



Preparation and Characterization of Binary Blend Films Containing Chitosan and Vanillin

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ABSTRACT

The present study explores the preparation of binary blend films of chitosan and vanillin using solution casting and solvent evaporating technique. The prepared binary blend films were analyzed by using Universal Testing Machine (UTM), Scanning Electron Microscope (SEM), FT-IR spectroscopy and Thermogravimetric analysis (TGA). With increase in concentration (wt %) of vanillin, tensile strength (Ts), young's modulus (Ym) and elongation at break (Eb) has displayed increased value compared to the pure chitosan film. The SEM micrographs of binary films with different ratio showed smooth homogeneous surface. The results of FT-IR study explains the presence of good interaction between chitosan/vanillin films. TGA confirms that, at lower temperature range blend films were stable. This may be attributed to the effect of crystalline nature of vanillin in chitosan films. The review of the study summarizes, the prepared binary films can find applications in pharmaceutical drugs and in food packaging applications.

Keywords: Chitosan, Vanillin, Mechanical properties, Phase morphology.

INTRODUCTION

Currently there is a much interest in the biopolymers to prepare novel materials towards betterment of food safety and shelf life of food. An expensive polymer property spectrum is higher than is needed for a specific application. Also an individual

polymer can exhibit poor property can be effectively overcome by blending it with another, which show higher property value. Thus by selecting appropriate polymers and blended it to produce the expected properties, without having to develop new

polymers and no investment in new plants can be done away with. The suitable properties can be attained by proper selection of blend constituents accompanied by control of morphology by appropriate methods of compatibilization, compounding and processing. Blending is known to improve mechanical properties to enhance processing ability. Improvement is becoming the most significant criteria as the emphasis is shifting to high performance.

Chitosan is one of the perspective natural polysaccharide and has biocompatible and biodegradable property¹. It possesses free amino and hydroxyl groups which facilitate its reaction. Chitosan is a semicrystalline, high molecular weight, nontoxic², linear amino, cationic³ polysaccharide with glucosamine and N-acetyl glucosamine units. It is prepared by excessive hot alkaline deacetylation of chitin (poly (N-acetyl-D-glucopyranose) of shrimp shells and crab⁴. Chitosan exhibits strong antimicrobial and antifungal activity⁵⁻⁹. The high sensitivity and low water barrier property of chitosan¹⁰ towards the moisture makes the use of chitosan based films in food packaging materials and biomedical applications^{11,12}. Pure chitosan films are brittle and exhibit good tensile properties. Further these properties were enhanced and self life of foodstuffs can be extended by modify with other polysaccharides.

Vanillin (4-hydroxy-3-methoxy-benzyldehyde) is used as a flavoring agent derived from bean or pod which finds application in industry as a food, drinks and cosmetics. Vanillin is considered as a nutraceutical molecule because it bears antimutagenic, anticlastogenic and antitumor property^{13,14}. Vanillin is a safe GRAS additive which exhibits bioactive properties like antioxidant and antimicrobial activity versus bacteria, moulds and yeasts¹⁵⁻¹⁸. The antimicrobial property of vanillin is due to phenolic group in chemical structure which

makes vanillin good in suppressing bacteria, yeasts and moulds. The goal of the research work was to prepare a series of vanillin modified blend films by casting technique. The work was mainly concentrated to study influence of vanillin on mechanical thermal and phase morphology of the chitosan films which are capable of food packaging and pharmaceutical drug release applications. It was reported that vanillin modified chitosan was employed to remove heavy metal ion¹⁹, preparation of nanoparticles by crosslinking with vanillin and preparation of microspheres of chitosan²⁰. To the best of our knowledge no information is available for the study of thermogravimetric, scanning electron microscope and mechanical properties to evaluate stability, phase morphology and tensile strength. In this study we will prepare the prepare vanillin modified chitosan blend films and study the morphology and mechanical behavior of binary films.

MATERIALS

Chitosan was supplied from (TCI) Tokyo, Japan, (degree of deacetylation 75%). Vanillin GR was procured from Merck Specialties Private Ltd. Acetic acid was received from spectrochem, Mumbai, and was used as received. Double distilled water used throughout the experiment. (See figure 7.)

Preparation of film

Vanillin modified chitosan films were prepared by solvent casting technique. The composition of chitosan/vanillin is presented in Table (1). Different concentration of (wt %) chitosan prepared by dissolving in 1% acetic acid with constant stirring overnight and different concentration (wt %) of vanillin solution prepared in double distilled water at room temperature. Then different weight ratios of vanillin solution (5, 15, 25, 35 wt %) were mixed with chitosan solution and stirred

till the solution becomes homogeneous, subsequently highly viscous and bubble free solutions were poured onto dry and cleaned Petri dishes. The solvent was evaporated at room temperature. Then films were dried at 45°C in an oven. After drying all films were peeled off from Petri dishes and preserved in vacuum desiccators.

Thickness measurements

Film thickness was measured using a Mitutoyo Dial Thickness Gauge (made in Japan). At different locations thickness measurements were taken and averaged. The thickness of all blend films found to be around 0.12mm.

Mechanical behavior

On mechanical properties microstructure has strong influence and they could provide important information about internal structure of materials²¹. Tensile strength (T_s), percentage of elongation at break (E_b) was measured by universal testing machine (UTM) LLOYD Instrument according to ASTM D882²². The films strips were cut into 2.5x10cm and film strips were loaded into the testing machine which was set at 50 mm/s and cross head speed at 50 mm/min^{23,24}. The interaction between polymers is the main constitutes of the film which influences the mechanical behavior.

