THE ANNALS OF “DUNAREA DE JOS” UNIVERSITY OF GALATI
FASCICLE IX. METALLURGY AND MATERIALS SCIENCE
N^{o}. 4 - 2022, ISSN 2668-4748; e-ISSN 2668-4756
Article DOI: https://doi.org/10.35219/mms.2022.4.08

INVESTIGATION OF STRUCTURAL CHARACTERISTICS BY FTIR SPECTROSCOPY OF CHITOSAN DERIVATIVE WITH N HETEROCYCLIC COMPOUND

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ABSTRACT

Chitosan and chitosan derivatives are biomaterials of great scientific interest for biomedical applications although some of the properties are limited as their solubility in the aqueous medium. N-heterocyclic compounds are of interest for chitosan alkylation and new derivatives can be obtained by modification of his structure resulting thus new structures of biomedical interest Chitosan samples from two sources were analysed, in which the dissociation is favoured in the aqueous medium by the presence of a bipyridine salt, N,N'-bis(phenacyl)-4,4'-bipyridinium dibromide. The synthesis of chitosan’ derivate was conducted in aqueous medium at 60 °C, by magnetic stirring for 24 hours. The dissociation is better and reduced the pH, to values lower than pH 6, which suggests the achievement of favourable structural arrangements between chitosan and dibromide bipyridinium. The FTIR spectra parameters indicate the presence of characteristic bands of the bipyridinium salt in the chitosan derivates structures, with structural differences in their composition.

KEYWORDS: functionalized chitosan, bipyridinium salt, structure, FTIR spectrometry

1. Introduction

Chitosan is a biomaterial with a very similar structure to cellulose, which ensures the structural integrity and protection of plants and animals, but it is also useful in the medical field in formulations with different functions and properties [1-6]. The only difference between the chitosan and cellulose structure is the amine group (NH_2) in the C-2 position of the chitosan structure instead of the hydroxyl group (-OH), present in the cellulose structure [4].

Chitosan, the most widespread biomaterial is considered a biopolymer that consists of N-acetyl-2-amino-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose and is a compound that is easily alkylated with several chemical compounds. After purification, chitosan has a rigid crystalline structure, through intermolecular bonding and intramolecular hydrogen bonding, showing polymorphism. However, unlike plant fiber, chitosan possesses net positive ionic charges, which enable it to chemically bond with negatively charged substances such as fats, lipids, cholesterol, metal ions, proteins and macromolecules etc., making it a biomaterial of wide interest [7-13].

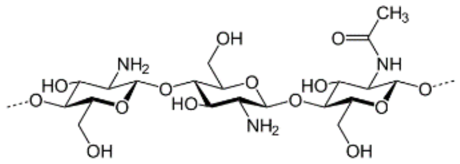
By alkylation with chemical compounds (Schiff bases, N-heterocyclic compounds) at the functional groups in the structure formed by N-acetyl-2-amino-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose, many reports have been made structures of new derivatives of chitosan with relevant properties and functions in medical and biomedical applications, as well as in pharmaceutical and industrial applications [6-7, 10, 13-16].

N-heterocyclic compounds are studied with the aim to obtain new structures with chitosan, each new compound confirming properties of biomedical or pharmaceutical interest [18].

In this work we propose the informal structural exploration in the obtaining of new derivative by synthesis between chitosan from two sources and an organic compound, N-heterocyclic salt, from the class of bipyridinium. The Fourier Transform Infrared Spectroscopy (FTIR) has been performed for analysis samples. The structure of chitosan is differentiated, depending on the manufacturer, from which materials it is obtained, and what degree of alkylation the final product has, characteristics that can greatly influence the structure and properties of new chitosan derivatives.

2. Materials, synthesis and method of structural analysis

In the synthesis to obtain new chitosan derivatives, two different chitosan samples were used, a commercial one marked CH_c (Sigma Aldrich Chemie GmbH; flakes, 85% degree of deacetylation) and a pharmaceutical product CH_f (capsules, with a content of 1.5 g chitosan (93% degree of deacetylation, obtained from the chitin of crustacean shells), cellulose (filling agent), magnesium stearate and silicon dioxide) (Figure 1.a). The schematic structure of chitosan of N-acetyl-2-amino-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose is shown below:

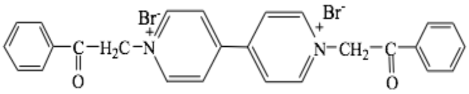


Chemical analysis of CH_c chitosan (fine grated) by fluorescence spectrophotometry (XRF Niton) indicates the presence of chemical elements such as: Ca (1875 ppm), K (450 ppm) Cr (139 ppm), As (2.5 ppm) and Zn, Ni, Cu, Fe, Mn, Se etc., as proof of the origin of chitosan from seaweed, chitosan being known as a biopolymer with a high degree of inhomogeneity [7].

