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Synthesis, Characterization and Antimicrobial activity of chitosan 4-chlorobenzaldebyde Schiff base

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Abstract

A chemical compound or mixture of compounds made up of repeated structural units formed through a process of polymerization is termed as polymer. In past decades, researchers were focused on biodegradable for its applications in the field of tissue engineering, gene therapy; wound healingand controlled drug delivery system. In our present work, Schiff base was prepared by reacting chitosan with p-clorobenzaldehyde. The derivative was characterized by FTIR spectroscopy, TGA, DSC, XRD, SEM and tested towards the cell uptake. The uncross linked chitosan Schiff base derivative was studied for its antimicrobial activities.

Keywords: Synthesis, characterization, antimicrobial, chitosan, chlorobenzaldebyde, schiff base.

Introduction

Schiff bases' or imines are most commonly used organic compounds. They are used as artificial intermediates and also in co-ordination chemistry[1].

The compounds with the structure of –C=N-(azomethine group) are known as Schiff bases, which are usually synthesized from the condensation of primary amines and active carbonyl groups[2].

As imines or azomethine group are privileged ligands , they can replace the carbonyl group of the aldehyde or ketone analogy in Schiff base compounds, because of their simple preparation in an one-pot condensation of aldehydes (or ketones) and primary amines in an alcohol solvent[3].

Degree of substitution (DS) is the extension of Schiff base formation. It is the number of free amino groups in relation to the Schiff bases on the substituted bio-polymeric matrix. This is an important factor that plays an important role in the application of Schiff bases.

Several chitosan Schiff base derivatives have been reported in literature. Thermal stability of chitosan Schiff bases was studied[4], where the reaction of chitosan with aromatic aldehydes was studied. The thermal process of the prepared polymer takes place at temperatures lower than that of chitosanindicating that all the Schiff base polymers are less stable than chitosan.

It seems that the instabilities of the Schiff base polymers compared with chitosan are due to the absence of the free amino group in the prepared polymers since they are replaced by the aromatic aldehyde.

Material and Methods

Chitosan was kindly gifted by Central Institute of Fisheries Technology, Willingdon Island, Cochin. p-clorobenzaldehyde from Sigma Aldrich, India and all other chemicals used are of analytical grade. Millipore water was prepared in the laboratory by double distillation of deionised water in quartz distillation.

Fourier transform infrared studies (FT-IR): Fourier transform infrared spectra of chitosan Schiff base derivative using KBr pellet method were recorded in the frequency range of 400–4000 cm^{-1} using SHIMADZU spectrophotometer.

X–ray diffraction studies (XRD): X–ray diffractograms of samples were obtained using an X – ray powder diffractometer (XRD – SHIMADZU XD – D1) with Ni – filter and Cu Kα radiation source. The relative intensity was recorded in the scattering range 2θ, varying from 10° to 90°.

Thermogravimetric analysis (TGA): Thermogravimetric analysis of the chitosan Schiff base derivatives was conducted using the instrument SOT Q600 V8.0 Build 95, to measure their weight loss at different temperatures in the heating range 20° - 850^0 C at a heating rate of 20^0C per minute.

Differential Scanning Calorimetric Analysis (DSC): The thermal behaviour of the chitosan Schiff base derivatives was studied using NET 2 SCH DSC thermal analyzer. The samples were inserted into the Aluminium pan and DSC scan was made from 30°–300^oC in nitrogen atmosphere at a heating rate of 20^oC per minute. The results were recorded and analyzed.

Scanning Electron Microscopy (SEM): SEM analysis was carried out in order to get surface morphology and cross section

morphology of chitosan Schiff base derivatives. With the help of the images obtained the compatibility of the mixtures of chitosan Schiff base derivatives was verified. To analyze the samples, the films were cut into pieces of different sizes and coated with a thin gold – palladium layer by a sputter coater unit (UG – microtech, UCK field, UK) and the cross section topography was analyzed with Cambridge Stereoscan 440 Scanning Electron Microscope (Leica, Cambridge UK).

Results and Discussion

FTIR studies: The structural and compositional information of concerning environmental samples can be obtained with the help of Fourier Transform Infrared (FT-IR) spectroscopy[5].

