitosan in waste treatment was investigated. The data showed that the modified chitosan was selective for some metal ions, depending on the type of chelating centre furnished with the modification process, e.g., N-N, N-O, O-O, and N-S. The metal ions Fe, Co, and Cu preferred N-O, while Pb, Cr, Ca, Ni, Cd, and Pd preferred the O-O chelating centre. The copper ion preferred the N-S chelating centre more than N-N in the modified chitosan. The removal capacity by modified chitosan ligands for Ca²⁺, Cd²⁺, Fe³⁺, Co²⁺, Pb²⁺, and Mn²⁺ exceed 80%. The modifications and complexations have a significant impact on the morphology of the

produced products. Some of the modified chitosan and their complexes showed a significant effect on fungi and Gram positive and Gram negative bacteria.

ACKNOWLEDGEMENT

The authors kindly acknowledge the financial support of this project by the Research Administration of the Kuwait University through research project grant SC 012 / 15. The analytical services provided by the ANALAB and SAF in the faculty of Science through the grant no. GS-01/01, GS 01/05 and GS 03/08 are also gratefully acknowledged.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Alexander P, Svetlana B. Chitosan and its derivatives as highly efficient polymer ligands. *Molecules*. 2016;21:330-335.
- Pestov A, Bratskaya S. Chitosan and its derivatives as highly efficient polymer ligands. *Molecules* and references cited therein. 2016;21:330-365.
- Cheung RCF, Ng TB, Wong JH, Chan WY. Chitosan: An update on potential biomedical and pharmaceutical applications. *Mar. Drugs*. 2015;13:5156-5186.
- Li B, Fang Y, Shan CL, Ibrahim M, Xie GL, Wang YL, Sun GC. Differential effect of metal ions on antibacterial activity of chitosan against *Burkholderia cenocepacia*. *Asian J. Chem*. 2013;25: 891-894.
- Hadi AG Adsorption of Cd (II) ions by synthesize chitosan from fish shells, *British J. Sci*. 2012;5:33-38.
- Mekahlia S, Bouzid B. Chitosan-copper (II) complex as antibacterial agent: Synthesis, characterization and coordinating bond-activity correlation study, *Physics procedia (Proceedings of the JMSM 2008 Conference)*. 2009;2:1045-1053.
- Yin X, Zhang X, Lin Q, Feng Y, Yu W, Zhang Q. Metal-coordinating controlled oxidative degradation of chitosan and antioxidant activity of chitosan-metal complex. *ARKIVOC*. 2004;ix:66-78.
- Prashanth KVH, Tharanathan RN. Chitin/chitosan: Modifications and their unlimited application potential—an overview, *Trends in Food Science & Technology*. 2007;1:117-131.
- Badawy MEI, El-aswad AF. Insecticidal Activity of chitosans of different molecular weights and chitosan-metal complexes against cotton leaf worm *Spodoptera-littoralis* and *Oleander Aphid Aphis nerii*, *Plant Protect. Sci*. 2012;48:131-141.
- Shigemasa Y, Minami S. Applications of chitin and chitosan for biomaterials, *Biotechn. Genet. Eng. Rev*. 1996;13:383-420.
- Wang X, Du Y, Liu H. Preparation, characterization and antimicrobial activity of chitosan-Zn complex, *Carbohydr. Polym*. 2004;56:21-26.
- Higazy A, Hashem M, Elshafei A, Shaker N, Abdel Hady M. Development of antimicrobial jute packaging using chitosan and chitosan-metal complex, *Carbohydr. Polym*. 2010;79:867-874.
- Wang X, Du Y, Fan L, Liu H, Hu Y. Chitosan metal complexes as antimicrobial agent: Synthesis, characterization and structure-activity study. *Polym. Bull*. 2005; 55:105-113.
- Sy Q, Daj W, Forster Cf. The use of sago waste for the sorption of lead and copper, *Water SA*. 1998;24:251-256.
- Ahmad A, Ghufuran R, Faizal WM. Cd(II), Pb(II) and Zn(II) Removal from Contaminated water by biosorption using activated sludge biomass. *CLEAN – Soil, Air, Water*. 2010;38:153-158.
- Zhang L, Zeng Y, Cheng Z. Removal of heavy metal ions using chitosan and modified chitosan: A review. *J. Molecular Liq*. 2016;214:175-191.
- Guibal E. Interactions of metal ions with chitosan-based sorbents: A review. *Sep. Purif. Technol*. 2004;38:43–74.
- Varma AJ, Deshpande SV, Kennedy JF. Metal complexation by chitosan and its derivatives: A review. *Carbohydr. Polym*. 2004;55:77–93.
- Guibal E, Vincent T, Navarro R. Metal ion biosorption on chitosan for the synthesis of advanced materials. *J. Mater. Sci*. 2014; 49:5505–5518.
- Kurita K. Controlled functionalization of the polysaccharide chitin. *Prog. Polym. Sci*. 2001;26:1921–1971.
- Kurita K. Chitin and chitosan: Functional biopolymers from marine crustaceans. *Mar. Biotechnol*. 2006;8:203–226.
- Wang J, Chen C. Chitosan-based biosorbents: Modification and application

