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Preparation of chitosan/different organomodified clay polymer nanocomposites: studies on morphological, swelling, thermal stability and anti-bacterial properties

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In the current study, chitosan films were prepared by dispersing different commercially-modified nanoclays, such as C-Na, C-10A, C-15A, C-30B, and C-93A. The exfoliation and morphology were studied using XRD and SEM. The C-15A, C-30B and C-93A nanoclays/Cts BNCs (Bionanocomposites) showed very good uniform exfoliation compared to that of other clays. The thermal analyses were evaluated using DSC and TGA. These results also confirmed that because of exfoliation, the thermal properties were improved in the case of C-15A, C-30B and C-93A nanoclays/Cts BNCs. The swelling capacity of a chitosan/clay films were studied. Increasing the chitosan content in the film increased the swelling capacity significantly; the decreasing order of swelling capacity of Cts/Clay films is in accordance with the decrease in clay content. Greater swelling capacity is shown by films Cts, C-Na and C-10A is because of the presence of greater hydrophilic agencies in the film makeup, which assist in improving the swelling characteristics of the films. The antibacterial activities of Cts/clay were also investigated against Gram-negative and Gram-positive bacteria (*E. coli* and *S. aureus*) according to the zone of inhibition in the disc diffusion method.

Keywords: chitosan, clay, bio-nanocomposites, swelling property and anti-bacterial property.

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1. Introduction

In the most recent decade, polymer/clay composites have received much attention because of their astonishing ability to improve most of the properties and are an essential alternative to standard polymer composites. The intercalation of polymers into the gallery of the clays brings greater characteristics to hybrid materials [1–4]. In comparison to the vast research on polymer/clay composites, the quantity of biopolymer/clay composites research is comparatively smaller [5–7]. Chitosan (Cts) is a polysaccharide that consists of N-acetyl-glucosamine and N-glucosamine units and it is largely insoluble at neutral and alkaline pH, given that its pKa within the variety of 6.2–7.0 is one of the second most plentiful natural polymers after cellulose in the world, naturally obtained and is derived by deacetylation of chitin present in crustaceous species [8, 9]. In addition to its unique properties such as biocompatibility, biodegrability and non toxicity, it is widely used in biotechnology, pharmaceuticals, cosmetics, textiles agriculture fields due to its antifungal and antimicrobial activities [10]. Unmodified chitosan is not antimicrobially energetic at pH 7, since it does not dissolve or include a large number of charges on the amino groups. The antimicrobial activity of chitosan also increases with increasing degree of deacetylation, due to the increasing number of basic amino groups.

Montmorillonite (MMT) is a member of the smectite group minerals which has a layered shape or platelets. Because of its high aspect ratios and excessive surface area, if clay particles are properly dispersed in a polymer matrix at a loading level of 1–5 % (w/w), precise mixtures of physical and chemical properties may be enhanced, and in turn those composites become more attractive for making films and coatings for a multiplicity of commercial applications, such as drug delivery systems and antimicrobial systems due to their natural abundance and the

propensity with which they can be chemically and physically modified for drug delivery applications [11, 12]. Montmorillonite has been appreciably implemented for prolonged release of drugs as it could hold large amounts of drug due to its excessive cation exchangeability. The surface adsorption of various drugs like griseofulvin, indomethacin and prednisone to montmorillonite clay improves their dissolution rate. The hydrophilic and swelling properties of montmorillonite in aqueous media help to facilitate the wetting of hydrophobic drug substances. This clay ultimately improves the bioavailability of drugs.

