INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SALICYLOYL CHITOSAN AND STARCH BLEND

S. Jafar sathik[1*], S. Raja Mohamed Kamil[1], M. Aboobucker Sithique[1] and H. Abdul Jaffar Ali[2]

[1]PG & Research Department of Chemistry, Islamiah College (Autonomous), Vaniyambadi – 635752, Vellore District, TamilNadu, India.
[2]PG & Research Department of Biotechnology, Islamiah College (Autonomous), Vaniyambadi – 635752, Vellore District, TamilNadu, India.

ABSTRACT

Salicyloyl Chitosan/Starch blend (SCSB) was prepared in two steps. In the first step, chitosan was modified as Salicyloyl chitosan (SCS) by treating with Salicyloylic acid and in the next step the prepared Salicyloyl chitosan (SCS) was blended with starch. Thus, synthesised blend was characterized by FTIR, XRD and TGA. The antibacterial activities were assessed against *Escherichia coli (E.coli)*, *Staphylococcus aureus (S.aureus)* and *Proteus Mirablis* by Inhibition Zone method. The antifungal activities against *Candida albicans*, *Aspergillus niger* and *Aspergillus flaus* were also assessed by the same method. The structure activity relationship of SCS and SCSB for the anti-microbial activities was also discussed.

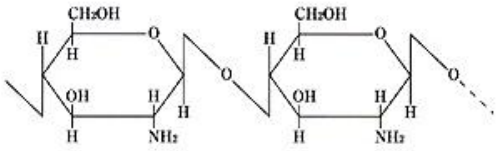
Keywords: Chitosan, Salicyloyl chitosan, Salicyloyl chitosan, antibacterial activity, antifungal activity.

1. INTRODUCTION

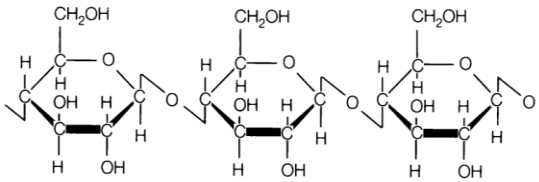
Chitosan is a potential bio macromolecule which has received much attention due to the tailor made properties and shall be used in many fields including pharmaceutical applications[1]. Since it is inexpensive, nontoxic and possessing potentially reactive amino functional groups, chitosan has been widely used in the fields of medicine, food, cosmetics, agriculture, waste water treatment, and so on[2-5].

Starch is one of the major renewable polysaccharide which usually contains about 30% amylose, 70% amylopectin and less than 1% lipids or proteins derived from plants. Biodegradable starch based plastics such as blends of starch with polyolefin or polyvinyl alcohol have recently been widely investigated due to the great market demands for agricultural foils, composting bags, food packaging as well as materials for biomedicine, pharmacy and cosmetics[6,7]. Blending of Chitosan with natural biopolymers is considered as a new class of materials that is expected to possess better physicochemical properties. There have been many studies on the blends of chitosan with various kinds of polymers in order to obtain some improved properties. Polymer blending is an attractive alternative for producing new polymeric materials with tailored properties instead of synthesising totally new materials[8].

Chitosan



Starch



The present work involves synthesising novel salicyloyl chitosan/ starch blend, characterising the prepared blend by using FTIR, XRD and TGA and analysing the antimicrobial activities against various bacteria and fungi.

2. MATERIALS AND METHODS

Chitosan was purchased from Central Institute of Fisheries Technology, Cochin with viscosity average molecular weight mention about 3, 00,000 K Da. DMF, Salicylic acid, acetic acid and starch were purchased from SD Fine chemicals and purified as per standard procedure. The antimicrobial activity was assessed using well diffusion method.

2.1. FT-IR Studies

Fourier Transform Infrared (FT-IR) spectral analyses of CS, SCS and SCSB were performed with Thermo Nicolet AVATAR 330 spectrophotometer in 4000-400 cm^{-1} wave length range, using KBr pallet method.

2.2. X-Ray Diffraction studies

X-Ray Diffraction of CS,SCS and SCSB were studied using X-Ray powder diffractometer (XRD-SHIMADZU XD-D1) using a Ni-filtered Cu Kα X-ray radiation source. The relative intensities were recorded within the range of 10^{o}-90^{o} (2θ) at a scanning rate of 5^{o} min^{-1}.

