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Synthesis of Chitosan Derivatives with Protecting and Deprotecting (With Amino Group Protection) Approach for Versatile Class of Antimicrobial Agents and Adsorbents

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Abstract:
Chitosan and modified chitosan have been proved for numerous biological and water treatment applications including as strong antimicrobial agents. In this work the main target of functionalization of chitosan with amine group protection. Chemical modification of chitosan serves as a great opportunity to design and develop broad spectrum antibacterial agents. Chitosan derivatives were synthesised and tested for antimicrobial action against gram positive and gram-negative bacteria as well as for Fungi. The result of FTIR and NMR analysis disclosed successfully protection of the Amine group and covalent attachment of different functional groups with polymer backbone. The chemically modified polymers were found active against both bacteria and fungi with MIC value (MIC = 100-250 µg/mL). Moreover, the derivatives were shown higher activity with lower MIC values as compare to reported Chitosan derivatives with different organic synthetic protocol. Chemically modified chitosan was evaluated as an adsorbent for the removal of copper (II) and nickel (II) from water. Experimental data of Atomic Absorption spectroscopy shows higher affinity towards Nickel (II) as compared to Copper (II).

Keywords: chitosan derivatives, Antimicrobial Agent, adsorbent, heavy metals, AAS.

1. INTRODUCTION

Chemical alteration at different positions in chitosan offers enormous applications in biomedical, bio-sorbent, pharmaceuticals, anticancer, wound-healing agents [1-2]. Antibiotics have become essential for pathogenic infectious diseases; however, overuse of antibiotics threaten human health due to resistance development in bacteria. In addition, with weak immune system fungal infections are becoming a major problem in current pandemic situations, so that the design and development of new antibacterial agents has become a demand at this juncture [3-5]. Modification of chitosan provides versatile tool for finding new antimicrobial agent because chitosan is perfect raw material for Chemical modification and explore several biological applications due to its

properties like nontoxicity and biocompatibility [6-10]. It shows broad range of activity against gram-positive and negative bacteria and fungi. Highly modified chitosan become less soluble in water and results poorly effective against Gram-positive and Gram-negative bacteria. However, presence of amino groups helps to improve solubility by forming ammonium cation < 6 pH and likely to increase antimicrobial activity [11-15]. Close proximity of charged ammonium functional group to chitosan backbone and presence of spacing fragments also improve the antimicrobial activity. Working on this hypothesis, we have installed various fragments at hydroxyl group of chitosan without affecting amino groups [16-19].

We introduce amino group protection and followed by de-protection to allow selective alterations at the reactive centres as well as high degree of substitution in the products.

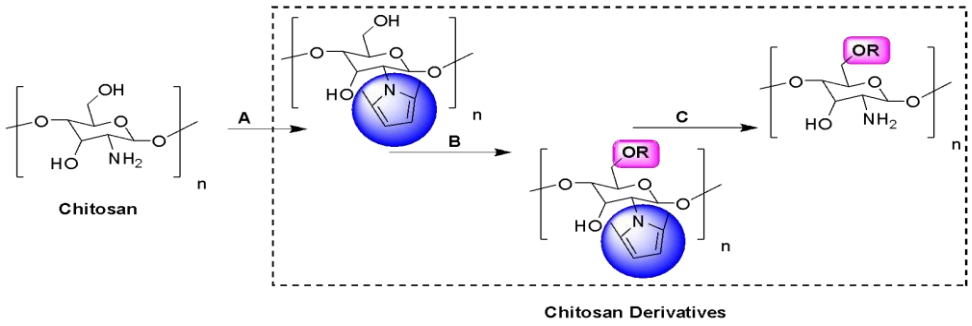


Figure 1 protection & deprotection of amino group in chitosan

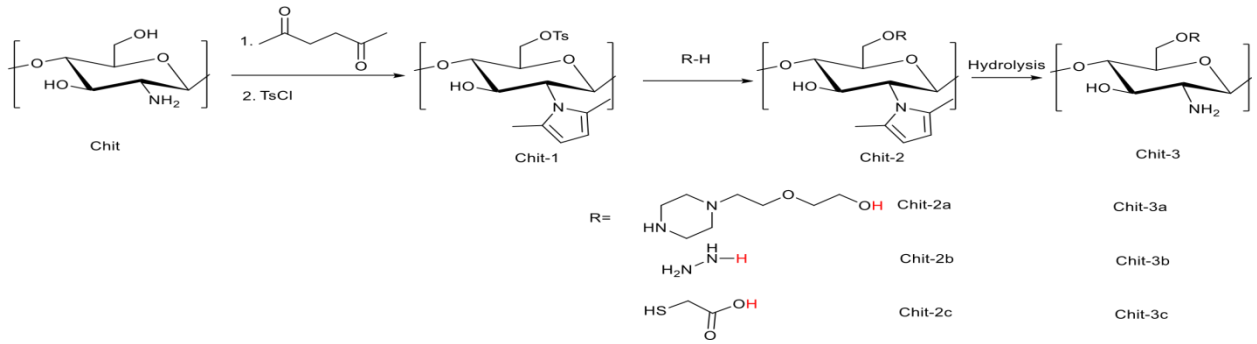
Utilising above synthetic protocol, we synthesised seven compounds, followed by purification, analysis and tested for antimicrobial activity. For this purpose, we chose a typical series of sample organisms, including a Gram-positive bacteria strain (Streptococcus sobrinus), a Gramnegative bacteria strain (Neisseriasubflava), and a fungus (Candida albicans) [16].

Chemically modified chitosan has drawn a lot of attention as an adsorbent for contaminants, because of its excellent adsorption potential and inexpensive cost. Chitosan's use in wastewater treatment is restricted by flaws such limited mechanical strength, low thermal stability, and sensitivity to pH. As per Scheme 1, the functional groups of chitosan can be modified to improve their performance via amino group protections, followed by easy removal of protected group. In the present study, the removal of copper, nickel, and other heavy metals by chemically modified chitosan with amino group protection is briefly discussed [20-24].

