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Research Article
Preparation, Characterization and Cross-linking of Chitosan by Microwave Assisted Synthesis

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Abstract

Background and Objective: The pH-dependent solubility of chitosan is a function of the amino groups in the molecule and its depolymerization in acidic medium is a major drawback for controlled oral delivery. This problem can be countered by irreversible chemical crosslinking with agents such as glutaraldehyde, formaldehyde, tripolyphosphate, genipin, vanillin etc. The objective of this study was to prepare cross-linked chitosan using vanillin and to study the factors involved therein. **Materials and Methods:** Cross-linked chitosan was prepared by refluxing and microwave irradiation method and then compared. Refluxing method required 6 h to complete the reaction, whereas microwave irradiation method required 1-5 min. Box Behnken method was employed to optimize cross-linking by the microwave irradiation method. Concentration of vanillin, microwave power and microwaving time were chosen as the independent variables and swelling in acidic and basic pH and degree of cross-linking as dependent variables. The cross linked chitosan was characterized by FT-IR analysis, elemental analysis, thermogravimetric analysis, ^{1}H NMR, solid state ^{13}C nuclear magnetic resonance (CP/MAS ^{13}C NMR) and Bradford assay. Statistically variables were evaluated by one way analysis of variance (ANOVA) at 0.05 level using design expert. **Results:** Results confirmed the formation of a Schiff base between chitosan and vanillin which in turn confirms the process of cross-linking. **Conclusion:** The microwave irradiation method be used as a rapid, reliable and economic method for cross-linking of chitosan.

Key words: Vanillin, cross-linking, microwave irradiation, schiff base, box-behnken

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Inter penetrating polymer networks (IPNs) are distinctive combinations of cross-linked polymers in which at least one network is synthesized and/or cross-linked in the presence of the other. IPNs aid in combining individual properties of two or more polymers by which a three-dimensional network structure was obtained which helps in the easy encapsulation of drugs[1]. Chitosan chemically is poly [β-(1-4)-2-amino-2-deoxy-D-glucopyranose]. It was a widely explored polymer, due to the presence of the amino functionality, which could be modified to impart desired properties and distinctive biological functions such as solubility, pH sensitivity, mucoadhesion, permeation enhancement and bio-adhesivity. The available amino and hydroxyl groups on chitosan are active sites capable of forming a number of linkages including amide and ester bonding, as well as Schiff base formation. Schiff base is a weak base and is insoluble in water and organic solvents, but soluble in dilute aqueous acidic solutions. The pH-dependant solubility of chitosan is a function of the amino groups in the molecule and its depolymerization in acidic medium is a major drawback for controlled oral delivery. This problem can be countered by irreversible chemical crosslinking with agents such as glutaraldehyde, formaldehyde, tripolyphosphate, genipin, vanillin etc[2].

Cross-linking influences properties such as mechanical strength, chemical stability, swelling, aqueous permeability, solubility and drug release. Cross-linked chitosan exhibit pH-sensitive swelling and drug release by diffusion through their porous structure and can be used in the formulation of novel drug delivery systems[3]. Various therapeutic agents such as anti-inflammatory agents, antibiotics, anticancer drugs, steroids, proteins, amino acids, anti-diabetics and diuretics have been investigated using cross-linked chitosan to achieve controlled release or for drug targeting[4-6]. Cross-linking agents are broadly classified as physical and chemical cross-linking agents. Some of the physical cross-linkers are citric acid, dextran sulfate or phosphoric acids. Chemical cross-linking agents includes glutaraldehyde, formaldehyde, vanillin and genipin. Cross-linkers used for chitosan cross linking are glutaraldehyde, formaldehyde, tripolyphosphate and polyaspartic acid sodium salt. However, glutaraldehyde and formaldehyde are considered to be toxic in certain concentrations and raise health concerns and cause undesirable side effects. Therefore, it was necessary to use a non-toxic and effective cross-linker for the cross linking of chitosan[7,8].

Vanillin (4-Hydroxy-3-methoxybenzaldehyde) obtained from vanilla pods of the tropical vanilla orchid are widely used as a flavoring agent in food stuffs, drinks and cosmetics. The cross linking of chitosan with vanillin was based on Schiff reaction between the aldehyde group of vanillin and amino groups of chitosan, leading to the formation of an imine bond (C=N). Vanillin has been used as a cross linker in chitosan nanoparticles[6,7], controlled release Resveratrol microspheres[8] and as an absorbent of heavy metal ions in waste water treatment[9]. Conventionally Schiff bases have been prepared by refluxing mixtures of the amine and the carbonyl compound in an organic solvent or by refluxing the mixture in heptane in the presence of acetic acid or azeotroping the mixture with benzene in the presence of acid[10]. However, microwave-assisted reactions, are being been widely used in organic synthesis because of its advantages like rapid heating, increased rate of reaction, shorter reaction time, simple reaction conditions, reduced hazards, reduced pollution, higher yield, simplicity in processing and handling[11-17]. The process can be optimized using design of experiments approach for the better utilization of resources. In the current study, Box Behnken design was employed to study the factors that influence the formation of cross linked chitosan. This design has fewer design points and can be less expensive than other designs with the same number of factors.

The objective of the present study was to carry out cross-linking of chitosan using vanillin by microwave irradiation method and to compare this method with the conventional method of refluxing in terms of increase in yield of cross linked chitosan.

MATERIALS AND METHODS

Materials: The study was executed in 2015 at the Department of Pharmaceutics, M.S Ramaiah College of Pharmacy, Bengalore.

Chitosan (85% DA) was procured from Marine Chemicals, Cochin, Kerala. Vanillin was purchased from Merck Chemicals Corporation Ltd., Mumbai, India. All chemicals and reagents used were of analytical grade.

Preparation of cross-linked chitosan: Preliminary trials were carried out to determine the ability of vanillin to cross-link chitosan. Various batches of cross-linked chitosan were prepared by two different methods:

Refluxing method: Chitosan (85% DAC) dissolved in 1% acetic acid and vanillin (dissolved in 10 mL of acetone) was refluxed together for 6 h at 100°C. The product was then washed with 5 mL of ethanol (95%), dried in a vacuum oven at 45°C for 12 h, pulverised, sieved using BSS# 60 and stored in a desiccator for further analysis.