The FT-IR spectrum of chitosan 4-chlorobenzaldebyde Schiff base was represented in the table-1 and figure-1. It showed some additional peaks to that of chitosan. A peak at 1570 cm^{-1} was due to the deforming vibration of NH_2 and the C-O absorption peak of hydroxyl group became stronger and shifted to 1062 cm^{-1}. A band at 3537.45 cm^{-1} is noticed due to the O-H stretching vibration. In addition, the characteristic absorbance of NH_2 disappearance. The results in table 3.3 indicate that NH_2 group reacted with 4-clorobenzaldehyde. Further new peaks at 1629 and 1408 cm^{-1} appeared in the spectra which are the characteristic bands for the imine group (-N=CH) and aromatic ring (C=C), respectively. A weak peak at 779 cm^{-1} is due to the stretching vibration.

TGA studies: Thermogravimetric Analysis (TGA) provides the information about the amount and rate of change in the weight of a material as a function of temperature or time in a controlled atmosphere.

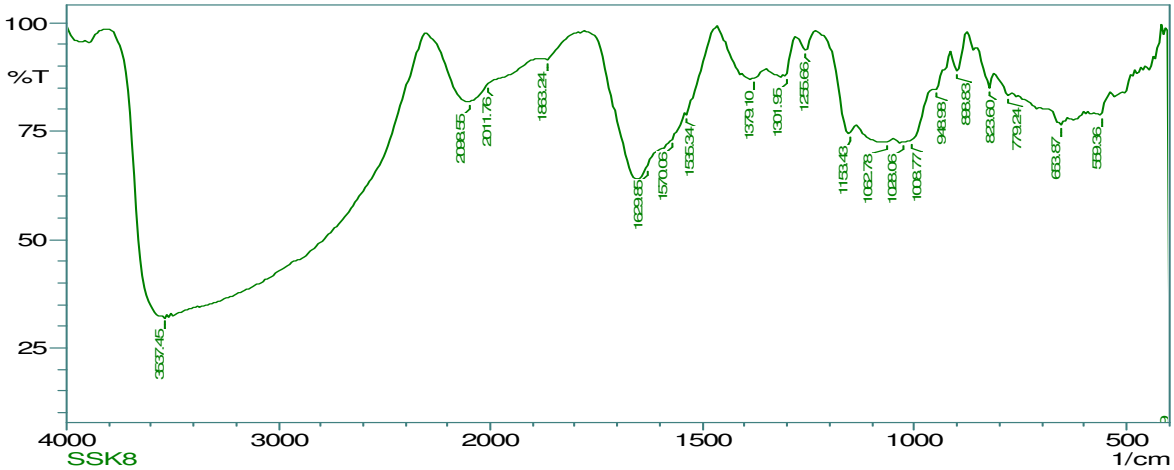

Figure-1
FTIR spectral of chitosan 4-chlorobenzaldebyde
3537.45(s),2098.56(w),1629.85(m),1570.06(m),1535.34(m),1408.04(w),
898.83(w),1379.10(w),1153.43(m),1062.78(m),1028.06m(w),779(w),823.60(w),653.87(w),589.56(w)

Table-1
FTIR spectral

Absorption Frequency (υ cm^{-1})	**Responsible Functional Groups**
3537.45	Intermolecular hydrogen bonded OH stretching and NH stretching
2098.56	C=NH group vibration
1629.85	Amide I band (C=O stretching in secondary amides) and C=N stretching (Schiff base)
1570.06	Amide II band (-NH bending in secondary amides)
1535.34	Amide-III band (CH_3 bending)
1408.04, 898.83	OH in plane bending and CH deformation
1379.10	CH deformation vibration in aldehydes
1153.43	Symmetrical CH bending in CH_3 (wagging)
1062.78	combined ring and C-Cl stretching vibrations - para- substituted
1028.06	C-O-C stretching
779,823.60	C-Cl stretching, N-H out-of-plane bending vibrations
653.87	N-H out-of-plane bending vibrations
589.56	aromatic C-Cl stretching vibration and ring deformation vibration

