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Preparation, characterization and antimicrobial studies of chitosan/silica hybrid polymer

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ABSTRACT

Organic/inorganic hybrid polymer systems based on chitosan/silica have gained much importance due to their application in enzyme immobilization, sensors and as novel biomaterials. The present study involves the preparation, characterization and antimicrobial studies of chitosan/silica hybrid polymers incorporated with disodium salt of ethylene diamine tetraacetic acid (EDTA) as non-reactive cross-linker, propylene glycol as plasticizer to enhance film forming properties and glutaraldehyde as cross-linking agent. The free standing films were studied by infrared spectroscopy, thermal studies, scanning electron microscopy and X-ray diffraction. The studies indicate that hybrids with 30% silica are the most compatible with chitosan and their physical and thermal properties are dependent on the amount of silica, EDTA, propylene glycol and gluteraldehyde. The films and hybrid polymer coated cotton fabrics were found to have good antimicrobial performance against *Bacillus cereus* and *Escherichia coli*.

Keywords: *Biopolymers, biocomposites, hybrid polymers, chitosan/silica, sol-gel, antimicrobial activity*

1. Introduction

Organic/inorganic hybrid materials based on chitosan and silica are promising systems due to their synergistic properties, resulting from the two different building blocks and their potential uses in enzyme immobilization, porous ceramics, and as electrochemical sensors [1-4]. Chitosan is primarily composed of glucosamine and N-acetylglucosamine residues with a 1,4- β -linkage, which can be obtained by deacetylation of chitin from shells of crustaceans, insects and other sources [5, 6]. It is considered an attractive biopolymer due to its biocompatibility, biodegradability, heat resistance and adsorption properties and used in several versatile applications that include cell culture media, optical materials, chelating agents, biomaterials, etc. [5]. The antimicrobial activity of chitosan against a wide variety of microorganisms including fungi, algae and some bacteria has been reported [7-9]. Previous studies [10] have shown that the preparation of chitosan with inorganic moiety like silica further improves its chemical and mechanical properties. Chitosan/silica hybrids are homogeneous transparent film-forming materials that are compatible through a certain composition range [11]. By evaporating chitosan, porous silica with a high specific area are formed [12]. Suzuki

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et al. conducted a fibroblast cell culture study to develop a novel bio-implant material by preparing the chitosan/silica hybrid membrane, which contained up to 50% silica (wt/wt) [13]. Chitosan/silica hybrid materials provide an alternative way as novel biomaterials for haemostatic dressing applications [14]. A similar study to prepare this type of membrane has been carried out by Liu et al. [15]. Ternary blends from chitosan/silica/poly(monomethyl itaconate) were formed and used in a functional coating application [16].

The present work was undertaken to prepare chitosan/silica hybrid polymers as free standing films and coatings on cotton fabric for their potential use in biomedical applications. Disodium salt of ethylene diamine tetracetic acid (EDTA) was incorporated as chelating agent. Propylene glycol was added as plasticizer to enhance film forming properties and glutaraldehyde as chemical cross-linking agent and reactive compatibilizer. The miscibility of hybrid polymers was determined by DSC. The films were evaluated for structural, morphological and antimicrobial properties.

2. Experimental section

Chitosan was received from M/s Bayer Chemicals, Bangalore, with a deacetylation percentage of 86% (average molecular mass, 140-310 kDa). Tetraethoxy silane (TEOS), disodium-EDTA, propylene glycol (PG), glutaraldehyde (GA), ethanol, acetic acid, citric acid, sodium hydroxide and sodium bicarbonate were purchased from e-Merck, Germany of reagent grade and used as received. Bacterial cultures namely *Escherichia coli* MTCC 40 and *Bacillus cereus* MTCC 1305 were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India.

