IOSUD - „Dunărea de Jos" University of Galaţi
Doctoral School of Fundamental Sciences and Engineering

FUNCTIONAL CHARACTERIZATION STUDIES FOR NEW CHITOSAN DERIVATIVES

DOCTORAL THESIS
THESIS SUMMARY

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Scientific leader,
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With great respect,
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INTRODUCTION

In today's modern technologies, there is an increasing concern for obtaining new smart and ecological products and materials, which must fulfill the needs of our modern society, a society which is constantly changing and trying to find solutions for a friendly environment.

Biomaterials are of great interest in many fields of study due to their numerous properties they possess. Those properties are represented by: biocompatibility and biodegradability, non-toxicity and possibility of being used as drug carriers with the aim of improving and preventing the health of the population.

Many polymers have been studied with the aim of developing new biomaterials of wide technical interest, but chitosan mainly occupies one of the first places after cellulose and can be successfully used in different forms such as:solutions, hydrogels, nano/microparticles with different degrees of deacetylation.

Chitosan is a biopolymer that can be obtained from natural sources and possesses unique biological properties such as biocompatibility, biodegradability, non-toxicity having many applications. Chitosan and the chitosan derivatives are considered promising biomedical products that can be included in microencapsulation techniques and enhancing investigations for the delivery of drugs, biological compounds and vaccines, etc. [Ibrahim M.A. et al., 2023]. Due to its antimicrobial properties, chitosan is a promising biomaterial, possesing a cationic nature and being a compound that is easily alkylated with several chemical compounds.

Chitosan and its derivatives include a variety of physico-chemical and biological properties, with specific and diverse uses (pharmaceutical industry, biomedical applications, dentistry, food industry, wastewater treatment, agrochemistry, environment and industrial uses). Although there are many studies on chitosan and its derivatives, the examination of better ways to obtain new functional derivatives are still strategies that remain of interest,

especially regarding the use of biopolymer as a matrix in natural development and biological derivatives as antimicrobial agents for a growth of its efficiency and specificity.

The personal motivation in choosing this topic was the desire to contribute to reasearching studies regarding the development of new solutions for natural products that could improve human health by increasing the solubility of chitosan and by modifying its chemical structure in order to obtain new products with antibacterial properties.

For this reason, the main objective of the doctoral thesis *("Studies regarding the functional characterization for new chitosan derivatives"*) was to obtain new products, to characterize new compounds and derivatives of functionalized chitosan, having specific properties, which can also be used as biomaterials, such as hydrogels.

The research which was carried out in the doctoral programme had the following specific objectives:

- Obtaining and characterizing compounds with chitosan, using metal ions Cu (II), Zn(II) and respectively Fe(III), studies on new metal complexes, with specific applications.
- Synthesis and physico-chemical and structural characterization of new chitosan derivatives using some 4,4' dipyridyl quaternary salts for functionalization.
- Evaluation of the influence of *4,4' dipyridyl quaternary salts* and synthesis factors, for obtaining of new functionalized chitosan derivatives with biological properties, antioxidant and improved antibacterial properties, which can be a solution for future medical applications.

Thus, the doctoral thesis brings new results due to the diversity of some studies which to our knowledge are unreported, regarding chitosan derivatives with heterocyclic N ligands, from the 4,4'-dipyridyl class, carefully selected quaternary salts.

The doctoral thesis entitled "Studies regarding the functional characterization for new chitosan derivatives" includes six main chapters, focused on the objectives of interest of the topic that were proposed in the doctoral programme.

The first two chapters represent the documentary part.

The first chapter *(Studies on obtaining functionalized materials based on chitosan)* presents the current state of research and is a synthesis of many studies on chitosan, the polymer with remarkable diverse applications and the characterization of chitosan derivatives. Many of the reported studies are related to biomaterials, their role and importance. The second chapter *(Viologens, heterocyclic compounds, of class 4,4, dipyridyl)* refers to organic, N-heterocyclic compounds that are of great current interest in obtaining new materials and as revolutionary technologies.

The experimental part of the scientific thesis is presented starting with chapter 3 *(Chitosan derivatives obtained by complexation with metal ions*) and includes the early stages of the doctoral programme, the first syntheses of new compounds based on chitosan which were the complexes of chitosan with metal ions. Thus, new derivatives were synthesized and characterized:complexes of chitosan with copper ions Cu(II) and zinc ions, Zn(II), then with Fe(III) ions, from solutions. The new complexes were obtained by different methods and under different conditions.The influencing factors in the obtaining process wre: temperature, pH of solutions, contact time, mass ratio between compounds and ultimatly, the samples werw analysed by spectrophotometric (UV-Vis), structural (FT-IR), and morphological (SEM) analysis) and by kinetic study of Fe(III) ions in acidic medium.

Chapter 4 includes the results of innovative research and the original personal contribution regarding the synthesis, chemical composition and physico-chemical, structural and biological properties for the new products obtained. The new functionalized chitosan derivatives were obtained using organic compounds included in *4,4 dipyridyl* class (N-heterocyclic salts). Initially, four N-heterocyclic salts named also as viologens (organic

compounds of major interest for materials engineering and modern technologies) were obtained and structurally characterized, through their specific properties, with redox reversibility, antifungal, antibacterial and redox activity. The salts synthesized and used for obtaining new chitosan derivatives were: *N,N'-diphenacyl 4,4'-dipyridylium dibromide* and *N,N'-bis(p-nitrophenacyl)-4,4'-dipyridylium dibromide* which has two NO_2 groups in addition to the first salt. *N,N'-diphenacyl-1,2-bis(4-pyridyl)ethane dibromide* and *N,N'-bis(p-nitrophenacyl)-1,2-bis(4- pyridyl) ethane.*

Chapter 4 (*Research on the functionalization of chitosan with N-heterocyclic compounds, in aqueous solution and influencing factors)* presents the results of research on the functionalization of chitosan with these salts, N-heterocyclic compounds, in aqueous solution, and the influencing factors (contact time, temperature of synthesis, agitation, mass ratio between chitosan:salt) for the synthesis of new chitosan derivatives, analytical and physico-chemical characterization, through modern analysis methods to highlight the structures of the products (UV-Vis, XRD, FT-IR, SEM -EDX, cyclic voltammetry). Promising results were obtained for chitosan derivative with *N,N'-diphenacyl-4,4'-dipyridylium dibromide.*

Chapter 5 (Research on the functionalization of chitosan with diquaternary N-heterocyclic salts, in an acidic environment) presents the research on the functionalization of chitosan with the same N-heterocyclic salts, in an acidic environment (acetic acid 2%).The influencing factors in the synthesis of new chitosan derivatives are discussed and there are some methods for characterizing materials, structures and their biological properties against pathogens presented in order to track the effectiveness of derivatives for future medical applications.

Chapter 6 (Chitosan hydrogels as biomaterials for medical applications) presents the importance and relevance of chitosan-based hydrogels for medical and pharmaceutical applications and the exploratory results regarding the new functionalized chitosan hydrogels which were characterized by structural (FTIR) and morphological methods (SEM). The antibacterial activity against *E. coli*, was also studied in order to establish the importance of biomedical practice.

The thesis includes final conclusions that emphasize the most relevant results from the research and the original contributions, followed by the presentation of dissemination of the research results.

The doctoral thesis with the title *"Studies regarding the functional characterization for new chitosan derivatives"* contains 180 pages and is structured in two parts: the documentary part represents 24% of the entire thesis o(without bibliographic references), and the experimental part represents which 76%, with the interesting results of the thesis, original personal contributions in the field of new materials. Bibliographic references are presented at the end of the chapters; being over 410 references in total that were studied; 3 scientific articles are published (ISI & BDI), with the results obtained regarding the topic of the thesis, in 2 articles I am the first author, in one article I am co-author. The work includes 17 schemes, a number of 76 figures and 24 representative tables.

The research activities were carried out using the modern infrastructure of Dunafrea de Jos University of Galați (www.ugal.ro), Materials Science and Engineering Department and Physical and Environmental Chemistry Department , as well as the accredited research centers ("ECEE", " BioAliment" and "Moras"). We believe that the results obtained and presented in the thesis can be of great interest and initiation that will encourage the carryng out of new studies on chitosan with new chitosan derivatives, using N-heterocyclic salts, in order to obtain other hydrogels with these compounds that could have biological activity, in finding viable solutions to support human health.

1.STUDIES RELATED TO OBTAINING OF FUNCTIONALIZED MATERIALS BASED ON CHITOSAN

This chapter presents the current phase of research regarding chitosan-based biomaterials, the parameters and special properties and their importance. Chitosan, next to cellulose, is the most widespread biopolymer in nature, a natural polysaccharide obtained by deacetylation of chitin which being a biomaterial with multiple applications in many fields.It has been higly pursued in the medical field, due to its important biological properties such as biocompatibility, biodegradability and low toxicity, or in pharmaceutical technology, as an antimicrobial and antitumor agent [Chylinska M. et al., 2019; Kritchenkov A.S. et al., 2020]. Obtaining of chitosan derivatives due to its abilities it is also discussed for many other fields of study.Chitosan has a great capacity to be chemically modified but it has a main drawback due to its limited solubility in water which limits its therapeutic use [Liu C.et al., 2019; Wang W. et al., 2020]. Although there are many studies on chitosan and its derivatives, searching for better ways to obtain new functional derivatives are still of great interest, especially when the biopolymer could be used as a matrix in natural development and biological derivatives as antimicrobial agents for a growth of its efficiency and specificity.

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3. VIOLOGENS-HETEROCYCLIC COMPOUNDS OF THE 4,4'-DIPYRIDYL CLASS

Heterocyclic compounds are important organic molecules used in medicinal chemistry as drugs for various diseases, with various therapeutic applications [Sirbu R. et al., 2019, Carver P.L. et al., 2019]. The structure of viologenes are making them perfect candidates in the synthesis of new compounds such as chitosan derivatives with pharmacological or medical properties[Costea (Nour) I.F. et al.2022; El-Naggar M.M. et al., 2020]. Using N-heterocyclic salts (viologens) with cationic chitosan can be obtained new functional chitosan derivatives by introducing the appropriate heterocyclic salt for the addition or substitution reactions of the already formed N-containing functional group [Botezatu (Dediu) et al., 2023; **Costea(Nour) I.F. et al., 2022**].

EXPERIMENTAL PART

4. CHITOSAN DERIVATIVES OBTAINED BY COMPLEXING WITH METALLIC IONS

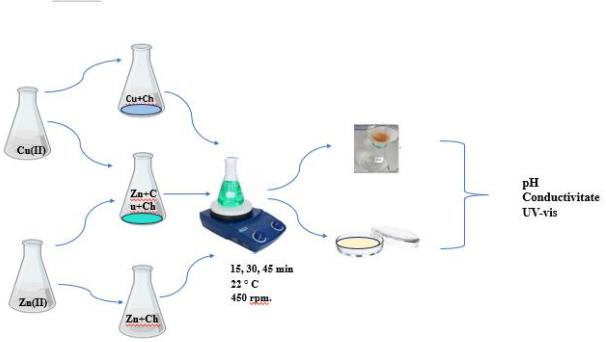
Due to its functional groups on the chemical structure, chitosan can facilitate the tion with cations and anions, increasing thus his ability in binding metal ions [Chen Z. et al., 2022; Ali M.A. et al., 2021].

Different studies present different complexation systems using metal ions, copper and zinc, cadmium, manganese, important ions for the pharmaceutical industry and the medical field [Maia M. et al., 2020; Luo X.Y. et al., 2022]. The results obtained by various researchers indicate that the presence of metal ions in the solution brings changes to the chitosan adsorption complex [Carver P.L et. al., 2019; Maia M. et al., 2020].