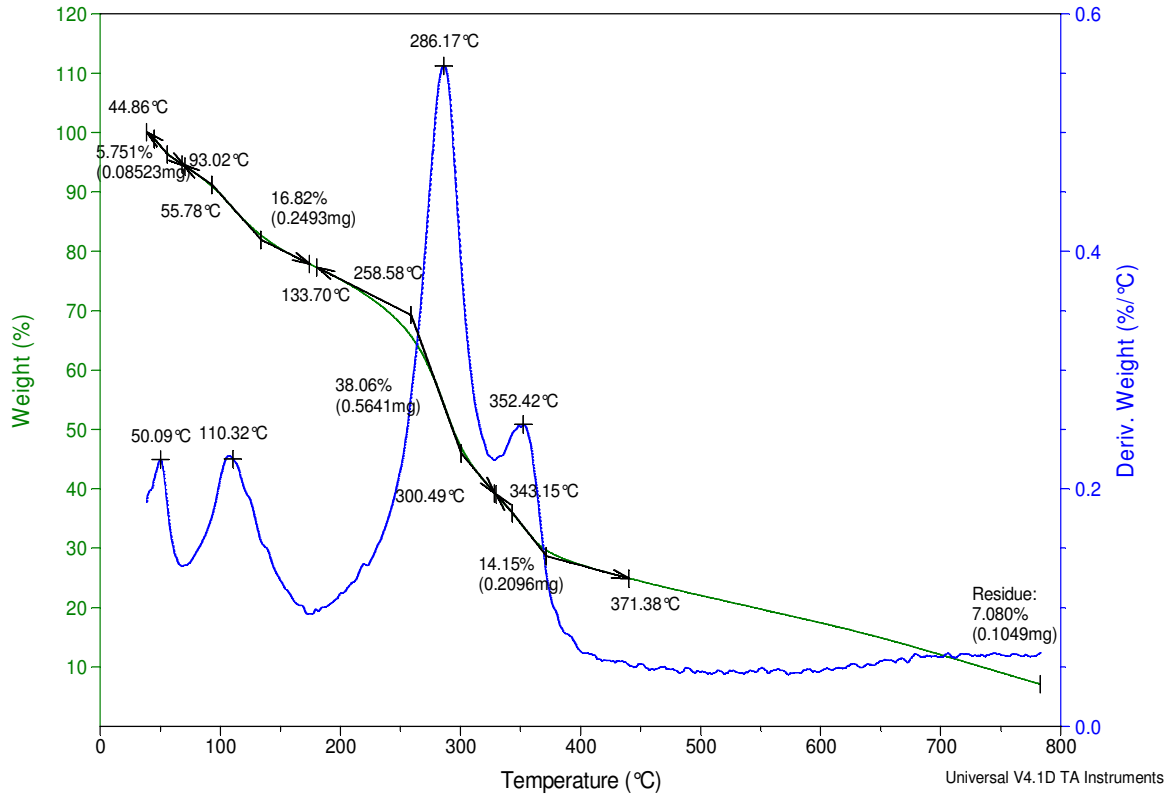

Figure-2
TGA of chitosan 4-chlorobenzaldebyde

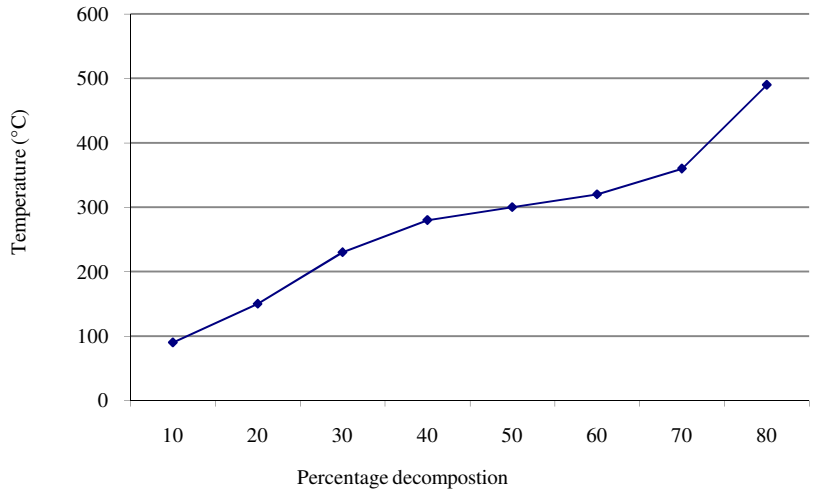

Figure-2(a)
TGA Thermal studies of chitosan 4-chlorobenzaldebyde

TGA thermal details of chitosan 4-chlorobenzaldebydeare presented in table-2, figure-2 and 2(a). Around 93% of the sample was disintegrated at 800^{0}C leaving behind 7.08 % of the sample as a residue at the end of the experiment. The sample was stable up to 40°C. The first stage has taken place in the range 40°-170°C, due to the elimination of water and the dopant molecules. The second stage was in the temperature range of 200°-400°C due to the breakage of the polymeric linkages (depolymerisation). The third stage was due to the decomposition of the polymer backbone. Maximum weight loss occurred in the temperature range 200° to 450°C.

Table-2
TGA thermal studies

Percentage decomposition (%)	Decomposition temperature (^{0}C)
10	90
20	150
30	230
40	280
50	300
60	320
70	360
80	490

DSC studies: The glass transition temperature of polymers, polymer blends and polymer composites can be determined using DSC. The heat capacity change is known as Glass transition temperature (T_g), while the melting temperature (T_m) and crystallization temperature (T_c) are the maximum of endothermic peak and the minimum of exothermic peak, respectively[6].