2.3. Thermo Gravimetric Analysis

Thermogravimetric Analysis was conducted to measure the thermal weight loss of the CS, SCS and SCSB on SDT Q600 V20.9 Build 20 instrument at a heating rate of $10^{o}C$ min^{-1} in nitrogen atmosphere. The weight losses at different stages were analysed.

2.4. Synthesis of Salicyloyl chitosan (SCS)

Salicyloyl chitosan was synthesised as follows, 1 g of dry chitosan was dispersed in 20 ml of DMF, and 1.71g of Salicylic acid (dissolved in DMF) was added, the reaction mixture was stirred at $60^{0}C$ for 2hrs under nitrogen atmosphere. Finally the whole mixture was filtered and solid products were purified by Soxlet method and dried under vacuum until a constant weight and the yellowish powder product was obtained[9]. The molar ratio of Salicylic acid to chitosan was fixed as (2:1).

2.5. Synthesis of Salicyloyl chitosan /Starch blend (SCSB)

The prepared SCS was dissolved in 1% acetic acid solution. Then starch powder was added and the mixture was heated about 80ºC till starch get completely dissolved. The obtained mixture was cast into petri plates and cured at room temperature and finally vacuum dried to remove the moisture. The resultant film was peeled off and used to further studies.

2.6. Antimicrobial Activity

Antimicrobial analysis was performed using standard agar well diffusion method to study the antibacterial and antifungal activities of the compounds[10]. Each bacterial and fungal isolate was suspended in Brain Heart Infusion (BHI) broth and diluted to approximately 105 colony forming unit (CFU) per ml. They were flood-inoculated onto the surface of BHI agar and then dried. Five-millimetre diameter wells were cut from the agar using a sterile cork-borer and 30 µL of the sample solutions were poured into the wells. The plates were incubated for 18 h at $37^{o}C$ for bacteria and at room temperature for fungi. Antimicrobial activity was evaluated by measuring the zone of inhibition in mm against the test microorganisms. Acetic acid was used as solvent control. Ciprofloxacin and Ketoconazole were used as reference antibacterial agent and reference antifungal agents respectively. The tests were carried out in triplicate.

3. RESULTS AND DISCUSSION

3.1. FT-IR

Fig(1),(2) and (3) show FT-IR spectrum of Chitosan, SCS and SCSB respectively. In Chitosan spectrum, the broad band in the range of 3000-3600cm^{-1} of all samples is attributed to OH stretching which overlaps the -NH stretching in the same region. The characteristic chitosan absorption bands at 2922 cm^{-1} represent -CH_2-stretching vibration attributed to pyranose ring[11]. The bands at 1656cm^{-1} and 1637cm^{-1} attributed to the amide bands. The adsorption band at 1151cm^{-1} is the adsorption of C-O-C stretching vibration in the glucopyranose ring and the bands at 880cm^{-1} had been assigned to the glucoside bridge structure[12,13]. In comparison of with the chitosan

FTIR spectrum, the new absorption bands at 1487 and 1460cm^{-1} (C=C stretching vibration), 650-850cm^{-1} (C-H deformation stretching) and 1254cm^{-1} (C-O stretching vibration) were assigned to the phenolic structure of SCS. The peak at 1593cm^{-1}(amide band) also indicated that the acylate reaction took place at the N-position and –NH-CO- groups have been formed[14]. In the salicyloyl chitosan-starch blend spectrum, the significant decrease of amide peak at 1591cm-1 was noticed which is connected with smaller content of chitosan in the sample of mixed polymers.

3.2. X – Ray Diffraction Analysis

XRD patterns of chitosan, SCS and SCSB are shown in Fig (4). Chitosan showed characteristic peak around 20 degree indicating the high degree of crystalline structure of chitosan. For SCS the peak at 20 degree became weaker and wider as its crystalline nature decreased due to the interaction of $-NH_2$ group of chitosan with the –COOH group of Salicylic acid. In SCSB the peak at 20 became much weaker than the SCS which indicates the interaction of - NH-CO- group with polymer chain of starch. These results confirmed the formation of SCS and SCSB.

