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Jafar Sathik S
PG & Research Department of Chemistry, Islamiah College (Autonomous), Vaniyambadi, Vellore District, Tamilnadu. India.

Raja Mohamed Kamil S
PG & Research Department of Chemistry, Islamiah College (Autonomous), Vaniyambadi, Vellore District, Tamilnadu. India.

Aboobucker Sithique M
PG & Research Department of Chemistry, Islamiah College (Autonomous), Vaniyambadi, Vellore District, Tamilnadu. India.

M. Yaseen Mowlana
PG & Research Department of Chemistry, Jamal Mohammed College, Trichirappalli, Tamilnadu. India.

Ravichandran C.T
Post Graduate and Research Department of Chemistry, Arignar Anna Govt.Arts College, Cheyyar, Tamilnadu. India.

Correspondence:
Jafar Sathik S
Assistant Professor of Chemistry, Islamiah College (Autonomous), Vaniyambadi-635752, Vellore District, Tamilnadu, India.

O-(2-hydroxy benzoyl) chitosan / starch blend: preparation, characterisation and antimicrobial activities

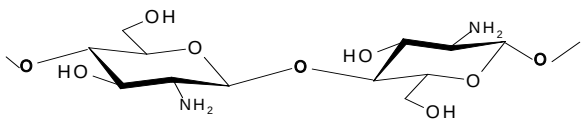
Jafar Sathik S, Raja Mohamed Kamil S, Aboobucker Sithique M, M. Yaseen Mowlana, Ravichandran C.T

Abstract
Modification of Chitosan into its derivative is a convenient method to improve its properties like metal ion adsorption, antimicrobial activity, etc. In this work the hydroxyl group of chitosan was modified by reacting with 2-hydroxybenzoic acid to obtain O-(2-hydroxybenzoyl) chitosan (OSCS). Further, the prepared derivative was blended with starch in 1:1 ratio using glycerol as a film forming material. It was named as OSCSB. The derivatives were characterised by FTIR, XRD and TGA to confirm their formation. XRD and TGA results showed the decrease in crystalline nature and thermal stability of the blend. The antibacterial activity was investigated against *Escherichia coli (E.coli), Staphylococcus aureus (S.aureus)* and *Proteus mirabilis* (*P. mirabilis*) by Zone of Inhibition method. The antifungal activities against *Candida albicans* (*C. albicans*), *Aspergillus niger* (*A. niger*) and *Aspergillus flavus* (*A. flavus*) were also assessed by the same method. This study clearly showed that both ester derivatives and its blend having a significant effect against the tested microbes.

Keywords: Chitosan, O-(2-hydroxybenzoyl) chitosan/ starch blend, Antimicrobial activity

1. Introduction
In the last few years, there has been growing interest in bio based polymer packaging products made from raw materials and originating from natural, agricultural, marine and livestock raising and renewable sources. Edible films and coatings prepared from polysaccharides, proteins and lipids have a variety of advantages over synthetic materials due to their biodegradability, edibility, biocompatibility and environmentally friendly [1]. These packaging materials moreover can serve as a carrier for nutrients, anti - browning agents, flavours and colourants to improve food quality and functionality and other active ingredients such as antimicrobial and antifungal compounds for extending product shelf life and reducing the risk of pathogen growth. These aims have been achieved with maintaining effective concentration of active compounds on food surfaces [2]. This type of packaging that is an innovative concept in food industries is named 'Active Packaging' [3, 4].
In the view of the predictable dwindling of fossil resources and of the severe environmental problems caused by the massive use of conventional packaging there is a considerable and growing interest in biodegradable materials obtained from natural polymers. In particular, polysaccharides, such as cellulose, chitin, chitosan, starch, etc., are considered as the key raw materials for the production of chemicals and materials for the biorefineries of the future [5].
Chitosan is a natural biopolymer, cationic polysaccharide, produced by the partial deacetylation of chitin isolated from naturally occurring crustacean shells. Chitosan is one of the most abundant naturally occurring polysaccharide. It is commercially available from a stable renewable source that is shellfish waste (shrimp and crab shells) of the sea food industry. This biopolymer has revealed to be useful in formation of biodegradable films and preservation of foods from microbial deterioration. The potential of chitosan to act as a food preservative of natural origin has been widely reported on the basis of *in vitro* trails as well as through direct application on real complex matrix foods [6].

