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Chitosan sodium benzoate complex as a hydrocolloid and its application for nutraceuticals

Rajesh Kannan

Research Scholar, Meenakshi Academy of Higher Education and Research, Chennai, TamilNadu

Revathi Kasturi*

Research Scholar, Meenakshi Academy of Higher Education and Research, Chennai, TamilNadu

*Corresponding author

Anandhi Dhanavel

Assistant Professor, Meenakshi Ammal Dental College and Hospital, Chennai, TamilNadu

Abstract---Hydrocolloids have been a potential use in food, nutraceuticals, and pharmaceuticals for their viscoelastic properties, such as thickening and gelling. The increasing demand for nutraceuticals and pharmaceuticals has led to the development of new hydrocolloids. Chitosan has been investigated for pharmaceutical excipient, drug delivery agent, and food applications could be a potential hydrocolloid. This study focuses on the physiochemical characterization of chitosan- sodium benzoate hydrocolloid using Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), Differential Scanning Calorimetry (DSC), and Thermogravimetric Analysis (TGA). From FTIR and XRD, evident changes were observed in the structure and crystallinity of chitosan-sodium benzoate from chitosan, sodium benzoate, and physical mixture. The thermal decomposition temperatures and weight losses were analyzed using DSC and TGA. In addition to that, antibacterial and antioxidant assays were evaluated.

Keywords---Chitosan, Hydrocolloid, Antioxidant, Nutraceutical.

1. Introduction

Hydrocolloids as solid, liquid, and semisolid forms are also extensively used in drug formulation for drug delivery systems (Manzoor et al., 2020). They are widely

used in pharma products due to their reduced price, nontoxic nature, biodegradability, and effective drug compatibility (Medina-López et al., 2022). They are crucial elements of numerous pharmaceutical formulations and impart various functionalities in the delivery of drugs. Thickening and gelling is the significant reason for the inclusive utility of hydrocolloids in pharmaceuticals and nutraceuticals (Williams and Phillips, 2021). It involves the interaction of the polymer with the solvent that includes a non-specific entanglement of polymer chains that are conformationally disoriented, resulting in a discontinuous rise in viscosity (Mahmood et al., 2017). They are utilized as dental adhesives and as bulk laxatives where these products are vicious and specifically applicable (Munir et al., 2021). These hydrocolloids are hydrophilic and valuable in the formulation and preparation of tablets (Zuleger and Lippold, 2001), disintegrants (Jin et al., 2015), emulsifying agents, suspension aids, stabilizers, and thickening additives (Tanna and Mishra, 2019). These hydrocolloids are distinctly exercised as protective colloids in suspension and sustaining agents in tablets (Li and Nie, 2016). They are also exerted as adjuvants in pharmaceutical formulations to obtain various functionalities such as encapsulation, sustained release of drugs, mucoadhesiveness (Cook et al., 2017), and transdermal delivery (Zou et al., 2021).

Natural hydrocolloids have been a profound source for active biological encapsulation, controlled release, edible coating, carrier, and binding in nutraceuticals. The natural hydrocolloids may originate from proteins or carbohydrates as they are the two main macromolecules naturally available (Yemenicioğlu et al., 2020). These two macromolecules are significant food nutrients for human beings and are consumed as a staple food. While plant-based hydrocolloids have been gaining the spotlight for their advantage as a hydrocolloid, their availability differs based on climatic and seasonal conditions (Hamman et al., 2015). Moreover, the extraction and purification of the hydrocolloids are complex that requires significant expertise and capital (Amiri et al., 2021). These obstacles would hinder their utility commercially since these are complex processes that would have a negative impact on mass-level production. There are numerous limitations such as cost, utility, functionality, and scalability compared to conventional hydrocolloid materials. The other conventional hydrocolloids that majorly are resourced from animal sources are reported to cause allergies and are susceptible to microbial contamination and putridity (Bisht et al., 2022).