3.3. Thermal Studies

The TGA curves of CS, SCS and SCSB were shown in the Fig (5) and (6). The thermogram of CS shows three consecutive weight loss steps. The first weight loss step was about 6.733 wt% at 55 – 191^{o}C which was responsible for the loss of moisture content indicating its hygroscopic nature. The second weight loss was about 38.35 wt% in the range of 230 to 327^{o}C, which was due to scission of the ether linkage in the chitosan backbone. In the third stage, the weight loss was about 24.85 wt % in the range of 327 - 840^{o}C, which corresponds to the thermal decomposition of glucosamine residues[15,16]. The moisture vaporisation causes a mass loss of about 14% between 90-140^{o}C in SCS and also it shows a large mass loss of about 46% in between 240-350^{o}C. This is mainly due to the thermal degradation of SCS. The SCSB curve shows three stage degradation due to evapouration of water (13 %), breaking of SCS and starch linkages (26.7%) and degradation of SCS and starch into smaller components (33%) in between 90-140^{o}C, 210-270^{o}C and 270- 400^{o}C respectively.

3.4. Antimicrobial activity

The antibacterial and antifungal activities of SCS and SCSB are shown in table (2) and table (3) respectively. These result showed the antibacterial activity of both SCS and SCSB were stronger than the reference compound ciprofloxacin for *E.coli* and almost equal for *Proteus mirablis* and *S.aueres* as well as the antifungal activity of SCS and SCSB were also stronger than Ketoconazole for *Candida albicans* and more or less equal to *A.niger* and *A.flavus*. The antimicrobial mechanism of SCS might be due to its phenolic structure which could alter microbial cell permeability interface with membrane function and interact with membrane proteins causing deformation in structure and functionality[17].The SCSB blends also show the stronger antibacterial activity similar to SCS. Even though the SCS blended with starch, its antimicrobial activity was not reduced. This could be explained as the SCS/starch blend is closely related to the composition of SCS and starch. As for the 50:50 ratio SCS and starch are miscible and SCS molecule chains would have more chance to combine with cell wall of the bacteria and kill them[18].