CHITOSAN



The antimicrobial activities of chitosan and its derivatives have aroused considerable recent interest [7-13]. The chitosan derivatives mentioned in the literature showed that one can differentiate specific reactions involving the $–NH_2$ group at the C-2 position or the non-specific reactions of –OH groups at the C-3 and C-6 positions, especially, esterification and etherification [14]. Therefore, acylation can occur on the hydroxyl groups to obtain chitosan ester and occur on the amino groups to obtain acyl amide. Much attention has been paid on the synthesis of N-aryl chitosan and N-benzyl chitosan which is often used as interim outcome to make some other derivatives relying on the strong point of the high rate of condensation and production, also the reactive condition and process of the synthesis appears not to be so strict and complicated. In addition N-benzoylation of chitosan is easily reverted. The number of O- substituted derivatives of chitosan is much lesser. In many cases, modification of chitosan through hydroxyl groups has an advantage because of free amino groups in the products and possible less influence on the fundamental skeleton [15]. Starch is a water soluble polysaccharide with well-known biodegradable and an edible film forming properties. Starch based packaging materials are derived from a variety of botanical sources such as corn, wheat, potatoes and tapioca and can be produced at low cost and at large scale from different surplus of harvesting and raw material industrialization [16].

Starch based films exhibit physical characteristics similar to synthetic polymers: transparent, odourless, tasteless, semipermeable to CO_2 and resistant to O_2 passage.

To improve the physical and functional properties of starch films, which find applications in functional food packaging, they are blended with other biopolymers and substances having antioxidant and antimicrobial activities. Due to the enormous potential of starch – chitosan films in the field of preservation and packaging technology, a lot of research works have been carried out in the preparation and characterization of this blend material [17-19]. This is mainly due to the fact that chitosan exhibits high antimicrobial activity against pathogenic and spoilage microorganisms, including both gram-negative and gram-positive bacteria and fungi [20].

The scope of this study is to synthesis new blend derivative of chitosan having better microbiological activities. In this work, the ester derivative of chitosan is prepared by reacting chitosan with 2-hydroxybenzoic acid in the presence of sulphuric acid and blended with starch in the ratio of 1:1. All the derivatives are characterized by FT-IR and XRD to conform their formation. Their thermal stability is analysed by TGA. Their antimicrobial activities are assessed against the three bacteria (*E.coli, S.aureus* and *P. mirabilis*) and three fungi (*A. Niger, A. flavus* and *C. albicans*).

2. Materials and Methods

2.1 Materials

Original chitosan was purchased from Central Institute of Fisheries Technology, Cochin. Sulphuric acid, salicylic acid, acetic acid, glycerol and starch were purchased from Galaxy scientific company, Vellore. FTIR spectra were recorded at DST-FIST sponsored laboratory, Islamiah College (Autonomous), Vaniyambadi, Vellore district. Antibacterial activity application was performed at Micro labs, Tiruchirappalli. All the spectra were taken at room temperature. All others chemicals were of analytical grade and were used without further purification.

2.2 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.

2.3 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 0^o-90^o (2θ) at a scanning rate of 5^o min^{-1}.

2.4 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 ^oC min^{-1} in nitrogen atmosphere. The weight losses at different stages were analysed.

2.5 Preparation of chitosan ester derivative

3 g of chitosan was suspended in 50 mL of 5M sulphuric acid. To this solution, 2.5 g of 2-hydroxybenzoic acid was added. The mixture was refluxed for 4 Hours and was cooled subsequently to room temperature. The pH was adjusted to 7.0 by neutralization with sodium bicarbonate. The desired compound was precipitated in acetone, filtered and washed with acetone to remove the unreacted acid. Finally, the precipitants were soxhlet extracted with acetone for two days and then oven dried overnight at 60 ^0C, to obtain the desired product.

2.6 Synthesis of chitosan derivative-starch blend

Chitosan derivative-starch blend was prepared by mixing 50 mL of 2% (w/v) solution of chitosan derivative with 2% (w/v) solution of starch. Glycerol was added as 30% (w/w) of the total solid weight. The solution was stirred at 50 ^0C for 30 minutes. The mixture was cast on to a plate and vacuum dried for three days.

2.7 Antimicrobial Activity

Antimicrobial analysis was performed using standard agar well diffusion method to study the antibacterial and antifungal activities of the compounds. 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 ^oC 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 and antifungal agents respectively. The tests were carried out in triplicate.