(a) (b)

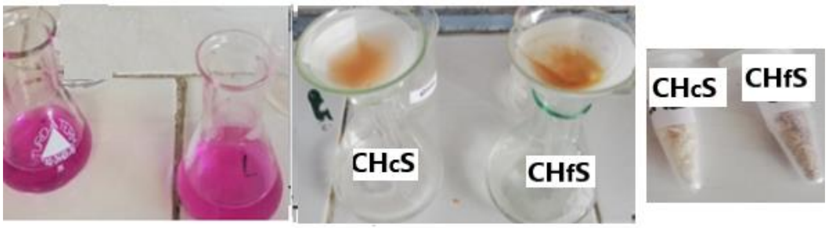
Fig. 1. *Chitosan ‘samples (a) and Bruker Alpha-P ATR FTIR equipment (b)*

The bipyridinium salt (S) used in the synthesis is N,N'-bis(phenacyl)-4,4'-bipyridinium dibromide (M = 554 g/mol) obtained in our laboratory according to the reference [19], the molecular structure being reproduced below:



- 0.2 g of chitosan (CH_c and CH_f respectively) were weighed in two glasses and 50 mL of distilled water was added. The procedure was followed by heated samples to 60 °C, by mechanical stirring (700 rpm) for 2 h, in duplicated samples for each type of chitosan. It was observed that the chitosan from both types of samples does not completely dissolve, and the pH analysed in the samples was 7.42 ± 0.04. Chitosan solutions were evaluated conductometric and pH-metrically, but also at 24 h and after 72 h, and there are no essential changes.
- 25 mL were measured from the clear chitosan CH_c solution, 0.01 g of the chemical compound, N,N'-bis(phenacyl)-4,4'-bipyridinium dibromide salt was added (ratio between chitosan: salt of 10:1) and the mixture was heated to 60 °C, by mechanical stirring (700 rpm), for 2 h. The resulting samples were made in duplicates and marked CH_cS_1. The pH and electrical conductivity for solutions are measured using the Constant C862 multiparametric analyser.
- at each remaining CH_c chitosan solution, of 25 mL hydrogel (with undissolved chitosan) was added 0.01 g of bipyridinium salt, obtaining the solution/sample marked CH_cS_2. The samples are heated to 60 °C, by stirring at 700 rpm, for 2 h. The pH and electrical conductivity of the solutions are measured also.

After the addition of the bipyridinium salt, the solutions showed an intensive red colour, as an effect of the protonation reaction of the N-heterocyclic salt, but colour disappears after 40 minutes of stirring, and the pH change over time is obtained (value of 5.35 ± 0.02 at sample CH_cS_1, and respectively of 6.70 ± 0.01 at CH_cS_2).



- the same synthesis procedure is followed for the sample of the pharmaceutical product and the samples were marked CH_f, obtaining the chitosan derivative with the bipyridinium salt, in the sample marked CH_fS_1, respectively CH_fS_2. The solutions have a pH of 5.70 ± 0.02 at sample CH_fS_1 and respectively of 6.50 ± 0.02 at sample CH_fS_2.

The samples synthesised marked CHcS2 and CH_fS_2 were filtered, resulting solid contents dried in the oven at 100 °C, weighed and the overage masses were: $m_{CHcS2} = 1.2566$ g ($\eta = 62.5\%$), $m_{CHfS2} = 1.1555$ g ($\eta = 57.5\%$).

Fourier transform infrared (FTIR) spectra of the chitosan powder, salt and chitosan derivates were recorded using a Bruker Alpha-P ATR (Germany) with a Diamond Crystal ATR (Attenuated Total internal Reflectance) accessory, over the range between 4000 and 400 cm^{-1} with a spectra resolution of 4 cm^{-1} (Figure 1.b).