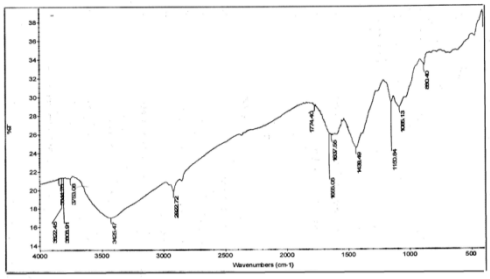


Fig. 1: FT-IR spectrum of Chitosan

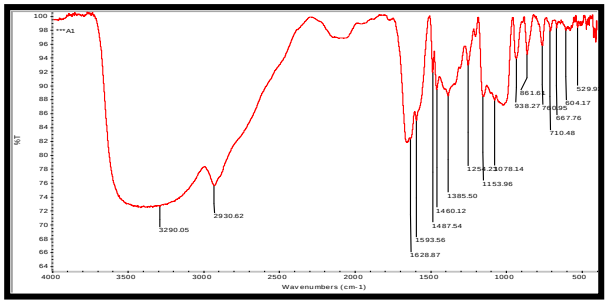


Fig. 2: FTIR spectra of SCS

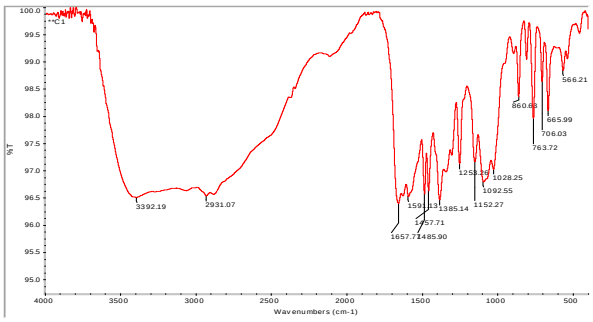


Fig. 3: FT-IR spetra of SCSB

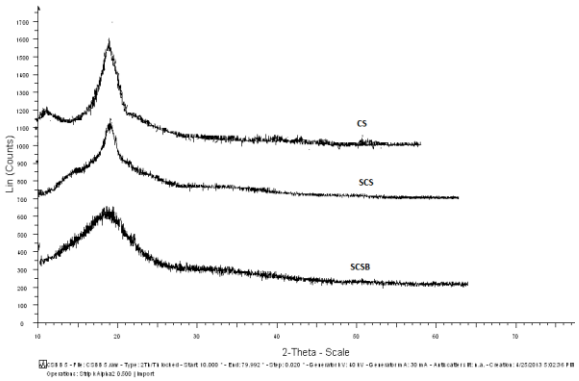


Fig. 4: XRD pattern of CS,SCS and SCSB

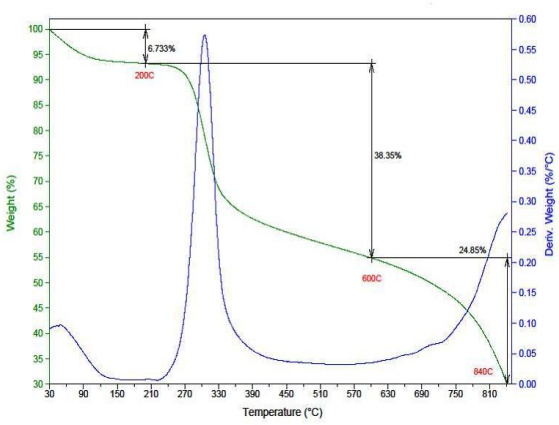


Fig. 5: TGA thermogram of chitosan

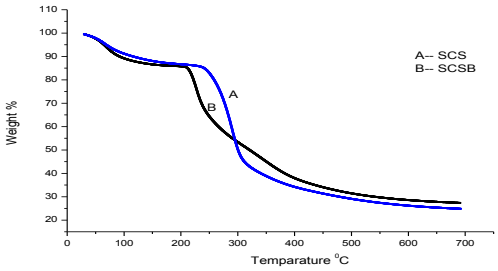
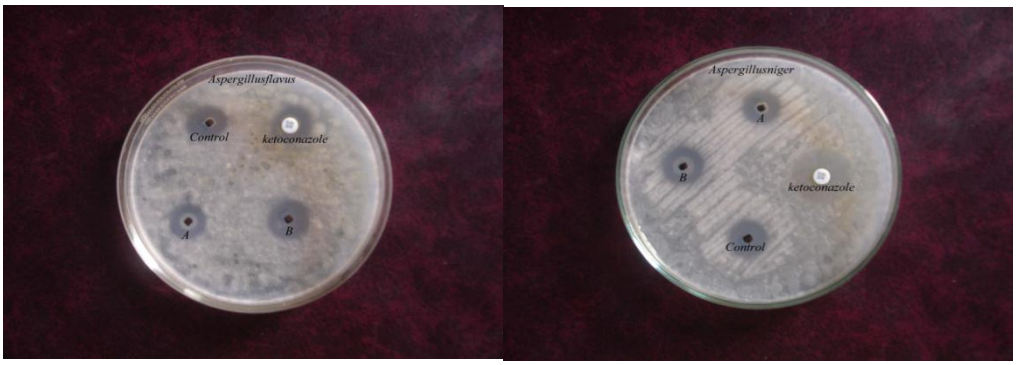


Fig. 6: TGA thermograms of SCS (A) and SCSB (B)

E.coli. ***Proteus Mirablis***

S. Aureus ***Candida Albicans***



Aspergillus Niger ***Aspergillus Flavus***

Fig. 7: Antimicrobial activity of the compounds A(SCS), B(SCSB), Control(Acetic acid) and Reference compounds(Ciprofloxacin & Ketoconazole)

Table 1: Thermal studies of CS, SCS and SCSB

Percentage of Decomposition (%)	Decomposition temperature (°C)		
	CS	SCS	SCSB
10	276	92	116
20	300	222	259
30	330	236	277
40	450	266	289
50	715	320	300
60	800	383	340
70	840	540	478

Table 2: Antibacterial activity of the compounds

S.No	Compounds	Zone of Inhibition (mm)		
		Escherichia Coli	*Proteus mirablis*	*Stapylococcus aureus*
1	Control (Acetic acid)	15	14	15
2	Ciproflaxacin	12	21	19
3	SCS	24	19	18
4	SCSB	26	20	20

Table 3: Antifungal activity of the compounds

S.No	Compounds	Zone of Inhibition (mm)		
		Candida albicans	*Aspergillus niger*	*Aspergillus flavus*
1	Control (Acetic acid)	18	10	10
2	Ketoconazole	20	15	12
3	SCS	20	11	10
4	SCSB	24	13	12

4. CONCLUSION

The new salicyloyl chitosan/starch blend was prepared successfully and it was characterised by FTIR, XRD, TGA. The FT-IR and XRD studies confirmed the formation of new blend and TGA studies showed its thermal stability. The antimicrobiol studies were done for three bacteria namely *E.coli*, *Proteus mirablis* and *S.aueres* and three fungi *C.albicans A.niger* and *A.flavus.* The new blend exhibited excellent antimicrobial activity for the bacteria *E.coli* and the fungi *C.albicans* rather than the reference compounds used in this work and showed significant acticity to other microorganisms. On the basis of our result, the newly synthesised Solicyloyl Chitosan/Starch blend can be used as a good antimicrobial film in wound healing and food packaging applications after further study.