Cts are capable of engaging with negatively charged clay, which has a silicate layer shape. When Cts dispersions have been blended with clays, the zeta potentioability of clays and the viscosities of the composite dispersion have been changed [13]. The dry fabric acquired from those composite dispersions is called a nanocomposite if the Cts intercalates into the silicate layer of the clay. Different types of clays, such as montmorillonite and rectorite, have all been used to prepare nanocomposite substances with Cts [14–17]. Within the practice method of these substances, it is necessary to use a heating treatment on the composite dispersion to initiate the nanocomposite formation [15, 18] Furthermore, the clay content material stimulated thermal stability and mechanical properties of the nanocomposites [16, 18]. Cts-clay nanocomposites have been developed and characterized for use as biosensors, packaging materials and superabsorbent substances [19–21]. Furthermore, Cts–clay films should retard the release of a bioactive agent integrated into the films. The current objective of these studies, in particular, targeted the impact of various organo-modified nanoclays on the properties of chitosan (Cts) based Bio-Nanocomposites (BNCs).

2. Experimental

2.1. Materials

Low molecular weight chitosan (CS) used in this work was bought from Sigma Aldrich Chemicals, Bangalore, and Karnataka, India. The Clays such as C-Na, C-10A, C-15A, C-30B, and C-93A were procured form Southern Clay Products, USA. Acetic acid and sodium hydroxide were obtained from Sigma Aldrich Chemicals, Bangalore, and Karnataka, India.

2.2. Preparation of clay/chitosan nanocomposites

The 2 % wt/vol of chitosan solution was prepared by dissolving it in 1 % acetic acid (pH=4.0) with continuous magnetic stirring for 30 min. and then allowed to sit overnight for complete dissolution, or alternatively, 3 % wt/vol ratio of clay (based on the chitosan wt.) was used. Therefore, an appropriate quantity of different clays were dispersed in 10 ml of 1 % acetic acid and magnetically stirred continuously for 24 hours at room temperature and ultrasonicated for about 30 minutes, for this solution, the as already prepared chitosan solution was transferred very slowly and this mixture was stirred for an additional 4 hours using magnetic stirrer. The resulting solution was carefully poured into Teflon-coated glass plates and kept overnight in a 60 °C oven. The resulting composite films were soaked in 1 % NaOH solution for 30 minutes to remove excess acidic content, followed by washed with distilled water several times to effect the removal of NaOH. Then, the composite films were peeled off from the glass plates and the resulting films again oven dried. Films were packed in air tight polyethylene pouches for further characterizations. A similar procedure was followed for the other clay sample dispersion in a chitosan matrix.

3. Results and discussion

3.1. X–Ray diffraction

The Fig. 1 shows the XRD patterns of neat Cts, Cts/C-Na, Cts/C-10A, Cts/C-15A, Cts/C-30B and Cts/C-93A nanocomposites with addition of 3 wt% of above commercial clays. The XRD pattern of Cts suggests the characteristic crystalline peaks at round 20 °. In line with the literature, after incorporation of clay inside Cts matrix the basal plane of Cts/C-Na at 2θ=7.2 ° which disappears and which will be substituted by new peak at around 6 ° whose d-spacing d=1.47 nm. The shift of the basal reflection of C-Na to a lower angle indicates the formation of an intercalated nanostructure, even as the peak broadening and lower depth most likely indicate a disordered intercalated or partially-exfoliated structure, in the case of unmodified C-Na, the inter space distance (d001) turned into enlarged from 1.23 to 1.47 nm (information required for specific composites Cts/93A, 15A, 30B)(approximate d-spacing values for C93A, C30B, C15A, C10A are 3.68, 3.53, 3.4, 3.27 respectively in accordance with graph). According to the XRD report, partial exfoliation is achieved only in the sample containing 3 % C-Na unmodified clay but no uniform exfoliation was noted for the composites of C-15A, C-30B and C-93A. However, according to SEM reports, partial exfoliation was achieved for the sample Cts/C-10A and same trend was observed in Fig. 1.