Table 1: Levels of Independent variables taken for optimization

	Levels used	
Independent variables	Low	High
Concentration of vanillin (% w/v)	1	4
Microwave power (%)	60	100
Microwaving time (min)	1	5

Table 2: Optimization trials as per DOE

Code for cross-linked chitosan	A: Concentration of vanillin (%)	B: Microwave power (%)	C: Microwave time (min)
CL1	1.0	60	3
CL 2	1.0	100	3
CL 3	1.0	80	1
CL 4	2.5	80	3
CL 5	4.0	100	3
CL 6	2.5	80	3
CL 7	4.0	60	3
CL 8	4.0	80	5
CL 9	2.5	80	3
CL 10	2.5	100	1
CL 11	4.0	80	1
CL 12	2.5	60	1
CL 13	1.0	80	5
CL 14	2.5	80	3
CL 15	2.5	80	3
CL 16	2.5	60	5
CL 17	2.5	100	5

Microwave irradiation method: Chitosan (85% DAC) dissolved in 1% acetic acid and vanillin (dissolved in 10 mL of acetone) was microwaved together at varying microwave power and time. After completing the reaction, the mixture was cooled and left overnight to obtain thick slurry. It was then washed with 5 mL of ethanol (95%), dried in a vacuum oven at 45°C for 12 h, pulverised, sieved using BSS# 60 and stored in a desiccator for further analysis.

Both the methods were tried out in order to make a comparative study in terms of yield, reaction period and ease of reaction. The reaction carried out using conventional method required about 6 h, while microwave irradiation method required only 1.0-5.0 min.

Optimization of the process variables: Based on the results of the preliminary trials, it was considered appropriate to optimize the microwave irradiation method for preparation of cross- linked chitosan. The experimental runs were based on Box-Behnken design using response surface methodology. The responses were subjected to multiple regression analysis to find out the relationship between the factors used and responses obtained. Three formulation variables at two levels were chosen for the study. Concentration of vanillin, microwave power and microwaving time were selected as the independent variables. Chitosan was soluble in dilute aqueous solution due to the polymerization of the amino groups. Cross-linking of chitosan would reduce its solubility in acidic medium to a great extent. Hence, swelling index in acidic pH and swelling index in basic pH were chosen as the dependent/response variables (Table 1). The effect of formulation variables on the response variables was statistically evaluated by one way analysis of variance (ANOVA) at 0.05 level using Design Expert 9.0.3.1 trial version (Stat Ease, USA). The design was evaluated by Quadratic model which bears the form of following equation:

$$Y = b0+b1X1+b2X2+b3X3+.....$$

The 17 trials were suggested with all possible combinations of variables (Table 2). Cross linking of chitosan for these 17 trials were carried out by microwave irradiation method by the procedure as mentioned earlier.

Characterization of cross-linked chitosan: All the 17 trials were analyzed for their swelling behaviour in acidic and basic pH and their degree of cross linking in order to generate an optimized solution.

Swelling studies: Swelling studies for cross-linked chitosan was carried by the tea bag method in acidic and alkaline buffer solutions. A tea bag (i.e., a 100 mesh nylon screen) containing an accurately weighed powdered sample (0.1 g), with average particle sizes of 40-60 mesh (250-350 µm), was completely immersed in pH 1.2 buffer (100 mL) and pH 6.8 buffer and allowed to soak for 2 h at room temperature. The tea bag was hung up for 15 min in order to remove the excess fluid. The % swelling index was determined using the following equation[18]:

$$\% \text{ Swelling index} = \frac{W2-W1}{W1}\times 100$$

where, W1 and W2 are the weights of the dry and swollen samples, respectively. The experiment was carried out in triplicate.

Degree of cross linking: The extent of cross linking of chitosan was determined by Bradford assay. The amount of free amino groups in the chitosan material before (Ci) and after cross-linking (Cf) is proportional to the optical absorbance of the solution. The % cross-linking index is calculated using the following equation[19,20]:

$$\% \text{ Cross-linking index} = \frac{\text{Ci-Cf}}{\text{Ci}} \times 100$$

Where:

Ci = Amount of free amino groups in chitosan before cross-linking

Cf = Amount of free amino groups in cross-linked chitosan

Preparation of bradford reagent: Fifty milligrams of coomassie brilliant blue G-250 was dissolved in 25 mL of 95% ethanol. To this solution, 50 mL of phosphoric acid was added. The resulting solution was diluted to 500 mL with distilled water.

Preparation of sample: To 1 mL of 1% cross-linked chitosan solution in 2% v/v acetic acid, 9 mL of Bradford reagent was added and mixed. The absorbance of the resulting solution was measured at 595 nm using UV Visible spectrophotometer (Shimadzu 1601, Japan). A solution containing 1 mL of 2%v/v acetic acid and 9 mL of Bradford reagent was taken as blank.

Characterization of optimized cross-linked chitosan (OPT-CV)

Fourier-transform infrared spectroscopy: Solid sample of approximately 2-3 mg was mixed with about 0.5-1 g of KBr (which is transparent to IR) and then thoroughly ground in a mortar. The mixture was pressed in a pellet die and placed in a fourier transform infrared (FTIR) spectrophotometer (FTIR 8400S, Shimadzu, Japan). The FTIR spectrum of the all the experimental runs were recorded.

Elemental analysis: Elemental analysis data was used to confirm cross-linking, to support FTIR, ^{1}H NMR and ^{13}C NMR studies. The elemental analysis was carried out using an Organic Elemental Analyzer (2400 Series II CHNS/O System, Perkin Elmer, USA).

Nuclear magnetic resonance (NMR)[21-23]:

- ^{1}H NMR spectra was recorded using a NMR spectrometer (Avance III HD, Bruker, USA) at a resonance frequency of 400 MHz and temperature 301°K. All the chemical shifts (d) were reported in parts per million (ppm) using tetramethylsilane (TMS) as internal standard
- Solid state CP/MAS ^{13}C NMR measurements were made using a JEOL ECX400 spectrometer (9.389 T). The samples were packed in a ZrO rotor with an outer diameter of 3.2 mm. The CP/MAS spectra were recorded with a contact time of 3.5 msec. The chemical shifts (d) were reported in parts per million (ppm) using Tetramethylsilane (TMS) as internal standard

Thermal gravimetric analysis (TGA): The thermal behaviour of chitosan and cross-linked chitosan was tested using a Thermal Analyzer (STA 6000, Perkin Elmer, USA). The operating conditions were as follows: Temperature range of 40-730°C, with a heating rate of 10.00°C min^{-1}.