2. EXPERIMENTAL SECTION:

Materials: Chitosan (medium molecular weight, > 75% deacetylated) was obtained from Acrosoganics. Chitosan). All other used reagents were purchased from TCI, India. NMR, Mass, IR and Uv instruments;

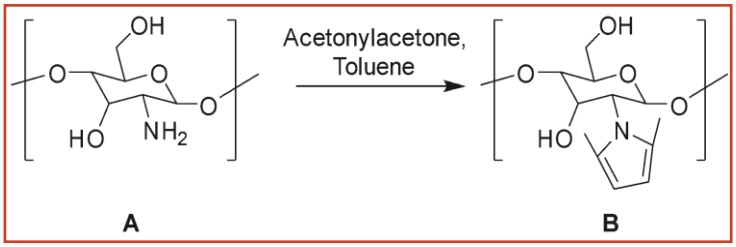
2.1 Chemical modification of chitosan



Scheme-1 Chemical modification of chitosan for possible applications

2.1.1 Compound-B

Paal Knorr reaction is widely is used to protect amine groups, amine group protection in chitosan backbone achieved by 2,5-hexandione . Primary amine groups of chitosan transform into stable 2,5-dimethylpyrolle moiety. Conversion of -NH2 group confirm by HNMR analysis



2.2.2 Compound-C

In general,primary hydroxyl group is much more reactive compared to secondary hydroxyl group with p-toluenesulfonyl chloride. Compound-B is converted into compound-C with ptoluenesulfonyl chloride, in the presence of triethylamine. The structure of product confirmed by NMR analysis. Conversion is calculated by HNMR, considering ratio of primary and secondary hydroxyl groups.

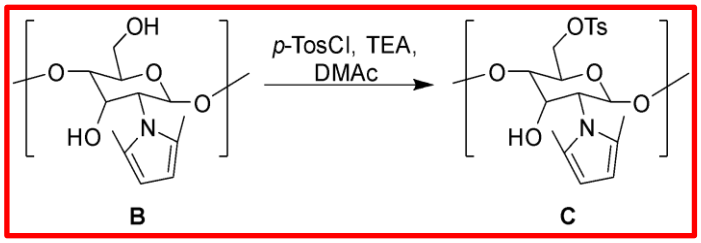


Figure 3 Structural motif of compound B and C

2.1.3Compound-D1

Tosyl group is good leaving group, therefore, it can be replaced by desired group easily. Compound-C was transformed into dark brown coloured compound-D1, by treating it with compound-1. Compound-1 like structures reported effective in antimicrobial activities. Product is isolated, washed and dried

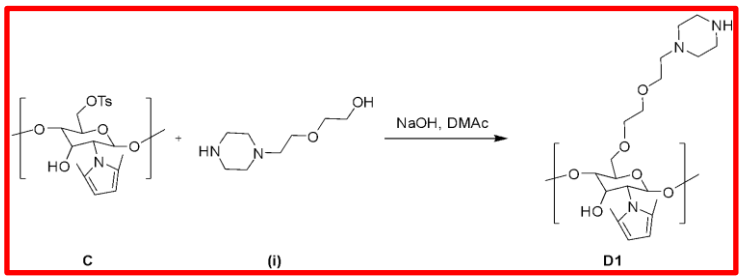


Figure 4 synthesis of Compound-D1

2.1.4 Compound-E1

Compound-D1 (700 mg) in EtOH (150 mL) was taken in 250 mL 3 necks RBF with glass condenser. Hydroxylamine hydrochloride (5gm) and water (20mL) was added to reaction mixture and allowed to stir at 90°C for 16 hour. The reaction mixture converted into light brown coloured. After cool the mixture, EtOH filtered, washed with chloroform (CHCl3) and vacuum drying with a rotary evaporator. Dry product is collected.

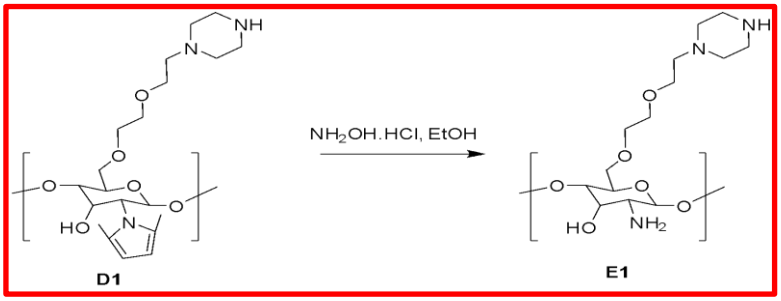


Figure 5 Structural motif of compound D1 and C1

2.1.5 Compound-D2

Compound-C (1g) in DMAc (100 mL) was taken in 250 mL 3 necks RBF. Compound-(ii) (3g) was dissolved in aqueous solution of NaOH (20mL) and added dropwise into RBF. The reaction mixture was stirred at 120°C temperature for 24 hour. During this reaction compound(C) converted into light yellow coloured. Reaction mixture cooled and directly filtered to remove solvent and washed with chloroform (CHCl3) to remove excess solvent and impurities.

After vacuum drying with a rotary evaporator, dry product is collected.