2.1. Preparation of Chitosan/Silica/GA films. Chitosan/silica hybrid of 80:20 (% wt) composition was prepared by dissolving 0.8 g of chitosan in 20 ml of 1% acetic acid. The solution was stirred well using a magnetic stirrer for about 2 to 3 hours. Partially co-condensed silica was prepared from 214 μl of TEOS by keeping TEOS: Water: Ethanol sol mixture in the molar ratio of 1:4:1. Hydrolysis was initiated by adding 2 drops of 0.1% HCl as the catalyst, and the solution mixture was then kept in an ultrasonic bath for 20 minutes to obtain a clear solution of silica sol. It was then added dropwise to the chitosan solution and stirred for 1 further hour. To the resulting solution, 37.74μl of GA (5%w/w of chitosan) was added, stirred for 1 hour and then poured into poly propylene (PP) petri dishes of 80 mm diameter and allowed to dry overnight at 80 ^{o}C to obtain the film. By following the above procedure, other compositions of chitosan- silica, i.e., 90:10, 70:30 and 60:40 were also prepared.

2.2. Preparation of Chitosan/Silica/EDTA/Proplylene Glycol/GA films. Chitosan-silica solution prepared as above was mixed with 0.04 g of EDTA (5 % w/w of chitosan) followed by 77.3 μl of propylene glycol (10% w/w of chitosan). The solution mixture was stirred for 20 minutes and partially co-condensed silica prepared as above was added to the solution dropwise and allowed to stir for 1 hour to obtain a clear homogenous solution. GA (37.74μl, 5%w/w of chitosan) was added to the reaction mixture, stirred for 1 hour and the film was casted in the same way as mentioned above. The other composition i.e. 90:10, 70:30 and 60: 40 were prepared following the same procedure. Similarly chitosan/silica/EDTA/GA films were prepared without addition of propylene glycol as above.

2.3. Coating on Cotton fabrics. In to the solutions of chitosan-silica (70:30)/EDTA blend prepared as above, a pretreated cotton fabric was dipped, to reach a wet pickup of 95%. The fabrics were pretreated by scouring and mercerization as reported elsewhere [17]. The coated fabric was dried at 100 ^{o}C and used for in-vitro antimicrobial studies.

2.4. Structural Characterization. The films were characterized by using FT-IR instrument (Bruker Tensor 27) and the spectra were collected and analyzed using OPUS software. The glass transition temperature (Tg) was measured by using model 2010 (TA Instruments, New Castles, USA) between the temperature range of 30-350°C at heating rate of 5 °C/min, with nitrogen gas flow rate of 60 ml/min. The DSC instrument was calibrated by using indium prior to the experiment. The distribution of silica particles within the hybrid films were examined by electron microscopy by JEOL JSM- 5200 microscope (JEOL Ltd., Tokyo, Japan), at an operating voltage of 25 kV. The samples were previously coated with a thin layer of gold deposited by sputtering under vacuum. X-ray diffraction (XRD) spectra were collected on a Phillips diffractometer (PANalytical B.V., ALMELO, The Netherlands) using Cu Kα radiation (k = 0.1505945 nm).

2.5. Antimicrobial Studies. The test organisms namely *Escherichia coli* MTCC 40 and *Bacillus cereus* MTCC 1305 were cultured over night in nutrient broth at 37°C. 100 µL of the overnight culture was uniformly spread on the nutrient agar medium. Circular discs of 1 cm diameter were cut from the test films and coated fabric and placed on the bacterial lawns and incubated at 37°C for 24 hours. The plates were visually examined for zone of inhibition around the discs, and diameter of the zone of inhibition was measured using a vernier caliper [18].

3. Results section

The films formed by the reaction of partially co-condensed silica prepared by sol-gel method from TEOS [19] through acid catalysis with chitosan solution [20] were clear and transparent. This indicates the complete miscibility of silica in chitosan matrix, where the miscibility or compatibility may be attributed to the interaction of silanol group of partially co-condensed silica and alcohol group of chitosan [21]. Moreover, acid hydrolysis mechanism of TEOS leads to the formation of a diffused network of silica in chitosan matrix [22, 23]. Further reaction leads to the formation of the Si-O-C bond between silica and chitosan and uniform dispersion of silica in chitosan matrix, which is shown in Figure 1. An increased percentage of silica in inorganic/organic hybrid polymers leads to the formation of phase boundaries between organic and inorganic polymers [24-26]. In our studies, it was also observed that the films with the silica composition greater than 30% of silica (based on the weight of starting TEOS) in hybrid polymer were brittle, which may be due to starting of phase separation effect. However the brittleness of the films can be reduced by adding a plasticizer, namely PG. The role of plasticizer is probably to decrease the phase separation and to increase miscibility between silica and chitosan.