REFERENCES

1. Patel VR and Amiji MM. Preparation and Characterisation of freeze dreid Chitosan

Poly(ethylene oxide) hydrogels for site – specific antibiotic delivery in Stomach. Pharmaceut.Res. 1996;13:588 – 593.
2. Khor E and Lim LY. Implantable Applications of Chitin and Chitosan. Biomaterials. 2003;24(13):2339-2349.
3. Yuan S and Wei T. New Contact lens Based on Chitosan/Gelatin Composites. Journal of Bioactive and Compatible polymers. 2004;19(6):467- 479.
4. Crini G. Non-Conventional Low – Cost Adsorbents for Dye Removal: A review. Bioresoure Technology. 2006;97(9):1061- 1085.
5. Esam A El-hefian, Mohamed Mahmoud Nasef and Abdul Hamid Yahya. Rheological and Morphological Studies of Chitosan/Agar/Poly(Vinyl Alcohol) Blends. Journal of Applied Science and Research. 2010;6(5):460- 468.
6. Mucha M, Ludwiczak S and Kowalcyzk M. Influence of Modified Starch on Photo stability and Adsorption properties of Low Density Polyethylene composite. Mol Crystl Liq Cryst. 2008;484:167-184.
7. Dagmara Bajer and Halina Kaczmarek. Study of the influence of UV radiation on biodegradable blends on chitosan and starch. Progress on Chemistry and its Application of Chitin and its Derivatives. 2010;15:17- 24.
8. Chandur VK, Badiger AM and Shambashiva Rao KRS. Characterising Formulations containing Derivatized Chitosan with Polymer Blending. IJRPC. 2011;1(4):950-967.
9. Wu X and Hu J. Preparation and Characters of Chitosan-g-salicylic acid. Journal of China Pharmaceutical University. 2006;6:503-506.
10. Bagamboula CF, Uttendaele M and Debevere J. Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linolool, and p-cymene towards *Shigella sonnei* and *S. flexneri.* Food Microbiol. 2004;21:33 – 42.
11. Pawlak A and Mucha M. Thermogravimetric and FTIR studied of Chitosan blends. Thermochimica Acta. 2003;396:153-166.
12. Gomes P, Gomes CA, Batista MKS, Pinto LF and Silva PAP. Synthesis, Structural Characterisation and properties of water soluble N-(γ-proponyl-amino acid)-chitosans. Carbohydrate polymers. 2008;71:54-65.
13. Wang T, Turhan M and Gunasekaran S. Selected properties of pH sensitive biodegradable chitosan-poly(vinyl alcohol) hydrogel. Polymer International. 2004;53:911-918.
14. Guangua He, Xu Chen, Yihua Yin, Hua Zheng, Xiong Xiong, Yumin Du. Synthesis, Characterisation and Antibacterial activity of Salicyloyl Chitosan. Carbohydrate Polymers. 2011;83:1274 – 1278.
15. Chen–Ho C, Fang–Yu WCF, Wei –Tung L and Ching–Dong H. Studies of Chitosan:II. Preparation and Characterisation of Chitosan/Poly(vinylalcohol)/ Gelatin ternary blend films. Int J Biol Macromol. 2008;43:37 – 42.
16. Ramya R, Sudha PN and Mahalakshmi J. Preparation and Characterisation of Chitosan Binary Blend. IJSRP. 2012;2(10):1 – 8.
17. Tiwari BK, Valdramidis VP, O'Donnell CP, Muthukaruppan K, Bourke P and Cullen PJ. Applications of Natural Antimicrobials for Food Preservation. Journal of Agricultural and Food Chemistry. 2009;57:5987 – 6000.
18. Svetlana Zivanovic, Jiajie Li, Michael Davidson P and Kevin Kit. Physical, Mechanical and Antibacterial Properties of Chitosan/PEO Blend Films. Biomacromolecules. 2007;8:1505 – 1510.