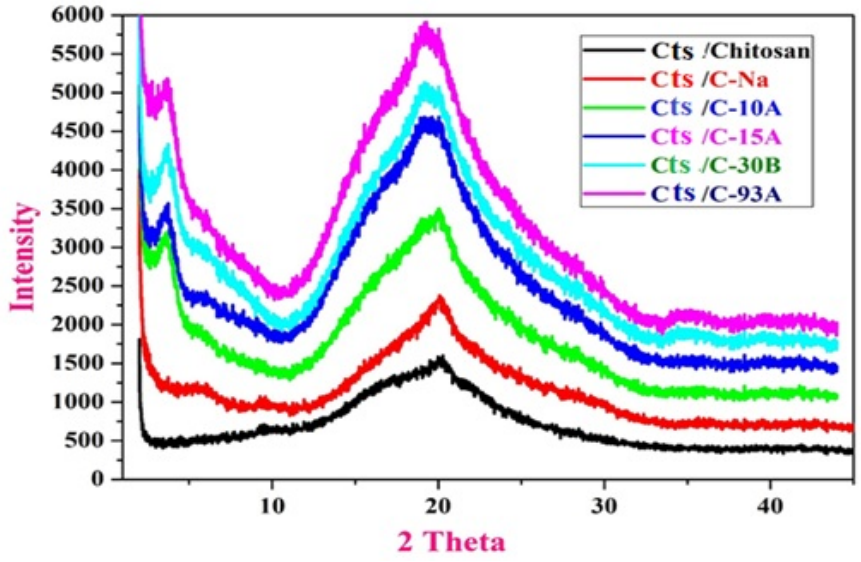

FIG. 1. XRD Pattern of chitosan with different organo modified clay

3.2. Fourier transfer infrared spectroscopy

FTIR was used to characterize the chemical interactions with Cts and clay in the nanocomposites. The organic cations in the clay may additionally comprise diverse useful functional groups that react with chitosan resin to improve interfacial adhesion among clay monolayer and polymer matrix, hence, this cation exchange effect is characterized by the enhancement of the magnitude of the 3193–3187 cm^{-1} band in conjunction with a reduction of intensities because of Si-O and Al-O. The enhanced intensity of the 3193–3187 cm^{-1} band displays the increased hydrogen bonding among the lattice hydroxyls and organic groups. Whilst the protons inside the chitosan are hydrogen bonded oxygen species of Si-O and Al-O segments, Si-O and Al-O bonds might be weakened and the tetrahedral symmetry of these moieties may be distorted. The FTIR spectrum of the clay shows the characteristic bands at 1662 cm^{-1} because of H-O-H bending, 1000 and 1182 cm^{-1} due to Si-O stretching 819 and 670 cm^{-1} due to (Al Mg)-OH vibration modes, while the IR spectra of Cts/C-10A showed additional peaks, including 1725 cm^{-1} (N-H bending), 1590 cm^{-1} (N-H bending) and 1455 cm^{-1} (C-H bending). The absorption band of the carbonyl(C=O) stretching for the secondary amide (amide first band) was observed at 1488 cm^{-1}. At 2356 cm^{-1}, the stretching frequency gradually increased from that of pure chitosan to C93A composite sample with the variation of the type of clay. FTIR was also used to study the polymer/clay interaction, since a shift in the δ_{NH3} vibration might be expected when $-\delta_{NH3}$ groups interact electrostatically with the negatively-charged sites of the clay. In fact, a shift of the δ_{NH3} band towards a lower frequency is noted in all the chitosan/clay films, as is shown in Fig. 2. However, this shift is higher for chitosan/clay films with the lowest amounts of chitosan, while the chitosan/clay films with the highest amounts of biopolymer show a frequency value the trend observed in the films of pure chitosan (Ct). This fact may be related to the $-\delta_{NH3}$ groups that do not interact electrostatically with the clay substrate (Fig. 2). The absorption frequency of pure chitosan in the range of 1721 to 1644 cm^{-1} was related to the vibrations of carbonyl bonds (C=O) of the amide group CONHR (secondary amide, ν_1 = 1721 cm^{-1}) and to the vibrations of protonated amine group (δ_{NH3}, $\nu_2 = 1644$ cm^{-1}).