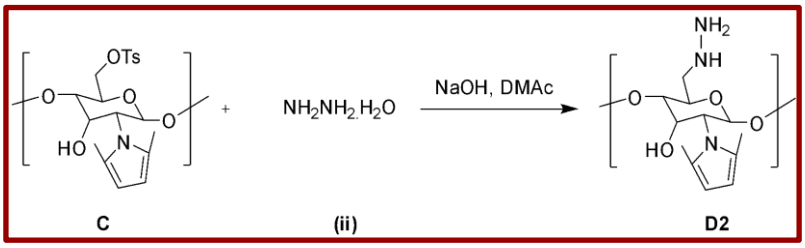


Figure 6 Structural motif of compound C and D2

2.1.6 Compound: E2

Compound-(D2) (700mg) in EtOH (150mL) was taken in 250mL 3 necks RBF with a glass condenser. Hydroxylamine Hydrochloride (5gm) and water (20mL) was added to the reaction mixture and allowed to stir at 90°C for 16 hours.The reaction mixture converted into Light brown

coloured. After cooling the mixture, EtOH filtered, washed with Chloroform(CHCl3) vacuum drying with a rotary evaporator. Dry product is collected.

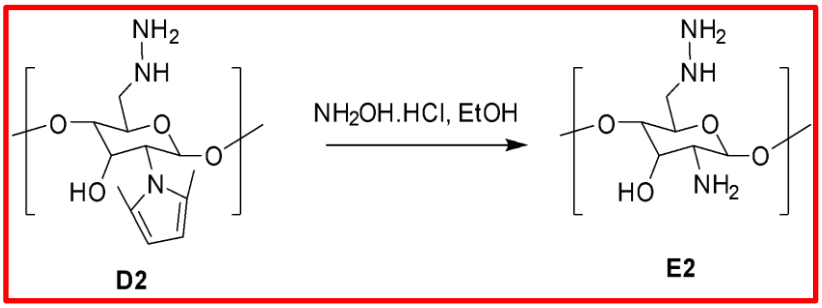


Figure 7 Structural motif of compound D2 and E2

2.1.7 Compound: S1

A four neck glass R.B.F charged with compound B and thioglycolic acid. Above mixture cooled to 0 °C, added con. sulphuric acid and allowed to stir at 90 °C overnight with precure to safety shield for 16 hours. The reaction mixture converted black-brown coloured. Resulting mixture diluted by water and filtered through cotton-cloth. Filtered black precipitates were washed with excess water to remove acidity and dried under reduced pressure to obtain S1 as black powder.

2.1.8Compound: S2

A single neck glass R.B.F charged with S1 in ethanol. To the above mixture, add hydroxylamine hydrochloride in excess amounts (10 eq.) and water, and the resulting mixture is allowed to stir at 120°C overnight with an assembled condenser. The reaction mixture converted black to brown coloured. Progression of reaction was checked by TLC. Upon completion, the mixture is diluted by water and basified with NaHCO3, filtered through cotton-cloth. Filtered black precipitates, which was washed with excess water wash to remove acidity and dried under reduced pressure to obtain S2 as black powder.

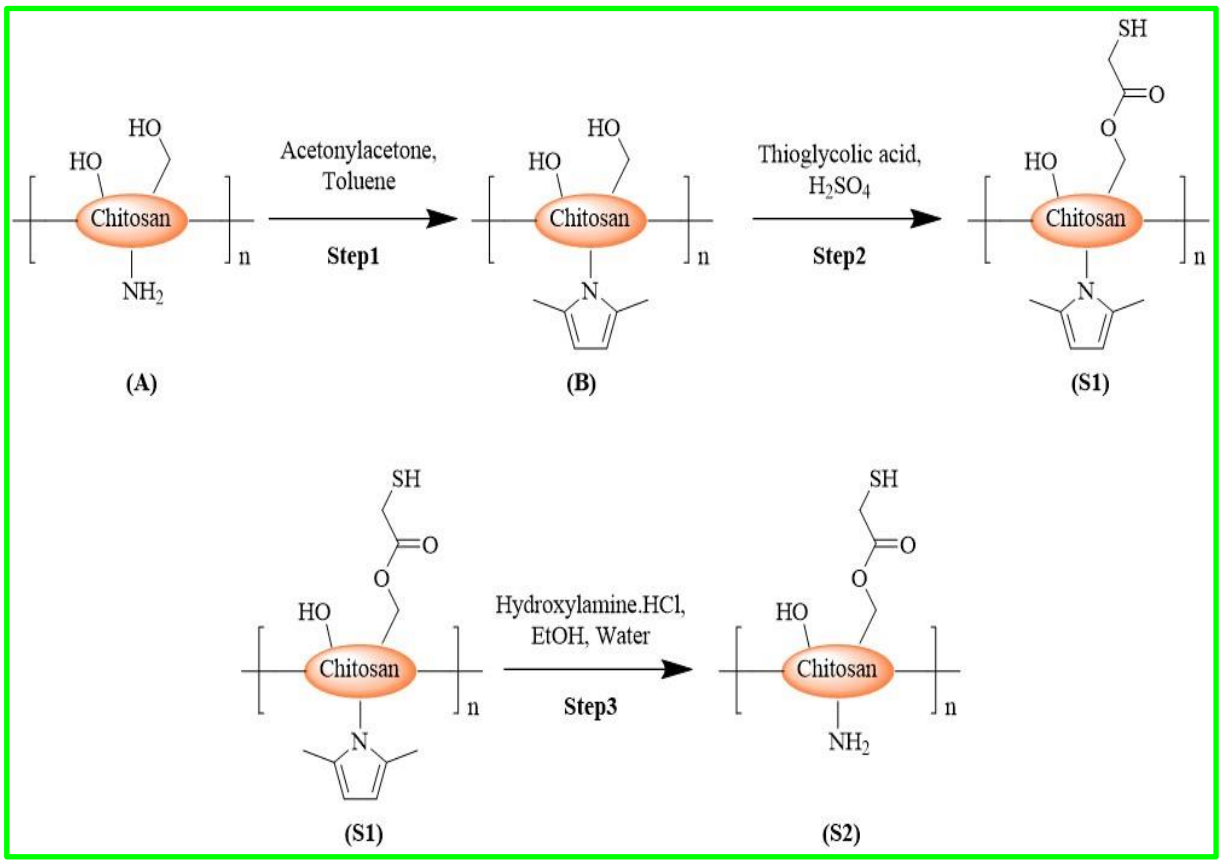


Figure 8 SEQ Figure * ARABIC 8 Reaction scheme for S1 and S2 synthesis

2.2 Adsorption experiments of chemically modified chitosan.

Each experiment on metal adsorption was carried out in batch mode using 125 mL Erlenmeyer flasks holding 50 mL of trace metal solution. Stock solutions were prepared at 1000 mg/L of Copper (II), Nickel (II). The experiments were carried out at a neutral pH range. All the synthesised adsorbents were compared with chitosan for Cu (II) and Ni (II) removal with different concentration range of synthesised water samples from the stock solution. All of the synthesised adsorbents were compared to chitosan for the removal of Cu (II) and Ni (II) in synthesised water samples from stock solution across a range of concentrations.