3.3. Absorption complexes of Cu(II) and Zn(II) ion with chitosan

A study was initiated in order to analyse the adsorption process of samples with metal ions. Chitosan granules are found in different forms and are microporous biopolymers, therefore the pores are large enough to allow the adsorption of metal ions such as Zn(II) and Cu(II) on the polymeric structure of chitosan [Wang M et al., 2019; Chen Z. et al., 2022].

Chitosan samples were produced using the metal ions Cu(II), respectively Zn(II), both in a mixture or only one ion, at the pH between 6-8, the optimal conditions for the adsorption of these ions [Cruz-Lopez L.P. et al., 2021]. The samples were stirred for a contact time of 15, 30, and 45 minutes, at 450 rpm (selected as optimal), at room temperature and then the remain product was filtered on quantitative paper. The supernatant solutions were analyzed spectroelectrochemically, using a UV-VIS T90+ spectrophotometer, at a wavelength between 200-900 nm, using quartz cuvettes (**Scheme 3.1).**



Scheme 3.1. Obtaining new chitosan complexes with Cu(II) and Zn(II) ions

It was found that the contact period influences the pH, the conductivity, as well as the absorbance in the UV-VIS spectra of the aqueous solutions after the complexation process **(Figure 3.4).** As the contact time increases, the absorbance also increases and two peaks are found at λ of 240 nm and in the samples obtained after 30 minutes at λ between 264-300 nm. Chitosan, being a polymer with a high retention capacity, has a faster absorption for zinc ions, compared to copper ions, which are more difficult to retain. An explanation would be that the retention of metal ions on chitosan is strongly dependent on changes in pH, on their molecular weight, on the specific properties of each metal ion. 30 minutes after contact, the absorbance decreases, when both ions are present simultaneously in the solution, it is an adsorption equilibrium between the components.

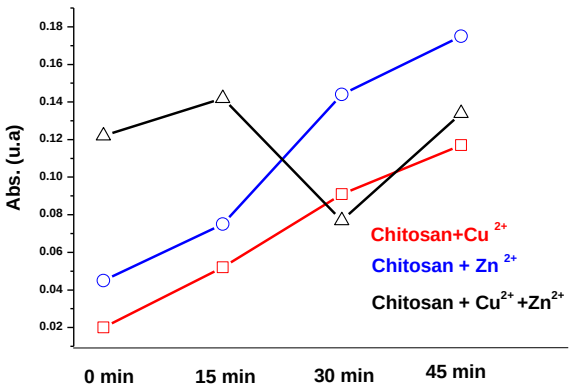


Figure 3.4. Variation of absorbance with contact time for the system chitosan-Cu(II) (red), chitosan-Zn(II) (blue), chitosan-Zn(II)-Cu(II) (black)

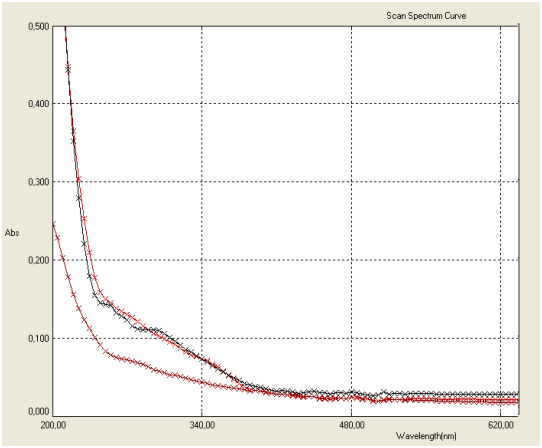


Figure 3.7. Absorption spectrum in UV-VIS for the chitosan - Zn(II) - Cu(II) complex system; contact time: 15 min. (grain), 30 min. (red) and 45 min. (blue)

Zn(II) ions adsorbed by chitosan were determined by the difference between Zn(II), initially in the samples with chitosan (10 mg/L) and Zn(II) in the filtrate. For the chitosan

complex with zinc ions, ions are adsorbed from the beginning of contact with chitosan.

Once the contact period increases, a decrease in retained Zn(II) is recorded, by exhausting the available bonds in the structure of the chitosan molecule. In the case of chitosan complexes with both metal ions (Zn(II) and Cu(II)), the absorbance values are higher than in the case of systems with chitosan and individual metal ions. The UV-VIS spectrum regarding the synergic action of the two metal ions on the chitosan structure **(Figure 3.7),** shows that both ions are absorbed on the chitosan molecule and with the increase of the contact period, two peaks are found, at λ of 240 nm, and after 45 min., contact time, one step at λ between 264-300 nm. In conclusion, for systems based on chitosan and copper ions, the adsorption equilibrium is established relatively quickly over time.

3.4. Fe (III) ion absorption complexes with chitosan

Chelation with Fe(III) ions indicates strong coordination with the functional groups, with pH-dependent interaction between them. At acidic pH, chitosan nitrogen groups are strongly involved in the formation of chelate bonds, favoring reaction rates for which iron metal ion uptake will involve a charge transfer complex [Wang M. et al., 2019; Chen Z. et al., 2022]. The adsorption efficiency on chitosan flakes was studied by changing the contact time and the concentration of ferric ions in acidic medium. The influence of some parameters in the absorption balance is of interest for explaining the ability of Fe (III) ions to form chelated bonds with chitosan [Wang M. et al., 2019; Mahir T. et al., 2018].

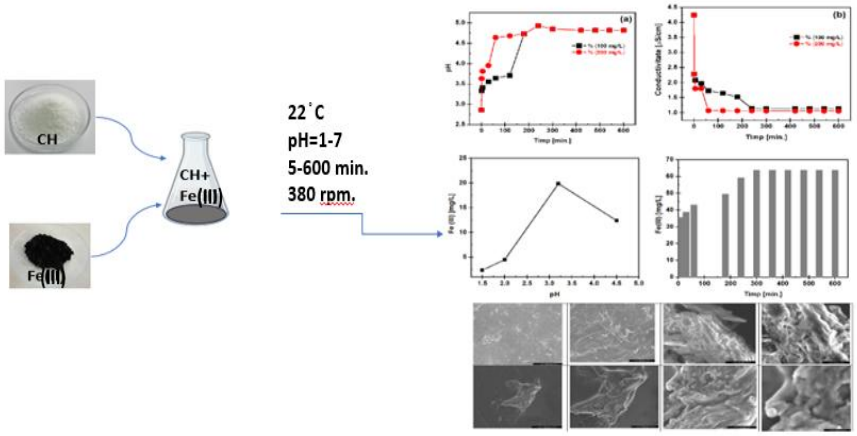
Adsorption experiments

Chitosan in contact with Fe (III) ions (1 mg/L, 50 mg/L, 100 mg/L and 200 mg/L Fe) was analyzed by changing some experimental parameters and each experiment was repeated twice, at a temperature of 22°C **(Scheme 3.2)**:

a) *contact time:* 100 mg chitosan flakes in contact with 50 mL contact from 100 mg/L and 200 mg/L Fe (III), at 380 rpm (mechanical stirring) and variable contact time between 5 and 600 minutes.

b) *the ratio of contact phases*: it has 100 mg and 200 mg chitosan, with 100 mg/L Fe (III) and 200 mg/L Fe (III), respectively, contact time of 540 min., 380 rpm.

c) *the effect of pH* between1-7, for the sample obtained from 100 mg chitosan with 100 mg/L Fe (III), contact time 60 min., at 380 rpm, using HCl 0.1 N and NaOH 0,1 N.



Scheme 3.2. Obtaining Fe (III) ion absorption complexes with chitosan

3.4.2. Results and discussions

Adsorption of metal ions to the surface of chitosan leads to surface complex involving nitrogen atoms [Burke, A. et al., 2020; Lapo B. et al., 2018].

When the agitation is increased above 200rpm, the retention efficiency of Fe ions also increases [Lapo B. et al., 2018; Ali M. A. et al., 2021]. After 300 minutes, a balance between the phases is established **(Figure 3.9).** This process of absorption is observed for 100 mg chitosan, up to a concentration of 66.6 mg/L Fe(III).

The adsorption capacity of chitosan for metal ions is much higher on a lower pH . For contact time of 300 min, for 100 mg of chitosan similar metal ion retention is observed, up to a concentration of 66.6 mg/L Fe(III) **(Figure 3.9).**

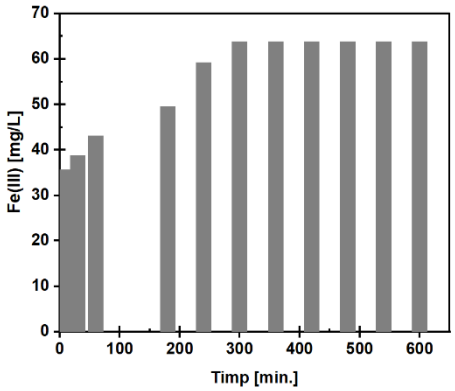


Figure 3.9. The effect of Fe (III) absorbed depending on contact time. Reaction conditions: 100 mg absorbant, 50 mL solution of 100 mg/L Fe (III),380 rpm, t= 22°C.

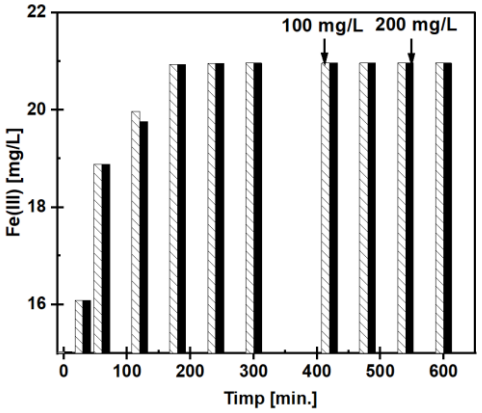


Figure 3.10. Fe (III) ions remaining in solution after adsorption on chitosan as a function of contact time. Reaction conditions: 100 mg adsorbent, 50 mL solution of 100 mg/L Fe (III) and 200 mg/L Fe (III), 380 rpm, t= 22°C.

In an acidic medium, iron ions are released with difficulty due to interaction with chitosan, forming stable bonds through the functional groups [Maia, M.T. et al., 2020, Ali M.A. et al., 2021]. The adsorption capacity of chitosan for metal ions is much higher on a lower. At contact time bigger than 300 min, using 100 mg chitosan leads to a similar retention of metal ions up to a concentration of 66.6 mg/L Fe(III) **(Figure 3.9)**

The balance of adsorption and complexation on chitosan, at different amounts of this and the same Fe (III) solution, is established after 60 min., wich is consideres as favorable as a contact time **(Figure 3.10).** For higher amount of chitosan (200 mg adsorbent), the equilibrium is more difficult to establish with variations of pH and conductivity of the solution. The amount of adsorbed iron(III) ions increases with the contact time, reaching a stable equilibrium in 5 hours, in both samples. After 240 min., as a contact time between the components, the pH of 5.5 from the filtrate remains constant in both samples with 100 mg/L Fe and respectively with 200 mg/L Fe **(Figure 3.11.a).** The speific conductivity of the solutions shows the same trend; a decrease in values until the balance between the phases is established **(Figure 3.11.b).** The retention of Fe(III) ions on chitosan is strongly dependent on pH changes. If the pH is acidic, then chitosan is dissolved, while for pH higher than 4.5, the formation of a colloidal system was observed. In more acidic solutions, more protons will be available to protonate amino groups in chitosan to form -NH3+ protonic groups, reducing the number of bonds available for Fe(III) adsorption.