Scanning electron microscopy

The phase morphology of CH/Vn blend films were investigated by using JSM-6360 Scanning Electron Microscope (JEOL, Germany). Prior to the examination, films were dried overnight in a hot air oven at 45°C and mounted on a metal stub with double side sticky tape. The films were coated with a thin layer of gold and images were examined at an accelerating voltage 10 kV.

FT-IR spectroscopy

The blend films were recorded on IR Prestage-21 Spectrophotometer Shimadzu (Japan) made up of KBr pelleting method with smart orbit high performance diamond ATR accessories. Measurements were conducted between 400-4000 cm^{-1} .

Thermogravimetric analysis (TGA)

The stability and thermal behavior of the films were determined by Thermogravimetric analysis (SDT 600QDT V20.9 Build 20 simultaneous thermal analyzer system). The samples of 5 mg were taken and warmed up until 700°C at a heating rate 10°C min^{-1} under dynamic nitrogen atmosphere. The percentage weight loss during heating was calculated by using associated software.

RESULTS AND DISCUSSION

Mechanical properties

Mechanical properties reflect the important factors and their ability to assessing the mechanical integrity of films. The maximum T_s , Y_m and E_b data was analyzed by using NEXOGEN Plus software. Figures (1, 2 & 3) represent the T_s , Y_m and % of E_b of the blend films with different proportions

In comparison with pure chitosan film, the T_s of the blend films show significant increase with incorporation of vanillin. The highest T_s were observed in different wt% ratio of 85%/15% and 75%/25%. It confirms the strong hydrogen bond between chitosan and vanillin. The presence of vanillin in films caused significant increase in T_s . The similar results were observed in effect of glycerol on basil seed gum²⁴ and effect of glycerol on chitosan²⁵. At the same time Y_m and E_b show increased value as the vanillin increased in the chitosan film. The blend ration containing 95% chitosan/5% vanillin has highest (%) of E_b value. This could be attributed to the lower wt % of vanillin in chitosan less vanillin

molecules available for bonding. But as the concentration of vanillin increased in the % of Eb shows decreased value, due to strong interaction between chitosan and vanillin. When equal concentration of chitosan/vanillin was mixed no film formation observed, because recrystallization of vanillin occurred and it strongly affected film by reducing the viscosity of the solution. Generally films required high mechanical properties values. The results of the study suggest the chitosan/vanillin could be utilized to improve the process ability with small shift in the Ts of films.

Scanning electron microscope (SEM)

Scanning electron microscope can furnish a better understanding of mechanical and structural characteristics²⁶⁻²⁸. Figure 4 shows the phase morphology of CH/Vn blend films. The SEM images confirm, films having uniform and homogeneous surface without disruption indicates presence of strong bonding. The result was in consistent with Mali and Grossmann (2002)²⁹. With increase in vanillin, the small white straps present on the surface of the pure chitosan were reduced. No phase separation was observed, that indicates complete miscibility and compatibility of two components. The results describe, micrographs of films were uniform, continuous and completely miscible. The all blend films were rigid and the brittleness of the chitosan is reduced with increase in concentration of vanillin.

FT-IR spectroscopy

The FT-IR spectra of pure chitosan and blend films were shown in Figure (5). Taking into account of SEM results, FT-IR analysis suggests, a strong interaction between chitosan and vanillin. The stretching frequency observed at 3347 cm^{-1} , clearly suggests the -OH group of chitosan involved in strong hydrogen bonding with hydroxyl and aldehyde group of vanillin due to this

absorption shifted from 3457 cm^{-1} to 3347 cm^{-1} . The primary NH_2 group absorbs at 3429 cm^{-1} was due to asymmetric stretch and 3373 cm^{-1} was due to NH_2 symmetric stretching in chitosan and both appeared as sharp bands. A small shift of 3429 cm^{-1} to 3386 cm^{-1} observed due to asymmetric N-H stretching band and shift of 3373 cm^{-1} to 3268 cm^{-1} was due to N-H symmetric frequency. A decrease of 43 cm^{-1} and 105 cm^{-1} in stretching vibration could be observed, suggesting that NH_2 group of chitosan involved with vanillin through hydrogen bonding by small decrease in the absorption frequency of NH_2 group. Overlapping of absorption frequency of hydroxyl and primary amine group appear as a broad peak in blend films.

The carbonyl group of vanillin occurs at 1695 cm^{-1} . After blending with chitosan, this value shifted from 1695 to 1646 cm^{-1} . The decreased value of 49 cm^{-1} for C=O group confirms the carbonyl group could be involved with -OH and NH_2 group of chitosan through hydrogen bonding. The ethers and derivative alcohols normally show characteristic C-O-C absorption bands. In chitosan a strong band appears at 1125 cm^{-1} for asymmetric C-O-C stretching and symmetric stretching band at 1064 cm^{-1} . In blend film asymmetric stretching frequency occurs at 1152 cm^{-1} increased by 27 cm^{-1} . This confirms the strong interaction between chitosan and vanillin. As bond length has relation with viscosity, could be attributed that bond length of C-O-C increased the viscosity show reduced value. The viscosity of chitosan lowered with addition of vanillin.

Thermogravimetric analysis

Thermogravimetric analysis was performed in order study the effect of temperature which promotes changes in material. The weight loss of chitosan based films was reported in figure (6). The moisture vaporization of about 18 % in chitosan and 20% in chitosan/vanillin films was detected in

the temperature range of 100-210°C. Chitosan showed a large mass loss of 46% between 210-470°C. This mass loss could be attributed to thermal degradation of polymeric chains with removal of volatile compounds. The third weight loss of 36% showed a complete decomposition of chitosan at 700°C.