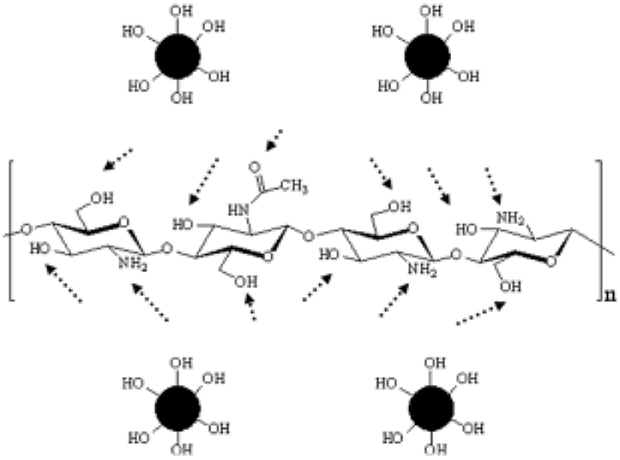

Figure 1: Molecular level interaction of silanol with chitosan polymer

3.1. FT-IR Studies. Figure 2 shows the FT-IR spectra of hybrid polymer with varied silica percentage, cross linked with GA. Major regions of the FT-IR spectra of chitosan and chitosan/silica hybrids showed absorption regions of chitosan with O-H (stretching, 3261 cm^{-1}), C-H (2924 cm^{-1}), C=O (1630–1670 cm^{-1}) and N-H (hydrogen-bonded 1557 cm^{-1}) [15]. As silica was incorporated in-situ in the chitosan solution, most of typical absorption bands of chitosan remained largely unchanged. With the increase of TEOS ratio, the intensity of absorption at 795 cm^{-1} of the resulting hybrid films increases in proposition whereas the intensity around 1250 cm^{-1} decreases. The trend of increased intensity around 795 cm^{-1} is due to the increased formation of Si-O-Si bonds, while the decrease in peak intensity around 1250 cm^{-1} is due to co-condensation of -OH groups of chitosan with silica moiety. These observations confirm the formation of chitosan-silica hybrid polymer and the molecular level bonding interaction between chitosan and silica [21].

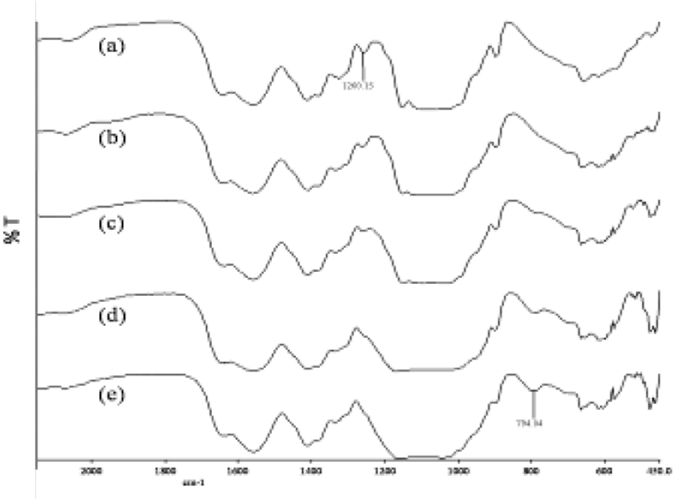

Figure 2: FT-IR spectra for hybrids of compositions: (a) Pure chitosan; (b) Chitosan/silica(90:10)/GA; (c) Chitosan/silica(80:20)/GA; (d) Chitosan/silica(70:30)/GA and (e) Chitosan/silica(60:40)/GA

3.2. Thermal Studies. In DSC thermogram, the width of crystalline melting endotherm (ΔT) gives the extent of compatibility/miscibility of the two components [27]. The lower ΔT indicates sharp melting and hence more compatibility of the composition. Figure 3 gives the plot of ΔT verses silica and chitosan composition. It can be observed from the plot that 30 % silica composition gives the minimum ΔT, which indicates that this composition of silica is the most compatible with chitosan.