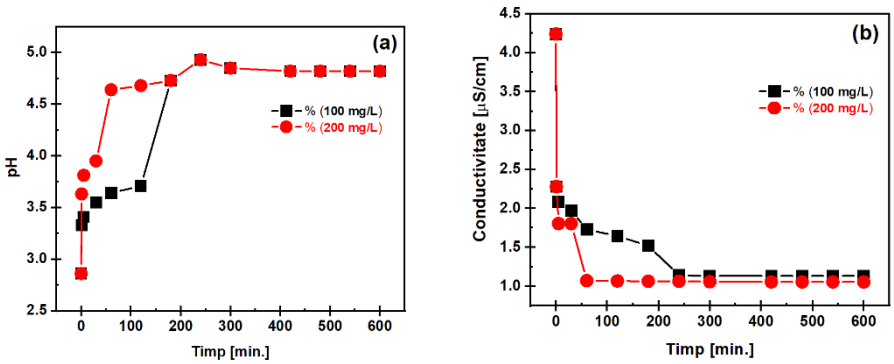


Figure 3.11. The effect of Fe (III) absorbed in chitosan as a function of the adsorption time following the pH variation (a) and the specific conductivity of the filtrates (b). Reaction conditions: 100 mg adsorbent, 50 mL 100 mg/L Fe (III) solution, 380 rpm, t= 22°C.

The optimum ferric ion adsorption of 19.87 ± 0.15 mg/L was obtained at pH between 3.0-3.2 Chitosan adsorption capacity after a contact time of 60 min. decreases when the pH is bigger than 3.2, the adsorption of iron ions is efficient, the total surface area of chitosan being occupied. Free amino groups are involved in the binding of Fe (III) ions on the surface of chitosan, but hydroxyl groups and carbonyl groups can also participate in the coordination process with amino groups.

B. Adsorption isotherm analysis

The optimal conditions of the equilibrium process were: 100 mg chitosan adsorbent, amount kept constant, Fe (III) ions between 25-200 mg/L, pH 3.20, contact time 600 min. The results are presented in **Figure 3.13** and **Figure 3.14**. The obtained results indicated that, at room temperature, the amount of iron (III) ions adsorbed on the chitosan molecule increases with the contact time, reaching an equilibrium between the components of approx. 5 hours.

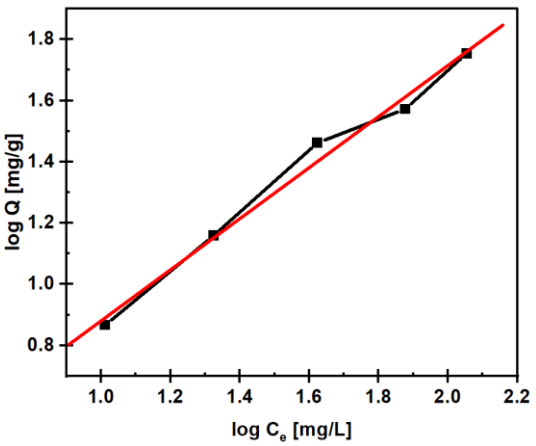


Figure 3.13. Adsorption isotherm of Fe (III) ions on chitosan

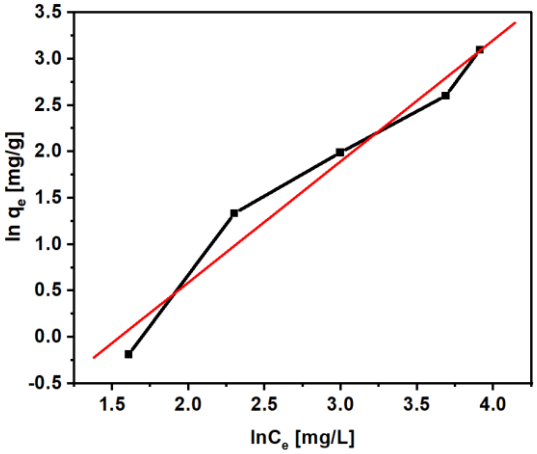


Figure 3.14. Freundlich adsorption isotherm

From the Langmuir isotherm, the equilibrium data from the last value of the adsorption capacity on chitosan indicates 82.30 mg Fe/g chitosan[Ali M.A. et al., 2021], the absorbing mechanism being due to ionic interactions and complexation with chitosan functional groups. The adsorption capacity for Fe (III) is higher compared to the value obtained for other ions and other absorbents [Ali M.A. et al., 2021]. Equilibrium adsorption

isotherms for iron ions indicated after 60 min. over 82 % Fe (III) (mg Fe /g adsorbent) retention capacity. The sorption process is fitted to Freundlich and Langmuir isotherms, but the equilibrium isotherm was more appropriate with the Langmuir model. [Burke A. et al., 2000, Lapo B. et al., 2018].

Chitosan flakes are microporous biopolymers, large enough to allow the absorption of Fe(III) ions. SEM analysis provides an indication that the mechanism of Fe (III) ion absorption on chitosan is a complex phenomenon and involves the formation of nodules on the chitosan structure by the adsorption of metal ions **(Figure 3.16).**

SEM images provide evidence that the sorption mechanism of Fe(III) ions on chitosan involves the formation of nodules on the chitosan structure through strong coordination with chitosan functional groups (amine and hydroxyl), being an effective adsorbent for the retention of heavy metal ions useful for medicine and environment.

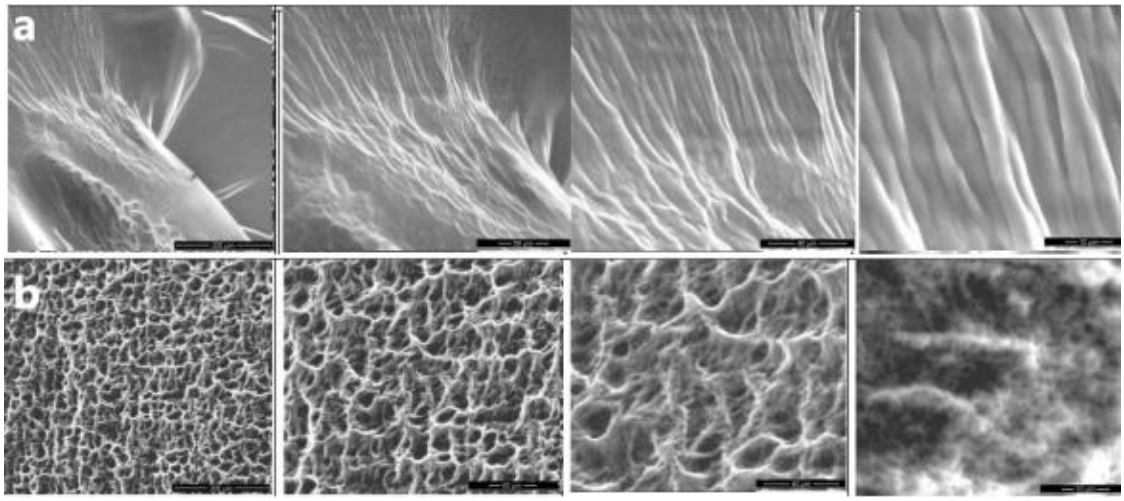


Figure 3.16. SEM images of modified chitosan absotion of Fe (III) ions: 200 mg adsorbent with 1 mg/L Fe (III) (a) and 500 mg adsorbent with 50 mg/L Fe (III) (b).

4. RESEARCH ON THE FUNCTIONALIZATION OF CHITOSAN WITH N-HETEROCYCLIC COMPOUNDS, IN AQUEOUS SOLUTION AND INFLUENCE FACTORS

This chapter presents the synthesis in aqueous medium to obtain new chitosan derivatives, using quaternary dipyridylium salts. The results for the chitosan derivative, using the *N,N'-diphenacyl-4,4'-dipyridylium dibromide* **(CHS)** are published in the Journal of Physics: Conference Series; Bristol 2021, Vol. 1960, No. 1 (DOI:10.1088/1742-6596/1960/1/012001) and confirms that the functionalization of chitosan is influenced by several factors.

Chitosan samples, from different sources, show dissociation in aqueous medium as a result of a slightly basic pH, which indicates limited solubilization for chitosan, both chitosan from commercial samples and chitosan from pharmaceutical samples.

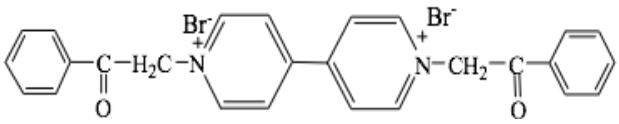
4.4. Functionalization of chitosan in aqueous medium with N,N'-diphenacyl-4,4'-dipyridylium dibromide

Chemical modifications of chitosan are necessary to improve its low solubility in aqueous solutions and to obtain new materials with distinct properties by using N-heterocyclic compounds being a solution of interest for research for this purpose [El-Naggar M.M. et al., 2020; Kritchenkov A.S. et al., 2020]. After having confirmed the formation of new derivative between *N,N'-diphenacyl-4,4'-dipyridylium dibromide* salt and chitosan biopolymer (chapter 4.3 - structural analysis by FTIR spectra [**Costea (Nour) I.F. et al., 2022**] was

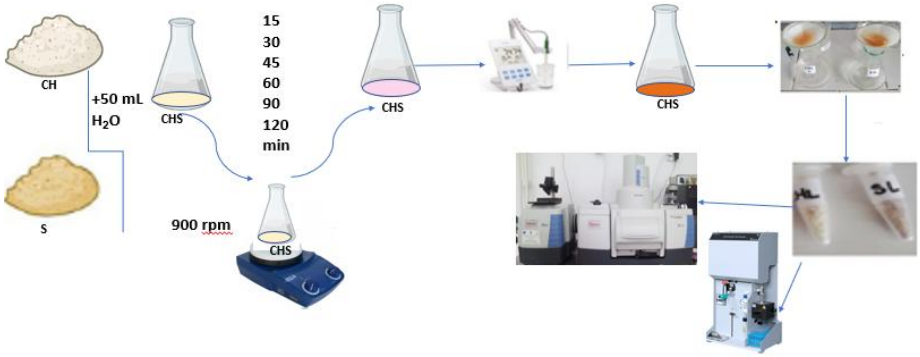
experimented new chitosan derivatives, following the influence of several factors in the synthesis process.

4.4.1. Materials and syntesis stages

Chitosan powder **(CH),** $C1_2H_{24}N_2O_9$, deacetylated poly(D-glucosamine) chitin (M=340 g·mol-1) purchased from Sigma-Aldrich and the N-heterocyclic salt, N,N'-diphenacyl dibromide were used - 4,4'-dipyridylium ($C_{28}H_{26}Br_2N_2O_2$,; M = 554 g·mol-1) (**S**), synthesized and purified in the organic chemistry laboratory according to the reference [Dinica R.M.,2007;Furdui B. et al., 2016], with the molecular structure:



The working stages in the functionalization of chitosan with the quaternary dipyridyl salt are schematically presented in **Scheme 4.2** and Images from the stages of the synthesis process **(Figure 4.9).**



Scheme 4.2. Experimental work for the functionalization of chitosan (**CH**) with salt, N,N'-diphenacyl-4,4'-dipyridylium dibromide (**S**), the chitosan:salt mass ratio being 20:1

Figure 4.9. Images of synthesis process for the chitosan derivative **CHS**

Some parameters have been changed, such as the contact time between the compounds, the synthesis temperature, and also the mass ratio of chitosan:dipyridyl quaternary salt was changed [**Costea(Nour) I.F. et al., 2022**].

The working parameters tested for three different syntheses, in order to obtain new chitosan derivatives, are presented in **table 4.1**.

Tabelul 4.1. Experimental conditions in the synthesis of chitosan derivatives **CHS**, with N,N'-diphenacyl-4,4'-dipyridylium dibromide (**S**), synthesis variant (a) - (c)

Parameters	Syntesis (**a**)		Syntesis (**b**)		Syntesis (**c**)
	Contacting time (min.)		T (°C)		
CH - chitosan (g)	0.20	15	0.20	20	0.20
S - dipyridylium (g)	0.01	30	0.01	30	0.01; 0.02
stirring speed (rpm)	900	45	900	40	900
		90	120 min. contacting time	60	90 min. contacting time
		120		70	
		22°C		80	70°C

4.4.4. Factors of influence in the process of synthesis of derivatives of CHS

4.4.4.1. Influence of contact time

The dried samples of **CHS** chitosan derivatives indicated yield that varies depending on the contact time in the synthesis process, between 67 – 77 % **(Figure 4.10.a).** The highest yield of 77% was registred after 90 min. of contact time between compunds and the lowest yield of 67% was registred after 120 min.