The prepared films displayed a lower mass loss of 34-46% between 200-490°C due to thermal degradation of chitosan/ vanillin films. The second weight loss of 46-54% occurred in between 490-690°C was due to degradation residual mass of blend films. This is worth noting that blend films were stable below the temperature range of 490 °C comparatively pure chitosan as it showed 46% of weight loss at 470°C. With raise in temperature large weight loss was observed for blend films. Regarding thermogravimetric analysis chitosan/vanillin films showed almost 90% weight loss between the ranges of 690°C. Utmost weight loss observed at 490-690°C for blend films. At the temperature range of 700°C only 0.901 % of mass was remained. TGA concludes that decomposition temperature of blend was higher than that of pure chitosan. The prepared blend films were stable at lower temperature range. This could be assigned to the effect of crystalline nature of vanillin in chitosan films. In equal concentration (wt %) of chitosan/vanillin no film formation was observed owed to recrystallization of vanillin.

CONCLUSION

The results of this work demonstrate that with addition of vanillin a reduced viscosity was observed in chitosan film. That confirms the strong intermolecular hydrogen bonding and also uniform distribution of vanillin. Incorporation vanillin in chitosan film showed significant enhancement in tensile strength, young's modulus and elongation at break, this could be attributed to strong interaction between two components. The films showed uniform, continuous and

homogeneous surface which was confirmed by SEM micrographs of binary blend films. The binary films were rigid, less brittle and stable at lower temperature compare to chitosan film. From the study it was concluded that binary films could be used as promising implantable devices to release pharmaceutical drugs in controlled manner and also as a potential material for development of food packaging and coating films.

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REFERENCES

1. Pangburn SH, Trescony PV, Heller J. Lysozyme Degradation of Partially Deacetylated Chitin, its Films and Hydrogels. *Biomat.* 1982; 3:105-108.
2. Leceta I, Guerrero P, de la Caba K., Functional Properties of Chitosan-based Films. *Carbohy Polym.* 2013; 93: 339–346.
3. Lambertus AM, van den Broek, Rutger JI, Knoop Frans HJ, Kappen Carmen G Boeriu. Chitosan Films and Blends for Packaging Material. *Carbohy polym.* 2015; 116: 237–242.
4. No HK, Meyers S P. Preparation of Chitin and Chitosan in *Chitin Handbook*, R.A.A. Muzzarelli and M.G. Peter, (*European Chitin Society*), Italy. Eds. 475: (1997).
5. Darmadji P, Izumimoto M. Effect of Chitosan in Meat Preservation. *Meat Sci.* 1994; 38:243-254.

6. Jo C, Lee JW, Lee KH, Byun MW. Quality Properties of Pork Sausage Prepared with Water-Soluble Chitosan Oligomer. *Meat Sci.* 2001; 59:369-375.
7. Fernandes JC, Tavaría FK, Soares JC, Ramos OS, Monteiro MJ, Pintado ME. Antimicrobial Effects of Chitosans and Chitooligosaccharides, upon *Staphylococcus Aureus* and *Escherichia coli*, in Food Model Systems. *Food Micro.* 2008; 25:922-928.
8. Yen MT.; J.H, Mau JL. Physicochemical Characterization of Chitin and Chitosan from Crab Shells. *Carbohydr Polym.* 2009; 75:15-21.
9. Akbuga J. A Biopolymer: Chitosan. *Int J of Adv Pharma Sci.* 1995; 1: 3-8.
10. Bonilla J, Fortunati E, Vargas M, Chiralt A, Kenny JM. Effects of Chitosan on the Physicochemical and Antimicrobial Properties of PLA Films. *J Food Eng.* 2013; 119: 236-243.
11. Wong DWS, Gastineau FA, Gregorski KS, Tillin SJ, Pavlath AE. Chitosan Lipid Films: Microstructure and Surface Energy. *J Agric Food Chem.* 1992; 40: 540-544.
12. El Ghaouth A, Arul J, Ponnampalalan R. Use of Chitosan Coating to Reduce Water Loss and Maintain Quality of Cucumber and Bell Pepper Fruits. *J Food Proc Pres.* 1991b; 15: 359-368.
13. Shyamala BN, Naidu M, Sulochanamma G, Srinivas P. Studies on the Antioxidant Activities of Natural Vanilla Extract and its Constituent Compounds through *in vitro* models. *J Agri Food Chem.* 2007; 55:7738-7743.
14. Sinigaglia M, Reguly ML, de Andrade HH. Effect of Vanillin on Toxicant-induced Mutation and Mitotic Recombination in Proliferating Somatic Cells of *Drosophila Melanogaster*. *Environ Mol Mutagen.* 2004; 44:394-400.
15. Sinha AK, Sharma UK, Sharm N. A Comprehensive Review on Vanilla Flavor: Extraction, Isolation and Quantification of Vanillin and other Constituents. *Int J Food Sci Nutri.* 2008; 59: 299-326.
16. Burri J, Graf M, Lambelet P, Lolige J. Vanillin: more than a Flavouring Agent - A Potent Antioxidant *J Sci Food Agri.* 1989; 48:49-56.
17. Mourtzinis I, Konteles S, Kalogeropoulos N, Karathanos VT. Thermal Oxidation of Vanillin Affects its Antioxidant and Antimicrobial Properties. *Food Chem.* 2009; 3:791-797.
18. Triantafyllou V I, Akrida-Demertzi K, Demertzis P.G. A Study on the Migration of Organic Ppollutants from Recycled Paperboard Materials to Solid Food Matrices. *Food Chem.* 2007; 101: 1759-1768.
19. Cestari AR, Vieira E F S, Mattos C R S. Thermodynamics of the Cu(II) Adsorption on Thin-Vanillin Modified Chitosan Membranes. *Chem Thermodynamics.* 2006; 38:1092-1099.
20. Wang G, Li P, Peng H M, Kong L. Formulation of Vanillin Cross-linked Chitosan Nanoparticles and its Characterization. *Adv Mat Res.* 2011; 335-336:474-477.
21. Paul DR, Barlow JW. Polymer Blends (or alloys). *J Macromol Sci Rev Macromol Chem.* 1980; 18:109-68.
22. ASTM, Annual Book of ASTM Standards, 2010, Philadelphia, PA.
23. Leila N, Abdorreza MN. Antibacterial, Mechanical, and Barrier Properties of Sago Starch Film Incorporated with Betel Leaves Extract. *Inter J Bio Macromo.* 2014; 66:254-259.
24. Naimeh K, Mohsen E, Zahra ED, Mehran G, Mohammad J. Characterization of New Biodegradable Edible Film Made from Basil Seed