3.3. Thermal analysis of Cts/clay composites using DSC and TGA

DSC showed two broad peaks for Cts/clay composites, one is an endothermic broad peak and the other one is an exothermic broad peak. The endothermic broad peaks for Cts, Cts/C-Na, Cts/C-10A, Cts/C-30B and Cts/C-93A composite films (as shown in Fig. 3) are: 273, 281, 297.5, 300, 271 and 292 °C respectively. The first temperature region is situated at high temperature in DSC curves because of heating rate used was higher 20 °C min^{-1}. The first peak could be assigned to the water loss, while the second to the decomposition of system components. It is bearing related to the thermal stability of nanocomposites. From these data, it can be concluded that nanocomposites of Ct/C-Na, Ct/C-10A, Ct/C-15A, Ct/C-30B and Ct/C-93A improved the thermal stability of chitosan. Additionally, for the degradation and deacetylation of chitosan and remaining residue at 270 °C and above, the decomposition in air is highly exothermic for the samples Ct/C-30B, Ct/C-93A. This may be due to

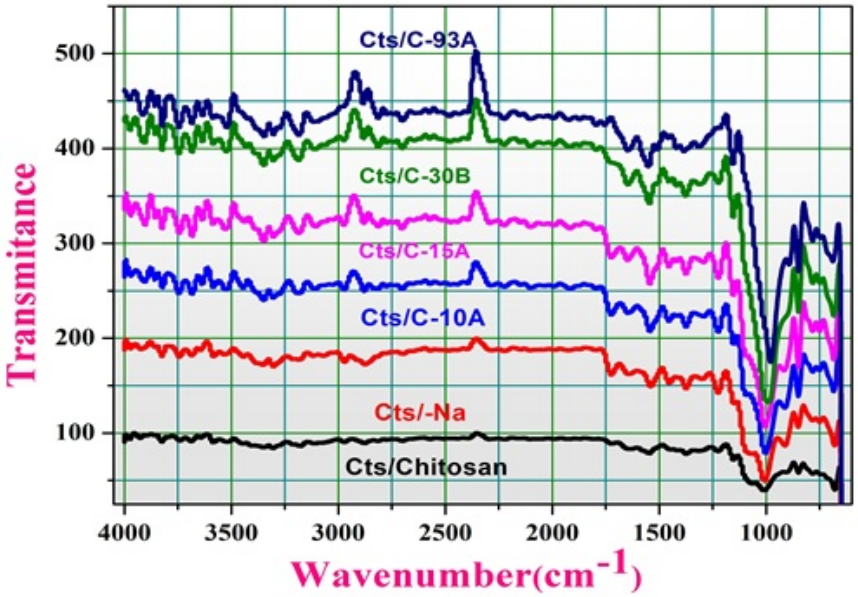

FIG. 2. FTIR patterns of chitosan with different organo modified clay composites

the surfactants used during the modification of these cloisites (C-30B, C-93A) and the same phenomenon was observed in the Fig. 4 (decomposition of intercalated Ct/C-30B and Ct/C-93A layers occurred in the vicinity of 270 °C with significant weight loss). It has been observed that the melting points and exothermic broad peaks all the composite films were 90, 75, 88, 95, 92, 83 °C respectively. The TG, glass transition behavior of Cts and Cts/clay bio-composite films were measured by the DSC evaluation. The TG and melting endothermic height of Cts 3 wt% of Cts/clay has been shifted from approxi. 50–130 °C. The DSC does not detect any traces of thermal transitions for 3 wt% of MMT/clay. The melting endothermic peak of Cts was observed at 140 °C at the same time as the height of Cts/Clay bio composites shown at 150–250 °C, the melting temperature expanded with increased clay content (3 wt%) within the Cts. For these composites, one can find two peaks in TGA curves. In the first stage, a smaller loss in weight 10–12 % was observed compared to the second stage (40 %).