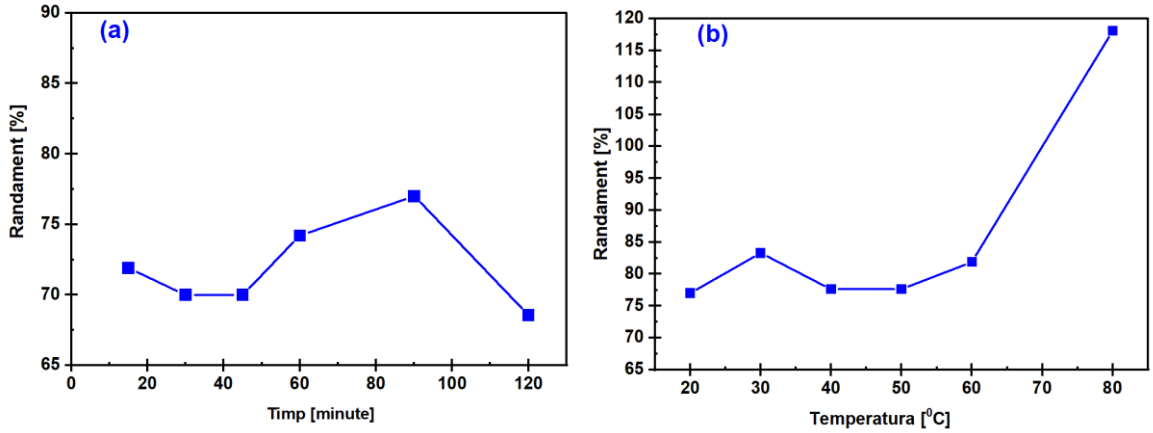


Figure 4.10. Evolution of the synthesis yield (η%) of **CHS** chitosan derivatives: (a) - synthesis at t =22°C, with different contact time, between 10 min. and 120 min.; (b) - synthesis with modified temperature, between 20°C and 80°C, contact time of 120 min.

The synthesis for the **CHS** derivative at room temperature, for the filtered solutions, indicated a pH between 6.0 - 6.5 **(Figure 4.11.a).** For variable contact time (15 - 120 min.), a slight increase in pH was registred, from 6.56 ± 0.02 (at 15 min.) to 6.72 ± 0.06 at 120 min. After 72 hours it was observed that all the filtered solutions showed a slight decrease in pH, between 0.10 ÷ 0.5 pH units, as a result of the balance between the functional groups.

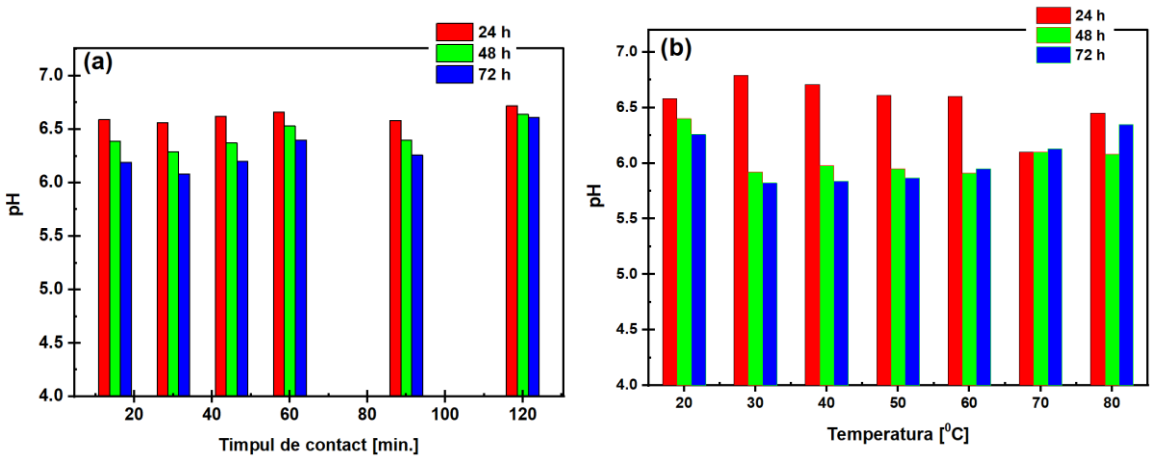


Figure 4.11. Evolution of pH in filtrates for the synthesis of chitosan derivatives CHS: (a) synthesis at t=22°C with contact time between 10 min. and 120 min.; (b) - synthesis with modified temperature, between 20°C and 80°C, contact time of 120 min.

The new functionalized chitosan compounds showed a decrease in pH(acidic Ph), in aqueous solution, after addition of dipyridyl salt, which suggests the achievement of favorable structural organizations between the polymeric chitosan and the salt; the pH values remain between 6.3±0.2. The electrical conductivity of the solutions, for the filtrates resulting from **CHS** derivatives, showed values between 329 ±3 μS/cm duet o the increasing of the ionic charge in the solutions in the presence of bipridylic quaternary salt compared to chitosan without added salt, which showed values between 270 ±2 μS/cm.

4.4.4.2. Influence of temperature

Keeping the contact time constant, the temperature of the synthesis process was changed. For the **CHS** chitosan derivative samples for a contact time of 120 min., when the temperature was changed, between 20°C ÷ 80°C, an increase in yield was obtained, η > 76% **(Figure 4.10.b).** For the temperature of 20, 40, 50°C, the efficiency is maintained in the same values. The optimal temperature suggested for the synthesis process in obtaining the **CHS** derivative is between 50÷70°C, but structural changes occur as otherwise in any change of parameters in the synthesis process. By adding N-heterocyclic salt, the synthesis yield for **CHS** chitosan derivatives shows a moderate increase, depending on the change in contact time between the compounds and by changing the temperature in the synthesis process, η=70% for dry samples, at room temperature **(Figure 4.10.b).**

4.4.4.3. The mass ratio of dipyridylium salt

Samples of chitosan-quaternary dipyridylium salt mixtures in distilled water, consisting of 0.2 g of chitosan with 0.01 g of salt (mass ratio between chitosan and salt being 20:1) and respectively consisting of 0.02 g of salt (ratio chitosan-salt being 10:1) were maintained at a temperature of 70°C, and a contact time of 90 min. It was observed that when the dipyridyl quaternary salt increases, salt remains in the solution, and the influence on the degree of chitosan substitution is reduced, due to the limited absorption efficiency in the chitosan chain.

The conductivity of the samples with salt (the chitosan-salt ratio being 10:1) indicates an increase of approximately 50%, which confirms that it is not an advantage to have a larger amount of salt, no stable bonds are made in chitosan structure.

4.4.5. Structural caracterisation of chitosan derivatives CHS

The solid samples were structurally characterized by X-ray diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR).

4.4.5.1. Spectrele de difracție cu raze X (XRD)

The X-ray diffraction (XRD) spectra registred for the functionalized chitosan derivative **CHS** and the chitosan sample **CH**, obtained under the conditions of synthesis c **(Table 4.1)** are shown in **Figure 4.14**. From the spectra, a certain change in the crystallinity of the **CHS** chitosan derivative is observed due to the adsorption reaction on the chitosan polymer structure, the short and flat peaks indicate that the samples have very low crystallinity and good adsorption factor. Regarding the crystallinity of the chitosan derivative **CHS** there are changes more obvious between the angle of 30 -40 Θ due to a high molecular mass and functional groups in the structure.

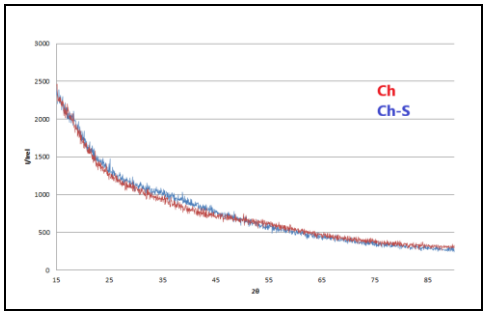


Figure 4.14. X-ray diffraction (XRD) spectra for the chitosan derivative CHS (blue) compared to chitosan (red)

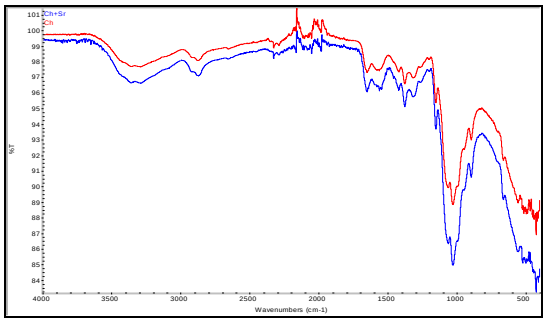


Figura 4.15. Comparative FTIR spectra for chitosan derivative CHS (blue) and chitosan CH (red), samples obtained under the same synthesis conditions (synthesis c - table 4.1), chitosan:salt mass ratio of 20:1.

The analysis carried out by XRD is, however, insufficient to determine the structure of the new product CHS, because it is necessary to use another, much more sensitive equipment that indicates diffraction spectra at an angle smaller than 10 Θ[**Costea (Nour) I.F. et al., 2022;**].

4.4.5.2. Structural evaluation by Fourier Transform Infrared Spectroscopy (FTIR)

Various other methods are used to characterize the new structures [De Masi A. et al., 2019; Ibrahim M.A. et al., 2023], but also FTIR, being useful to identify the chemical structure of the new chitosan derivatives obtained in **Figure 4.15.** The samples analyzed by FTIR for the newly synthesized CHS derivative showed characteristic bands identified in the spectra of chitosan but also other new bands, confirming new structures as well as the stability of the compounds. In the first zone, the vibration frequency corresponds to the hydroxyl -OH functional groups and the C-C bonds, from the chitosan structure, with a decrease in transmittance, to the CHS derivative. From the 3288 cm^{-1} position in the CH chitosan sample, displacements of 1 cm^{-1} occurred, up to a maximum of 4 cm^{-1} to the right for the **CHS** chitosan derivative **(Figure 4.15).**

The positions of the representative spectral parameters are presented in **Table 4.2**, associated with the main vibration characteristics of some types of bonds, from the

investigated structures that can be associated with some functional groups in the chitosan structure and to the new compounds of the synthesized derivatives.