3. Results and Discussions

3.1 Fourier Transform Infrared spectral analysis

The broad band in the range of 3000-3600 cm^{-1} range in the spectra of all samples is shows broadband range attributed to OH stretching, which overlaps the -NH stretching in the same region. The characteristic chitosan absorption band at 2922 cm^{-1} represents $-CH_2-$ aliphatic group. The bands at 1666 cm^{-1} and 1637 cm^{-1} attributed to the amide bands. The band at 1150

cm^{-1} is due to the absorption of C-O-C stretching, vibration in the glucopyranose ring. In the FT-IR spectrum of OSCS, the new absorption peak at 1701 cm^{-1} was attributed to the characteristic band of the ester bond. This bond was brought by the benzoylation of chitosan with 2-hydroxy benzoic acid. The characteristic absorption band at 1637 cm^{-1} was still found. It clearly indicated that the acylation mainly occurred on the hydroxyl group of chitosan. In the OSCSB spectrum, the peaks corresponding to amine groups were slightly shifted and considerably decreased. It showed that some interactions were occurred between the OSCS and starch.

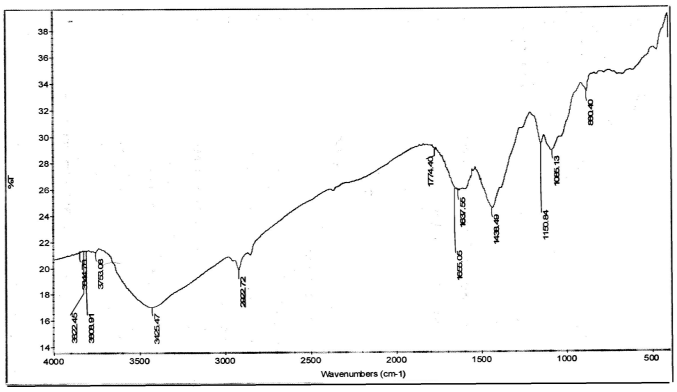


Fig 1: IR spectrum of chitosan

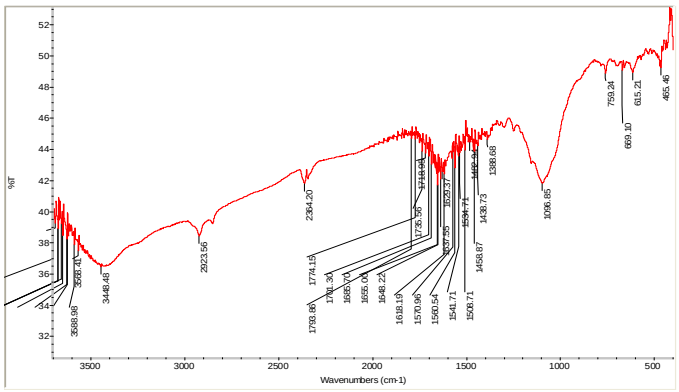


Fig 2: IR spectrum of OSCS

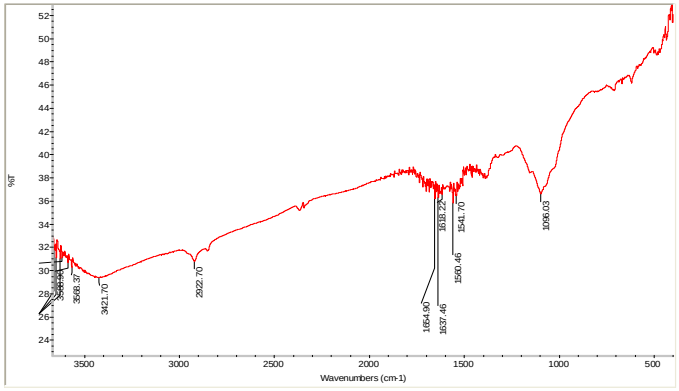


Fig 3: IR spectrum of OSCSB

3.2 X– Ray Diffraction studies

The XRD pattern of chitosan in Fig: 4 showed two characteristic peaks around 2θ = 10 and 20. This indicated the high degree of crystalline nature of chitosan. For the derivatives (Fig: 5 and 6), the peak at 20 became much weaker and wider. These changes suggested that the substitution and blending led to decrease in the crystalline nature of chitosan.