The advantage of natural hydrocolloids in the food and pharmaceutical industry is their abundant accessibility in nature and minimal cost of processing (González-Henríquez et al., 2019). The hydrocolloids are uniquely employed in pharmaceutical, food, and nutraceutical products due to their rheological and structural functionalities. These factors play a pivotal role in improving the shelf life and quality attributes relative to thickening and gelling properties (Razavi, 2019). Hydrocolloids are heterogeneous polymeric compounds with substantial hydroxyl groups (OH) with an intensified affinity for binding water molecules, making them hydrophilic compounds. This hydrophilic nature renders the property of constituting viscous dispersions or gels when dispersed in water (Li and Nie, 2016). The hydrocolloid interaction and the resulting thickened or gelled crosslinked polymer are due to intermolecular forces like hydrogen bonds, electrostatic forces, Van der Waals forces, and hydrophobic interactions (Banerjee

and Bhattacharya, 2012). These hydrocolloids are sourced from plants, animals, microbes (fermented products), and chemically modified plant-driven (synthetic gum) resources prevalently from nature. These hydrocolloids have a wide range of physicochemical features structural and metabolic functionalities in their natural form, which is explicitly tailored to a specific application. Despite their application in food industry they are also considered a potential element in the nutraceutical and pharmaceutical industry. The gelling and thickening properties are vital functions required in pharma or nutraceutical products. Apart from these properties, hydrocolloids also accredit therapeutic properties such as antioxidative (Ai et al., 2017, Hamdani et al., 2021), antihypertension (Bouaziz et al., 2017, Kolsi et al., 2016, Ali et al., 2011), anti-diabetic (Rosa-Sibakov et al., 2016, Wang et al., 2016), anticancer (Milani and Golkar, 2019), antimicrobial (Bilal et al., 2017, Roohinejad et al., 2017), and many more due to their chemical composition (Manzoor et al., 2020).

Despite these advantages, these hydrocolloids confront challenges such as a) preventing unwanted interactions of the bioactive elements with the environment and the other components in the formulation, b) limiting degradation of the active nutrient in the formulation throughout the processing until the final packing, c) stabilization of the nutrient elements during the entire shelf life of the packaged product, d) assure the functionality of the nutraceutical after consumption (Daliu et al., 2018).

Amongst other natural polymers, chitosan and its functionalized derivatives are gaining significance in high-value products of the pharmaceutical, biomedical, cosmetic, and food industries (Aranaz et al., 2021, Hu et al., 2021). This biopolymer is obtained by deacylation of chitin in an alkaline condition, and chitin is the second most abundant natural polymer constituting a structural component in the exoskeleton of crustaceans as ordered microfibrils (Kou et al., 2021). The polymer encompasses significant biocompatibility, biodegradability, antioxidative, and antimicrobial properties suitable for nutraceutical and associated derivatives. Chitosan has been reported as an effective excipient in the pharmaceutical application for tablets' direct compression and has shown controlled release properties with many tablet formulations (Nigalaye et al., 1990, Nunthanid et al., 2004). Chitosan in nanosize improves the bioavailability and stability of bioactive ingredients. Moreover, there is evidence of enhanced cell uptake of these positively charged polymeric nanoparticles by epithelial cells (Akbari-Alavijeh et al., 2020). Chitosan has effectively exhibited antimicrobial and antioxidant activity in pristine and functionalized forms making it a promising option for nutraceuticals due to its high capacity to bind fat in the gastrointestinal tract (Abd El-Hack et al., 2020). Chitosan as a hydrocolloid has been studied for the release of lidocaine and effectively attained slow and sustained release attributed to the chitosan content concerning the degree of reacylation (Kristl et al., 1993).

These advantages make chitosan a paragon hydrocolloid that could be extensively used in nutraceuticals and pharmaceuticals. The availability, ease of extraction, inherent physicochemical properties, cost, and proven ability to be a drug delivery agent have shown an equivalent choice material as the most used gelatin. They are even advantageous over gelatin in many aspects such as abundance,

processing, cost, and a versatile polymer that could be functionalized by modifying the functional groups of the compound. Therefore based on these rationales, this study compares the gelling ability of chitosan composited with sodium benzoate, a commonly used preservative in food, pharmaceuticals, personal care products, and industrial products, with the gelling ability of gelatin. Since thickening and gelling properties are the principal functions of the hydrocolloids, the chitosan-sodium benzoate composite is examined for its gelling ability with the most consumed hydrocolloid in versatile industries. We believe this is the first study to synthesize chitosan sodium benzoate composite to apply the gelling agents to the best of our knowledge. The study would be a platform to develop chitosan-based hydrocolloids for numerous therapeutic applications and an economical option for several nutraceuticals and food industries.

2. Materials and Methods

Chitosan with a molecular weight of 3.8 – 20 kDa with 75 % Degree of Deacetylation (HiMedia, India), acetic acid, sodium benzoate, and gelatin was obtained from Sigma Aldrich. The rest of the chemical used in the study were analytical grade, and all the solution was prepared in Nanopure water.