The addition of nanoclays produces a thermal barrier and decreases the weight loss, thus inorganic particles are found to enhance the thermal stability of the chitosan composites. As can readily be seen, better thermal stability was observed for Cts/C-10A, Cts/C-15A, Cts/C-30B, Cts/C-93A composites. This is linked with more intercalation and exfoliation of the chitosan and inorganic fillers (clay). In air flow, another degradation step at 63 °C for the samples Cts/C-10A and Cts/C-93A was observed, the cause of which may be assigned to the oxidative degradation of carbonaceous residue formed during second step. The carbonaceous residue is greater in these samples because of the nature of the modifiers/surfactants which were used in the modification of cloisites.

3.4. Morphological study using SEM

The morphology of surfaces with different surface structure between MMT (clay) and chitosan films are shown in Figs. 5(a-f) using SEM data. The surface of Cts exhibits a smooth laminated structure; comparatively, the surface of composite films seems to be course, indicating an improved intercalation of Cts chains in the clay platelets. Thus, SEM analysis results agreed with the XRD analysis results that produced increase in basal spacing value which was relatively small. This small increase indicated that most of chitosan were on clay (MMT) surface because of formation of hydrogen bonds and intermolecular interactions of these polymers. The exfoliated structure was observed in XRD results. Those outcomes have been corroborated with the aid of SEM effects. The homogenous dispersion of nanoparticles and homogenous matrix in SEM photographs give proof for an exfoliated structure.

3.5. Swelling study

The swelling capacity of a chitosan/clay films performs an essential function regarding the antibacterial activity, wound recovery potential and for biomedical packaging because of their excessive water/solvent retaining potential. The Cts, Cts/C-Na, Cts/C-10A showed higher swelling capacity than Cts/C-15A, Cts/C-30B and Cts/C-93A films, and, among the all these samples, Cts/C-15A showed lowest swelling capacity. This may be because of more