RESULTS AND DISCUSSION

Preliminary trials were carried out to determine the ability of vanillin to cross-link chitosan. Various batches of cross-linked chitosan were prepared by two different methods: Refluxing method and microwave irradiation method. The reaction carried out using conventional method of refluxing required about 6 h, while microwave irradiation method required only 1.0-5.0 min. Hence, microwaving method was chosen for the preparation of cross-linked chitosan. Based on the results of the preliminary trials, it was considered to be appropriate to optimize the microwave irradiation method for preparation of cross-linked chitosan using design of experiments (DOE).

Optimization of the process variables: Optimization was carried out using Box-Behnken design using Response surface methodology[24] by taking into consideration concentration of vanillin, microwave power and microwaving time as independent variables and the influence of these variables on the responses swelling index in acidic pH and swelling index in basic pH. This design was adopted to analyze the relationship between multiple variables with reduced number of experimental runs; according to which 17 formulations were prepared. All the 17 trials were analyzed for their swelling behaviour in acidic and basic pH and their degree of cross linking determined.

Swelling studies: The pH sensitive behavior of cross-linked chitosan (all 17 trials) was determined by carrying out swelling studies in 1.2 pH acidic buffer and 6.8 pH phosphate buffer and comparing it with unmodified chitosan[25]. The results showed that unmodified chitosan showed maximum swelling in pH 1.2 buffer and minimum swelling in pH 6.8 buffer. Cross-linked chitosan CL 10 prepared using 2.5% vanillin using 100% microwave power for 1 min, CL11 prepared using 4% vanillin using 80% microwave power for 1 min, CL 13 prepared using 1% vanillin using 80% microwave power for 5 min and

CL 16 prepared using 2.5% vanillin using 60% microwave power for 5 min showed higher swelling in pH 6.8 buffer when compared to pH 1.2 buffer (Table 3). Increased swelling in pH 6.8 buffer may be linked to a higher degree of cross-linking.

Degree of cross linking: To further support the results of FT-IR studies and to confirm the degree or extent of cross linking, Bradford assay was carried out on all the cross linked chitosan trials. The amount of free amino groups in the chitosan material before (Ci) and after cross-linking (Cf) was considered to be proportional to the optical absorbance of the solution[26]. All the runs showed cross-linking between 11.3-66.55%. Cross linked chitosan CL 8, 10, 11, 13 and 16 showed a cross-linking of 60.98, 63.93, 63.60, 66.55 and 65.57%, respectively (Table 3).

Table 3: Data for swelling studies and degree of cross-linking for trial runs

	Swelling index (%)		
Code for cross-linked chitosan	Acidic pH (pH 1.2)	Basic pH (pH 6.8)	Cross-linking index (%)
Chitosan (85% DAC)	1053	387	-
CL1	570	254	8.19
CL 2	500	355	4.59
CL 3	800	357	7.54
CL 4	617	404	11.30
CL 5	560	180	34.75
CL 6	617	404	17.06
CL 7	800	357	48.85
CL 8	550	458	60.98
CL 9	617	404	50.45
CL 10	400	877	63.93
CL 11	443	683	63.60
CL 12	696	650	59.01
CL 13	321	917	66.55
CL 14	617	404	11.30
CL 15	617	404	12.60
CL 16	350	854	65.57
CL 17	632	359	13.44

Effect of process variables on swelling index in acidic pH: The results of formulations as per design was fitted into various models and a linear model was found to be significant for swelling index in acidic pH with F-value 5.77 and p-value 0.0074. In this model factors A and B (concentration of vanillin, microwave power) had significant effect on the swelling index in acidic pH whereas factor C (microwaving time) did not have significant effect on the response. The model equation is as follows:

$$\%\ \text{Swelling in acidic pH} = +571.00+20.25\times A-40.50\times B-60.75\times C+146.50\times AC+144.50\times BC$$

The effect of both the factors A and B can be explained with 3D response surface plot as in Fig. 1. As the vanillin concentration and microwave power increased the swelling index also increased.

Effect of process variables on swelling index in basic pH: The results of formulations as per design was fitted into various models and a quadratic model was found to be significant for swelling index in acidic pH with F-value 9.84 and p-value 0.0032. In this model factors all the factors A, B and C