2.1 Preparation of chitosan sodium benzoate hydrocolloid

1% chitosan solution was prepared by continuously dissolving chitosan powder in 0.1M of acetic acid until we obtained a homogenous mixture. The solution was filtered through a Whatman filter to remove the impurities, and the obtained solution was used for the hydrocolloid preparation. 0.01g of sodium benzoate was dissolved in acetic acid and was filtered. To 100 ml of chitosan solution, 20 ml of sodium benzoate solution was added and stirred for a few minutes until the solution got viscous. The viscous solution gets thicker, and this gel is freeze-dried with liquid nitrogen and then lyophilized for 12 hours. The lyophilized sample was utilized for all the physicochemical analyses.

2.2 Gelation temperature

The gelation temperature of chitosan sodium benzoate hydrocolloid was determined by the modified visual tube inversion technique (Ur-Rehman et al., 2011). A 5 ml of chitosan solution was taken in a glass tube and was kept in a temperature-controlled water bath at a ramp rate of 1°C/min with two minutes of equilibration. A 2 ml of sodium benzoate solution was added to the tubes with chitosan and incubated in the water bath. After every temperature point, the glass tubes were tilted horizontally, and the surface of the hydrocolloid was observed. The samples, when tilted, were analyzed for immobilization of sample inside the glass tube for 30 secs continuously, and the temperature when the hydrocolloid remained inside the tube was noted and considered gelation temperature.

2.3 FTIR analysis

Fourier Transform Infrared Spectroscopy (FTIR) spectrum was recorded for the chitosan, sodium benzoate, and chitosan sodium benzoate lyophilized using FTIR

8400S SHIMADZU, Japan. The lyophilized samples were used, and the FTIR spectra were collected in a range from 4000 cm^{-1} to 400 cm^{-1} wavenumbers with a minimal resolution of 4 cm^{-1} to identify the functional groups of chitosan, sodium benzoate with chemical bonds present in the hydrocolloid samples.

2.4 XRD

The X-ray Diffraction spectra for chitosan, sodium benzoate, and hydrocolloid were recorded for analyzing the microstructure with a D8 Advance ECO XRD Systems with SSD160 1 D Detector **X-ray diffractometer (BRUKER)**. The lyophilized powdered samples of chitosan sodium benzoate hydrocolloid were utilized for obtaining the diffraction patterns. The voltage was held at 40kV with a consistent 40mA intensity. The scanning scope of 2θ angle was between 5° and 65° , and the counting time was 1.2 seconds at each angle step (0.05°).

2.5 DSC

Differential Scanning Calorimetric (DSC) of chitosan sodium benzoate hydrocolloids was done to assess the thermal behavior with NETZCH Germany & STA 449 F3 Jupiter. A 5mg lyophilized hydrocolloid was taken for analysis which is kept sealed in an aluminum pan. The glass transition temperature was analyzed with a heating rate of $20^\circ\text{C min}^{-1}$ within $0\text{--}400^\circ\text{C}$ under a nitrogen environment.

2.6 TGA

The thermogravimetric analysis of the lyophilized hydrocolloids was performed to analyze the characteristic temperature at a maximum decomposition rate. The analysis was done with NETZCH Germany & STA 449 F3 Jupiter at a heating rate of $20^\circ\text{C min}^{-1}$ within $0\text{--}400^\circ\text{C}$ with a consistent nitrogen purge. 5mg of sample was wrapped and sealed in the aluminium pan for the TGA analysis.

2.7 Antioxidant activity

2.7.1 ABTS

The free radical scavenging activity of the chitosan sodium benzoate hydrocolloid for stable free radical 2,2-Azino-Bis-3-Ethylbenzothiazoline-6-Sulphonic acid (ABTS) was determined (Pu et al., 2019, Yang et al., 2018). The ABTS stock solution was prepared in 100 ml of methanol, dissolving 12 mg of ABTS, and then stored at -20°C until experimentation. The working solution was made from the stock solution by adjusting the absorbance of the purple free radical solution to 0.5 ± 0.05 . This working solution was further used for analyzing the scavenging effect of the samples. Chitosan sodium benzoate hydrocolloid was diluted into various concentrations, and to these, about 2.85 ml of ABTS solution was mixed. This mixture was made up of a final volume of 3 ml which was incubated for ten mins, and absorbance was recorded at 742 nm. A standard ascorbic acid solution was used as the positive control for the assay.