- (*Ocimum basilicum* L.) Gum. *Carbohy Polym.* 2014; 102: 199–206.
25. Leceta I, Guerrero P, de la Caba K. Functional Properties of Chitosan-based Films. *Carbohy Polym.* 2013; 93:339–346.
 26. Jouki M, Mortazavi SA, Yazdi FT, Koocheki A, Characterization of Antioxidant-antibacterial Quince Seed Mucilage Films Containing Thyme Essential oil. *Carbohy polym.* 2013; 8:77.
 27. Ankita M, Bhushan H, Biophysico-chemical Characteristics & Applications of Nanoparticles. *Advanced Journal of Drug Delivery and Therapeutics.* 2014; 1:1.
 28. Khaled ME, Surface Features of Interfacial Layers of Solid-phase Gramicidin Antibiotic an AFM- Investigations *Advanced Journal of Pharmacology and Pharmacotherapeutics.* 2014; 1:3.
 29. Mali S, Grossmann MV E. Microstructural Characterization of Yam Starch Films. *Carbohy Polym.* 2002; 50:379–386.

Table 1. Composition of chitosan/vanillin films

Sample code	Compositions Chitosan/Vanillin (CS/ Vn)	Chitosan (Wt %)	Vanillin (Wt %)
CS	100	100	0
CV ₁	95/5	95	5
CV ₂	85/15	85	15
CV ₃	75/25	75	25
CV ₄	65/35	65	35

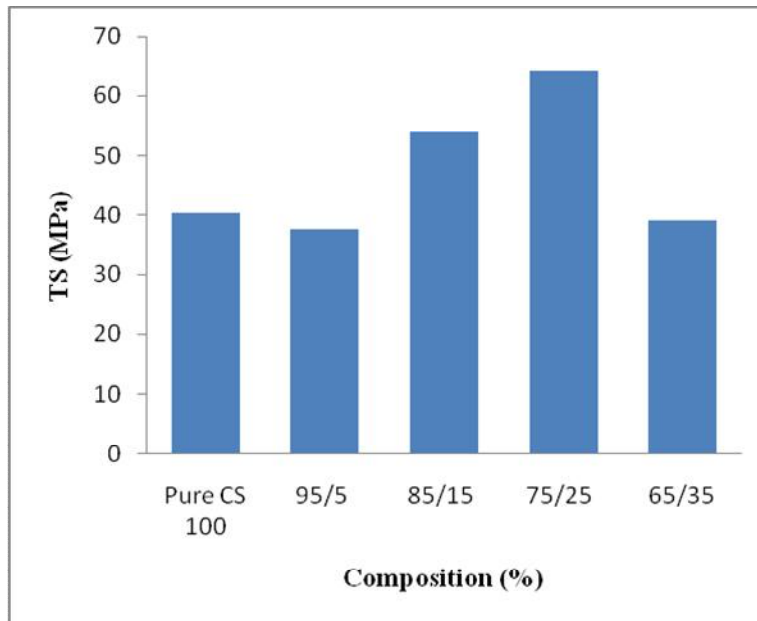


Figure 1. Tensile Strength (MPa) of pure chitosan and chitosan/vanillin blend films

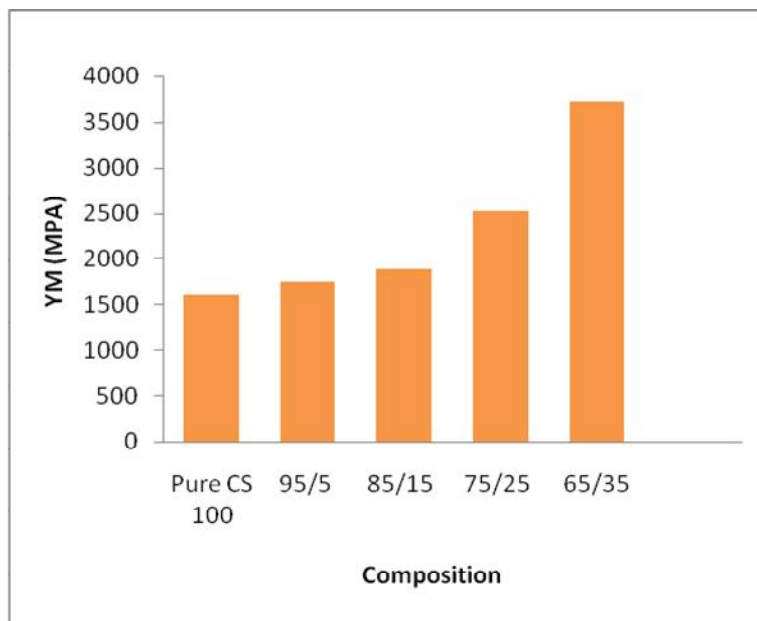


Figure 2. Young's Modulus (MPa) of pure chitosan and chitosan/vanillin blend films