The following equation was used to estimate the amount of metal ions per unit mass of the adsorbent:

$qe = (C0-Ce)*V/m$

where Ce (mg. L^{-1}) is the concentration of Ni (II) and Cu (II) after equilibrium with different (L) is the volume of the solution, C_0 is the concentration of Ni (II) and Cu (II) prior to the adsorption, and q_e is the adsorbed amount of metal ions per unit mass of the adsorbent (mg. g^{-1}) at equilibrium.

3. Results and Discussion:

3.1 NMR analysis of protection of amino group

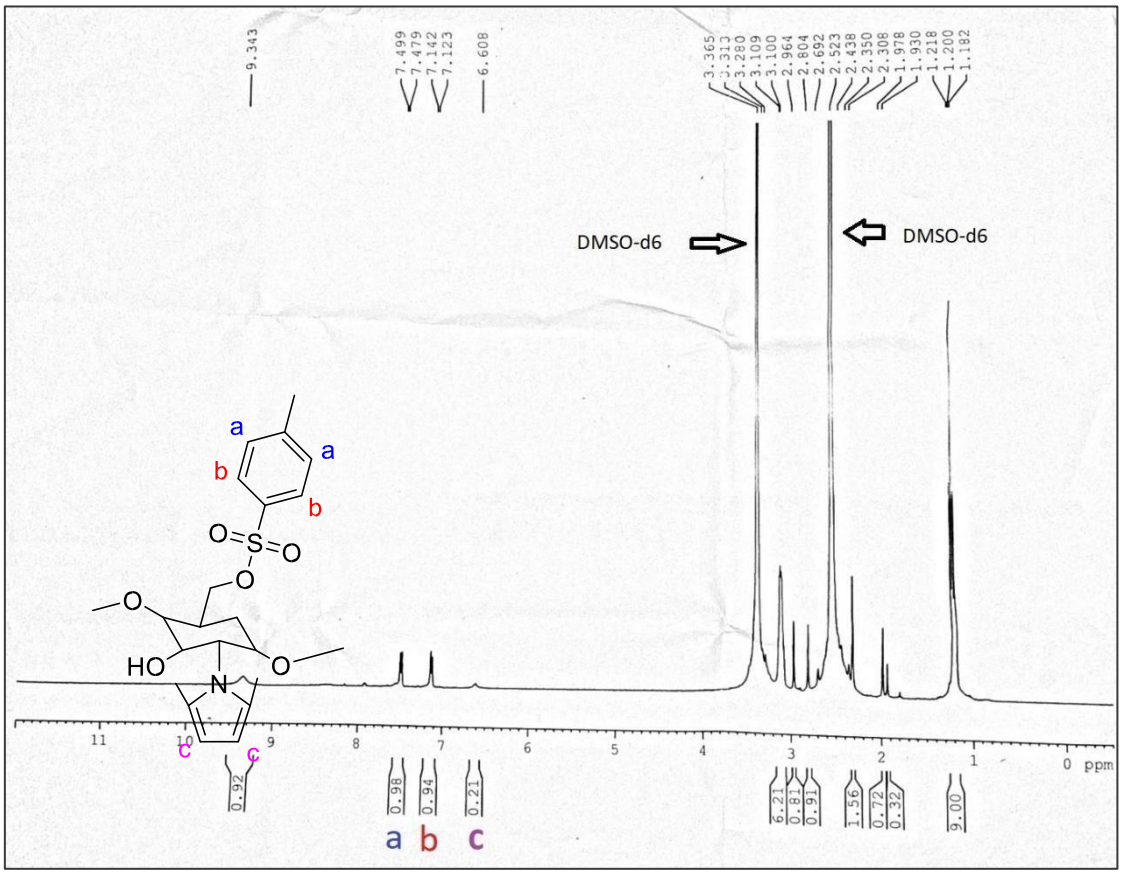
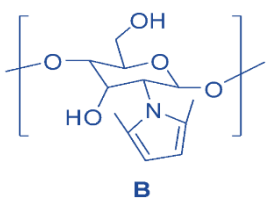
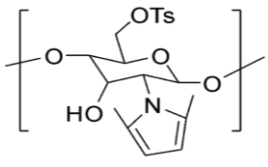
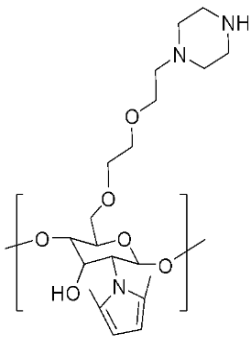
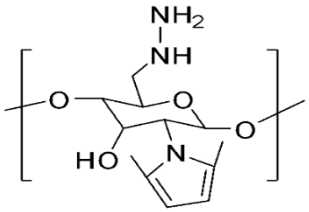
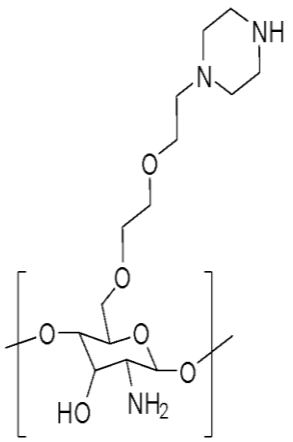