Figure-3 represents the DSC curve of chitosan 4-chlorobenzaldebyde Schiff base. The glass transition temperature of the polymer was observed at 203.25°C. In addition to this a broad endothermic peak was obtained at 94.63°C showing the crystallization of the polymer. The exothermic peak was obtained at 290.63°C showing the melting of the derivative. These values are in agreement with the values of Tirkiskani. The DSC curves of chitosan 4-chlorobenzaldebyde Schiff base showed an endothermic peak at 100º C. Another endothermic peak was observed at 206º C. This endothermic peak was followed by an exothermic peak at 270º C.

XRD studies of chitosan 4-chlorobenzaldebyde: It is mandatory to determine the structure of synthesised compound in order to known whether the compound possesses crystalline nature or not. This can be done by the X-ray diffraction (XRD) analysis [7].

The figure-4 shows the XRD spectrum of chitosan 4-chlorobenzaldebyde Schiff base. The XRD spectrum of the derivative showed one long broad peak at 2θ=30° indicating that the sample had amorphous nature. The intensity change and peak shift in the XRD showed good interaction which would have taken place between chitosan and aldehyde during blending.

SEM observation of chitosan 4-chlorobenzaldebyde: The surface morphology of chitosan Schiff bases was characterized by SEM. Scanning electron microscopy is an extremely useful method for visual confirmation of surface morphology and the physical state of the surface.