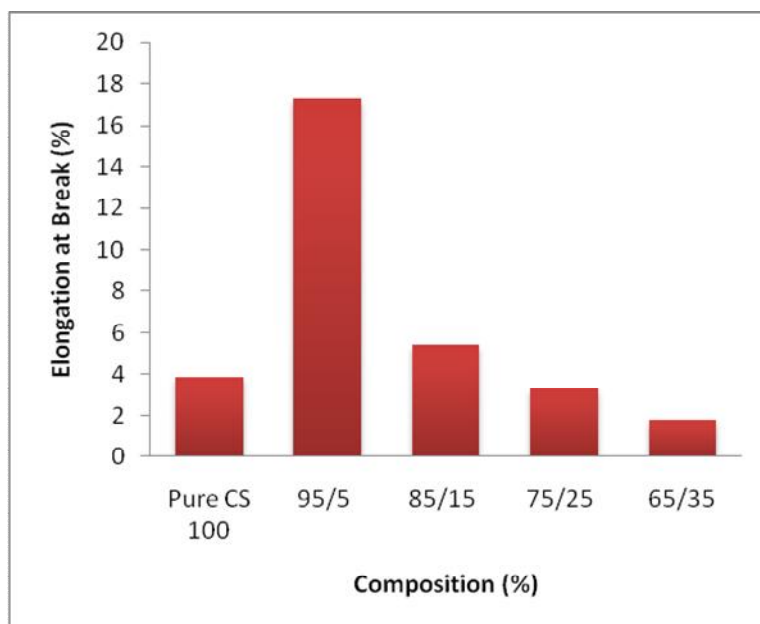


Figure 3. Elongation at Break (%) of pure chitosan and chitosan/vanillin blend films