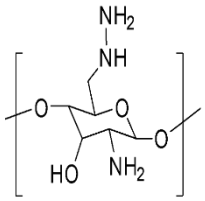
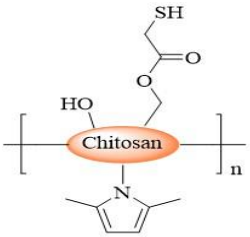
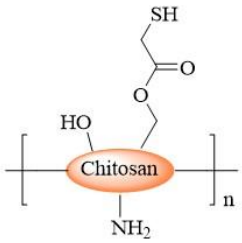
Figure 9 NMR of Compound-B

NMR analysis shows three different types of proton present between 6 and 8 ppm. It indicates the formation of a five-membered nitrogen atom-containing ring generated from chitosan, which has a free primary amino group at the polymeric backbone. NMR data also suggested partial protection of amino groups because chitosan polymer has a number of free amino groups, so they were partially convertedinto payroll rings and the rest of the groups still in unconverted form, which was the desire for free adsorption experiments [25-26].

3.2 Antimicrobial Activity Result:

S R. NO	CODE & Structure of Modified Polymers	*E. COLI* Gram-negative	*P. AERUGINOSA* Gram-negative	*S. AUREUS* Gram positive	*S. PYOGENUS* Gram positive
		MTCC 443	**MTCC 1688**	**MTCC 96**	**MTCC 442**
1	 **B**	62.5 microgram /ml	100 microgram /ml	125 microgram /ml	100 microgram /ml

2	 C	100 microgram /ml	125 microgram /ml	50 microgram /ml	62.5 microgram /ml
3	 D1	100 microgram /ml	250 microgram /ml	100 microgram /ml	250 microgram /ml
4	 D2	62.5 microgram /ml	100 microgram /ml	62.5 microgram /ml	50 microgram /ml
5	 E1	100 microgram /ml	125 microgram /ml	250 microgram /ml	500 microgram /ml

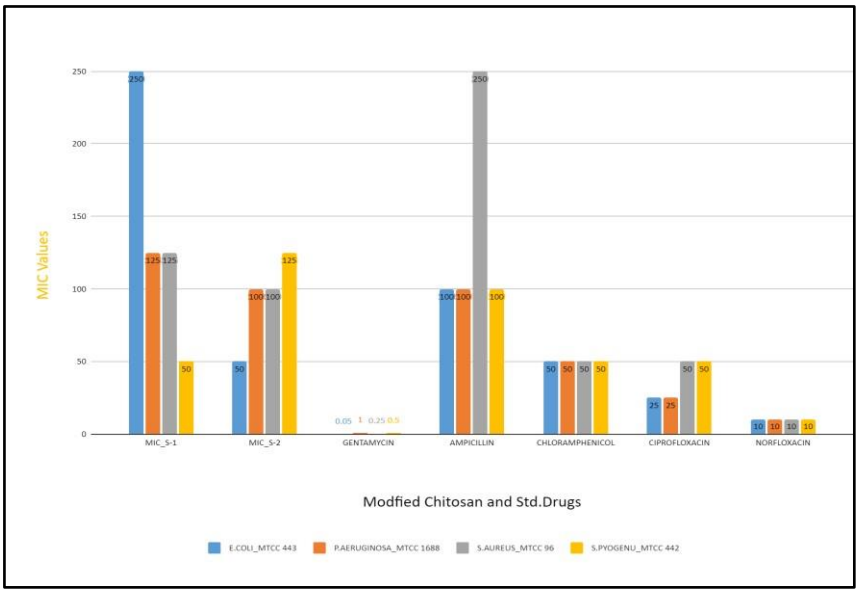
6	 E2	125 microgram /ml	100 microgram /ml	50 microgram /ml	62.5 microgram /ml
7	 (S1)	250 microgra m /ml	125 microgram /ml	125 microgra m /ml	50 microgram /ml
8	 (S2)	50 microgra m /ml	100 microgram /ml	100 microgra m /ml	125 microgram /ml

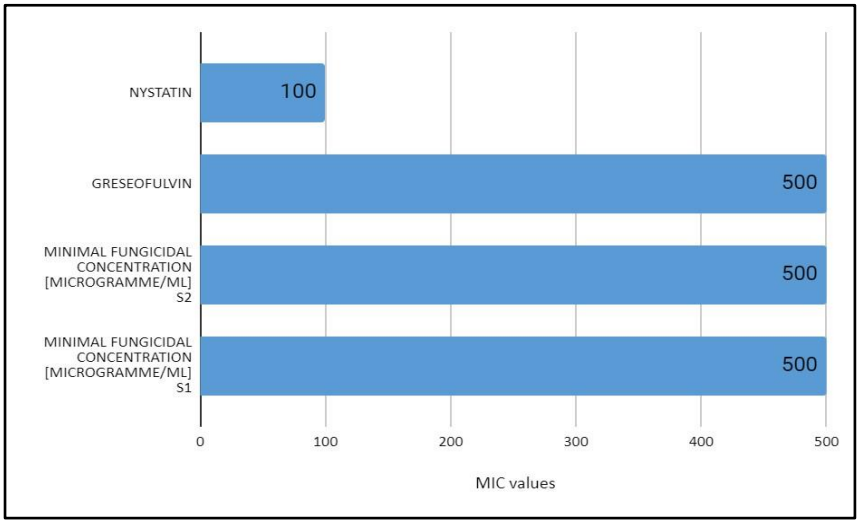
3.3 Comparison of Antimicrobial activity of various chitosan derivatives against *S. aureus, E. coli* and *P. aeruginosa:*