2.7.2 FRAP

The metal chelating of chitosan sodium benzoate hydrocolloids could reduce potassium ferricyanide (Fe^{3+}) to potassium ferrocyanide (Fe^{2+}) and eventually interact with ferric chloride to form ferric-ferrous (Huang et al., 2019). The reduction has a strong absorption at 700 nm and would be recorded for evaluation for all the samples, including pristine polymer and sodium benzoate (Pu et al., 2019). The assay was carried out to increase the chitosan sodium benzoate hydrocolloid concentration. The higher the absorbance shows stronger the reducing power. FRAP was made by mixing 300 mM acetate buffer of pH 3.6, 20 mM Ferric chloride (FeCl_3) and 10 mM 2,4,6- Tris (2-pyridyl)-s-triazine (TPTZ) in 10:1:1 ratio.

2.8 Antibacterial

The turbidimetric method determined the minimum inhibitory concentration (MIC) of chitosan, sodium benzoate, and chitosan-sodium benzoate hydrocolloid (Dananjaya et al., 2016, Stawski et al., 2017). In this method, several test tubes, each containing 5 ml of nutrient broth. All four samples were dissolved in 1% acetic acid, and the pH of chitosan-sodium benzoate was about 6.6. To 5 ml of broth, an equal number of samples were added. The test tubes were inoculated under aseptic conditions with 50 μL of the freshly prepared bacterial suspension. The positive control was bacitracin, and the blank control tubes contained only nutrient broth and 1% acetic acid. Then, the tubes were incubated at 37°C for 24 hours. The absorbance value was measured at 600 nm. The lowest sample concentration that inhibited bacteria growth was considered the minimum inhibitory concentration (MIC). A loopful from each test tube was inoculated on nutrient agar and examined for signs of growth.

3 Results and Discussion

3.1 Tube Inversion Method

The sol gel transition of the chitosan sodium benzoate hydrocolloid was assessed by tube inversion method was carried out observe flowability of the hydrocolloid for 5 ml in a 10 ml glass tube in a temperature controlled waterbath shaker. The flowability was analyzed between 5 to 55°C. Gelatin was used a known control since it was the most used hydrocolloid in functional foods and pharmaceuticals. forms gel when only external temperature up to 55°C is given, and then they are to be kept at 4°C. However, in the case of chitosan, gel formation could be observed within 5 sec at 25°C.