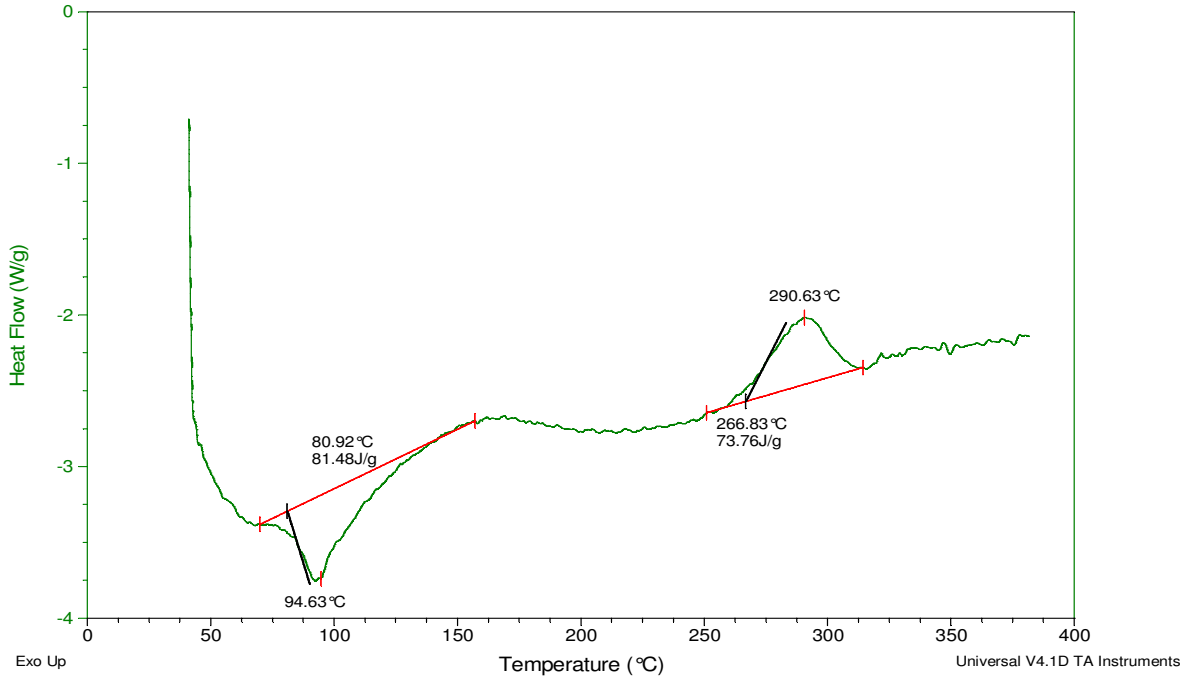

Figure-3
DSC of chitosan 4-chlorobenzaldebyde

The SEM images of chitosan 4-chlorobenzaldebyde showed a microporous and fibrous structure. The expected interactions between chitosan matrix and aldehyde was well exposed through hydrogen bonding and the formation of imine linkage which make the Schiff base derivatives stable with many hydrophilic sites at the surface. The cross sectional morphology of the same showed the fine interaction with pores and microvoids. In the Schiff base the pores are effective in increasing the functional surface, which enabled the same to be used as a promising material.

Antimicrobial activity of chitosan 4-chlorobenzaldebyde: The mode of inhibition of chitosan on the growth of bacteria and fungi might be due to the interaction of chitosan with membranes or cell wall components. The mechanism behind the inhibition of bacterial growth is due to the cationically charged amino-group combining with the anionic components on the cell surface. This may suppress bacterial growth by impairing the exchanges with the medium, chelating transition metal ions and inhibiting enzymes, due to the positive charge, resulting in increased permeability of the membranes and leakage of cell material from tissue or due to water binding capacity and inhibition of various enzymes by chitosan[8,9]. Bioabsorption activity is seen in chitosan and it can absorb nutrients of bacteria and many inhibit the growth.