Tabelul 4.2. *The position of the representative bands with the responsible vibrations in the chitosan derivative **CHS**, from FTIR spectra*

Sample	ν_{O-H}	ν_{C-C}	$\nu_{C=O}$	δ_{N-H}	δ_{C-H}	ν_{C-O}	ν_{C-O}
CH	3257	1879	1646	1580	1407	1162	1029
CHS	3882	1870	1647	1559	1374	1149	1024

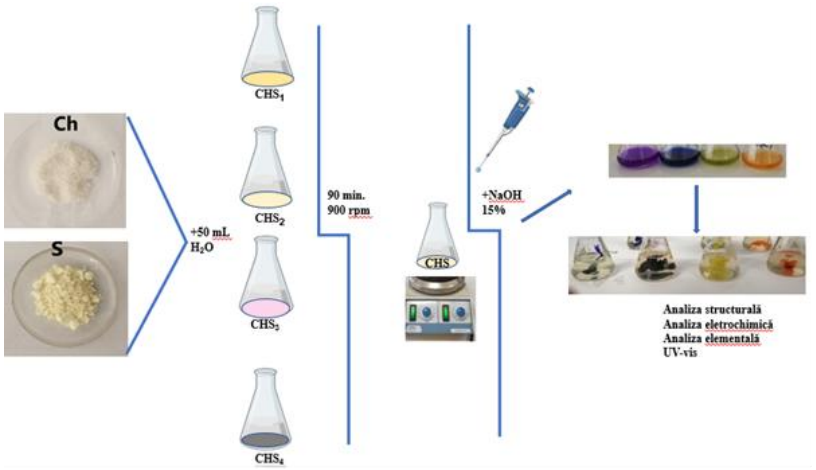
The band at 1559 cm-1, present in the chitosan derivative CHS is absent in the spectrum of the analyzed chitosan, being attributed in this case to the presence of the C-N bond in the structure of the heterocyclic cuaternary salt, but it can also be present in the FTIR spectrum of the chitosan samples, as proved for **CHc** and **CH_f** at 1557 cm^{-1} (chap. 4.3), was attributed to the amine structure. The position of the band at 1024 cm^{-1}, assigned to the C-O bond indicates a shift of -5 cm^{-1} (to the left) for the new structural complex of the chitosan derivative CHS, compared to the CH sample. The characteristic bands at 893 cm^{-1} and 422 cm^{-1} were similar in chitosan and the new chitosan derivatives, but the maximum intensity differed, being lower for the new compounds, which confirms the implication of N-heterocyclic salt bonds , by deforming the structures. Therefore, in the spectrum of the functionalized derivative chitosan **CHS**, a decrease in transmittance is observed, being a process of modification of the chitosan structure.

4.5. Functionalization of new chitosan derivatives with different dipyridyl salts in aqueous medium

There were used other N-heterocyclic salts, from the 4,4 dipyridyl class for new chitosan derivatives, to see if the structure of these organic compounds has influence in the alkylation process to chitosan, in the synthesis keeping constant temperature and contact time the same.

It was used for the syntesis **S1** *(N,N'-diphenacyl-4,4'- dipyridylium dibromide)* salt and also **S2** *(N,N'-bis(p-nitrophenacyl)-dibromide)*, **S3** *(N,N'-diphenacyl 1,2-bis(4-pyridyl) ethane dibromide)* and respectively, **S4** *(N,N' bis(p-nitrophenacyl)-1 dibromide ,2-bis(4 -pyridyl) ethane)*. The conditions of the process were established as being optimal ones to the results obtained with the derivative noted **CHS** (chapter 4.4). The mass ratio between chitosan and salt was 20:1. First,the solubilization for chitosan aws carried out, the samples being made in duplicate. The mixture in the aqueous solution is magnetically stirred (900 rpm) for 90 min., keeping a constant temperature of 70°C**.**

The samples were filtered (quantitative paper), but the process was difficult. Samples were dried in a desiccator for 48 h, weighed on an analytical balance and calculated the synthesis yield, the samples being then kept in covered Petri dishes until the elemental and structural analysis. The new functionalized chitosan derivatives are denoted **CHS1, CHS2, CHS3, CHS4 (Scheme 4.3)**



Schema 4.3. The syntesis of the new chitosan derivatives
CHS_1, CHS_2, CHS_3, CHS_4

4.5.4. Elemental composition of chitosan derivatives

As far as the total carbon content is concerned, C (%) it is observed that chitosan derivative sample marked **CHS_1** and **CHS_3** present the same value, but the chitosan sample marked **CHS_2** indicates an increase in C content of +0.73% comparing to the chitosan sample marked **CHS_4** which showed a decrease in C content by 0.71% (**Table 4.5).**

Table 4.5. *Analysis of the content of total nitrogen N (%) and total carbon C (%) at chitosan derivatives marked as* ***CHS1, CHS2, CHS3*** *and* ***CHS4***
4.

Chitosan Derivative	N (%)		C (%)	
	calculated	confirmed	calculated	confirmed
CH	8,00	7,44	42,35	59,80
CHS_1	9,56	5,99	43,06	60,09
CHS_2	8,03	6,36	42,63	60,82
CHS_3	10,17	10,72	43,08	60,08
CHS_4	7,97	5,85	42,71	59,37

Regarding the total nitrogen content, N (%), it is observed that the chitosan derivative marked **CHS_3** showed the highest value, of 10.72%, an increase in nitrogen content of +3.28% compared to the chitosan sample, without addition of heterocyclic N salt, and respectively an increase in nitrogen content of +4.73 % compared to the **CHS_1** deriv. An explanation would be that ethylene group in the structure of **S3** salt *(N,N' bis(phenacyl)-1,2-bis(4-pyridyl) ethane dibromide)* favors "wrapping", the interaction with the polymer chain of chitosan.

4.5.5. Structural Evaluation by Fourier Transform Infrared Spectroscopy (FTIR) în UV-Vis

The new synthesized chitosan derivatives can generate compositional networks with synergic effect, when they can be used as biomedical materials of great interest for future applications.

For the samples of chitosan derivatives and marked **CHS_1, CHS_2, CHS_3** and **CHS_4**, FTIR spectra were made as it is shown in **Figure 4.18**, where changes in the position of

some characteristic bands of chitosan derivatives are observed, with a reduction in transmittance, suggesting interactions in the polymer structure of chitosan.

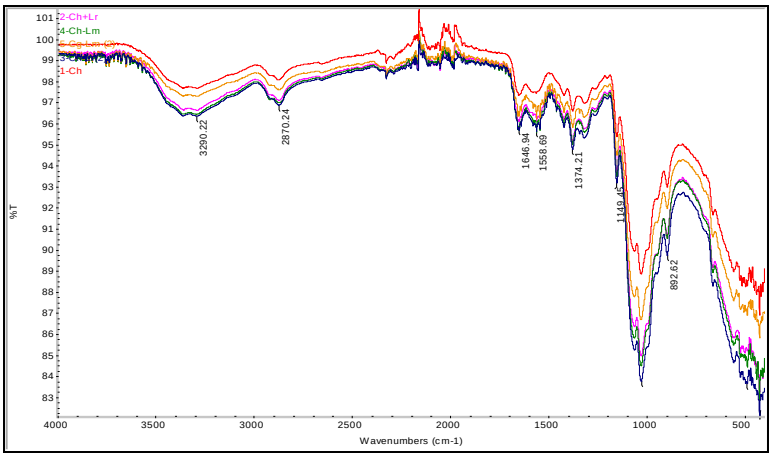


Figure 4.18. Comparative FTIR spectra for **CH** chitosan samples and chitosan derivatives **CHS_1**, **CHS_2**, **CHS_3** and **CHS_4**

The vibration frequency corresponding to reactive groups -OH, in the spectrum of chitosan is absent in the spectrum of salts, it is not present. The band appeared at 3288 cm-1 suggests the existence of hydrogen bond associations, which appears in aqueous solution in the structure of the synthesized chitosan derivatives and is due to the solvent effect. In the IR spectra of chitosan derivatives, the most intense bands appear at 1191 cm^{-1}, 1085 cm^{-1}, 1049 cm^{-1} and 1014 cm^{-1}. The FTIR spectral analysis highlights the fact that, for the same functional group in the spectral range 1400 – 1490 cm^{-1}, the characteristic bands are more numerous in the spectra of functionalized chitosan derivatives, as an effect of the interaction with dipyridyl quaternary salts. From the FTIR analysis, it is observed that there are characteristic bands at 1647, 1559, 1149 and 1374 cm^{-1}, similar to chitosan and the new chitosan derivatives, with small shifts between 1 cm^{-1} and max 4 cm^{-1} .

4.6. Conclusions

- ✓ Due to low solubility of chitosan, the synthesis strategy for new functionalized chitosan derivatives in aqueous solution focused on the appropriate choice of the chitosan source (high molecular weight) and on establishing some reaction parameters such as synthesis temperature or time contact between chemical compounds.
- ✓ The synthesis of functionalized chitosan in aqueous solution with *N,N'-diphenacyl-4,4'-dipyridyl dibromide* salt was carried out in the temperature range 20°C - 80°C, for a contact time of up to 120 min. (between 15 min. - 120 min.).
- ✓ It was observed that by adding N-heterocyclic salt, the synthesis yield for **CHS** chitosan derivatives shows a moderate increase, depending on the change in the contact period between the compounds and by changing the temperature in the synthesis process, yields >70% being obtained for dry samples.
- ✓ Although chitosan is not totally soluble in aqueous solution, in the presence of the N-heterocyclic salt used in the synthesis process, through mechanical agitation, some changes are observed in the polymer chain such as: changing of colour in the sample solutions, pH and conductivity variations of the filtrated solutions.

- ✓ Structural changes for **CHS** chitosan derivatives were confirmed by XRD analysis and FTIR spectra, in all samples, and also characterised by elemental composition (C% and N%).
- ✓ New functionalized derivatives are confirmed using other heterocyclic N salts (*N,N'-bis(p-nitrophenacyl)-4,4'- dipyridylium dibromide; N,N'diphenacyl-1 dibromide ,2-bis(4-pyridyl)ethane* and *N,N' bis(p-nitrophenacyl)-1,2-bis(4-pyridyl)ethane dibromide* respectively). These salts differ in structure by the presence of symmetrically positioned ethylene groups and the presence of nitro (NO_2) functional groups.
- ✓ The structural changes in the FTIR spectra of functionalized chitosan derivatives confirm variations in the position of the characteristic bands, compared to the spectrum of chitosan, without the addition of N-heterocyclic salt, obtained under similar synthesis conditions, and support the presence of quaternary salts in the new structures; the chitosan:salt ratio being 20:1, due to the high molecular weight of dipyridyl salts.
- ✓ Chitosan, this biopolymer, although insoluble in aqueous solution showed great solubility in the presence of the N-heterocyclic which confirmed a process of adsorption and interaction between the compound due to favorable positions of some functional groups.
- ✓ pH is an important parameter of the synthesis process, which cannot be neglected in obtaining new products, which will have distinct properties, solubilization and effective biological characteristics, or antibacterial potential, needed in biomedical applications.
- ✓ Investigation studies to find out the molecular mass for chitosan derivatives **CHS**, but also for chitosan derivatives functionalized with dipyridyl quaternary salts marked as **CHS_1, CHS_2, CHS_3** and **CHS_4** are of interest in obtaining new products, in order to follow their solubility in the aqueous environment, for the physiological pH, and to improve the antimicrobial activity of chitosan, a versatile material with multiple applications.
- ✓ The study of the mechanisms that involves obtaining new compounds, in aqueous solution with dipyridyl salts is of wide interest, the nature/type of chitosan samples being important, the molecular mass and degree of deacetylation of chitosan have an important role in the synthesis process.

5. RESEARCH ON THE FUNCTIONALIZATION OF CHITOSAN WITH N-HETEROCYCLIC SALTS IN ACID MEDIUM

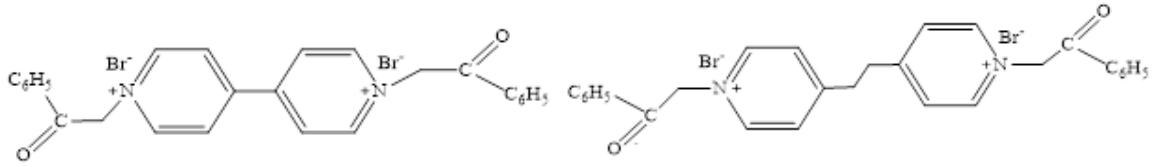
The present study describes the synthesis of new chitosan derivatives with *N,N'-diphenacyl-4,4'-dipyridylium dibromide* and *N,N'-diphenacyl-1,2-bis(4-pyridyl)ethane dibromide*, in acetic acid, for an effective solubilization and to improve the antibacterial activity of the products. The results of the study are published in Carbohydrate Research, **2023**, doi.org/10.1016/j.carres.2023.108964. The functionalization of chitosan is confirmed as being more efficient in the acetic medium and the influence of some factors is important in order to improve the properties of the new derivatives, especially the antibacterial ones. The research also expanded with other dipyridine salts from the same class.