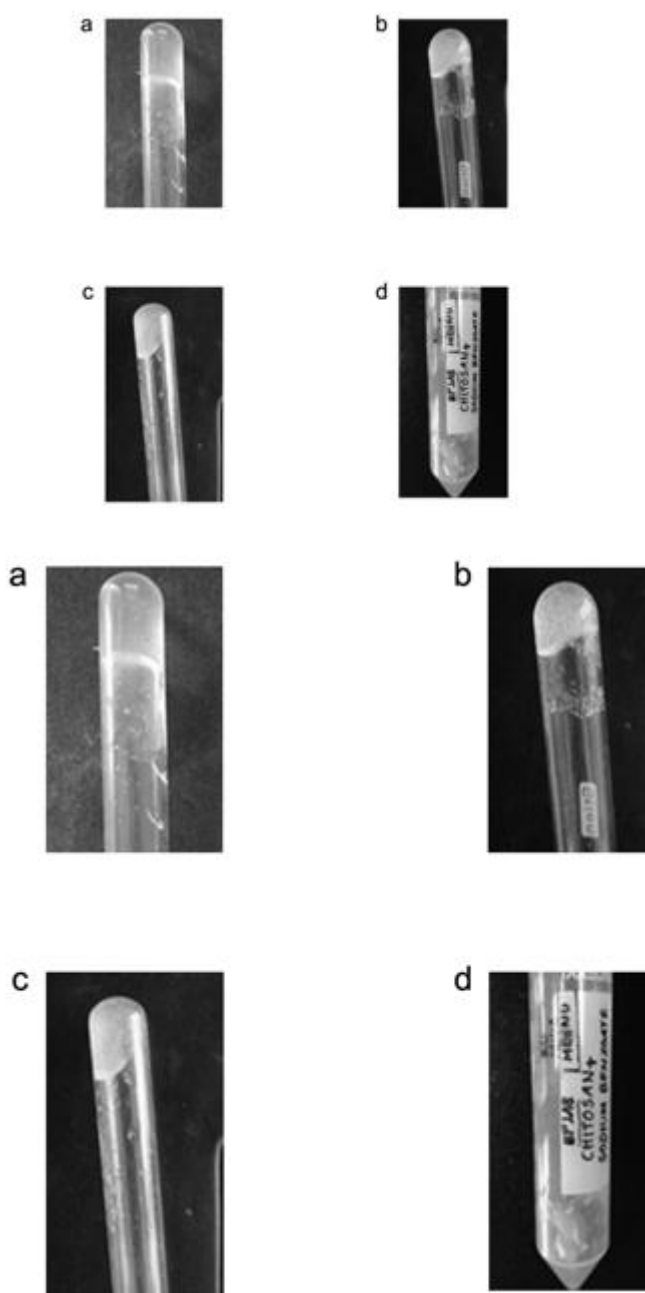


Fig. 3.1 a) Gelatin b) Chitosan c) Chitosan sodium benzoate) Lyophilized sample

The chitosan sodium benzoate composite formed a gel within 3 sec at 25°C, and the gel was stable compared with chitosan. The obtained gel was lyophilized for further characterization (Ilium, 1998).

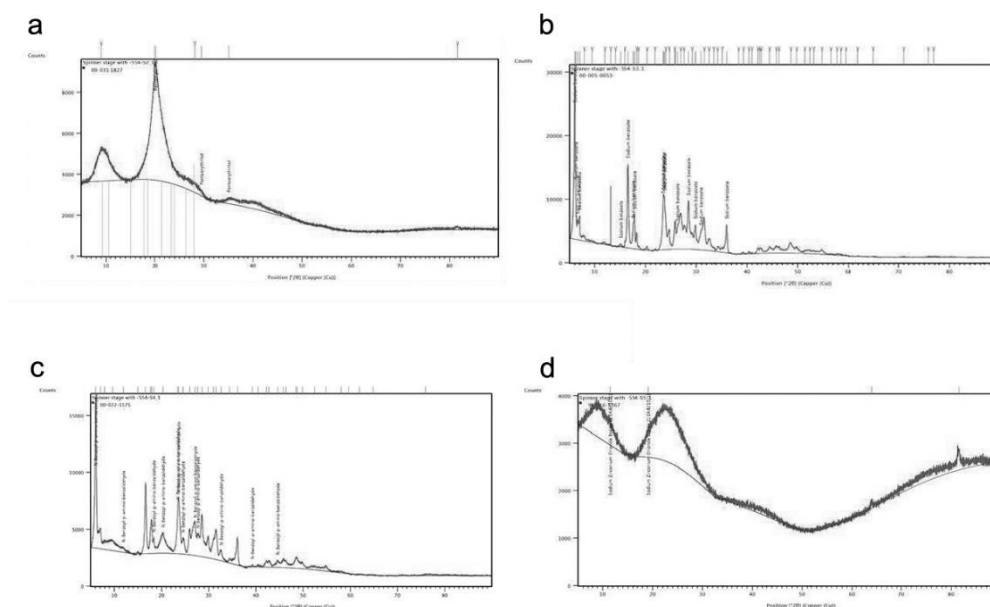
3.4 XRD analysis

X-ray diffraction patterns were performed for chitosan, sodium benzoate, physical mixture, and our sample. Significant diffraction peaks were observed in chitosan at $2\theta = 20$, $2\theta = 11.1$; the whole spectrum shows the compound in the semi-crystalline form. Similar spectrum for chitosan was reported starch sodium benzoate and chitosan composite formation (Wang et al., 2003).

Table 1. XRD interpretation of peaks

Samples	Peaks and their Description	Crystalline/ Semi Crystalline
Chitosan	Sharp – Broad	Semi-crystalline
Sodium Benzoate	Sharp – Narrow	Crystalline
Chitosan Sodium Benzoate Physical Mixture	Sharp – Narrow	Crystalline
Chitosan Sodium Benzoate composite	Blunt – Broad	Semi-crystalline

The X-ray diffraction peaks obtained for sodium benzoate are sharp and acute, depicting a crystalline form. Consequently, there are identical diffraction peaks in the chitosan and sodium benzoate physical mixture, which explains no interaction. In the composite, the observed diffraction peaks with peak values $2\theta = 23$, $2\theta = 12.4$. From the diffraction patterns of chitosan sodium benzoate composite, new diffraction peaks were identified, and they also had significant patterns where chitosan peaks were repressed (Sekar et al., 2018).