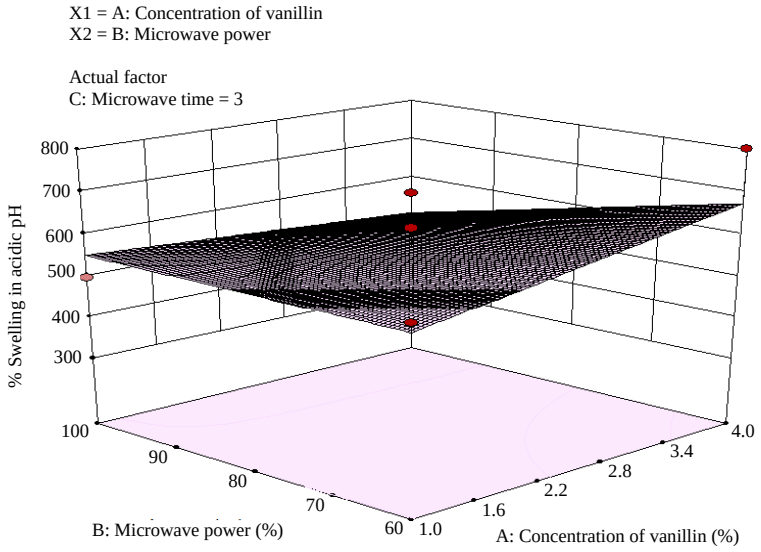


Fig. 1: 3D RSM graph of response swelling index in acidic pH

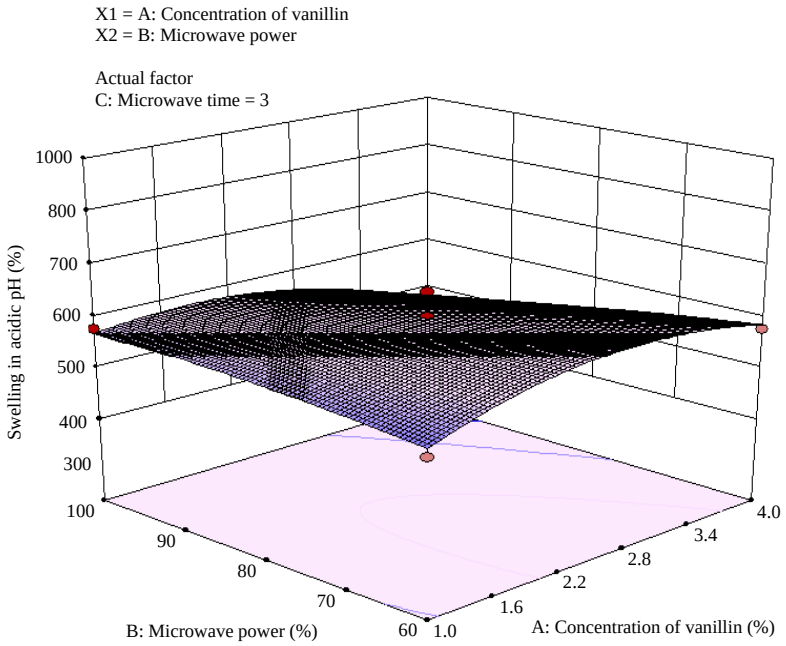


Fig. 2: 3D RSM graph of response swelling index in basic pH

Table 4: Summary of ANOVA for dependent variables from Box-Behnken design

Source	Sum of squares	df	Mean square	F-value	Probability
Response surface 2FI model for % swelling acidic pH					
Model	2.153E+005	5	43059.40	5.77	0.0074
A-Concentration of vanillin	3280.50	1	3280.50	0.44	0.5210
B-Microwave power	13122.00	1	13122.00	1.76	0.2117
C-Microwave time	29524.50	1	29524.50	3.96	0.0721
AB	85849.00	1	85849.00	11.50	0.0060
AC	83521.00	1	83521.00	11.19	0.0065
BC	82081.00	11	7461.91		
Quadratic model for % swelling in basic pH					
Model	7.303E+005	9	81142.11	9.84	0.0032
A-Concentration of vanillin	5253.13	1	5253.13	0.64	0.4510
B-Microwave power	14792.00	1	14792.00	1.79	0.2223
C-Microwave time	55.13	1	55.13	6.685E-003	0.9371
AB	19321.00	1	19321.00	2.34	0.1697
AC	1.541E+005	1	1.541E+005	18.68	0.0035
BC	1.303E+005	1	1.303E+005	15.80	0.0054

(concentration of vanillin, microwave power, microwaving time) had significant effect on the swelling index in basic pH. The model equation is as follows:

$$\%\ \text{Swelling in basic pH} = +404.00-25.63\times A-43.00\times B+2.63\times C-69.50\times AB-196.25\times AC-180.50\times BC-99.37\times A2-18.13\times B2+299.13\times C2$$

The effect of the factors A, B and C can be explained with 3D response surface plot as in Fig. 2. The results of ANOVA are shown in Table 4.

On analysis of the results, 85 numerical optimized solutions were generated based on the experimental design. A solution was selected randomly, coded as OPT-CV and considered as optimized cross linked chitosan. This was selected based on the basis of its % swelling in acidic and basic pH as per the trial runs. OPT-CV includes 1.84% of vanillin, 60 % microwave power for 5 min, showing swelling of 345% in acidic pH and 917% in basic pH. The results of predicted observation and actual experimentation are shown in Table 5, which confirmed the closeness of the observed responses with that of the responses predicted by design expert software. The results demonstrated a good relationship between the predicted and experimental values, confirming the validity of the model. This confirmed the capacity of chitosan to depolymerize in acidic pH is effectively reduced by cross-linking with vanillin.