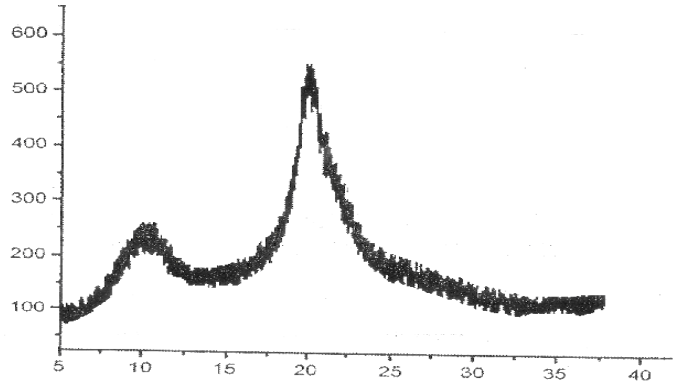


Fig 4: XRD pattern of Chitosan

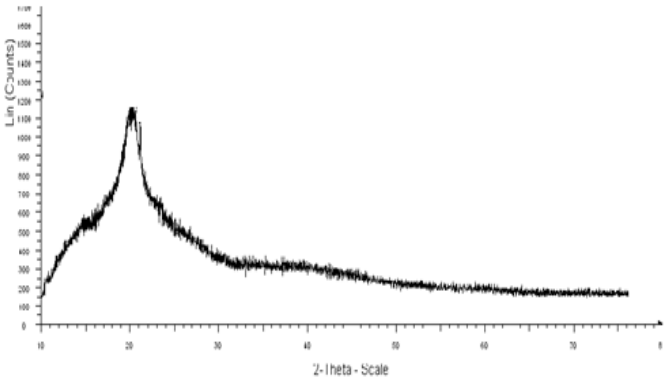


Fig 5: XRD pattern of OSCS

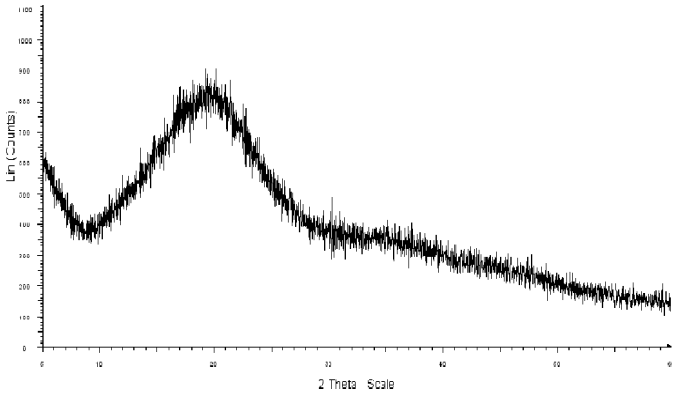


Fig 6: XRD pattern of OSCSB

3.3 Thermogravimetric analysis

TGA thermal details of chitosan is shown in Table: 1 and Fig: 7. about 50% of chitosan is decomposed at around 700 ^{0}C. Two weight losses were observed in the chitosan TGA curve. The weight loss at around 200-350 ^{0}C was due to the detachment of the entangled chitosan chains. The second degradation at around 450-700 ^{0}C may be due to the degradation of chitosan molecule. The thermal decomposition detail of OSCS given in Table: 2 and Fig: 8 represent the percentage decomposition of the sample at different temperatures. Around 97.8% of the sample was disintegrated at 82 5 ^{0}C leaving behind 2.2% of the sample as a residue at the end of the experiment. TGA thermal details of OSCSB is presented in Table: 3 and Fig: 9. At the end of the experiment nearly 30% of the sample remind as a residue. The first stage has taken place in the range 40-180 ^{0}C, due to the elimination of water and the dopant molecules. The second stage was in the temperature range of 200-400 ^{0}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 200-400 ^{0}C. The TGA thermograms of the chitosan derivative and their starch blends showed lower thermal stability than the pure chitosan. This could be related with crystalline and/or morphological variations in blend with respect to those of the pure polymers.