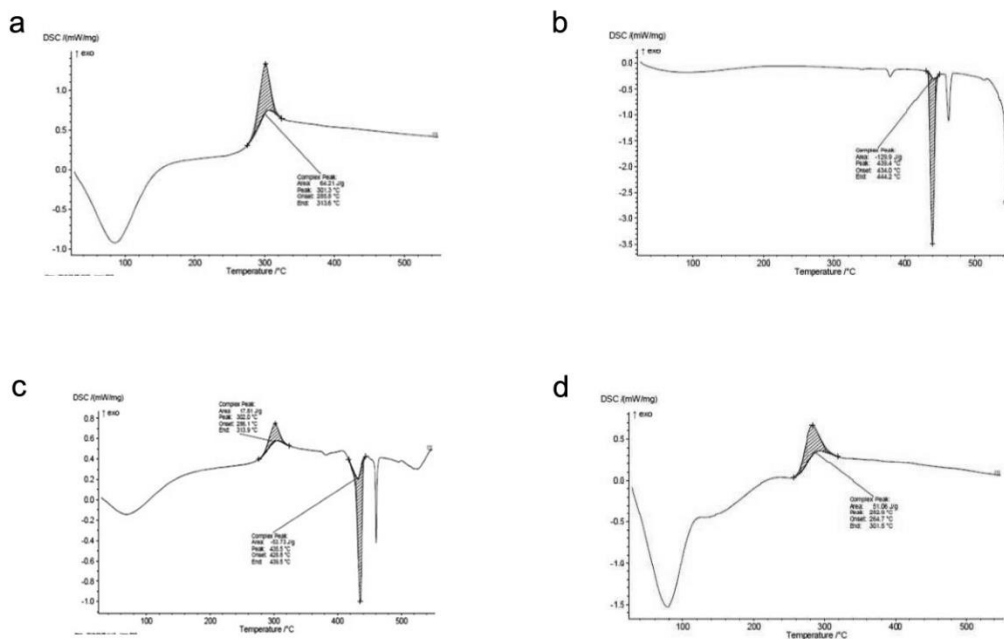
3.3 XRD spectra of a) Chitosan, b) Sodium benzoate, c) Chitosan sodium-benzoate physical mixture, d) Chitosan sodium-benzoate composite

3.5 DSC analysis

DSC analysis was done for chitosan, sodium benzoate, physical mixture, and sample to show the interaction between sodium benzoate and chitosan, leading to the changes in the molecular structure and crystalline form chitosan. In the thermogram, at 91.3°C in chitosan and 294°C in the sample would be attributed inbound water. Comparing the endotherms of the chitosan, sodium benzoate, their physical mixture, and composite, an evident peak shift in the position of peaks and change in the total area of the peak temperature. These changes in the peak shifts show that the macromolecules are altered in their water-holding ability and strength of water-polymer interaction.

Table 2. DSC interpretation of the onset and end of various peaks

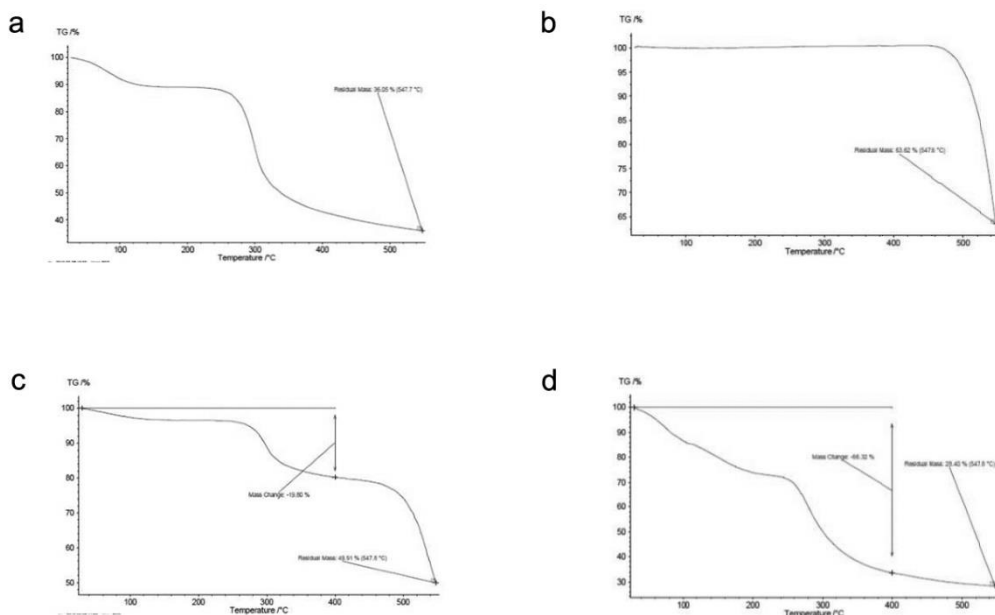
Samples	Peak Onset (°C)	Peak End (°C)	Area (J/g)
Chitosan	285.8	313.6	64.21
Sodium Benzoate	434.0	444.2	-129.9
Chitosan Sodium Benzoate Physical Mixture	CH = 286.1 SB = 426.8	CH = 313.9 SB = 439.5	CH = 17.81 SB = -53.73
Chitosan	264.7	301.5	51.06