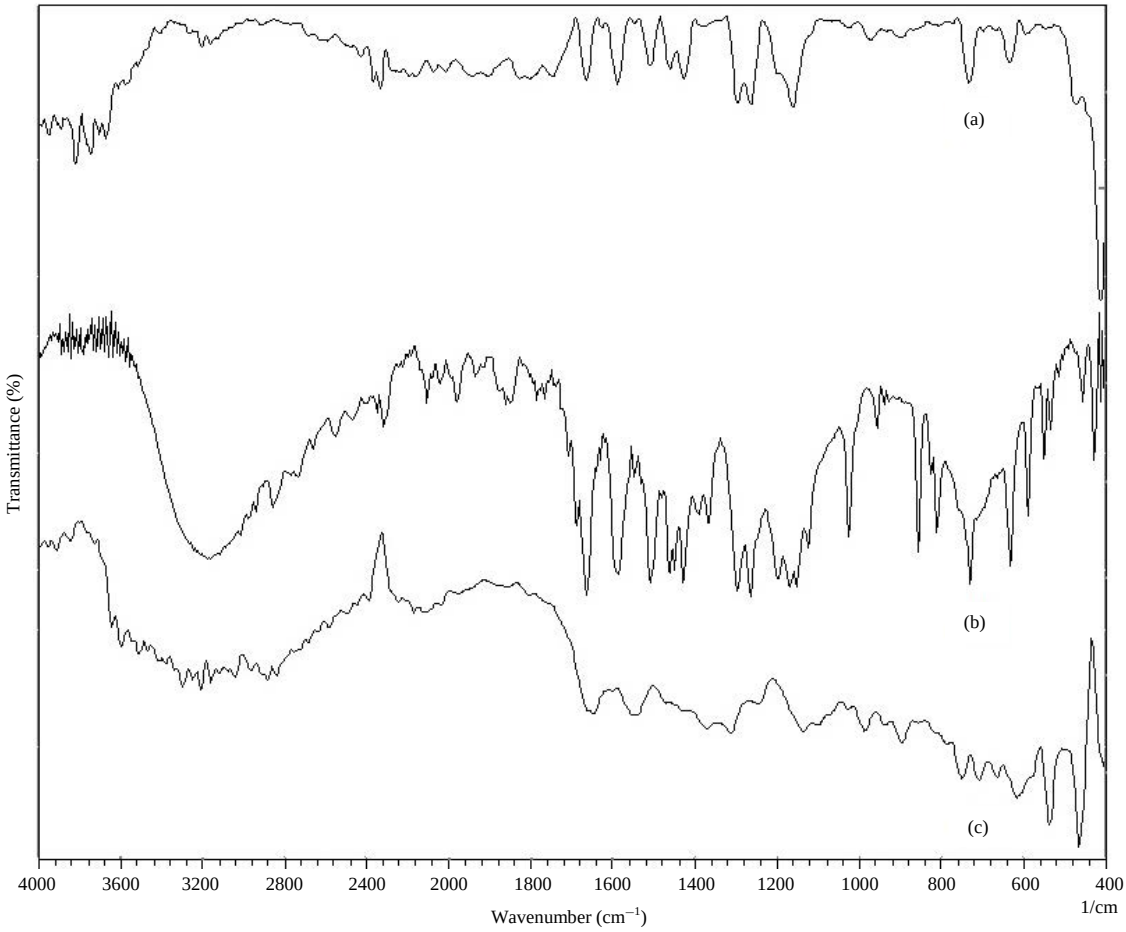


Fig. 3: IR spectrum of (a) OPT-CV, (b) Vanillin and (c) Chitosan

Table 5: Optimized formulation (Predicted v/s Actual)

	OPT-CV	
Observation	Predicted value	Observed value
Concentration of vanillin	1.84%	1.84%
Microwave power	60%	60%
Microwave time	5 min	5 min
Percentage of swelling in acidic pH	332	345
Percentage of swelling in basic pH	959	917

Table 6: Elemental analysis data

	Theoretical value			Experimental value		
Sample codes	C (%)	H (%)	N (%)	C (%)	H (%)	N (%)
Plain chitosan	39.68	7.85	7.59	39.92	6.819	6.62
OPT-CV (Optimized cross-linked chitosan)	53.67	6.11	4.47	54.93	6.163	3.29

Characterization of optimized cross-linked chitosan (OPT-CV)

FT-IR studies: The spectra of optimized cross- linked chitosan showed the characteristic bands of chitosan, vanillin along with new peaks (Fig. 3a-c). The formulation of vanillin cross-linked chitosan was based on Schiff base reaction between the aldehyde group of vanillin and amino group of chitosan. This can be confirmed by the presence of C=N (imine) group in the IR spectrum. The cross-linking in OPT- CV was confirmed by the detection of peaks in the range 1580-1680 cm^{-1}, which was attributed to the C=N (imine) group. The stretch vibration of C=N group was considered to be more prominent in the range 1600-1660 cm^{-1}. The frequency 1589 cm^{-1} in IR spectra of OPT-CV may be attributed to the benzene stretch of vanillin. The frequencies at 1660 cm^{-1} range may be due to the vibration of amide I and II groups of chitosan. The optimized cross linked chitosan has shown peaks at 1627 and 1637 cm^{-1} which can be considered as C=N (imine) groups[27]. This confirms the process of cross linking, which can be further supported by elemental analysis and NMR data.

Elemental analysis: The data of elemental analysis is shown in Table 6, in which the theoretical values were calculated according to the molecular formula. After cross linking with vanillin, the C/N value of cross-linked chitosan increased

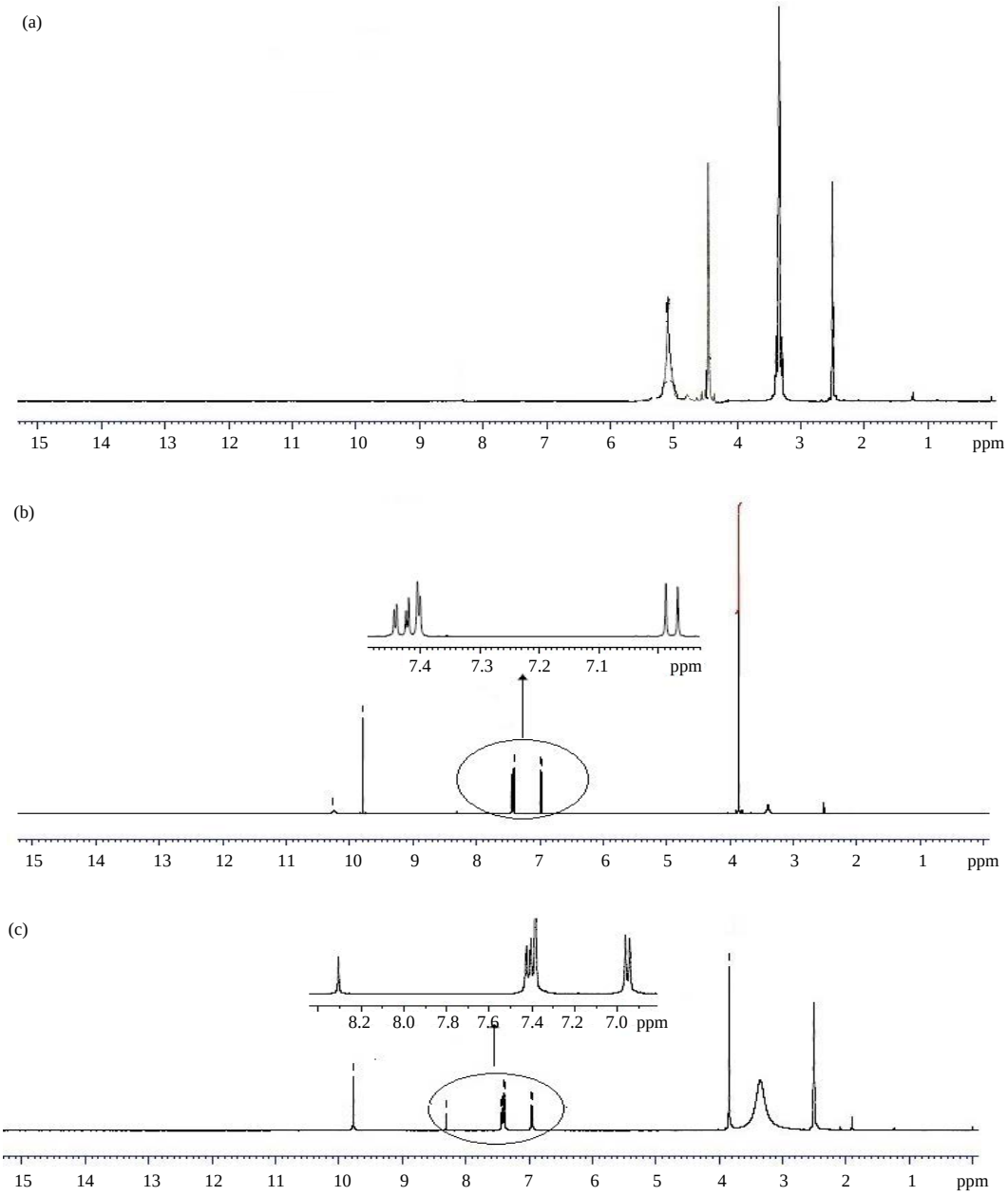


Fig. 4: ^{1}H NMR spectra of (a) Chitosan, (b) Vanillin and (c) OPT-CV

compared to that of pure chitosan, indicating that the cross linking reagent was introduced onto chitosan molecules[28].