3. Results and discussion

Synthesis experiments regarding the absorption of N-heterocyclic salt, N,N'-bis(phenacyl)-4,4'-bipyridinium dibromide (S) were carried out in chitosan solutions, in aqueous medium, samples purchased from two sources. The new compounds obtained were analysed physic-chemical and the investigation of structural characteristics was provided. The chitosan, samples of CH_c and CH_f in aqueous medium indicate a pH value higher than 6.3, with average values of 7.40 ± 0.04 being obtained, a pH that does not favour total solubilization of them, because the positive charge of the amino groups is lost and thus chitosan becomes insoluble in water [20-21]. Most of the characteristic properties for chitosan are due to the primary amino groups that at pK_a of 6.3 are found in large numbers in the polymer chain, for a lower pH, the ions of the NH_3^+ group transform chitosan into a water-soluble cationic polyelectrolyte [21-22].

Through the heating process at 60 °C, between chitosan and salt (ratio of 10:1) with mechanical stirring, after 2 h, pH values of 5.35 ± 0.02 are obtained for samples with CH_cS_1, which suggests that through the addition of bipyridine salt, bridges are created with the functional groups of the chitosan, the derived compound being a new structure. The pH value favours the solubility of chitosan in the aqueous medium. The synthesis procedure in this medium indicates a yield obtained of over 55%, with a higher value for samples with CH_c. There is possible that at temperatures over 60 °C there is also a deterioration of some properties.

Regarding the electrical conductivity analysis in solutions, CH_c shows values λ of 110 ± 10 $\mu S \cdot cm^{-1}$, and the CH_f solution indicates a less dissociation (52 ± 8 $\mu S \cdot cm^{-1}$), due to their excipients in structure. When adding an equal amount (0.01 g) of bipyridinium salt, in both types of samples, dissociation is favoured in aqueous solutions, the electrical conductivity value increases, with a greater tendency for samples with CH_c (780 ± 21 $\mu S \cdot cm^{-1}$), compared to samples with CH_f (530 ± 12 $\mu S \cdot cm^{-1}$), the explanation being the resistance of the excipients.

The samples of chitosan derivatives obtained were dried at 100 °C and structurally characterised, by using FTIR spectrophotometry, to identify the structural chemical characteristics, obtaining useful information regarding the spectral parameters, the vibration’s characteristics associations of the various types of bonds in structures, the existence and the strength of the chemical bonds between chitosan and the salt [23-24].

In Figure 2, the FTIR spectra of the chitosan showed different bonds, indicating some structural changes between the chitosan powders (CH_c, CH_f). The resulting spectra were compared with standard chitosan taken as a reference to confirm the structure, the results being consistent with the data reported by Puvvada *et al.* [22]. There are structural differences in the chitosan composition, the sample marked CH_f (with the excipients), suggests also the possibility of different structural arrangements, which are not found in the chitosan marked CH_c. This similarity of the spectra confirms the basic structure of the chemically chitosan extracted from a different source.