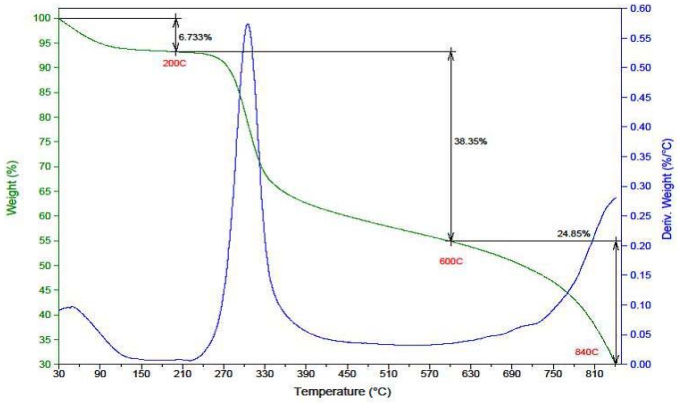


Fig 7: TGA thermogram of Chitosan

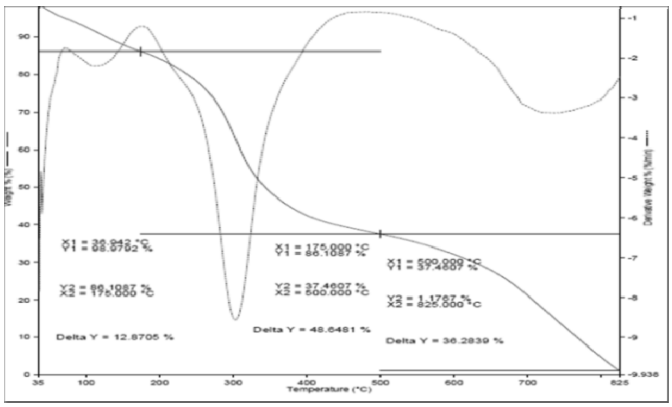


Fig 8: TGA thermogram of OSCS

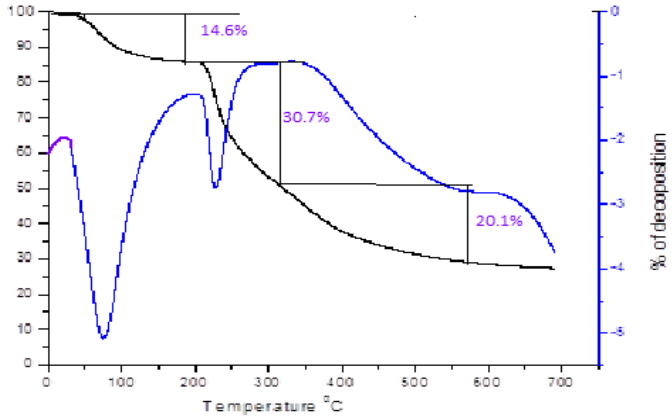


Fig 9: TGA thermogram of OSCSB

Table 1: TGA thermogram details of chitosan

(%) of Decomposition	Decomposition temperature (^{0}C)
10	159.15
20	277.35
30	322.75
40	340.95
50	368.25
60	445.50
70	622.75
80	704.55
90	754.60

Table 2: TGA thermogram details of OSCS

% 0f Decomposition	Decomposition temperature (^{0}C)
10	186.45
20	231.85
30	250.05
40	309.10
50	345.50
60	372.80
70	436.40
80	663.70

Table 3: TGA thermogram details of OSCSB

% of Decomposition	Decomposition temperature (^{0}C)
10	92
20	222.24
30	235.70
40	266.20
50	320.60
60	382.80
70	538.40

3.4 Antimicrobial Activity

The antimicrobial activity of the chitosan derivatives against some microorganisms was studied. The bacteria selected for the study were *Escherichia coli, Proteus mirabilis and Staphylococcus aureus* and the fungus were *Aspergillus niger, Aspergillus flavus and Candida albicans.* The results are shown in Table: 4. In the case of bacterial and fungus control, the synthesized drugs showed considerable activity but less than the standard drug. Among the tested microbes, chitosan blend showed higher activity against *S.aureus* (gram positive) bacteria and *A. niger*. Generally, the modified chitosan were found to be more active towards fungal and bacterial species. Between the OSCS and OSCSB, later one showed slightly better antimicrobial activity than the former one. This may be due to the surface modification of the blend during the blending process [21].

Table 4: Antimicrobial activity of chitosan, OSCS and OSCSB

ORGANISM	Zone of Inhibition(mm)		
	Control	OSCS	OSCS B
E.coli	38	13	14
Staphylococcus aureus	25	22	25
P. mirabilis	15	15	16
Candida albicans	23	10	15
A. niger	35	17	20
A. flavus	35	14	18

4. Conclusion

In the present study, chitosan was chemically modified to enhance its applications. The O-substituted chitosan derivative (OSCS) was successfully synthesized by treating chitosan with, 2-hydroxy benzoic acid. The derivative was blended with starch in 50:50 ratio. The prepared blend derivative was characterized by FT-IR. The result confirmed the formation of the derivatives. TGA result showed that the prepared derivative was thermally less stable than the chitosan. The crystal nature of the chitosan and their derivatives was studied by XRD. The XRD data revealed that the crystal nature of the derivatives was decreased by the substituent. The antimicrobial activity of the chitosan derivatives were investigated against some bacteria and fungi. It was found that the derivatives have a significant effect against all the microorganisms. This would encourage the use of these biopolymeric chitosan derivatives as effective antimicrobial

agents. This work clearly indicated that chitosan derivative and it's blend could be used as antimicrobial agents in food packaging materials after further studies.

5. References

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