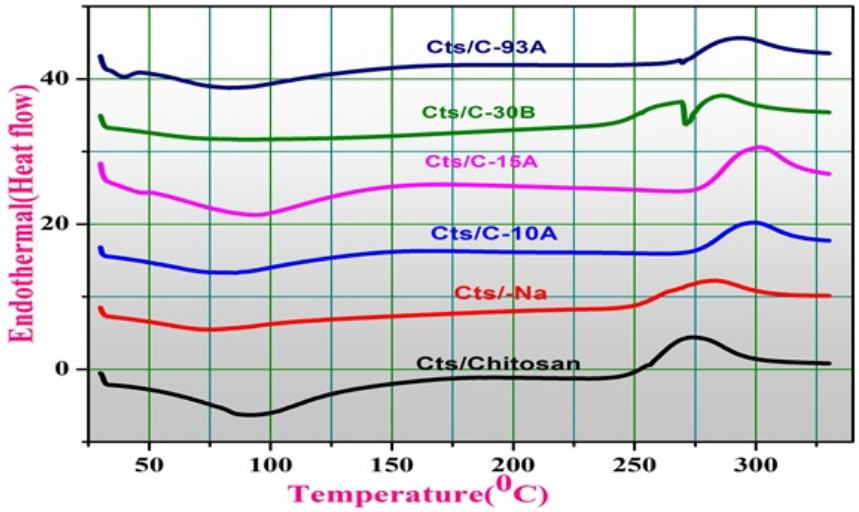

FIG. 3. DSC micrographs of chitosan with different organo modified clays

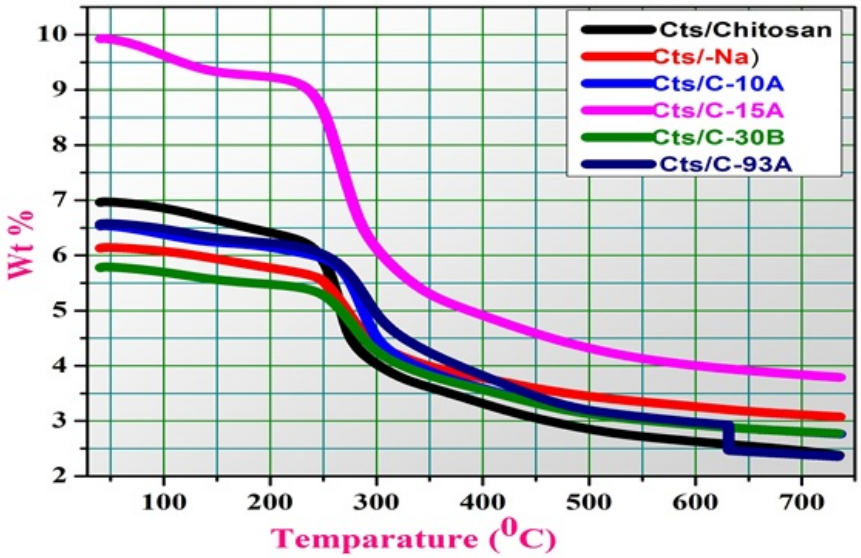

FIG. 4. TGA of chitosan with different organo modified clays

cross linking /interaction of clay with chotosan polymer chains for example, the swelling capacities of the samples were as follows: Cts-1.8 g/g; Cts/C-Na-1.85 g/g; Cts/C-10A 1.75g/g. The other films had the following values: Cts/C-15A-1.58 g/g; Cts/C-30B-1.5 g/g; Cts/C-93A-1.35 g/g respectively. The lowering of the swelling potential is attributed to the clay binding with Cts chains, i.e. the interplay of negative charges on the clay with 'O' and N–atoms of hydroxyl and amine groups present in Cts chains. This produces an additional cross links inside the chain networks. The higher degree of cross-linking with the film restricts the penetration of water for swelling. The increase in chitosan content in the film increases the swelling capacity significantly, the decreasing order swelling capacity of Cts/Clay films is in accordance with Cts/C-15A(1.35 g/g)< Cts/C-93A(1.5 g/g)< Cts/C-30B(1.58 g/g) ,Cts/C-10A< Cts/C-Na(1.7 g/g)< Cts(1.78 g/g) more swelling capacity is showed by films Cts,Cts/C-Na and Cts/C-10A is due to the presence of greater hydrophilic agents within the film network which assist in improving the films' swelling properties (Fig. 6).

4. Antibacterial tests

The antimicrobial activity of chitosan and chitosan-based nanocomposite films were examined qualitatively via an inhibition zone method. In this technique, specific pathogenic microorganism such as *S. aureus* and *Escherichia*