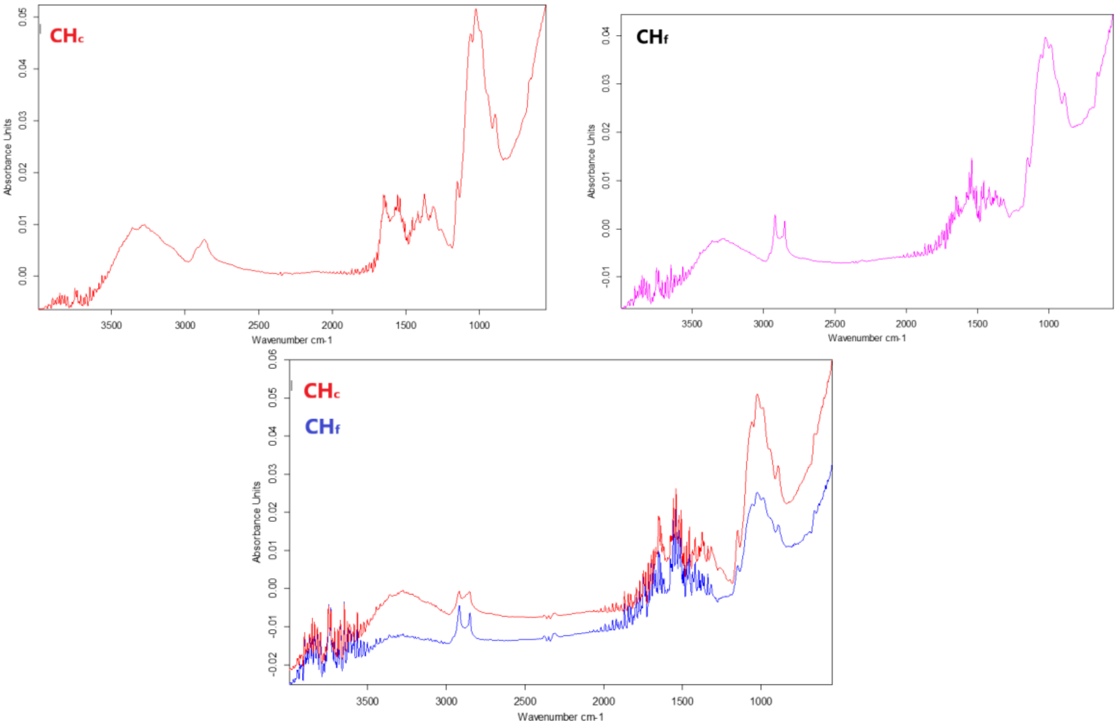


Fig. 2. *FT-IR spectra for chitosan powder and in aqueous medium (CH_c CH_f), at 60 °C, 700 rpm*

In the chitosan derivatives samples obtained with the N-heterocyclic salt, N,N'-bis(phenacyl)-4,4'-bipyridinium dibromide (S) (Figures 3-6), FTIR spectra indicate essential changes for the higher frequency area, up to 3500 cm^{-1} and around 1500 cm^{-1}.

The representative peaks for the chitosan samples and for the new compounds are mainly grouped in three regions. In the area of 3500-3300 cm^{-1}, the existence of absorption bands confirms the presence of hydroxyl groups (-OH), from the chitosan structure. The broadband aspect of absorption indicates association through hydrogen bonds. Sample CH_c, for example, showing a high absorption capacity of water molecules (WA_{24} = 355.29 %) (Figure 4). The broad-band aspect of the absorption for the chitosan derivative CH_cS_1 (Figure 5) indicates a somewhat more favourable association through hydrogen bonds compared to the sample CH_fS_2 (Figure 6).

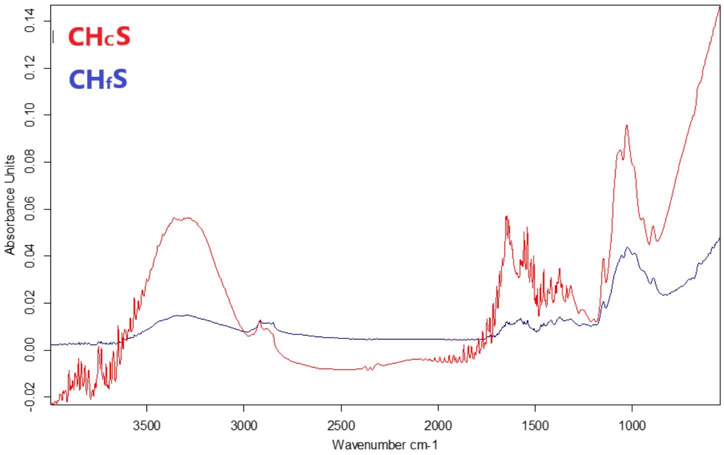


Fig. 3. *FT-IR spectra for chitosan solutions with addition of 0.01 g of N,N'-bis(phenacyl)-4,4'-bipyridinium dibromide (S), in aqueous medium, at 60 °C, 700 rpm*