5.3.1. Materials and synthesis of quaternary salts

The same type of chitosan was used for the synthesis of new chitosan derivatives as in aqueous medium (chapter 4; (C12H24N2O9), M of 340 g·mol-1, DD >75%). For the synthesis of new chitosan derivatives, the same salts used in chapter 4 were used:,

quaternary dipyridyl salts, *N,N'-diphenacyl-4,4'-dipyridylium dibromide* (**Sr**) and *N,N'-diphenacyl-1,2-bis(4-pyridyl)ethane dibromide* (**Sm**).The synthesis, purification and characterization of dipyridyl salts was carried out in the organic chemistry laboratory of the Faculty of Sciences and the Environment, from the Dunărea de Jos University of Galati, using all methods, as reported in previous studies **(Figure 5.2)** [Furdui B. et al., 2012].

The structural characteristics obtained are comparable to those obtained in the references [Cârâc A. et al., 2018]. The difference between the two dipyridyl salts is the ethylene group in the *N,N' diphenacyl-1,2-bis(4-pyridyl)ethan dibromide* compared to *N,N'-diphenacyl-4,4'-dipyridylium dibromide*, where this ethylene group is missing. In order to differentiate them structurally, we sometimes chose to simplify their notation in the text with the notation **Sr** - salt with a rigid bridge, respectively with **Sm** - salt with a movable bridge.



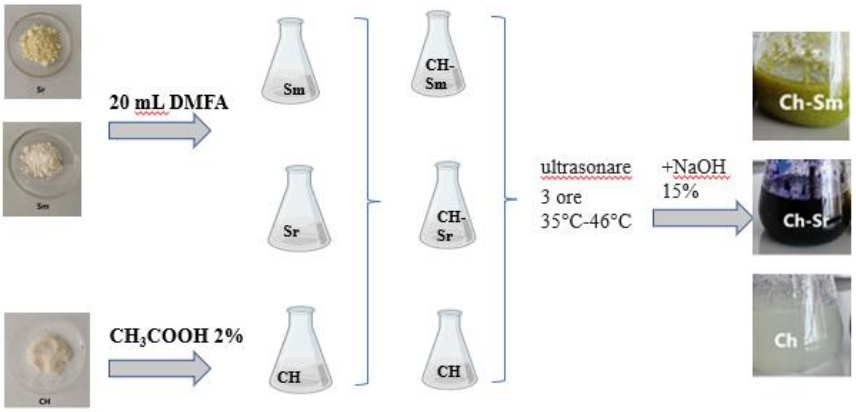
N,N'-diphenacyl-4,4'-dipyridylium dibromide (**Sr**) (M = 554 g·mol^{-1})

N,N'-diphenacyl-1,2-bis(4-pyridyl)ethane dibromide (**Sm**) (M =582 g·mol^{-1})

Figure 5.2. Structures of the quaternary dipyridyl salts used

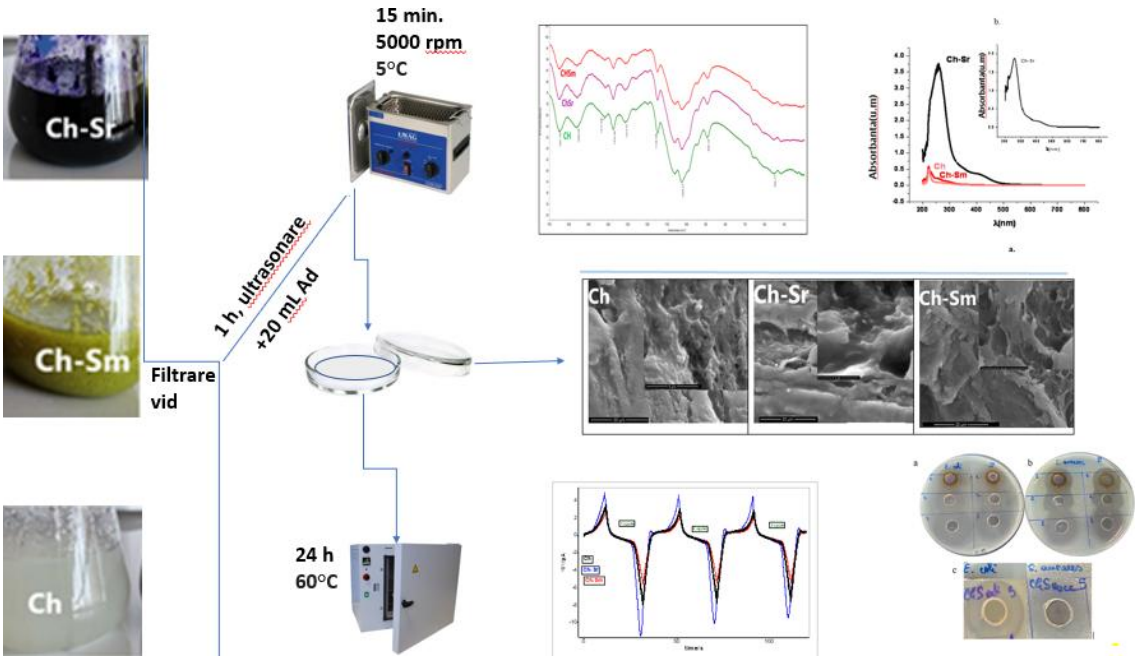
5.3.2. Experimental procedure for the synthesis of new chitosan derivatives

Initially, the N-herocyclic salts of interest, *N,N'-diphenacyl-4,4'-dipyridylium **(Sr)** dibromide* and *N,N'-diphenacyl-1,2-bis(4-pyridyl)ethane dibromide* **(Sm)** were syntetised (chapter 4.2.1). These purified salts were used in the experimental procedure of chitosan functionalization **(Scheme 5.1).**



Scheme 5.1. Obtaining of functionalized chitosan with dipyridyl quaternary salts

The synthesis of new functionalized chitosan derivatives using dipyridyl salts is shown in **Scheme 5.2.** 0.2 g of each bipyridine salt was weighed after being dissolved in 20 mL of DMFA (N,N-dimethylformamide). Samples were prepared from chitosan (2 g) dissolved in 200 mL of 2% acetic acid. Three sets of salt and chitosan mixture samples were prepared, in the chitosan:salt mass ratio of 10:1 using 0.2 g of each salt.



Scheme 5.2. Representation of the synthesis steps and characterization of new functionalized chitosan derivatives, **CHSr** and **CHSm**

Samples of chitosan and quaternary dipyridyl salt were kept in an ultrasonic bath for 180 min., at room temperature (35°C - 46°C - temperature recorded in the solution). At the end of the experiments, a few mL of 15% NaOH were added and specific colors were observed for the resulting hydrogels, depending on the chemical structure of the synthesized derivatives. The samples with the chitosan derivative functionalized with *N,N'-diphenacyl-4,4'-dipyridylium dibromide* **(Sr)** marked as **CHSr**, showed an intense purple color compared to the chitosan derivative with *N,N'-diphenacyl-1,2-bis(4-pyridyl)ethane dibromide* **(Sm)** marked as **CHSm** which showed a dark yellow colour. The chitosan sample without bipyridine salt remains silvery white. An explanation of the colors is the property of quaternary dipyridyl salts which can form ylide compounds when treated with strong bases [El-Gharably A.A. et al., 2022; Nadagouda M.N. et al., 2022].

The obtained samples of the new compounds were filtered under a vacuum filtration kit. 400 mL of distilled water was added to the obtained filtrates and the mixture was maintained to ultrasound for one hour, then to centrifugation for 15 min. at 5000 rpm, at 5°C. Finally, the resulting sediment was placed in Petri dishes and dried in an oven at 60°C for 24 hours.

Therefore, chitosan samples and functionalized chitosan derivatives were synthesized in 2% acetic medium and dried at 60°C. The synthesis yield for the functionalized chitosan samples was > 65 % (65.30 ±0.22, for all three sets of samples).

After drying, the physico-chemical and structural characterization, morphological of the samples were analyzed using equipment in **Figure 5.3** .The antibacterial potential was analysed on the same experimental conditions. All samples prepared for were kept in an ultrasonic bath for dissolution and homogenization, at least 60 min. At the end, the pH (3.5±0.1) and specific electrical conductivity (0.5 mS/cm) were measured for all the obtained solutions (using a Consort C862 pH meter).

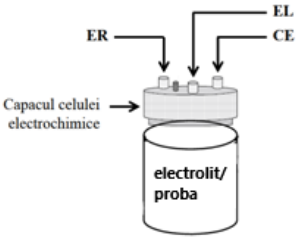
The UV-VIS T90+ spectrophotometer
1 cm quartz tubs

Primacs SERIES - Carbon/Nitrogen Analyzer

FTIR, Nicolet iS50 (Thermo Scientific)

SEM Quanta 200

Potentiostat galvanostat Biologic SP 150



Electrochemical cell

pH-multiparameter, Consort C862

Ubbelode Viscometer

Figure 5.3. Apparatus used for characterization of functional chitosan derivatives

5.3.6. Spectroelectrochemical characterization by UV-Vis

Samples of functionalized chitosan derivatives (**CHSr** and **CHSm**) were characterized in the UV-Vis in contrast with samples containing salt-free chitosan, although chitosan itself is transparent and difficult to structurally characterize by spectroscopic methods [**Costea (Nour) I.F. et al., 2022**; Furdui B. et al., 2014]. The spectra recorded for the samples of chitosan derivatives 1% ($m_{derivative}/V_{acetic\ acid\ 1\%}$) showed absorbance at λmax of 225 nm in contrast with chitosan which showed a maximum value of 0.49 a.u. and the derivative marked as **CHSm** the value of 0.59 a.u**. (Figure 5.4).**

In contrast, the spectra of the functionalized derivative marked as **CHSr** showed a much higher absorbance, about eight times more intense compared to the derivative marked **CHSm** and the chitosan sample, respectively **(Figure 5.4.a).** The sample for the derivative marked as **CHSr** showed a high intensity peak at λmax of 260 nm with absorbance of 1.9

a.u. in samples diluted in acetic acid **(Figure 5.4.b).** The recorded spectra confirm that the structure of chitosan showed changes, its structure was functionalized and the properties are expected to be different from those of non-functionalized chitosan.

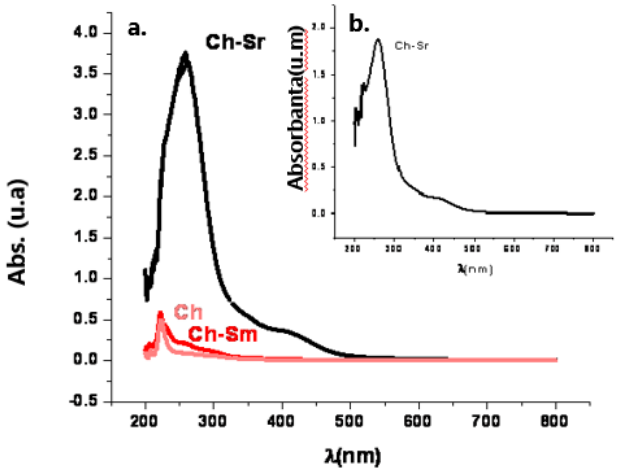


Figure 5.4. UV-VIS spectra registred for functionalized chitosan derivatives marked **CHSr** and **CHSm**, samples dissolved in 1% acetic acid

To obtain chitosan derivatives, pH plays a very important role in their synthesis. At lower pH the amino group (-NH2) is protected. In the case of the derivative marked **CHSr**, functionalization with the quaternary dipyridylium salt shows no pH changes in solutions. (3.50±0.03) [Botezatu A. et al., 2023]. Spectra were also registred in DMSO, the analysis being useful to explore the biocompatibility properties of new derivatives for the study human cells [Carac A. et al., 2018].