Nuclear magnetic resonance (NMR): The ^{1}H NMR spectra of chitosan, vanillin and optimized cross-linked chitosan (OPT-CV) is presented in Fig. 4.

The ^{1}H-NMR spectrum of chitosan (Fig. 4a) presents a peak at 2.5 ppm, corresponding to hydrogen bonded to the carbon atom C2 of the glucosamine ring, while the peaks between 3.30 and 4.00 ppm corresponds to the hydrogen atoms bonded to carbons C3, C4, C5 and C6 of the glucopyranose units. The anomeric carbon (C1) presents peaks between 4-5 ppm[21-23]. The spectrum of vanillin shows a characteristic peak at 3.857 ppm which can be attributed to -OCH_3 group and a peak at 6.968 due to -OH group. The aldehyde (-CHO) proton signal is very distinctive, appearing as a singlet at 9.785ppm and as a small peak at 10.259 ppm. Three peaks were observed at 7.401, 7.420 and 7.444 ppm (shown in the magnified image of Fig. 4b), which may be attributed to the presence of the aromatic ring. The peak at

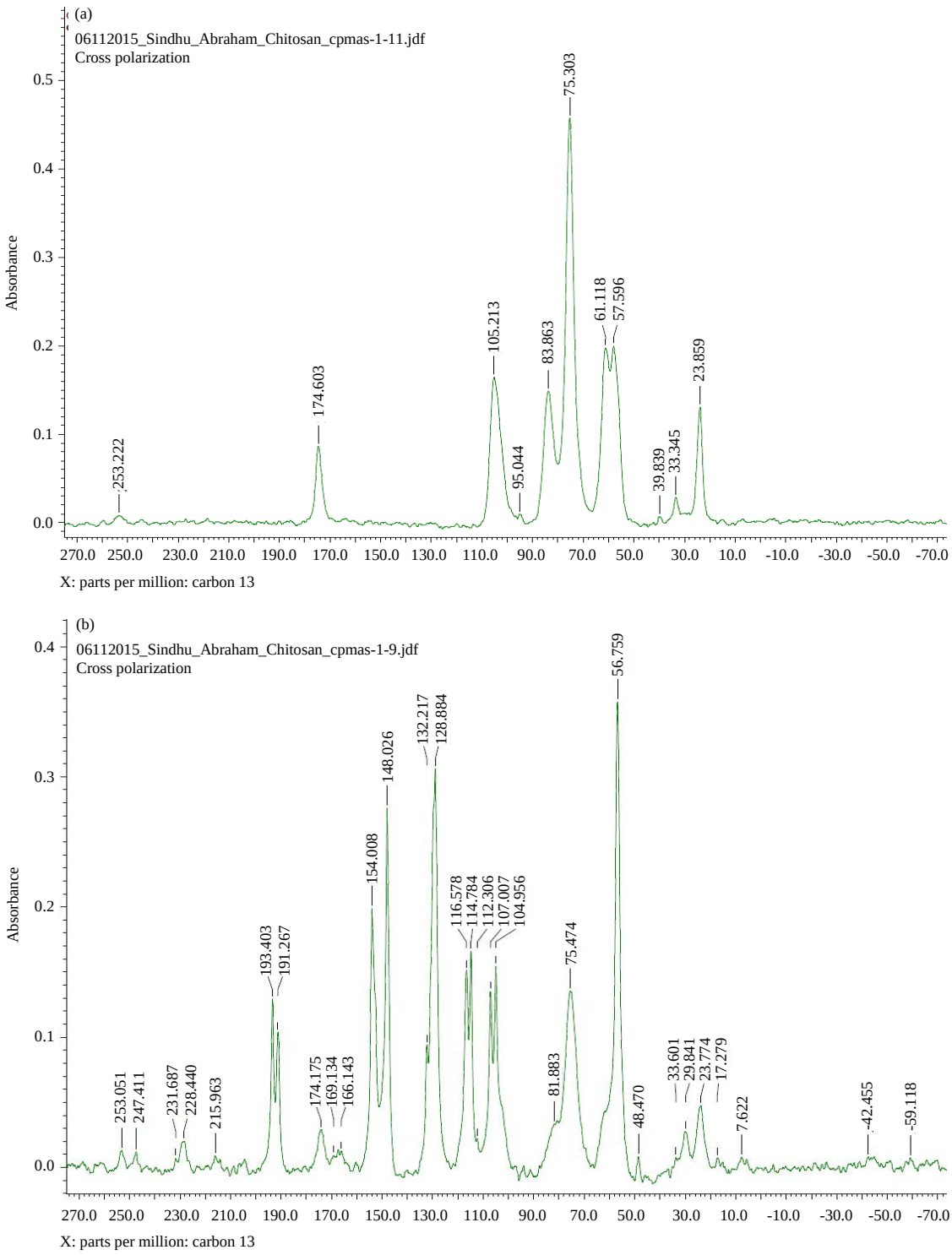
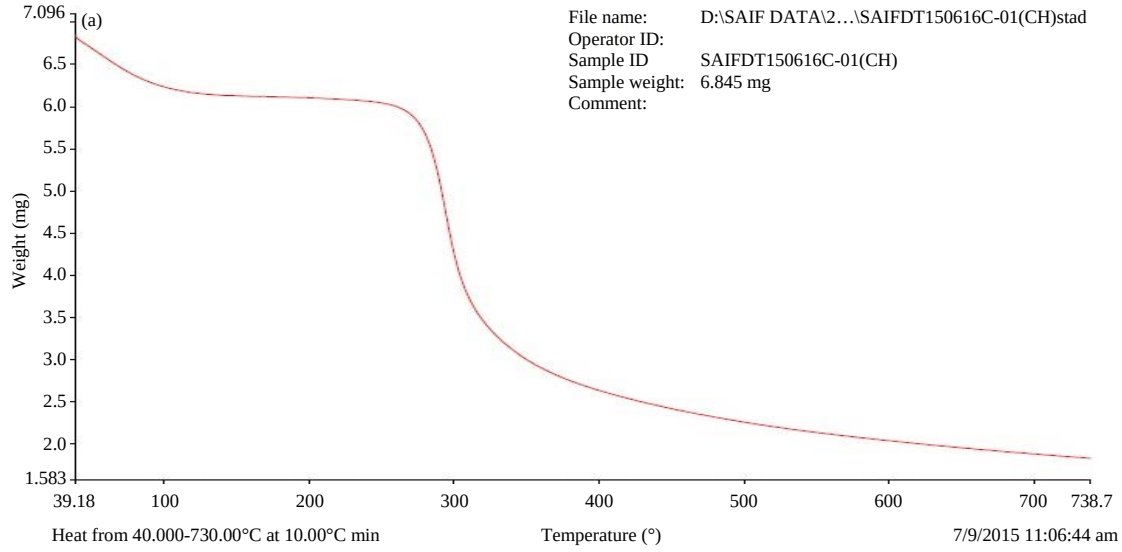