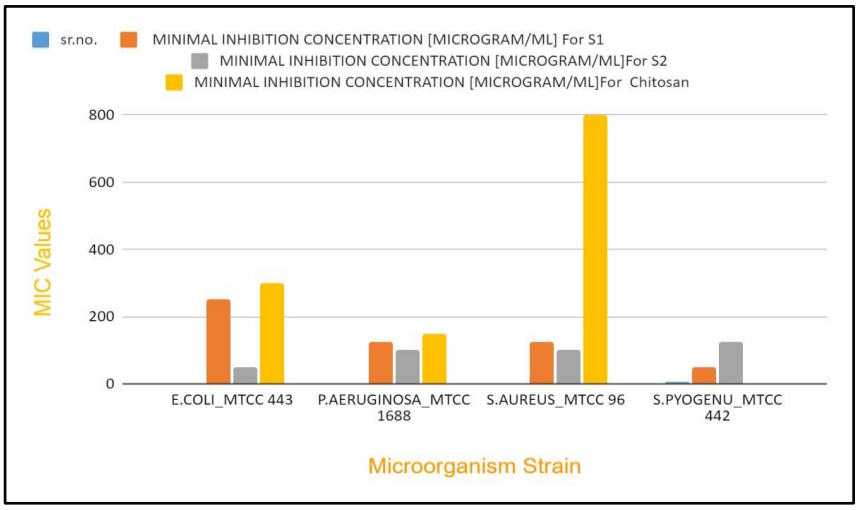
Antimicrobial activity of various chitosan derivatives against S. aureus, E. coli and P.aeruginosa

		Bacteria & MIC values Microgram/ml		
Sr.No.	Chemically modified Chitosan	*Escherichia coli*	*Pseudomonas eruginosa*	*Staphylococcus aureus*
1	Cyanoethyl Chitosan	312	Not reported	19
2	Aminoethylchitosan	62.5	7.8	125
3	Dimethylaminoethyl chitosan	62.5	15.6-32.5	125
4	Diethylaminoethyl Chitosan	250-500	62.5-125	250
5	N-ethyl chitosan	64	128	32
6	N-butyl chitosan	128	128	64

	N,N,N-trimethy chitosan	2000	Not Reported	2000
7	N-(2-(N,N,N-Trimethylammonium)acetyl) chitosan	128-16384	64	2000
8	N-(2-(1-Pyridinium)acetyl)chitosan	512-1024	512	8-1024
9	N-(3-Pyridylmethyl) chitosan	2000	NR	2000
10	N-dodecylchitosan	128	64	256
11	(Acetyl/Chloroacetyl/Benzoyl)Phenylthiosemicarbazone	7	NR	56->250
12	(O-carboxymethyl/N,Ocarboxymethyl) chitosan6	320/62.5	NR	320/31.3
13	Quaternary(Me/Et/Pr/But/Benz)carboxylethylchitosan	6.3-31.3	NR	6.3-31.3
14	(Acetyl/Chloroacetyl/Benzoyl(thioureacarboxymethyl chitosan	62.5-500	NR	7.8-62.5
15	**Compound_B**	**62.5**	**100**	**125**
16	**Compound_C**	**100**	**125**	**150**
17	**Compound_D1**	**100**	**250**	**100**
18	**Compound_D2**	**62.5**	**100**	**62.5**
19	**Compound_E1**	**100**	**125**	**500**
20	**Compound_E2**	**125**	**100**	**50**
21	**Compound_S1**	**250**	**125**	**125**
22	**Compound_S2**	**50**	**100**	**50**