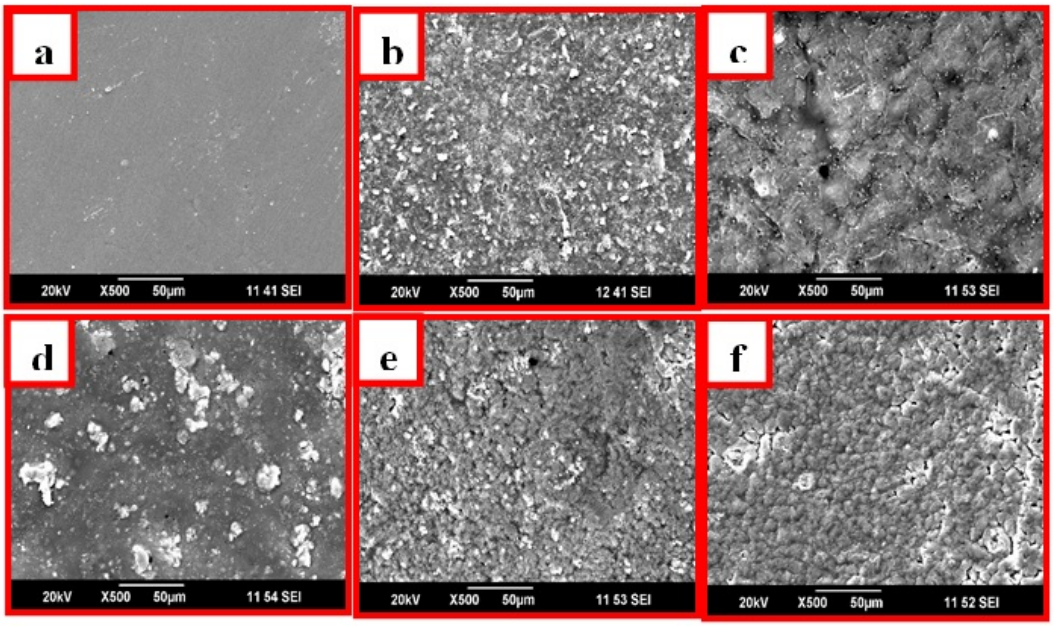

FIG. 5. SEM images of a) Pure Cts, b) Cts/C-NA, c) Cts/ C-10A, d) Cts/C-15A e) Cts/C-30B and f) Cts/C-93A

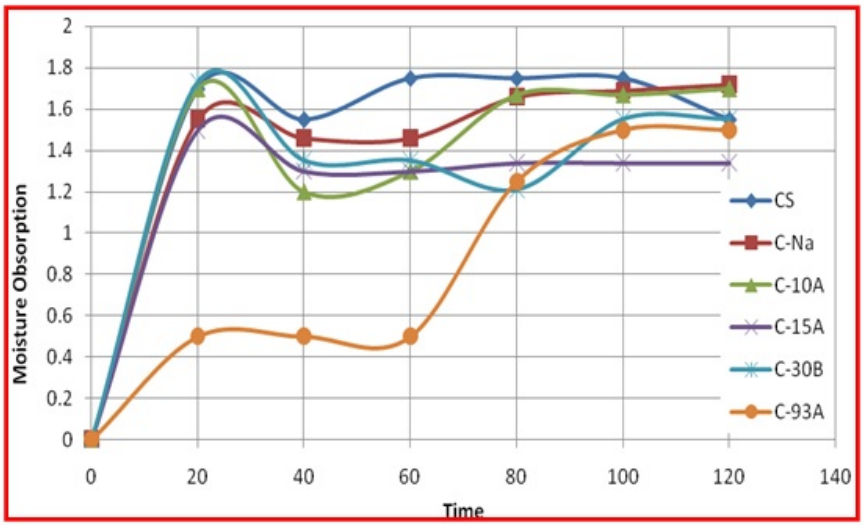

FIG. 6. Swelling behavior of chitosan with different organo modified clays

coli were used to assess the antimicrobial activity of the chosen two films. Both traces of *S. aureus* and *E. coli* were cultivated on tryptic soy (TS) agar (Difco Lab) at 30 °C for two days. All the stock cultures were saved at 4 °C. For the qualitative size of antimicrobial activity, the film samples were punched to make disks (diameter) 6 mm), and the antimicrobial activity present determined the use of a modified agar diffusion assay (disk check). The plates were examined for feasible clean zones after incubation at 37 °C for two days. The presence of any clear zone that shaped across the movie disk at the plate medium was recorded as an illustration of inhibition towards the microbial species. The qualitative antimicrobial activity of the films became determined the use of a quarter inhibition technique for testing pathogenic bacteria.