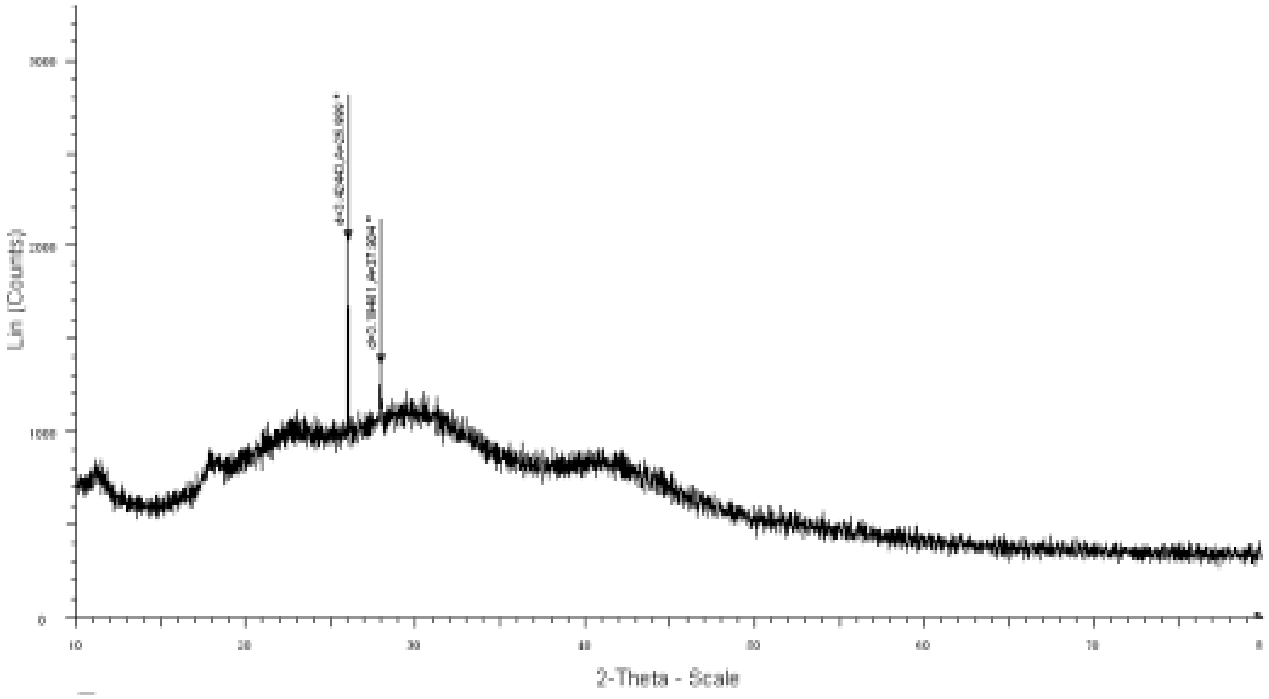

Figure-4
XRD of chitosan 4-chlorobenzaldebyde

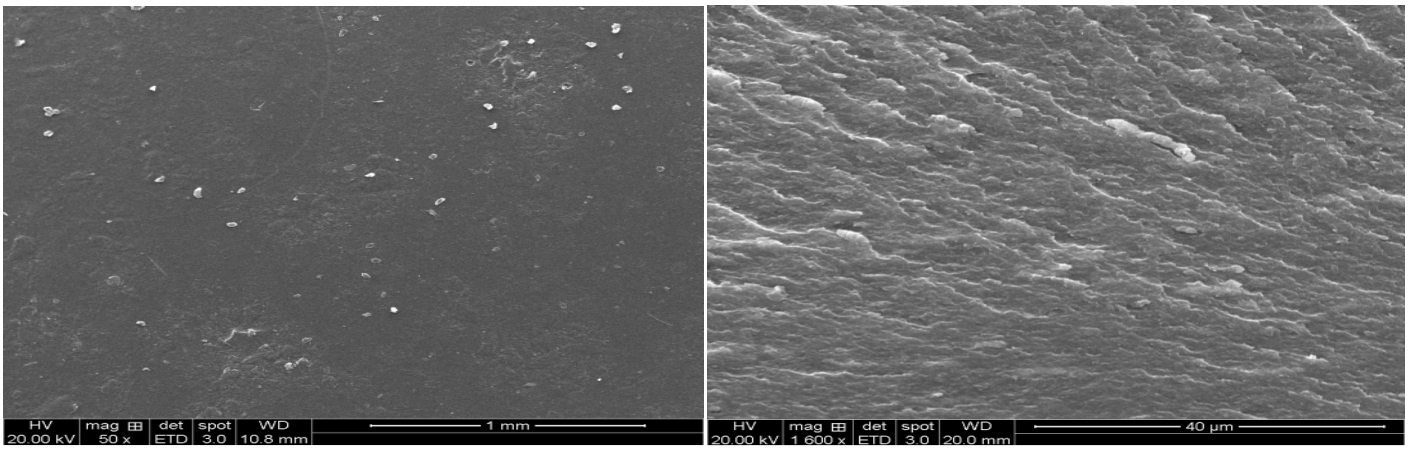

Figure-5
SEM Observation of chitosan 4-chlorobenzaldebyde

From the observations of the activities of chitosan 4-chlorobenzaldebyde against the bacterial species, it is seen that chitosan 4-chlorobenzaldebyde showed some activity but lesser than the standard drug, Ciprofloxacin. The chitosan 4-chlorobenzaldebyde which contains electronegative chloro group in para to imine bond decreases the antibacterial activity.