- for biosorption of heavy metals and radionuclides. *Bioresour. Technol.* 2014; 160:129–141.
23. Boamah PO, Huang Y, Hua M, Zhang Q, Wu J, Onumah J, Sam-Amoah LK, Boamah PO. Sorption of heavy metal ions onto carboxylate chitosan derivatives—A mini-review. *Ecotoxicol. Environ. Saf.* 2015;116:113–120.
 24. Fu F, Wang Q. Removal of heavy metal ions from wastewaters: A review. *J. Environ. Manag.* 2011;92:407-418.
 25. Ali RK, Bahare R, Azam K. Effective removal of a cobalt-tetrasulfonated phthalocyanine dye from an aqueous solution with a novel modified chitosan-based superabsorbent hydrogel. *J. Appl. Polym. Sci.* 2018;135:46167.
 26. Shariful MI, Sepehr T, Mehrali M, Ang BC, Amalina MA. Adsorption capability of heavy metals by chitosan/poly(ethylene oxide)/ activated carbon electrospun nanofibrous membrane. *J. Appl. Polym. Sci.* 2018;135(7):45851. DOI: 10.1002/app.45851
 27. Iyengar U, Avs PR. Hexavalent chromium interaction with chitosan. *J. Appl. Polym. Sci.* 1990;39:739-747.
 28. Findon A, McKay G, Hs. Blair Hs transport studies for the sorption of copper ions by chitosan. *J. of Environ. Sci. and Health.* 1993;A28:173-185.
 29. Schmuhl R, Krieg HM, Keizer K. Adsorption of Cu(II) and Cr(VI) ions by chitosan: Kinetics and equilibrium studies, *Water SA.* 2001;27:1-7.
 30. Kyoon H, Park NY, Lee SH, Meyers SP. Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. *Inter. J. Food Microbiology.* 2002; 74:65-72.
 31. Asano T. Proc. First Int. Conf. Chitin/Chitosan, R. A. Muzarelli and E. R. Pariser, eds, Cambridge, MA: MIT Sea Grant report. 1978;78-7:231-252.
 32. Allan GG, Altman LC, Bensinger RE, Ghosh DK, Hirabayashi Y, Neogi AN, Neogi S. In chitin, chitosan and related enzymes, J. P. Zikakis, ed. Academic Press, Inc. 1984;119 -133.
 33. Chen CS, Liao WY, Tsa GJ. Antibacterial Effects of N-sulfonated and N-sulfobenzoyl Chitosan and Application to Oyster Preservation. *J. Food Prot.* 1998;61:1124-1128.
 34. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *J. Biomed. Mater. Res.* 2000; 52:662-668.
 35. Thovhogi N, Park E, Manikandan E, Maaza M, Gurib-Fakim A. Physical properties of CdO nanoparticles synthesized by green chemistry via *Hibiscus Sabdariffa* flower extract. *J. Alloys and Compd.* 2016;655:314-320.
 36. Keri RS, Patil MR, Patil SA, Budagumpi S. Comprehensive review in current developments of benzothiazole-based molecules in medicinal chemistry *Eur. J. Med. Chem.* 2015;89:207-251.
 37. Hisamoddin SZK, Sanchaity P, Sharma PY, Patel NU Benzothiazole the molecule of diverse biological activity. *Pharma Sci. Monitor.* 2014;5:207-225.
 38. Yang Y, Cui M. Radiolabeled bioactive benzoheterocycles for imaging β -amyloid plaques in Alzheimer's disease. *Eur. J. Med. Chem.* 2014;87:703-721.
 39. Manoj NB, Mayuri AB, Hitesh DP. synthetic strategies for fused benzothiazoles: past, present, and future, *synth. Comm.* 2014; 44:2427-2457.
 40. Xie W, Xu P, Wang W, Liu Q. Preparation and antibacterial activity of a water-soluble chitosan derivatives, *Carbohydr. Polym.* 2002;50:35-40.
 41. Je JY, Kim SK. Chitosan derivatives killed bacteria by disrupting the outer and inner membrane, *J. Agric. Food Chem.* 2006; 54:6629–6633.

© 2018 Al-Fulajj et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://prh.sdiarticle3.com/review-history/23903>