The spectra obtained in DMSO (aprotic solution) also confirm a higher absorbance for the chitosan derivative **CHSr** compared to the chitosan derivative **CHSm** and respectively chitosan without added salt **(Figure 5.5),** but the absorbance has a lower intensity compared to the spectra recorded in the acetic acid medium **(Figure 5.4).** This result is a confirmation of the presence of ion pairs in the structure of **CHSr** chitosan derivatives, structural arrangements more favorable in the acetic environment than in DMSO (an apropic solvent).

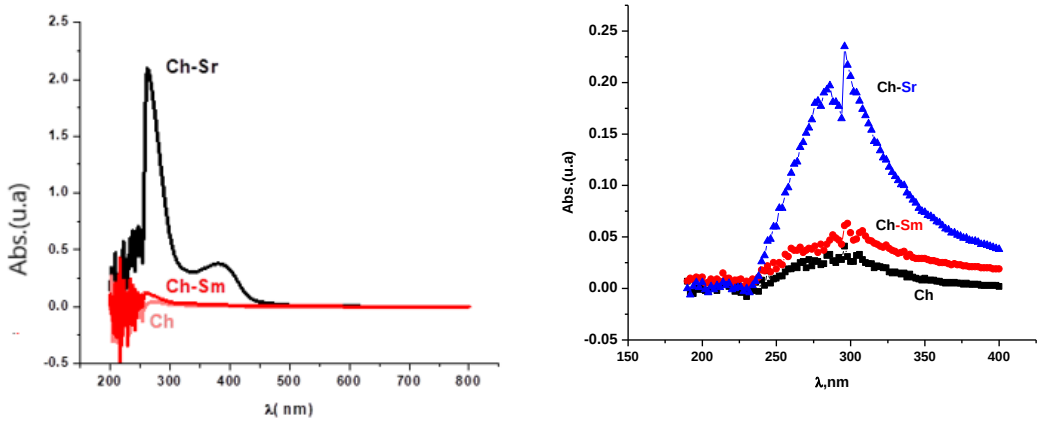


Figure 5.5. UV-VIS spectra of functionalized chitosan derivatives, marked **CHSr** and **CHSm**, samples dissolved in DMSO

5.3.7.Structural characterization by FTIR

The functionalization of chitosan (high mass HMW) with the two dipyridyl quaternary heterocyclic N-salts and obtaining new chitosan derivatives is also confirmed by Fourier

transform infrared (FTIR) spectroscopy **(Figure 5.6).** FTIR analysis is useful for the characterization of biopolymers, as it provides information about the molecular structure of chemical compounds [Sánchez-Cid P. et al., 2022; Wei H. et al., 2022]

The infrared spectra of the new functionalized derivatives showed characteristic bands for chitosan functional groups, glycosamide units and respectively for functional groups from the chemical structure of N-heterocyclic salts **(Figure 5.6).**

In the spectrum of pure chitosan, bands specific to its chemical structure could be identifie in the region 3320 - 3292 cm^{-1} which is a vibration specific to O-H stretching and N-H stretching. Also appearing in the spectrum is the overlap of the stretching of the O-H group in the carbohydrate ring, at 2875 cm^{-1} specific to the C–H stretching, at 1650-1655 cm^{-1}, the vibrational band specific to amide I and also the deformation vibrations of the amine, at 1558 cm^{-1}, bending vibrational bands, N-H from amine and amide II, at 1375 cm^{-1}, a band specific to CH3 symmetric deformation and at 1026 cm^{-1}, a band specific to C-O stretching skeletal vibration [Wei H. et al., 2022]. In the spectrum of the functionalized chiosan derivative denoted CHSr, the absorption band observed at 2934 cm^{-1} is specific to the C–H stretch, at 3292 cm^{-1} is specific to the O–H stretch, at 1655 cm^{-1} to N–H and the stretch from the functional group –CONH–, and the bands at 1201 cm−1 and 1313 cm^{-1} are specific to CH3 symmetric deformation and at 895 cm^{-1}, C–H out-of-plane bending vibrations, respectively. The presence of the aromatic ring is confirmed by several positions of the bands, which are more intense in the regions 1539-1560 cm^{-1} and 834-897 cm^{-1}.

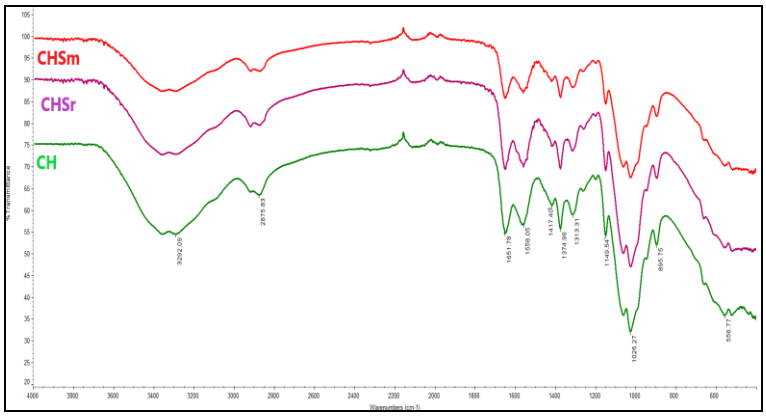


Figure 5.6. FTIR spectra of functionalized chitosan derivatives (**CHSr** and **CHSm**)

5.3.8. Determination of molecular mass by viscometric measurements

The rheological characterization of chitosan structures and derivatives is important in order to characterize the hydrodynamic properties of polymers and to determine the average molecular mass [Kandile N.G. et al., 2015].

The average molecular mass (MM) of the samples of functionalized chitosan derivatives (**CHSr** and **CHSm**), compared to chitosan, was analyzed from dry samples of the obtained derivatives, the chitosan:salt mass ratio being 10:1 (chapter 5.3.3), from series of five diluted solutions, dissolved in 1% acetic medium, samples kept in the ultrasonic bath, for one hour, for homogenization.

For the sample solutions, the kinematic viscosity and the intrinsic viscosity were calculated, and was represented by graphical representation **(Figure 5.8)**, using the equation $[\eta]=k\cdot M\cdot a$ (M is the average viscosity of the molecular weight, [η] the viscosity intrinsic, k (1.424 10^{-3} cm^3/g) and a (0.96) values, constants), the average molecular mass is obtained from the data presented. For the functionalized chitosan derivative **CHSr** a value of 692

g/mol (Da) and the chitosan derivative **CHSm** of 670 g/mol (Da) are obtained, compared to a value of 657 g/mol (Da) for chitosan (**CH**).

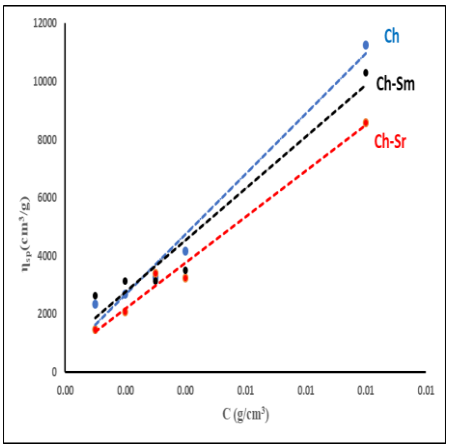


Figure 5.8. Intrinsic viscosity of functionalized chitosan derivatives denoted **CHSr** and **CHSm** compared to chitosan **(CH)**

Changes of the average molecular mass of chitosan derivatives are dependent on the structure of the N-heterocyclic salt used in the functionalization of chitosan, the values being higher when using a quaternary salt (**Sr**), compared to the salt denoted (**Sm)**, than for chitosan obtained without salt, under the same synthesis conditions.

5.3.9. Electrochemical evaluation of antioxidant activity by open circuit potential (OCP) and cyclic voltammetry (CV)

The antioxidant activity of the synthesized chitosan derivatives was evaluated by the electrochemical study, by recording results of the open circuit potential (OCP) and by cyclic voltammetry (CV) recording voltammograms, in the aprotic solvent DMSO, without the use of electrolyte support in the samples, experiments carried out for three sets of samples. The electrochemical evaluation of the working electrode (WE), the carbon electrode, was studied in the classical three-electrode electrochemical cell **(Figure 5.3),** where the applied potential range was between ±1 V/$E_{Ag/AgClsat.}$ The reduction and oxidation processes in the samples are fast enough that the equilibrium conditions are maintained on the surface of the working electrode (carbon).

For OCP measurements, for the chitosan derivative denoted **CHSr**, there are positive values and slight increase over time (from 0.04 V to 0.1 V/$E_{Ag/AgClsat}$), and the chitosan derivative denoted **CHSm** more positive potential values (0.2 ±0.02 V /$E_{Ag/AgClsat}$) compared to chitosan samples, marked **CH** (0.18 ± 0.01 V /$E_{Ag/AgClsat}$) **(Figure 5.9)**. It is expected that the samples of the chitosan derivative denoted **CHSr** exhibit redox exchange capacity, the results indicating the presence of several electroactive species in the samples. Through the functionalized structure of chitosan and the antioxidant activity of biopolymers can be improved.

Through cyclic voltammetry (CV) it is possible to obtain information on the mechanism of the electrochemical reaction, the identification of the species present in the solution, the determination of the diffusion coefficients of the electroactive species. The simplest possible mechanism is oxidation or reduction with the transfer of an electron between the species in the solution and the working electrode (the reversible reaction (Ox+e-↔Red) [Da Silva S.B. et al., 2018; Sato K. et al., 2018].

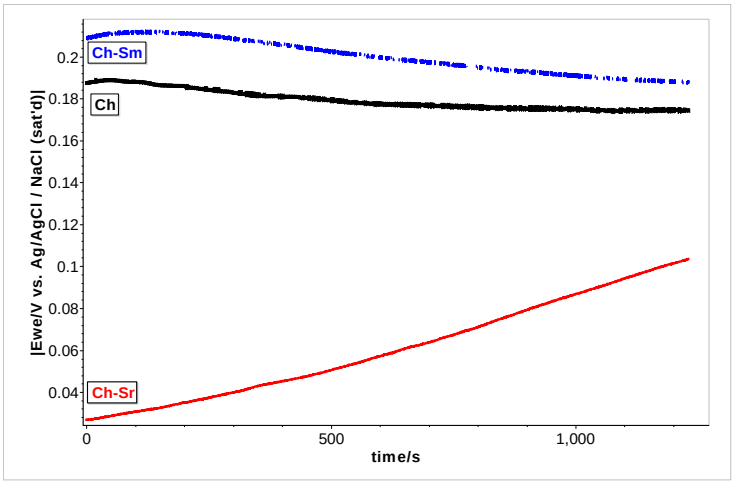


Figura 5.9. Evolution of Open Circuit Potential (OCP) values recorded for functionalized chitosan derivatives **CHSr** and **CHSm**, **CH** up to 20 min.

For the functionalized chitosan derivative, experiments were carried out at three different scanning speeds, at 20, 50 and 100 mV s-1, for 3 recording cycles, for the applied potential between ± 1 V/$E_{Ag/AgClsat}$ (**Figure 5.10; Figure 5.11**). Cyclic voltammograms recorded on sample solutions in DMSO indicated an increase in the anodic current (Ipa) when the scan speed increases. For the scanning speed of 100 mV s-1, for the chitosan derivative **CHSr** a maximum value of up to 4.4 μA is obtained for the anodic current Ipa, at the reversal potential. In the functionalized chitosan derivative, denoted **CHSm**, the anodic current value is lower, Ipa of about 3.2 μA, and for chitosan without salt it is Ipa of 3.8 μA (**Figure 5.10**).