Pure chitosan film did not show any antibacterial properties. This effect of chitosan may be related fact that chitosan does not diffuse through the adjusted agar media in the agar diffusion test method, so that the only organism in direct contact with the active site of chitosan are inhibited [22–24]. The inhibition zone diameters for the Cts/C-15A sample is better than those of Cts/C93A sample, and it has also been observed that *S. aureus* is more sensitive to Cts/C15A than *E. coli*. This may be attributed to the antimicrobial activity of the quaternary

ammonium silicate layer of Ct/C-15A incorporated film. The effectiveness of such groups bearing alkyl substituents in disrupting bacterial cell membranes (especially for *S. aureus*) and causing cell lysis has been well documented in the literature [25–28]. In the case of Cts/C93A, greater activity was noted against *E. coli* than *S. aeureus*. There are some suggestions that the antimicrobial activity of polymer clay nano composites depends on super molecular organization of the surfactants and the nanostructure of the components. Cts/C-93A has most activity against *E. coli* despite having the lowest surfactant content in the organoclay, that is, 90 % of the surfactants with respect to CEC(Cationic exchange capacity) whereas Cts/C-15A has highest surfactant content in the organo clay and has highest activity against *S. Aureus* (Fig. 7).

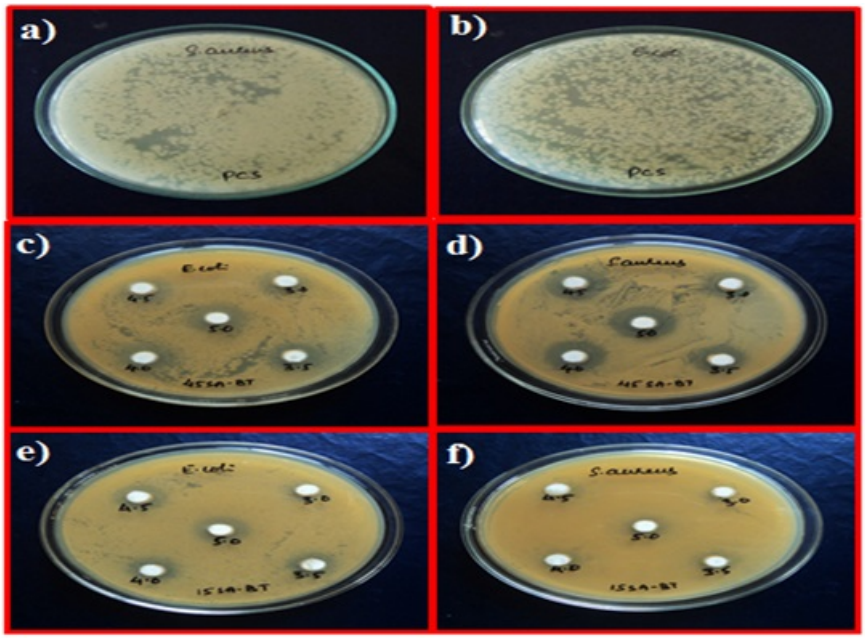

FIG. 7. Antimicrobial activity of a) pure Ct for E coli., b) Pure Ct for S aureus, c) Ct/C-93A for E coli, d) Ct/C-93A S aureus, e) Ct/C-15A for E coli, f) Ct/C-15A for S aureus

5. Conclusion

The effect of different organo-modified nanoclays on the properties of chitosan (Cts) based bio-nanocomposites (BNCs) was evaluated. The exfoliated structure was observed in XRD results. These results were corroborated by SEM results. The homogenous dispersion of nanoparticles and homogenous matrix in SEM images provided evidence for the BNC's exfoliated structure. The addition of nanoclays produces a thermal barrier and decreases the weight loss, thus inorganic particles are found to enhance the thermal stability of the chitosan composites. The increase in chitosan content in the film increased the swelling capacity significantly; the decreasing order swelling capacity of Cts/Clay films was due to the presence of more hydrophilic groups in the film network, which assist in improving the swelling characteristics of the films. The chitosan with different organo-modified clays exhibited optimum antibacterial activity against *E. coli.* and *S. aureus*.The inhibition diameters of these complexes were slightly smaller and the amount of antibiotic explains the smaller inhibition halos that the adsorption complexes showed in comparison to those of the antibiotic solutions.

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