Fig. 5(a-b): ^{13}C NMR spectra of (a) chitosan (b) OPT-CV

2.5 ppm is due to the solvent DMSO. The spectrum of OPT-CV (Fig. 4c) exhibits all the peaks belonging to chitosan and vanillin protons, in addition to a new peak at 8.306 ppm which is due to the imine (HC=N) bond formed due to the condensation reaction between the vanillin aldehyde group and chitosan amine group[27]. This can further be justified by the decrease in intensity of peak at 9.767 ppm and absence of a peak at 10 ppm (-CHO peaks of vanillin), since -CHO has formed HC=N bond[24].

The CP/MAS ^{13}C NMR spectra of chitosan, vanillin and composite film F1 are presented in Fig. 5. The spectrum of chitosan presents all the already known characteristic peaks reported in literature for aliphatic carbons in the chitosan, between 20 and 110 ppm, for example C1 ring carbon at 105.213 ppm, or methyl CH_3 carbon at 23.859 ppm (Fig. 5a). The spectrum of OPT-CV exhibits all the peaks belonging to chitosan and vanillin carbons, in addition to which one notices the presence of a new peak at 169.134 ppm, which could be attributed to the imine (CH=N) carbon of a new linkage formed by condensation reaction of the aldehydic group of vanillin with chitosan amine group (Fig. 5b).

Thermal gravimetric analysis (TGA): The TGA measures the amount and rate of change in the weight of a material as a function of temperature or time in a controlled atmosphere. TGA helps to assess the thermal stability of polymer and its blends. According to the TGA analysis, 2 stages of weight loss were observed in the chitosan sample (Fig. 6a). In the first stage, chitosan showed a 10% weight loss at 59.96°C due to the loss of water molecules that are adsorbed and bound to the polysaccharide. In the second stage, Chitosan showed 50-60% weight loss at 293.94°C which may be attributed to the partial decomposition of polysaccharide molecule[29].



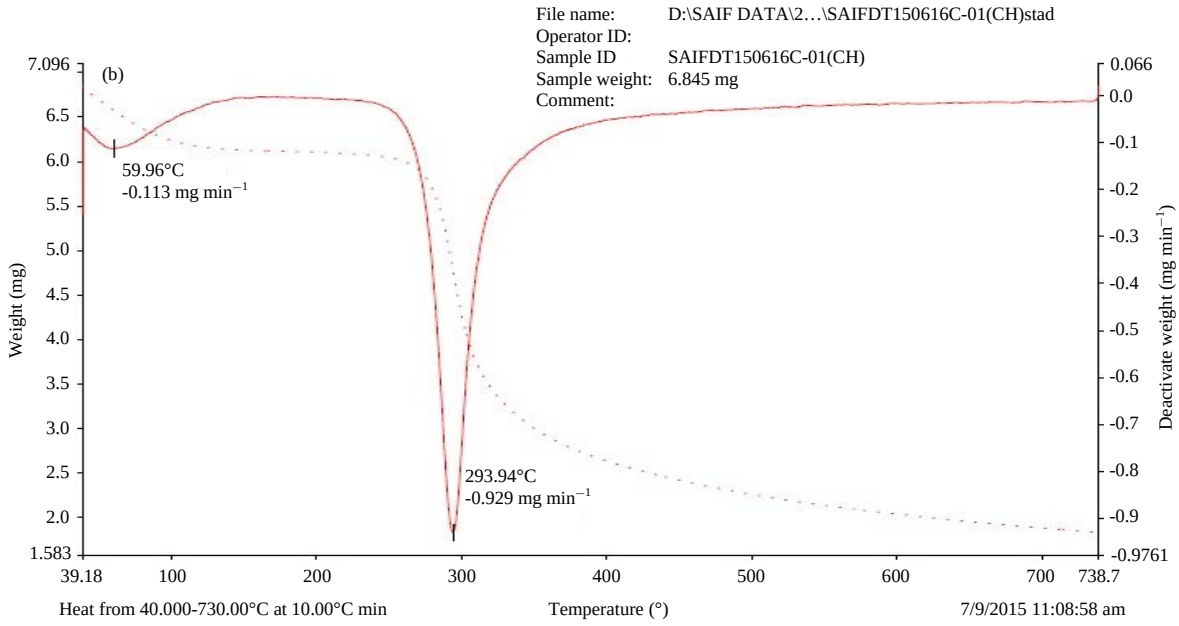
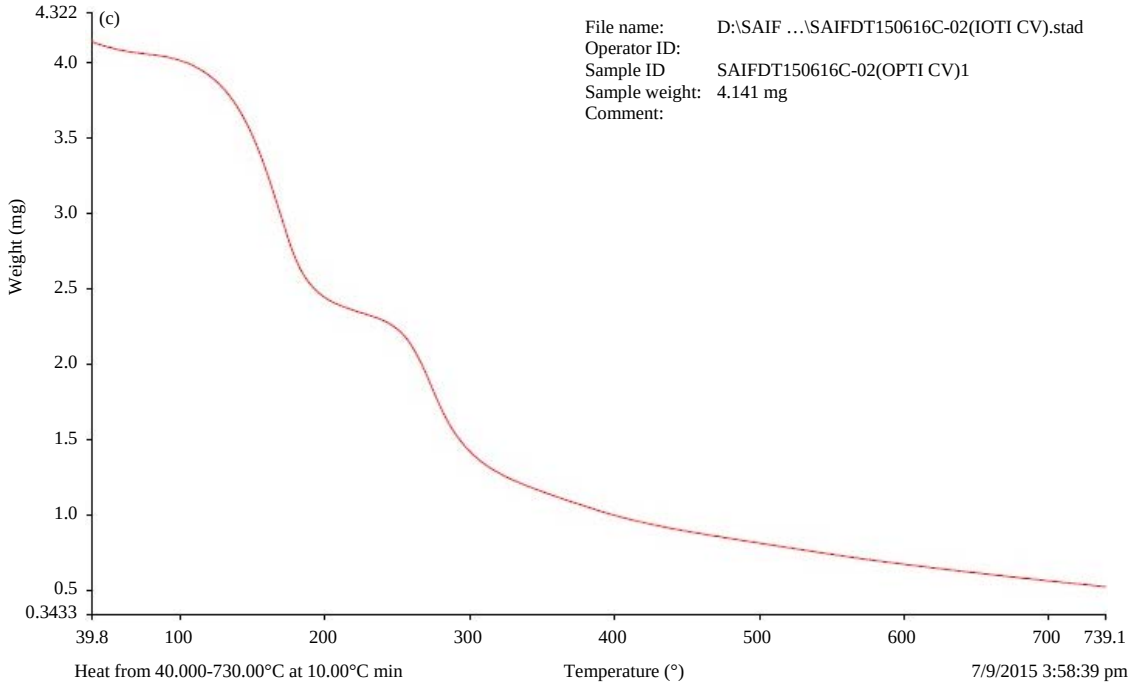