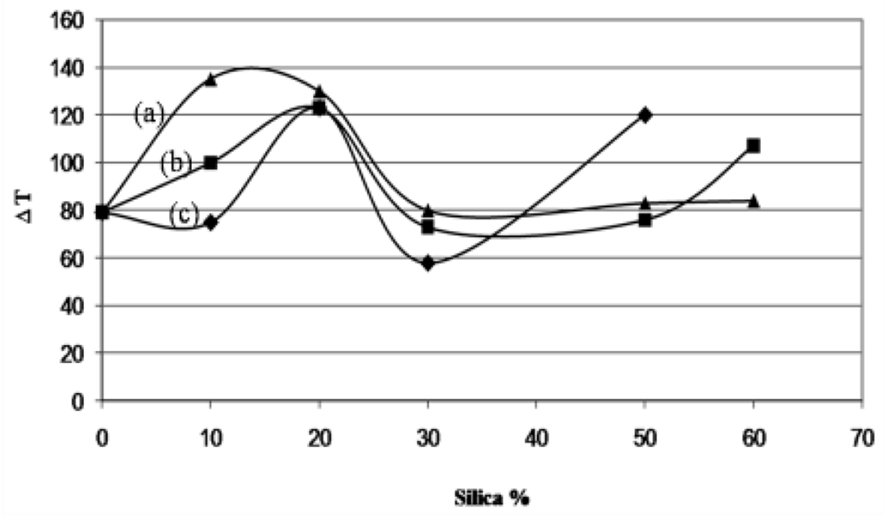

Figure 3: Plot of ΔT verses silica composition (a) Chitosan/silica/EDTA/PG/GA; (b) Chitosan/silica(70:30)/EDTA/GA and (c) Chitosan/silica(70:30)/GA

Figure 4 gives the DSC thermgram of 30% silica containing hybrids with reactive and non-reactive compatibilizer viz. GA and EDTA respectively. Figure 4 also shows the DSC thermogram of pure silica and chitosan. The glass transition temperature (T_g) was determined according to ASTM standard E1356 Standard Test Method for Assignment of the Glass Transition Temperatures by Differential Scanning Calorimetry. This gives the midpoint temperature between the extrapolated onset and endset of endotherm. Crystalline temperature (T_c) and T_g are complementary to each other; higher the T_g„ higher will be the T_c [23]. Table 1 gives the T_c and T_g of different 30% silica containing hybrid composition. Incorporation of silica in chitosan increases the T_g as it was reported in cases of silica containing polyimides [28]. Cross-linking agent increases both the T_c and T_g values. Gluteraldehyde is a reactive cross-linking agent, which increases the T_g from 86 ^oC to 98 ^oC and EDTA is an non-reactive compatibilizer, which further increases the T_g from 98 ^oC to 100 ^oC. As reported [29] that plasticizer decreases the T_g, in our studies, T_g is also decreased from 100 ^oC to 92 ^oC for PG incorporated hybrids.

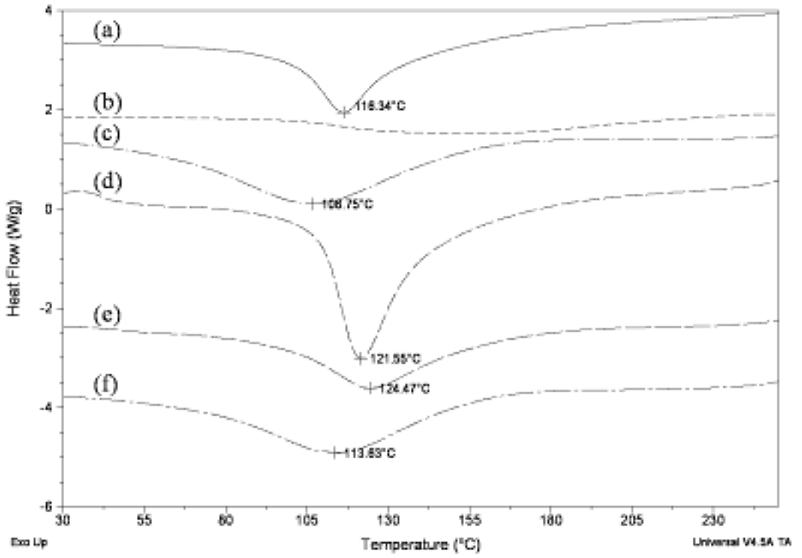

Figure 4: DSC thermographs for hybrids of compositions (a) Pure chitosan; (b) Pure silica; (c) Chitosan/silica(70:30); (d) Chitosan/silica(70:30)/GA; (e) Chitosan/silica(70:30)/EDTA/GA and (f) Chitosan/silica(70:30)/EDTA/ PG/GA