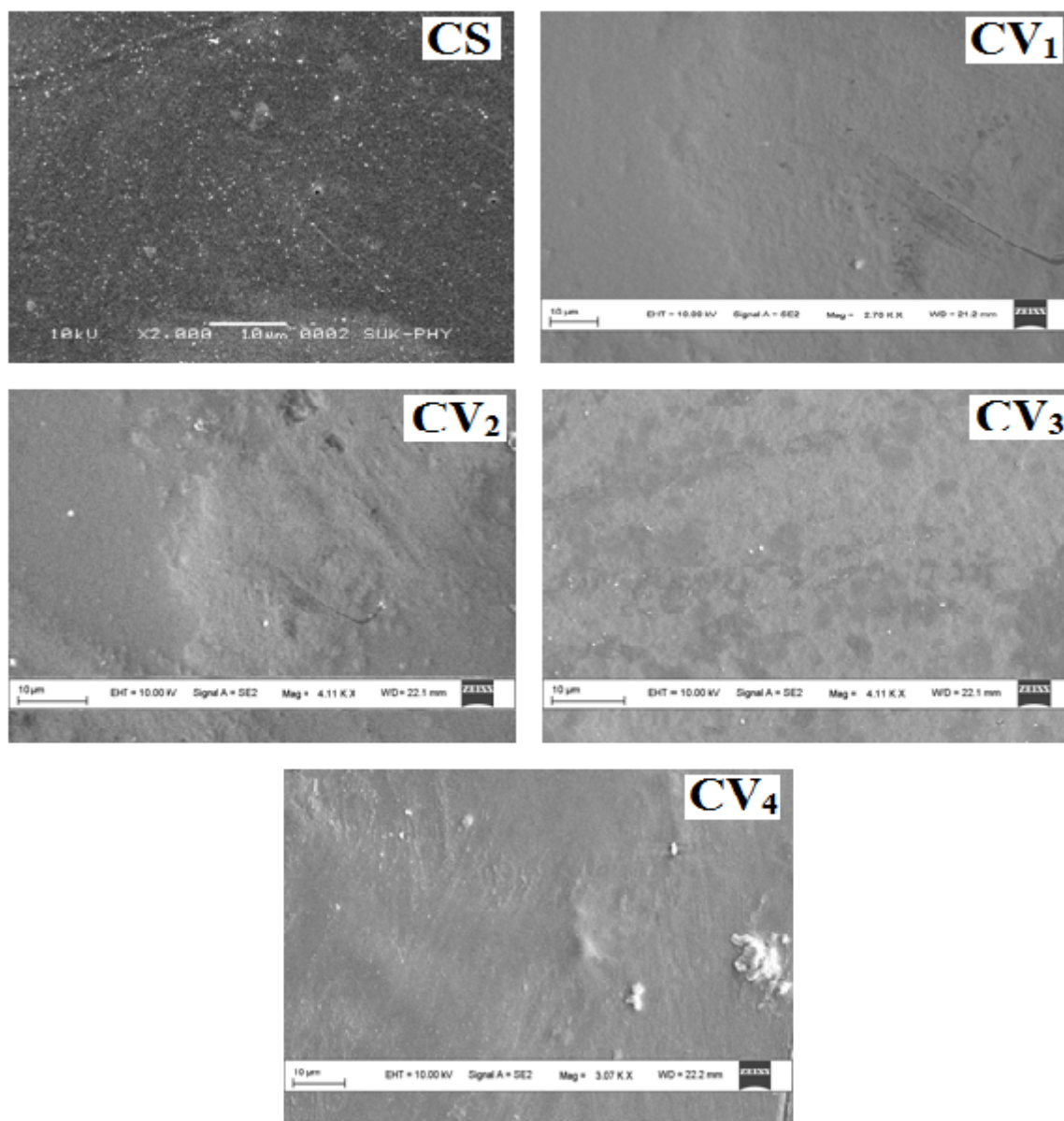
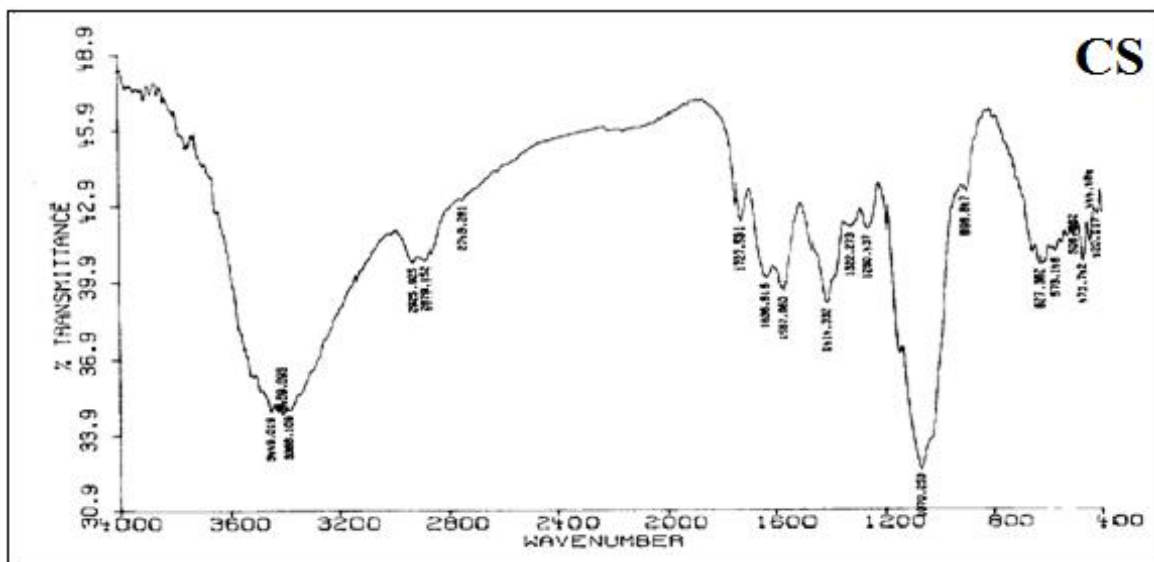
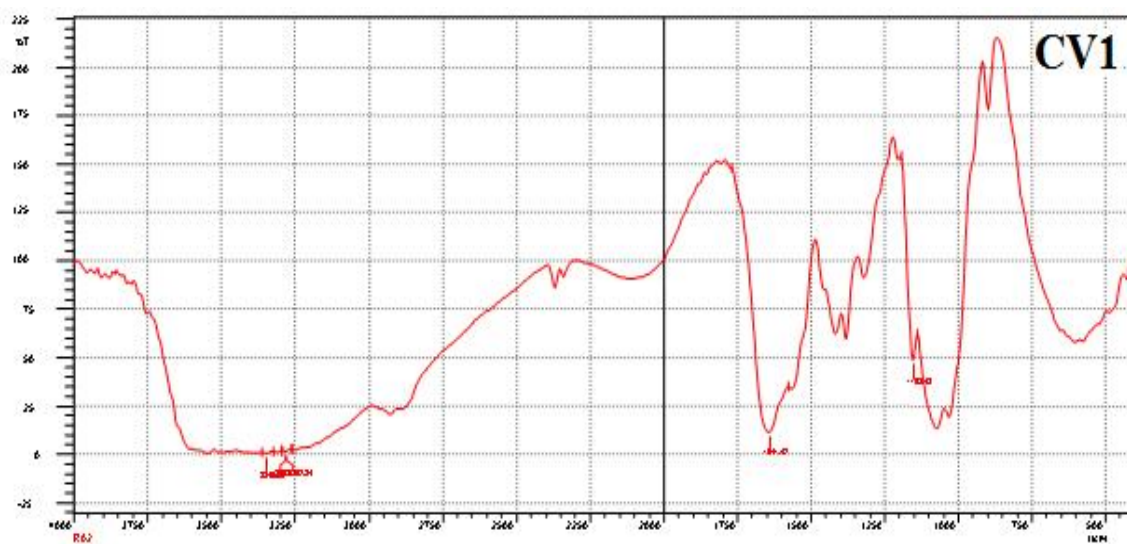


Figure 4. SEM micrographs of CS-Pure Chitosan, CV₁- 95%/5%, CV₂- 85%/15%, CV₃- 75%/25%, CV₄- 65%/35%