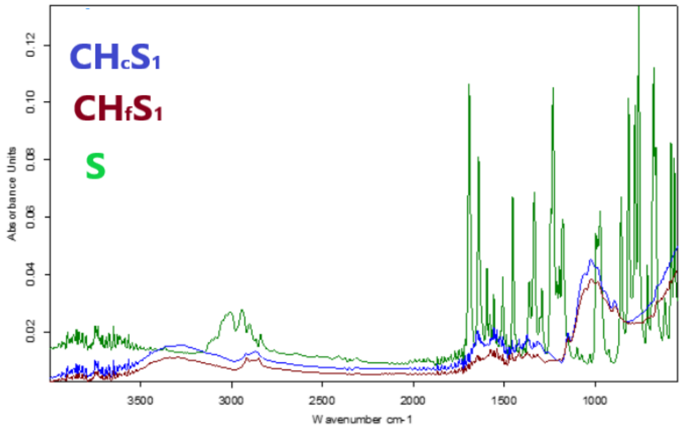


Fig. 4. *Comparative FT-IR spectra of clear chitosan solutions, with addition of 0.01 g of N,N'-bis(phenacyl)-4,4'-bipyridinium dibromide (S), in aqueous medium, at 60 °C, 700 rpm*

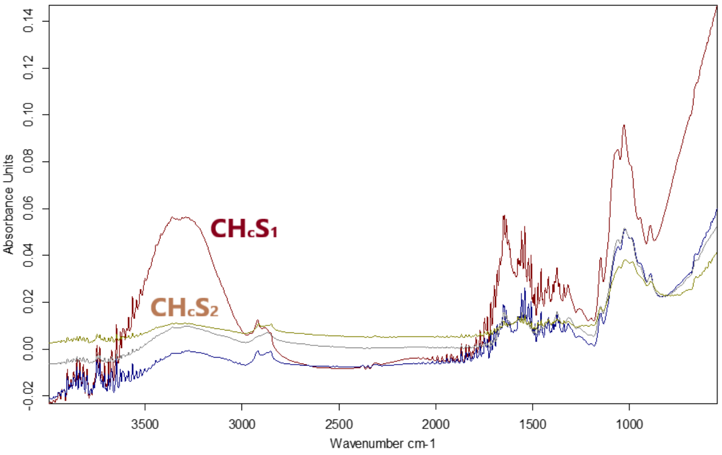


Fig. 5. *Comparative FT-IR spectra of the chitosan’ derivate CH_cS_1 and CH_cS_2, in aqueous medium, at 60 °C, 700 rpm*

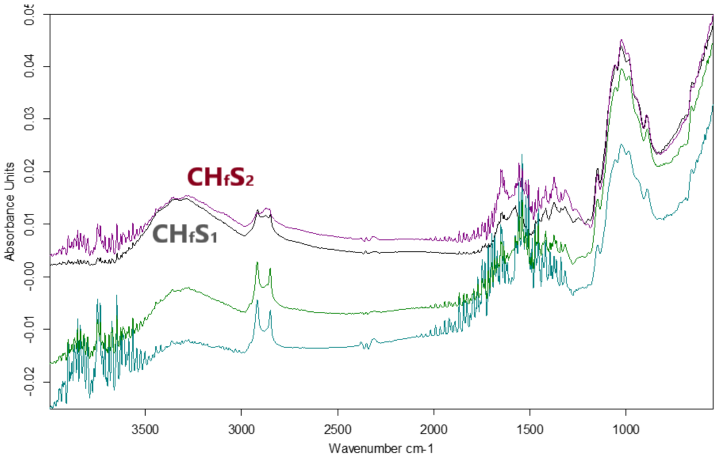


Fig. 6. *Comparative FT-IR spectra of the chitosan’ derivate CH_fS_1 and CH_fS_2, in aqueous medium, at 60 °C, 700 rpm*

The FTIR analysis of samples proved the presence of N,N'-bis(phenacyl)-4,4'-bipyridinium dibromide in structure of the new chitosan compounds (Figure 4) by the ν_{CHarom} absorption bands positioned at 3054-3061 cm^{-1}, with a slight shift to the left (2 cm^{-1}) compared to those find in the spectrum of the bipyridinium salt (Figure 7). The FT-IR spectra at N,N'-bis(phenacyl)-4,4'-bipyridinium dibromide (S) were made on solid samples (crystals) and is a confirmation a series of characteristic bands: 3040-3000 cm^{-1} (C-H$_{arom}$), 1730-1722 cm^{-1} (C-O$_{ester}$), 1700-1670 cm^{-1} (C=O), 1640-1630 cm^{-1} (C=N), 1200 and 1100 cm^{-1} (C-O-C) [24].