Table 1. T_c and T_g of different 30% silica containing hybrid films

Composition	T_c (^oC)	T_g (^oC)
Chitosan	116	84
Chitosan/Silica	107	86
Chitosan/Silica/GA	122	98
Chitosan/Silica/EDTA/GA	124	100
Chitosan/Silica/EDTA/PG/GA	114	92

3.3. Scanning Electron Microscopic Studies. Figure 5 shows SEM micrograph of particles of silica distribution in the chitosan matrix cross-linked with GA. It can be seen that the incorporation of EDTA agglomerates silica particles, whereas addition of PG as plasticizer reduces its agglomeration. It may also be noted that increasing silica content in the EDTA incorporated film, further increases the agglomeration of silica. The encapsulation of silica by EDTA in sol-gel derived optically transparent glass has been reported [30]. EDTA is a well known chelating agent with six potential sites composed of four carboxyl and two amino groups available for binding with a range of cations [31]. Under acidic conditions, the alkoxide group of TEOS is protonated in a first rapid step, which makes the silicon atom electrophilic, thus more susceptible to attack from water. This results leads to the formation of a pentacoordinate transition state with a significant SN2 character type [32]. The observed agglomerated morphology of silica particles, for hybrid films prepared with EDTA, may be attributed to preferential complexation and stabilization of electrophilic silica network by hexacoordinate EDTA ligand as compared to chitosan. Moreover, increasing proportion of TEOS leads to enhanced agglomeration of silica particles and conversely addition of propylene glycol decreases this effect.

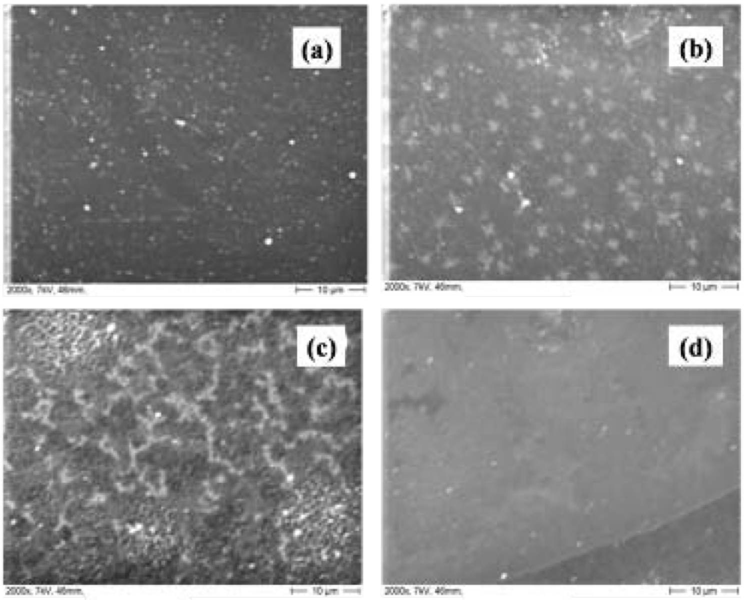

Figure 5: Scanning Electron Micrographs of hybrids of compositions (a) Chitosan/silica (70:30)/GA; (b) Chitosan/silica(70:30)/EDTA/GA; (c) Chitosan/silica(60:40)/EDTA/GA and (d) Chitosan/silica(70:30)/EDTA/ PG/GA.

3.4. X-Ray Diffraction Studies. The XRD patterns of the chitosan/silica hybrids are given in Figure 6. XRD is widely employed technique for the characterization of crystalline structure of the materials. All the patterns exhibit a main peak centered at ca. 20.02^{o} associated with the chitosan crystalline structure [33]. The results also suggest that EDTA promotes crystallinity of the hybrid, while the crystallinity is suppressed by the introduction of PG. Crystallanity due to EDTA may be attributed to its complexation of silica, the suppression of crystallanity by PG may be due to its plasticizing effect. These results are comparable to the trend obtained from DSC studies.