Fig. 6(a-d): Continue



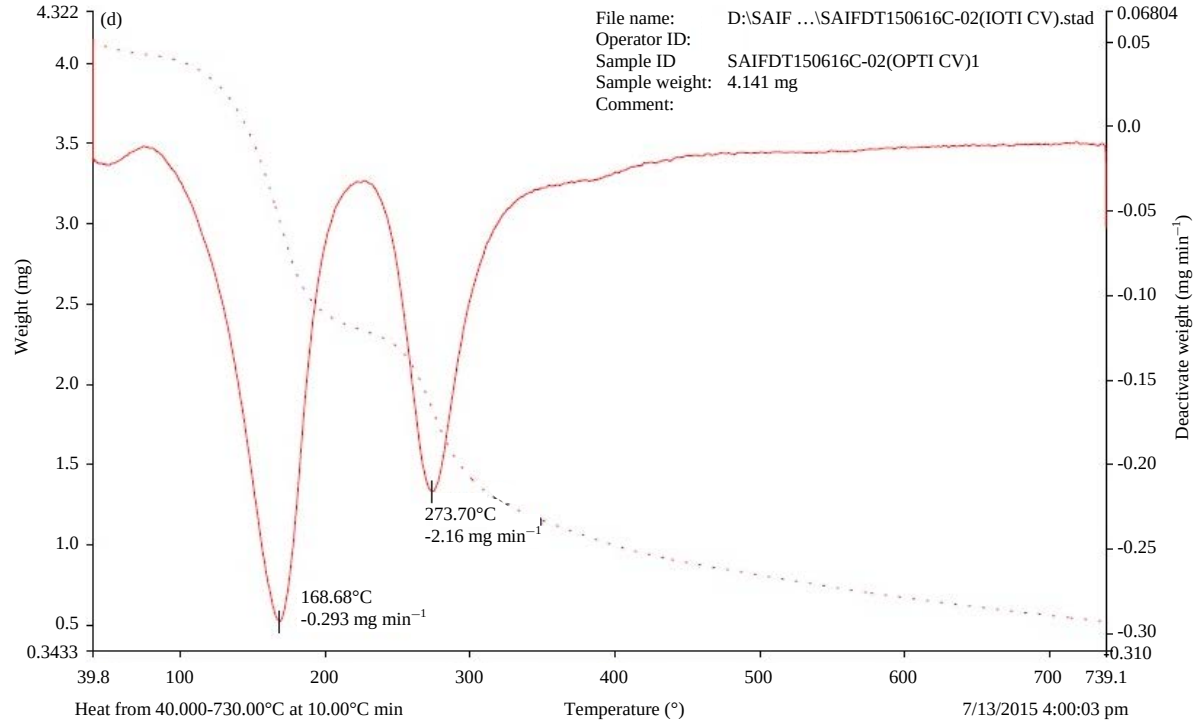


Fig. 6(a-d): TGA curve of (a) chitosan, (b) chitosan showing the points of weight loss, (c) OPT-CV and (d) OPT-CV showing the points of weight loss

Cross-linked chitosan exhibited 3 stages of weight loss (Fig. 6b). The first weight loss of 10% was at 50°C due to the removal of water molecules that are adsorbed and bound to the polysaccharide. A second weight loss of 30-40% was observed at 168.68°C, due to the thermal decomposition of the Schiff base formed between chitosan and vanillin (Fig. 6c).

The third stage of weight loss was at 273.70°C, (Fig. 6d) which may be attributed to the partial decomposition of polysaccharide[26].

Even though the TGA curve of chitosan was similar to that of cross-linked chitosan, there are still some differences. The peak due to the partial decomposition of chitosan was observed at 293.94 °C, which was a higher temperature than that observed in the case of cross-linked chitosan (273.70 °C). This decrease in temperature for cross- linked chitosan may be due to the presence of other functional groups such as -CHO, $-OCH_3$.

CONCLUSION

The optimized cross-linked chitosan was characterized by different methods to confirm the process of cross- linking. The results of the studies proved the formation of a Schiff base between chitosan and vanillin which in turn confirms the process of cross-linking. This study confirmed that microwave irradiation method could be used as a rapid, reliable and economic method cross-linking of chitosan and prepared chitosan could be used as a polymer for various drug delivery systems.

SIGNIFICANCE STATEMENTS

This study describes the usefulness of microwaving method in carrying out polymerization reactions. This study discusses the utility of microwave irradiation method for preparing cross linked polymers in a cost effective manner. This study will help all research groups to carry out polymer related work in an economical manner.

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