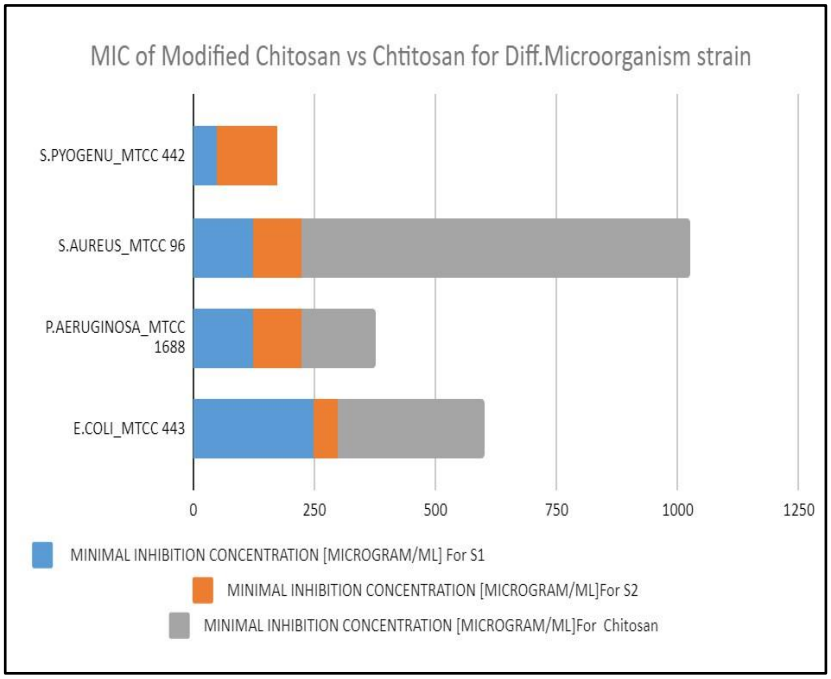






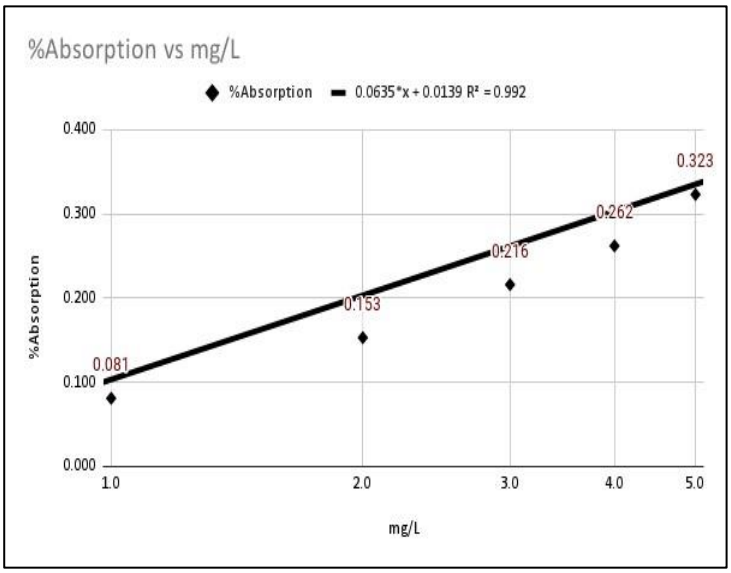
synthesized chitosan derivatives were tested for antibacterial and antifungal activity with different microbial strain with Microbial Type culture Collection MTCC-443, MTCC -1688, MTCC-96 and MTCC-442, experimental results show Compound (S1) and (S2) are effective against bacterial strain and for fungal strain. These Compounds have comparable activity against pathogens.

Modified chitosan compared with reported Chitosan as per Table 3.3. Above data shows Lower MIC value is found in modified chitosan with amine Protection,



3.3 Batch adsorption experiment results:

Compound-S1 and S2 were tested for Copper And nickel removal from aqueous samples. Concentration of nickel and copper at 120 and 240 minutes measured with atomic absorption Spectrometer. Modified Chitosan with thiol group Shown good Affinity to copper and nickel due to presence of -SH group and -OH groups at side chain of modified Chitosan. Present Study revealed that Compound -S2 have free amino group and Presence of -Sh group shows tremendous Selectivity towards nickel ions. for high concentration 125 ppm Compound-S2 can trap 98.6% nickel ions from aqueous medium and it will be confirmed by AAS with std.
method at wavelength.



for First 120 minutes q_{120}=22.5 mg/g and q_{240}=30.9mg/g and removal for 120 minutes 72% while for 240 minutes reached 98.6%, it shows comparative good capacity in class of modified polymer as adsorbent for nickel removal. current study result aligned with theory of Adsorption kinetics initially rate is higher due to availability of more nickel ions in solution but at higher contact time most of site of adsorbent is occupied so rate decrease as per time increases. Most removal is achieved in the first 2 hours but adsorbent is not reached at its equilibrium so with slow rate adsorption of nickel continues after 3 hours also but rate is very slow due to less availability of ions in aqueous medium. In present work experimental data tested for PSO, PFO, IPD and Elovich model for both linear and nonlinear models. Linear and Nonlinear PSO model gives excellent fit with following data

Kinetic Models	K	q_e	Experimental q_e
Linear Pseudo second order Kinetic model	0.002177474	30.86	30.82
Non Linear Pseudo second order Kinetic model	0.000133	48.83	30.82

The rate constant K in range characterizes the adsorption rate. The small value of K suggested a slow adsorption rate

time	q_t	t/q_t	slope	1/slope = q_e	Intercept	q^2_e	K=1/Inter cept*qe^ 2	R^2
0	0	0	0.03	30.86	0.48	952.6	0.002	0.96
120	22.47	5.33						
240	30.82	7.78						

The adsorption process seems to reach equilibrium over time, as evidenced by the increase in qe from 120 to 240 minutes. However, at 240 minutes, the experimental value qe = (30.825 mg/g) is closer to the calculated value. The experimental results at 240 minutes are consistent with the calculated qe, suggesting that the modified CS-2 is effective in nickel removal. Overall, considering the trend and proximity to the calculated value, it appears that the adsorption process is worth pursuing for practical applications.

Intercept value provides insight into the thickness of the boundary layer. A lower *C* value suggests that the boundary layer effect is minimal, implying that the external mass transfer resistance is not significant compared to the intra-particle diffusion.

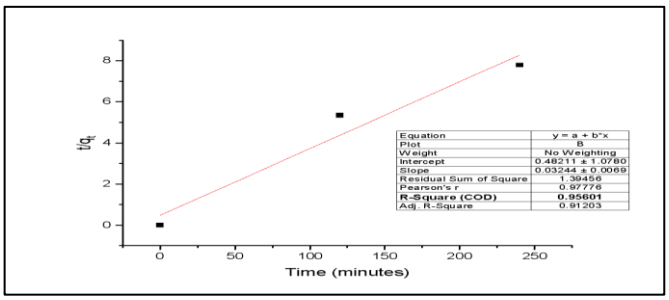
The constant value for the linear intra-particle diffusion rate constant Kdiff is already provided as: Slope = kdiff =2.0012 mg/g.This constant value is crucial as it quantifies the rate at which the adsorbate (nickel) diffuses into the pores of the adsorbent. Higher values of Kdiff typically indicate more efficient diffusion, which is desirable in adsorption processes for effective pollutant removal.

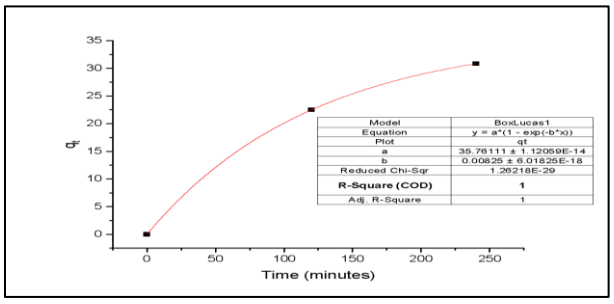
Comparison and Interpretation –IPD models:

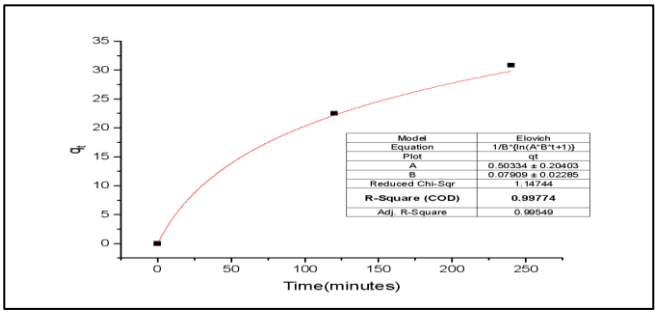
IPD model	Slope=Kdiff	intercept	Equation
Linear Model	2.00	0.1253	
Non Linear model	1.99	0.1568	

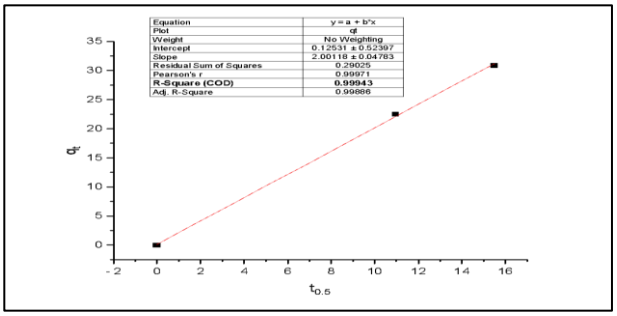
Both models suggest a relatively high rate of intra-particle diffusion for nickel removal. The linear model shows a slightly lower boundary layer effect compared to the nonlinear model. The diffusion constant is very similar in both models K_{diff}=2, indicating that the rate of intraparticle diffusion is consistent across different modelling approaches.

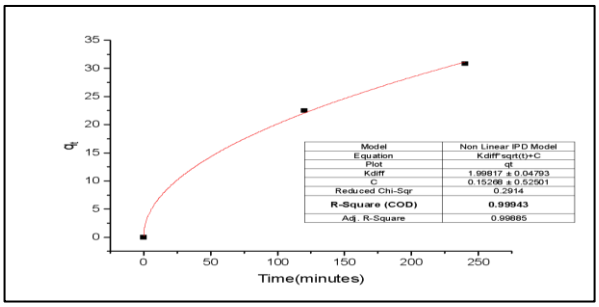
Elovich model: This model is based on the assumption of a chemisorption process with a decreasing rate of adsorption over time. Elovich model satisfied in nonlinear fashion with R2=0.99. Elovich model gives two constant values alpha and beta from this value interpretation gives inside valuable information regarding adsorbent and adsorbate interaction here Elovich model strongly supported that chemisorption is dominant process in nickel removal with thioglycolic acid modified Chitosan derivatives. α value of 0.50 suggests a moderately fast initial adsorption rate. It implies that at the beginning of the adsorption process, there is a significant number of active sites available on the surface of the chemically modified chitosan for nickel ions to adsorb onto. β is the coefficient associated with the exponential term in the Elovich equation. In this case, it is 0.079. This value reflects the influence of the coverage of the adsorbate on the surface on the rate of adsorption. β value of 0.079 indicates a moderate effect of surface coverage on the adsorption rate. It suggests that as the adsorption progresses, the rate of adsorption gradually decreases due to the increasing coverage of the adsorbent surface by the adsorbed nickel ions. However, this decrease in the adsorption rate is not very rapid, indicating that the surface saturation is not immediate.



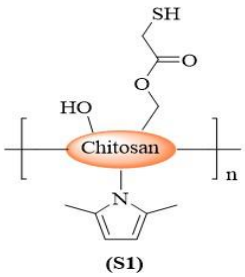


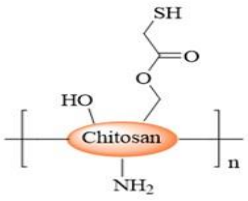






Comparison of Adsorbent capacity with Batch Adsorption experiment:

Adsorbent: S1 And S2	Contact Time (min)	Adsorbent Dose (gm)	C_0	C_i (ppm)	W (gm)	V (L)	$q_t = (C_0 - C_i) \times V / W$ (mg/g)
 (S1)	120	0.4	125	108	0.4	0.1	4.25

 (S2)	120	0.4	125	35.1	0.4	0.1	22.475

Compound (S1) is less efficient; this happens due to the protection of the amine group and the lower availability of this electron-removing -NH2 group. This fact is responsible for the lower removal efficiency of nickel Cation. In other cases, deprotection of amine further enhances the capability of nickel-capture of compound (S2). Effective protection and deprotection will be helpful to design a desired adsorbent with selectivity. In the present study, chemisorption is a rate-determining step, and it is also suggested that chemical interaction is enhanced due to the deprotection of the side chain NH2 group. Compound (S1) shows only 13.6% removal in 120 minutes, and (S2) shows more than 70%. This marginal difference in cation removal capacity is directly related to the availability of the free amine group of Chitosan.

Conclusion:

In this work, chemical modification of chitosan was achieved via protection of the amino group, and compounds B, C, D1, D2, E1, E2, S1, and S2 were synthesized and tested for antibacterial and antifungal activity. Chemically modified chitosan showed antimicrobial activity comparable with other reported chitosan derivatives. Both S1 and S2-thiolated chitosan showed better bacteriostatic action and also acted as antifungal agents. Both compounds have activity comparable to that of existing antifungal agents. In addition, these thiolated chitosan are promising adsorbents for nickel and copper removal. The deprotected S2 compound shows 22.5 mg/g removal capacity. Kinetic studies reveal that it follows a pseudo-second-order reaction model. Maximum removal capacity shown by S2 q_e = 30.86 mg/g, which is close to the experimental value of 30.82 mg/g. All other kinetic models are tested and satisfying, and the kinetic model parameter value is aligned with experimental results. Theoretically, S2 is more active due to the more available free site for binding cations due to deprotection and side chain substitution with sulfur-containing groups. above fact also experimentally supported by present experimental work with higher efficiency of S2-Compound.

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