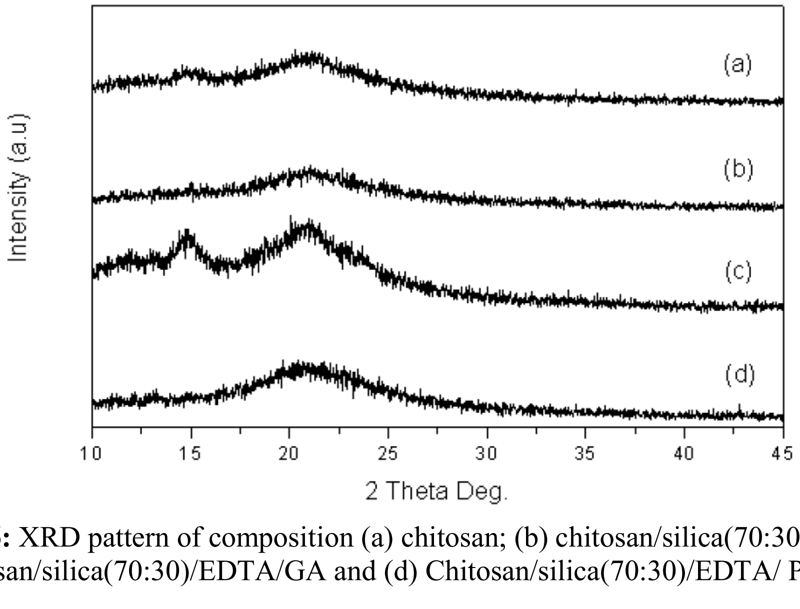

Figure 6: XRD pattern of composition (a) chitosan; (b) chitosan/silica(70:30)/GA; (c) Chitosan/silica(70:30)/EDTA/GA and (d) Chitosan/silica(70:30)/EDTA/ PG/GA

3.5. Antimicrobial Properties. The antimicrobial findings are based on the measurement of a clear zone of inhibition surrounding the samples, where the growth of the test microorganism was inhibited. Table 2 shows the antimicrobial activity of the hybrid films and coated textiles. At low pH, chitosan possess a lot of poly-cationic amines that interact readily with negatively charged substances at the cell surface of bacteria, such as proteins, phospholipids, fatty acids, and subsequently inhibit the growth of microorganisms [34]. The antimicrobial activity of the films and textiles, which were cross-linked with and without GA were tested against the test organisms, *E. coli* and *Bacillus cereus* The hybrids with EDTA showed better antimicrobial activity as compared to hybrids without EDTA since the divalent ion chelator loosens the structure of lipopolysaccharide [35]. The hybrids without GA cross-linking showed inhibitory zones against the test organisms, whereas the hybrids cross linked with GA did not show the zone of inhibition. Nevertheless, there was no over growth of the test organism in all samples. The reduced activity for hybrids cross-linked with GA may be attributed to the formation of a chemical bond between the amine group of chitosan and the aldehyde group of GA [19], which leads to reduced availability of amine group to interact with the microbial cell walls.

Table 2. Antimicrobial activity of hybrids against *B. cereus* and *E. coli*

Hybrids with EDTA and without GA cross linking	Zone of Inhibition ± SD (in mm)	
	Bacillus cereus	*Escherichia coli*
Chitosan/Silica (90:10) film	25±0.5	23±0.6
Chitosan/Silica (80:20) film	23±0.3	20±0.5
Chitosan/Silica(70:30) film	21±0.6	20±0.3
Cotton fabric treated with Chitosan/Silica (70:30)	25±0.5	18±0.4

4. Conclusions

Chitosan/silica hybrid polymers of varied compositions have been prepared as transparent self standing films. The FT-IR studies of the films show the molecular level interaction of silica with chitosan network. All the films showed antimicrobial activity against Gram-positive microorganisms. The addition of EDTA to the film makes it active against Gram-negative microorganisms also. DSC studies reveal that 30% silica composition gives the minimum ΔT, which indicates that this composition of silica is the most compatible with chitosan. XRD and DSC studies show that crystallanity of the hybrids were promoted by the incorporation of EDTA, whereas the addition of PG decreases it. While the film without GA cross-linking shows the inhibition zone, the GA cross-linked films did not show the zones of inhibition. These studies reveals the physical, thermal and antimicrobial properties of chitosan/silica hybrids that can be modified by incorporating silica, EDTA, PG and GA and can be potentially used for medical applications.