Antifungal activity: The investigated schiff base compound was tested against the following fungi namely *Candida albicans, Mucorsps* and *Rhizopussps* and shown in table-4

It is seen that in the case of activity against fungi the inhibition was stronger in the order, *Candida albicans*> *Mucorsps*> *Rhizopussps* as shown by chitosan 4-chlorobenzaldebyde Here for fungi also the electronegative plays a role in decreasing antifungal activity.

Cytotoxicity studies of chitosan 4-chlorobenzaldebyde: For cell survival and function, on the material surface the cell adhesion, growth, and proliferation are the sequential response. The mechanism of necrosis or apoptosis determines the cellviability. The destruction of the plasma membrane by external damage is the result of necrosis. Apoptosis, which is much slower than necrosis, leads to highly controlled cell death by biochemical and morphological events. The cellular response to a toxicant is determined by viability assays which are the vital steps in toxicity studies. Viability assays give information on cell death and metabolic activities.

The MTT assay used in this work is a quick and effective method for testing mitochondrial activity, which correlates quite well with cell proliferation.

It is seen that the absorbance index of the tested group increased with the increase of culture time. Significant differences were observed in the cell activity of chitosan 4-chlorobenzaldebyde derivative, in the first three days, which implied that the derivative was beneficial to cell development.

Alkaline Phosphatase assay: The apoptosis assays were performed to determine whether the action of the chitosan Schiff base film induce cell death (apoptotic), to quantify the percentages of the apoptotic cells. One such observed change is an up regulation of production of alkaline phosphatase (ALP). ALP is a hydrolase enzyme responsible for dephosphorylization of organic molecules including DNA, proteins and alkaloids.

The modification of chitosan as chitosan Schiff base derivative showed that the cells remained in the initial shape and could grow and proliferate.

Conclusion

The chitosan-based Schiff base with chlorobenzaldehyde have been synthesized and characterized by conventional methods. The thermal stability of chitosan 4-chlorobenzaldebydehas decreased significantly in comparison with chitosan. In addition, the crystallinity of Schiff base is quite different from that of their corresponding complexe. The difference of crystallinity and thermal stability is mainly attributed to the formation of chitosan Schiff base derivative with covalent bonds, hydrogen bond interaction and electrostatic interactions.

Table-3
Antibacterial activity of chitosan 4-chlorobenzaldebyde

Organisms (Bacteria)	**Ciprofloxacin**	**Zone of Inhibition (mm)**
		chitosan 4-chlorobenzaldebyde
Streptococcus faecalis Gram +ve	24mm	5mm
Staphylococcus aureus Gram +ve	23mm	4mm
Bacillus cereus Gram +ve	26mm	5mm
Escherichia coli Gram –ve	26mm	6mm
Klebsiellapneumoniae Gram -VE	22mm	8mm
Pseudomonas aeruginosa Gram -ve	25mm	5mm

Table-4
Antifungal activity of chitosan 4-chlorobenzaldebyde

Organisms (Fungi)	**Amphoteric in B**	**Zone of Inhibition (mm)**
		chitosan 4-chlorobenzaldebyde
Candida albicans	14mm	9mm
Mucorsps	18mm	7mm
Rhizopussps	14mm	2mm

Table-5
MTT assay MCF-7 (GDC055) Human Adenocarcinoma cell lines (540nm)

Sample	1 day		3 days		5 days		7 days		14 days	
	OD	% Viability	OD	% Viability	OD	% Viability	OD	% Viability	OD	% Viability
chitosan 4-chlorobenzaldebyde	0.03	1.2	1.01	40.4	1.44	57.6	2.3	92	2.4	96

Table-6
Alkaline Phosphatase assay

Sample	1 day	3 days	5 days	7 days	14 days
chitosan 4-chlorobenzaldebyde	3.0	9.2	15.1	20.8	21.4

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