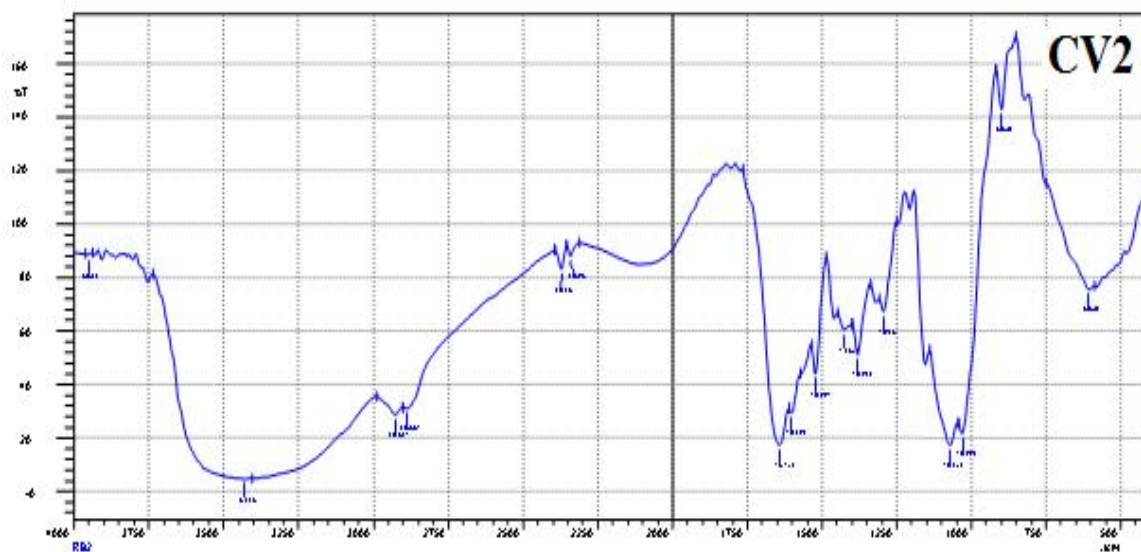


(a)

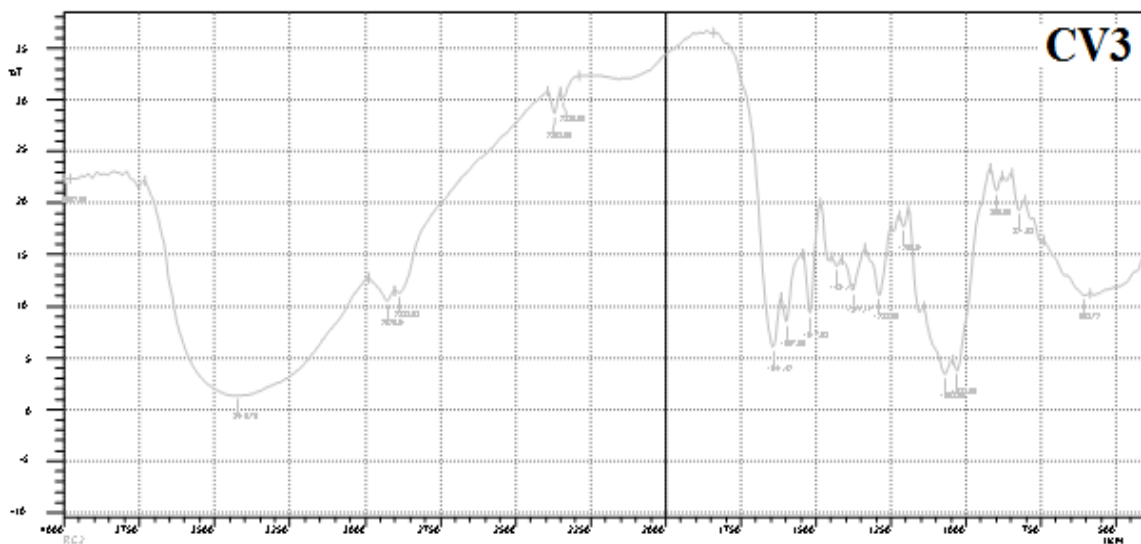


(b)

Figure 5.

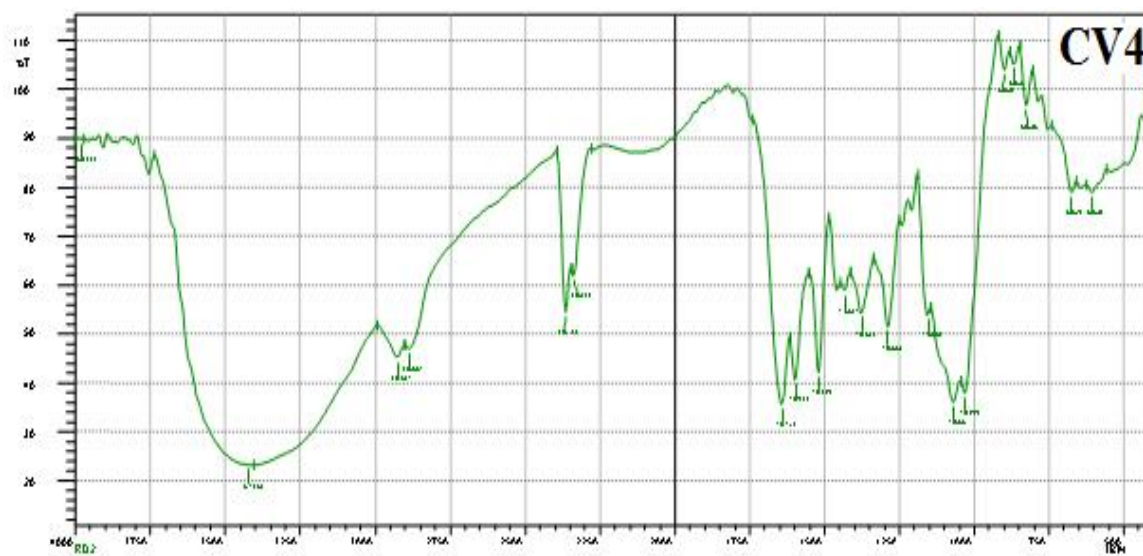


(c)



(D)

Figure 5.