5. References

[1] Peng F., Lu L., Sun H., Wang Y., Liu J., Jiang Z., Hybrid Organic−Inorganic Membrane: Solving the Tradeoff between Permeability and Selectivity, *Chem. Mater.,* 17, 26, 6790-6796, **2005**

[2] Risha B., Joseph M. C., Silicon Biotechnology: New Opportunities for Carbohydrate Science, *Aust. J. Chem.,* 56, 7-11, **2003**

[3] Smitha S., Shajesh P., Mukundan P., Warrier K.G.K., Sol-gel synthesis of biocompatible silica-chitosan hybrids and hydrophobic coatings, *J. Mat. Res.,* 23, 8, 2053-2060, **2008**

[4] Lee E. J., Shin D. S., Kim H. E., Kim H. W., Koh Y. H., Jang J. H., Membrane of hybrid chitosan-silica xerogel for guided bone regeneration, *Biomaterials.,* 30, 5, 743-750, **2009**

[5] Zong Z., Kimura Y., Takahashi M., Yamane H., Characterization of chemical and solid state structures of acylated chitosans, *Polymer.,* 41, 3, 899-906, **2000**

[6] Al Sagheer F. A., Al- Sughayer M. A., Muslim S., Elsabee M. Z., Extraction and characterization of chitin and chitosan from marine sources in Arabian Gulf, *Carbohyd. Polym.,* 77, 2, 410-419, **2009**

[7] Raafat D., Bargen K. V., Haas A., Sahl H. G., Insights into the Mode of Action of Chitosan as an Antibacterial Compound, *Appl. Environ. Microb.,* 74, 12, 3764-3773, **2008**

[8] Liu X. F., Guan Y. L., Yang D. Z., Li Z., Yao K. D., Antibacterial action of chitosan and carboxymethylated chitosan, *J. Appl. Polym. Sci.*, 79, 7, 1324-1335, **2001**

[9] Entsar I. R., Moham E. T. B., Christian V. S., Gay S., Walter S., Chitosan as Antimicrobial Agent: Applications and Mode of Action, *Biomacromolecule.,* 4, 6, 1457-1465, **2003**

[10] Al-Sagheer F., Muslim S., Thermal and Mechanical Properties of Chitosan/SiO_2 Hybrid Composites, *Journal of Nanomaterials.,* 2010, 1-7, **2010**

[11] Retuert J., Nuñez A., Martínez F., Yazdani-Pedram M., Synthesis of polymeric organic-inorganic hybrid materials. Partially deacetylated chitin-silica hybrid, *Macro. Rapid Comm.,* 18, 2, 163-167, **1997**

[12] Retuert J., Quijada R., Arias V., Yazdani-Pedram M., Porous silica derived from chitosan-containing hybrid composites. J. Mat. Res, 18, 2, 487-494, **2003**

[13] Suzuki T., Mizushima Y., Characteristics of silica-chitosan complex membrane and their relationships to the characteristics of growth and adhesiveness of L-929 cells cultured on the biomembrane, *J Ferment. Bioeng.,* 84, 2, 128-132, **1997**

[14] Tamaki R., Chujo Y., Synthesis of chitosan/silica gel polymer hybrids, *Copmpos. Interf.,* 6, 259-266, **1999**

[15] Liu Y. L., Su Y.H., Lai J. Y., In situ crosslinking of chitosan and formation of chitosan–silica hybrid membranes with using γ-glycidoxypropyltrimethoxysilane as a crosslinking agent, *Polymer.,* 45, 20, 6831-6937, **2004**

[16] Martinez Y., Retuert J., Yazdani-pedram M., Colfen H., Hybrid ternary organic–inorganic films based on interpolymer complexes and silica, *Polymer.,* 45, 10, 3257-3265, **2004**