3.4 DSC spectra of a) Chitosan, b) Sodium benzoate, c) Chitosan sodium-benzoate physical mixture, d) Chitosan sodium-benzoate composite

3.6 TGA analysis

The TGA curves can be compared with the residual masses. The residual mass of chitosan is 36.05%, 63.02%. The residual mass of chitosan was like that of the residual peaks found in (Tripathi et al., 2009). The residual mass of the physical mixture should be the exact mean of chitosan and sodium benzoate, and it is 49.91, which shows that both the compounds have some residual mass impact. The residual mass of the sample is 28.40% which is less when compared with chitosan, and the peak is more sensitive because it has lost its thermal stability. The weight loss at 40°C to 150°C is due to the moisture vaporization, and 200°C to 300°C is due to the thermal degradation of chitosan.



3.5 TGA spectra of a) Chitosan, b) Sodium benzoate, c) Chitosan sodium-benzoate physical mixture, d) Chitosan sodium-benzoate composite

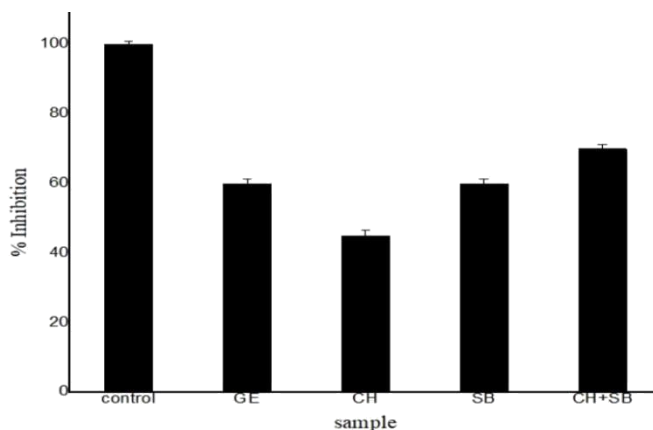
Our sample's TGA curve has two significant weight losses, like chitosan. One weight loss at 50°C to 170°C is due to moisture degradation and the second weight loss at 210°C to 320°C is due to thermal degradation of the sample.

Table 3. TGA interpretation concerning the residual mass

Samples	Residual Mass (%)
Chitosan (CH)	36.05
Sodium Benzoate (SB)	63.02
Physical Mixture (CH+SB)	49.91
Sample (CH+SB)	28.40

3.7 ANTIOXIDANT ASSAY

3.7.1 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) ABTS ASSAY

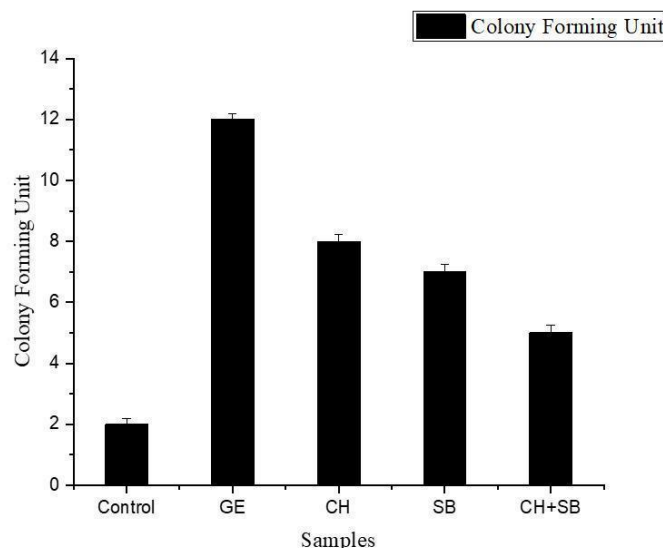


3.7.1 Antioxidant activity of chitosan benzoate complex against ABTS

Trolox is taken as control which has high antioxidant activity. Compared to gelatin and chitosan's antioxidant activity, gelatin had more antioxidative activity than chitosan. Sodium benzoate is a well-known antioxidative agent (Yetuk et al., 2014). Combining chitosan and sodium benzoate, relatively higher antioxidant activity was found in the chitosan benzoate complex than in gelatin. The outcomes show that the composite has resulted in enhanced free radical scavenging properties.