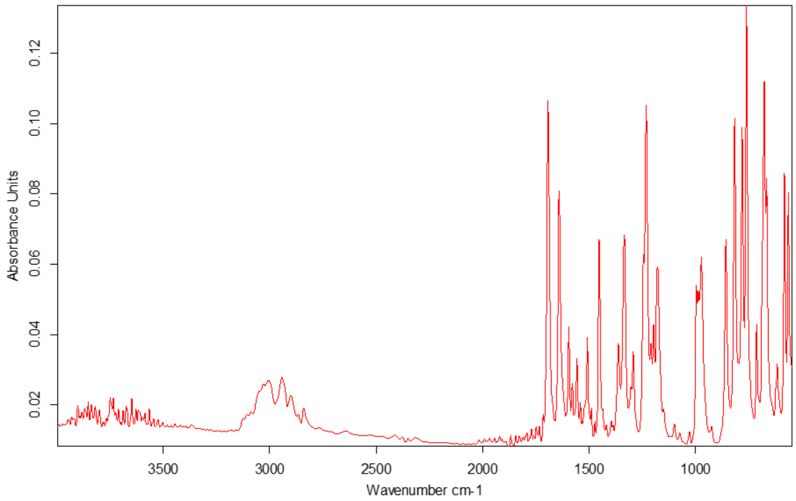


Fig. 7. *FT-IR spectra of N,N'-bis(phenacyl)-4,4'-bipyridinium dibromide (S)*

Of interest in the FTIR spectra is the region at 1650 cm^{-1}, as an effect of the intense absorption due in particular to the valence vibration of the heterogeneous double bond C=O, component part of the bipyridinium salt structure, position for samples and derivatives. And the peak position at 1646 cm^{-1} supports new positions for amide bonds forming δ_{N-H}.

The FT-IR spectral analysis highlights the fact that, for the same functional group in the spectral range at 1400-1490 cm^{-1}, the characteristic peaks are more numerous in the chitosan derivatives spectra, compared to the spectrum of the bipyridinium salt [20]. At the same time, small shifts of these peak positions to the right are highlighted, compared to the FTIR spectrum of the salt, as a result of the complex steric effects that appear in the new structures of the chitosan derivatives.

More intense peaks appear in the chitosan derivatives spectra, at 1191 cm^{-1}, 1085 cm^{-1}, 1049 cm^{-1} and 1014 cm^{-1}. The spectra also indicate the positions of the absorption band, at 1100-1050 cm^{-1}, which characterizes the molecular structure as a whole and can be attributed to C-O or C-N bonds, the peak position confirming the structure of the salt in the chitosan derivative, by comparing it with the spectrum of chitosan, when the position is shifted from 1162 cm^{-1} for ν_{C-0}, towards lower values. The position at 1029 cm^{-1}, assigned for ν_{C-0}, is similar in all spectra with chitosan and its derivatives, with a shift of 3-5 cm^{-1} to the left in samples with CH_f.

Spectra proved the presence of amino and carbonyl functional groups on derivates, available for binding with biological compounds. Chitosan CH_f, although it indicates links with the bipyridinium salt, presenting a higher degree of deacetylation, is not recommended for the continuation of our study, due to the presence of excipients in the structure, which affects the properties. Chitosan CH_c, although it has a lower degree of acetylation, structurally confirms a chitosan derivative, with bipyridinium salt, promising new properties, but there are studies that recommend much higher degrees of deacetylation. Derivates of chitosan are interesting biomaterials which could promise a potential in the biomedical applications [16, 18, 25-26].

4. Conclusions

The chitosan samples, from two sources, show dissociation in aqueous medium, for weak basic pH, which indicates less solubilization of their structure. The dissociation is favoured by the presence of the bipyridinium salt in the synthesis process for new derivates, with notable differences between the two samples of different origin. In the synthesis solutions, for new chitosan derivates, the pH is reduced when bipyridinium salt is added, which suggests the achievement of favourable structural arrangements between chitosan and N heterocyclic salt, in the aqueous medium, the values pH reaching 6.3. The FT-IR spectrum confirms the presence of the characteristic bands for the bipyridinium salt in the new structures, but indicates structural differences that appear in their composition, and excipients influence the data. The mechanism study of the reaction between chitosan with bipyridinium salt is of interest, the nature/type of chitosan samples is important and the degree of deacetylation of chitosan has a decisive role in the synthesis process. Results justify the usefulness of FTIR spectroscopy for chitosan derivates structural investigation.

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