[17] Seung I. K., Eui S. L., Heung S. Y., Mercerization in degassed sodium hydroxide solution, *Fibers and Polymers.,* 7, 2, 186-190, **2006**

[18] Jorgensen, J.H., Turnidge, J.D., J. A. Washington., Antibacterial suscepti-bility tests: dilution and disk diffusion methods. In 'Manual of clinical microbiology', 7th ed., American Society for Microbiology, Washington D.C, **1999**

[19] Braun S., Rappoport S., Zusman R., Avnir D., Ottolenghi M., Biochemically active sol-gel glasses: the trapping of enzymes, *Mater. Lett.,* 10, 1-2, 1-5, **1990**

[20] Rinaudo M., Pavlov G., Desbriers J.: Influence of acetic acid concentration on the solubilization of chitosan, *Polymer.,* 40, 25, 7029-7032, **1999**

[21] Lai S. M., Arthur M. Y. J., Chen W. C., Hsiao J. F., The Properties and Preparation of Chitosan/Silica Hybrids Using Sol-Gel Process, *Polymer-Plastics Technology and Engineering.,* 45, 5, 997–1003, **2006**

[22] R. K. Iler., The Chemistry of Silica, 1st ed., John Wiley & Sons, New York, 1979

[23] Aelion R., Loebel A., Eirich F., Hydrolysis of Ethyl Silicate, *J. Am. Chem. Soc.,* 72, 12, 5705-5712, **1950**

[24] Ragosta G., Musto P., Polyimide/silica hybrids via the sol-gel route: High performance materials for the new technological challenges, *Polymer Letters.,* 3, 7, 413–428, **2009**

[25] Morikava A., Kakimoto M., Iyoku Y., Imai Y., Preparation of a New Class of Polyimide-Silica Hybrid Films by Sol-Gel Process, *Polym. J.,* 24, 1, 107-113, **1992**

[26] Wang H., Zhong W., Du Q., Yang Y., Okamoto H., and Inoue S., Novel Polyimide/Silica Nanohybrids from Water Glass Novel Polyimide / Silica Nanohybrids from Water Glass, *Polym. Bull.,* 51, 1, 63-68, **2003**

[27] Sperling L. H., Glass-rubber transition behavior. in Introduction to Physical Polymer Science Edited by Sperling L. H., 3rd ed.,Wiley Interscience, New York, **1986**

[28] Nandi M., Conklin J. A., Salviati L. Jr., Sen A., Molecular level ceramic/polymer composites 1. Synthesis of polymer-trapped oxide nanoclusters of chromium and iron, *Chem. Mater., 2*, 6, 772-776, **1990**

[29] Jagannath J. H., Jayaraman K. S., Arya S. S., Dynamic mechanical thermal analysis of chapati and phulka (Indian unleavened bread), *Food Chem.,* 64, 4, 571-576, **1999**

[30] Oka K. S., Mackenzie J. D.: in Better Ceramics Through Chemistry VI, Edited by Cheetham A. K., Brinker C. J., Mecartney M. L. and Sanchez C. Mater. Res. Soc. Proc. 346, Pittsburgh, PA., **1994**

[31] Ogino H., Shimura M..: in Advances in Inorganic and Bioinorganic Mechanisms IV, Academic Press, Inc., London, **1986**

[32] Brinker C. J., Scherer G.W..: Sol-Gel Science: The Physics and Chemistry of Sol-Gel Processing, Hydrolysis and Condensation Ii: Silicates. Academic Press, Inc., New York, 1990

[33] Jaworska M., Sakurai K., Gaudon P., Guibal E., Influence of chitosan characteristics on polymer properties. I: Crystallographic properties, *Polym. Int.,* 52, 2, 198-205, **2003**

[34] Muzarelli R. A. A., Chitosan based dietary foods, *Carbohy. Polym.,* 29, 4, 309-316 **1996**

[35] Alakomi H. L., Paananen A., Suihko M. L., Helander I. M., Saarela M., Weakening Effect of Cell Permeabilizers on Gram-Negative Bacteria Causing Biodeterioration, *Appl. Environ. Microb.,* 72, 7, 4695-4703, **2006**

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