3.7.2 FRAP assay

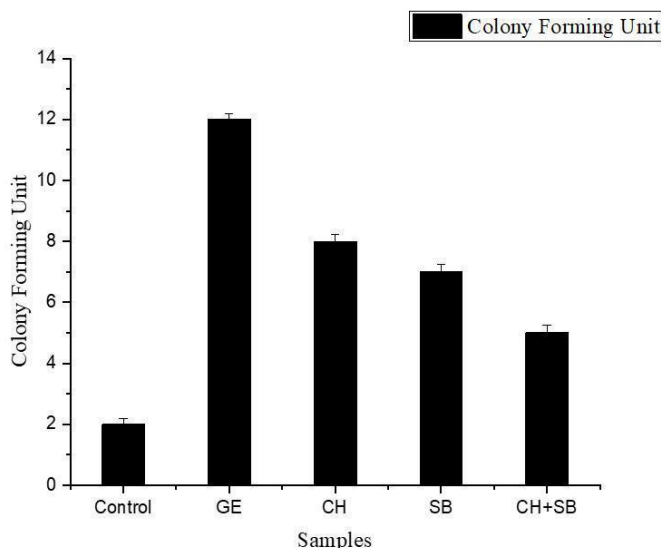
The antioxidant effect of gelatin, chitosan, sodium benzoate in our sample was determined by FRAP assay. FRAP measures the reducing power for samples. The outcomes of this assay were similar to the ABTS assay (Sekar et al., 2018). The concordance shows evident scavenging activity. The comprehensive antioxidant property of the chitosan sodium benzoate complex examined with scavenging activity of prominent free radicals depicts the antioxidative potentiality of the hydrocolloid appropriate for numerous healthcare applications.



3.7.2 Antioxidant activity of chitosan benzoate complex against FRAP

3.8 Antibacterial Assay

Control is ampicillin which has high antimicrobial activity. Gelatin shows less antibacterial activity than chitosan. Sodium benzoate has higher antibacterial activity compared to others. When comparing our sample chitosan and sodium benzoate with other samples, our sample has higher antibacterial activity. The bacterial cell wall is negatively charged, and the chitosan sodium benzoate considers as the target site (Abd El-Hack et al., 2020). The higher surface charge density of the complex, which is more significant than its pristine, interacts with the bacteria. The complex binds very strongly to the bacterial cell wall, which would have led to its instability, eventually leading to disruption of the cells wall (Pérez-Córdoba et al., 2018). This would result in leakage of intracellular components leading to the death of the bacteria.



3.8 Antibacterial activity of chitosan benzoate complex

4. Conclusion

The prepared chitosan sodium benzoate complex is colorless and has an effective gelling ability at a lower temperature than the gelatin. The ability to form gels at lower temperature and instantly in less than tens of seconds make the complex synthesized a better hydrocolloid than other natural animal source hydrocolloids in terms of thickening, which is the principal property of a hydrocolloid. FTIR, XRD, DSC analyze the composite formation and TGA, and the outcomes show a strong chemical interaction between sodium benzoate and chitosan. In FTIR, the functional group modification for our chitosan sodium benzoate composite was studied. In XRD analysis, the crystallinity behavior of our chitosan sodium benzoate composite was observed by diffraction peaks with peak values $2\theta = 23$, $2\theta = 12.4$. The chitosan sodium benzoate composite follows exothermic reaction in the DSC analysis and has the peak onset at 264.7°C and the peak-end at 301.5°C . In TGA analysis, the residual mass was less for chitosan sodium benzoate composite compared with chitosan and sodium benzoate. The study also has shown higher thermal stability than its pristine form with its semi-crystalline structure. The combination of chitosan and sodium benzoate has improved antioxidative and antibacterial properties. The study's outcome shows that chitosan sodium benzoate can be an effective hydrocolloid that can be modified and utilized in multifarious applications of nutraceuticals.

Moreover, chitosan and sodium benzoate's in-vitro and acute cytotoxicity are evaluated to determine the toxicity level specific to the gastrointestinal tract. Based on these outcomes, the molecular interaction of the composite with the potential protein targets in the gastrointestinal tract would also be examined through the insilico approach. This would render a possible mechanism on the interaction of chitosan sodium benzoate with the potential protein in the gastrointestinal tract.

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