(e)

Figure 5. FT-IR spectra of chitosan/vanillin blend films of (a) CS, (b) CV₁, (c) CV₂, (d) CV₃, (e) CV₄

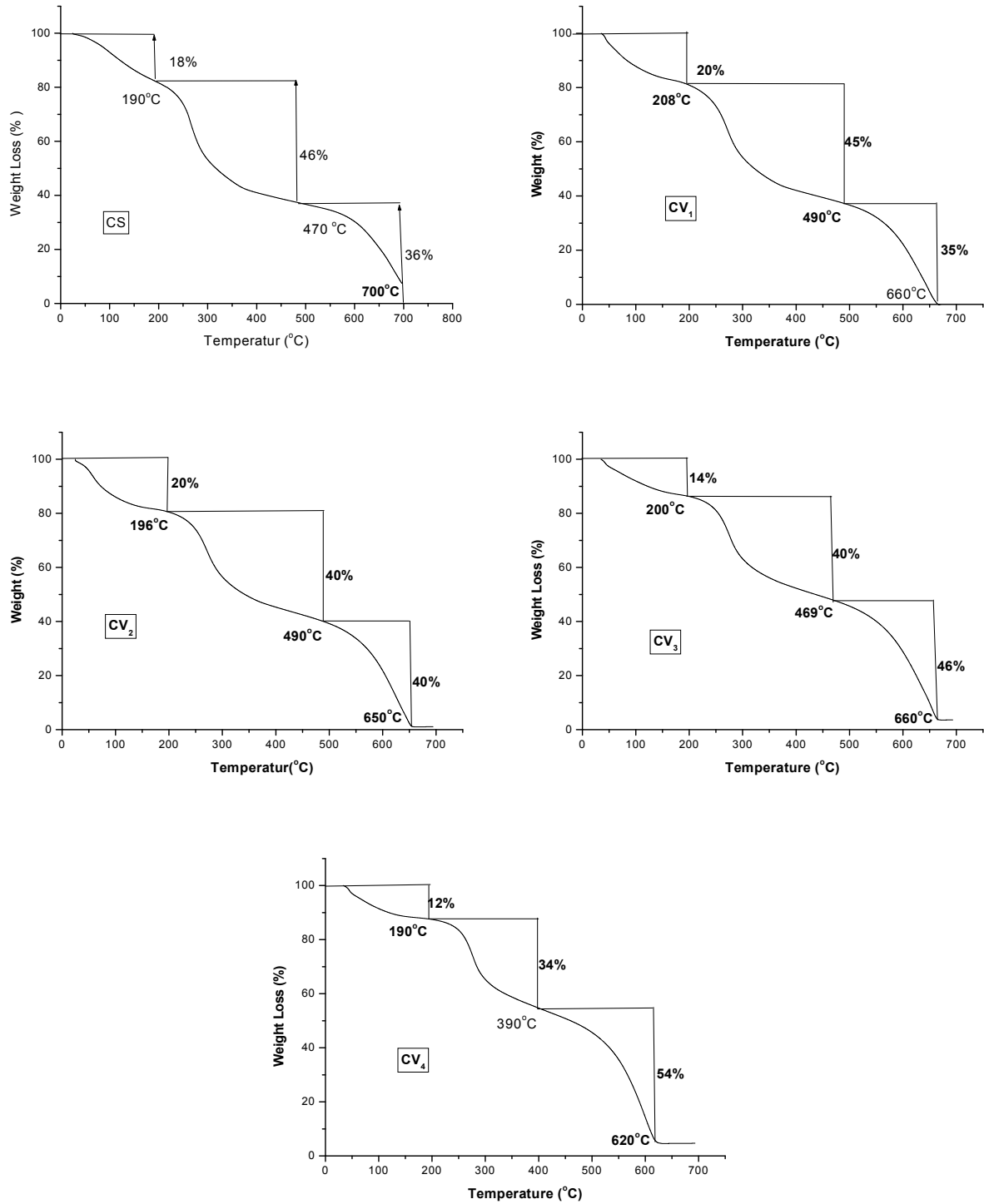


Figure 6. Thermogravimetric curve of pure chitosan and chitosan/vanillin blend films of CS, CV₁, CV₂, CV₃, CV₄

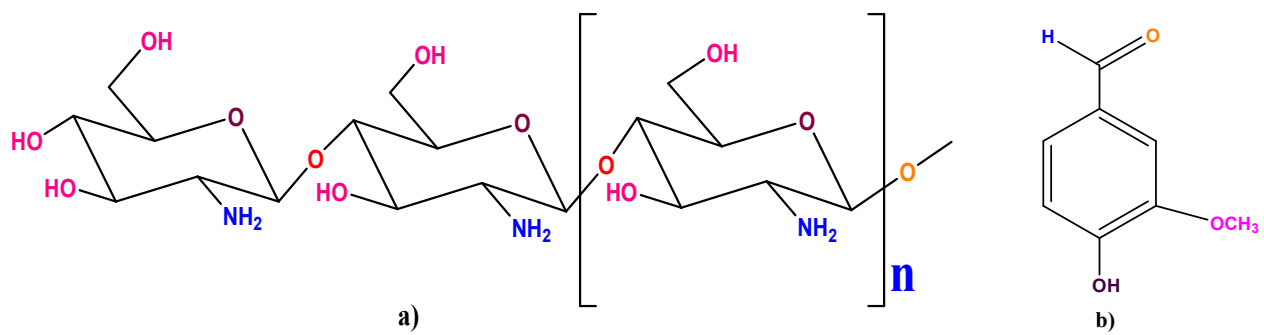


Figure 7. Chemical structure of (a) chitosan and (b) vanillin