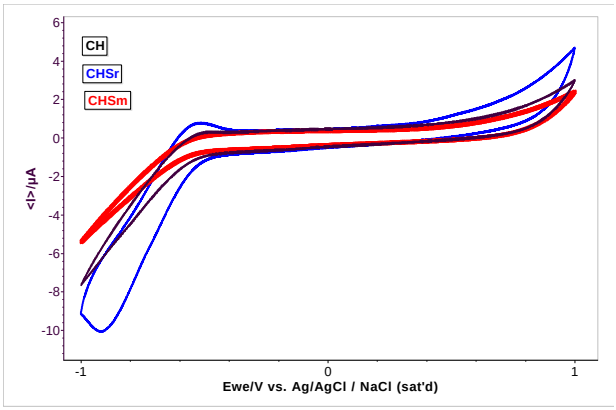


Figure 5.10. Cyclic voltammograms recorded for functionalized chitosan derivatives (**CHSr** and **CHSm**) in DMSO, $E_{Ag/AgClsat}$± 0.1 V, scan rate of 100 m

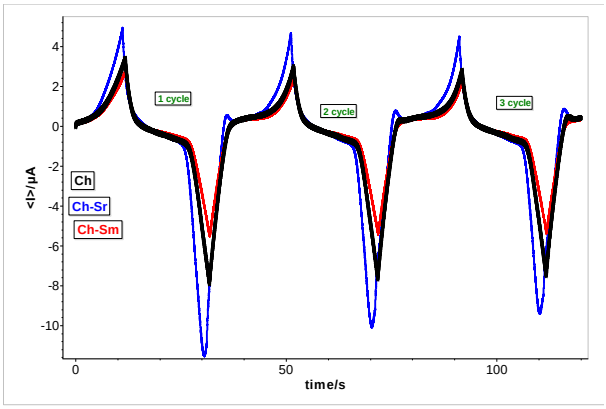
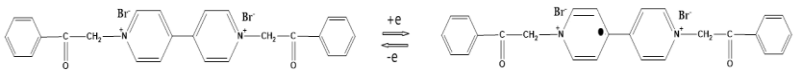


Figure 5.11. Voltammograms recorded for functionalized chitosan derivatives (**CHSr** and **CHSm**) in DMSO, $E_{Ag/AgClsat}$± 0.1 V, scan rate of 100 mV·s-1, three cycles

Tabel 5.3. *Ev Evolution of the anodic current intensity (Ipa) in chitosan and functionalized chitosan derivatives, upon changing the scanning speed and different recording cycles*

Scan speed ($mV \cdot s^{-1}$)	**CH**			**CHSr**			**CHSm**		
	$-Ip_a$ (μA)								
	1st	2nd	3th	1st	2nd	3th	1st	2nd	3th
100	7.87	7.59	7.40	11.54/ 10.89	10.07/ 9.20	9.36/ 8.46	5.49	5.42	5.39
50	8.66	7.85/ 7.20	7.62/ 6.93	8.47/ 6.94	7.77/ 6.40	7.57/ 6.28	6.09	5.88	5.76
20	5.55	5.06/ 4.99	4.71/ 4.55	5.84/ 4.70	5.49/ 4.37	5.48/ 4.40	5.03	4.87	4.81

An electrochemical reaction accompanied by changes in the structure of the heterocyclic core for the quaternary bipyridyl salt, denoted Sr/2Br− in its oxidized state (left) and reduced state (right) is shown below:



Sr^{2+} (oxidată) $\rightleftharpoons$ $Sr^{+\cdot}$(redusă)\ $Sr^{ox} \leftrightarrow Sr^{red}$

For one electron, for an irreversible reaction, the peaks of the cathodic and anodic current peaks are not equal, and where the oxidation is very slow, no cathodic electron peak is observed in the functional groups on the chitosan structure [Qin Y. et al., 2020].

By participating in the reaction with the delocalization of the anionic charge, in chitosan derivatives, the quaternary bipyridine salt thus contributes to the electrochemical reactions through the electroactive functional groups. After the CV experiments were completed, UV-Vis spectra were recorded on the samples, in DMSO, which indicated changes in the profile compared to the initially recorded spectra, and an increase in absorbance was generally observed **(Figure 5.13).** In the case of the functionalized derivative denoted **CHSr**, the absorbance increase is more obvious from λ of 255 nm to 300 nm, while for the derivative marked as **CHSm** it is a bit representative around λ of 260 nm, with a profile similar to that of the chitosan sample, without addition of salt.

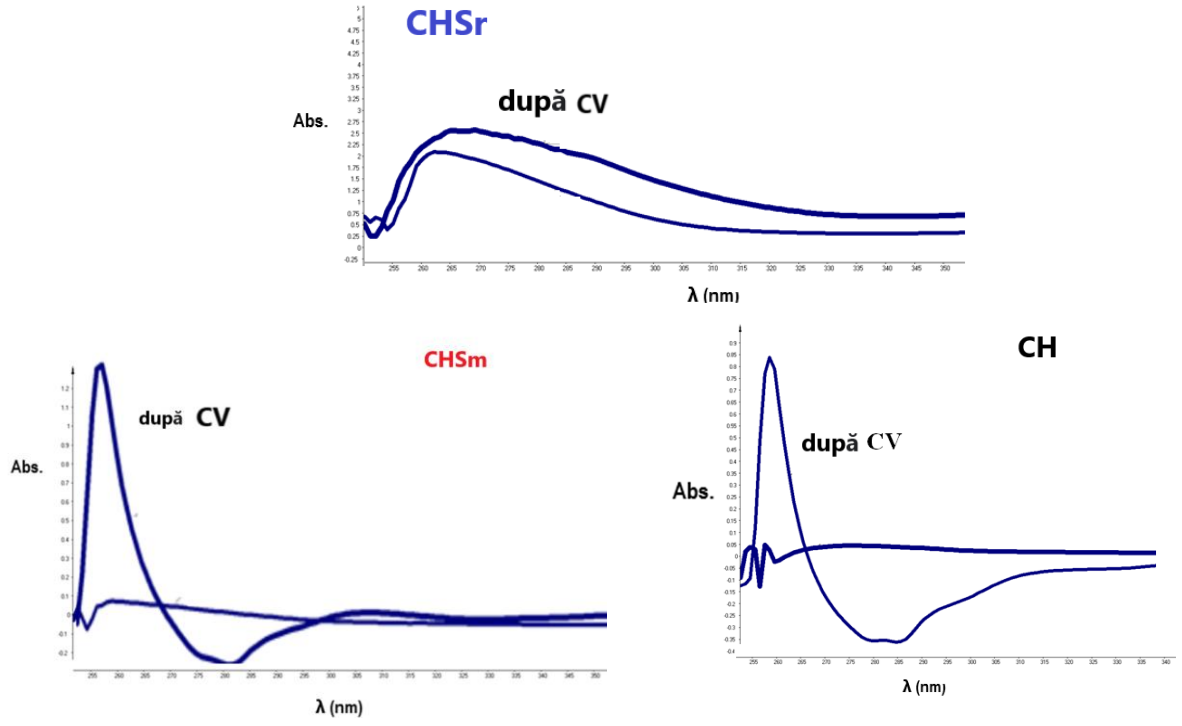


Figure 5.13. UV-Vis spectra of functionalized chitosan derivatives (**CHSr** and **CHSm**), samples dissolved in DMSO, before and after cyclic voltammetry experiments

Therefore, cyclic voltammetry (CV) is a useful tool to characterize the antioxidant activity of chitosan derivatives, because it provides useful information in the spectral, morphological properties regarding the electronic exchange between functional groups, from the polymer structure of functionalized chitosan derivatives.

5.3.11. Structural Morphological Analysis by Scanning Electron Microscopy (SEM)

The morphological study of the surfaces of chitosan derivatives functionalized with N-heterocyclic salts was carried out using images from scanning electron micrographs (SEM) (**Figure 5.15**). SEM images of the functionalized chitosan derivatives, **CHSr** and **CHSm**, showed a polyphase microporous structure. The pore sizes are uneven, thin-walled, with short and numerous microvilli. An aspect of bifunctional structure can be asserted for the derivative denoted **CHSr**.

The compositional analysis by EDX (Energy-dispersive X-ray spectroscopy), carried out on the SEM images, revealed the presence of the essential chemical elements carbon (**C**), oxygen (**O**) and nitrogen (N) **(Figure 5.16).**

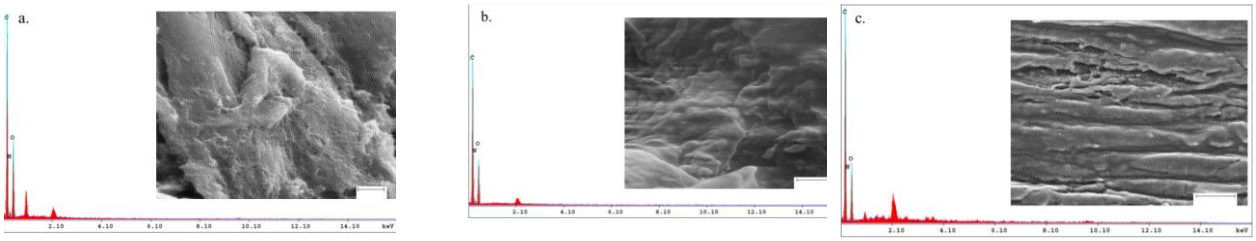


Figure 5.16. EDX spectra of selected SEM images for chitosan (a) and functionalized chitosan derivatives denoted **CHSr** (b), **CHSm** (c)

5.4. Evaluation of the antibacterial potential of chitosan derivatives with N,N'-diphenacyl 4,4'-dipyridylium dibromide and N,N'-diphenacyl 1,2- bis(4-pyridyl)ethane dibromide

Functionalized chitosan derivatives (**CHSr** and **CHSm**) samples were prepared in 1% acetic acid, on which the antibacterial activity against pathogenic (*Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*) and non-pathogenic microorganisms was tested (*Saccharomyces cerevisiae*, *Candida sp*., *Aspergillus niger*, *Penicillium sp., Bacillus subtilis*).The antibacterial potential and antipathogenic activity of the samples were tested.

5.4.1. Inhibition of pathogenic microorganisms

The antimicrobial activity of functionalized chitosan derivative samples was tested against one Gram-negative bacteria (*Escherichia coli* ATCC 25922) and two Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* Scott A). The non-selective medium, Brain Heart Infusion (Oxoid, Hampshire, United Kingdom) was used for cultivation of *Listeria monocytogenes* and Muller-Hinton medium (Scharlau, Barcelona, Spain) for *Escherichia coli* and *Staphylococcus aureus*, respectively. One colony from each strain was inoculated onto the specified medium and maintained at 37°C for 18 hours to reach stationary phase of growth. The in vitro antibacterial activity was tested by the diffusimetric method from wells cut into the agar medium [Verlee A. et al., 2017]. A layer of specific culture medium with 1.5% agar was poured into sterile Petri dishes, after its fluidization at 45°C. Then, 10 µL of each of the overnight cultures was inoculated into 7 mL of molten agar medium (42°C) and the second layer was poured over the first layer of medium to reach a final concentration of 107 cfu/plate . After solidification of the medium, wells with diameters of 9 mm were created in which 100 µL of sample was introduced. The incubation conditions for the samples were 37°C for 24 h.

Figure 5.17 shows the antimicrobial activity of chitosan and its derivatives against pathogenic strains of Escherichia coli and Staphylococcus aureus. The results showed that, for

the same concentration, the activity of the derivatives labeled **CHSr** and **CHSm** against both pathogenic bacteria is similar to that of the chitosan samples, the diameter of the inhibition zones is specified in **Table 5.4.**

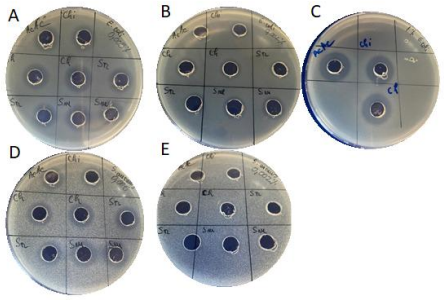


Figure 5.17. Antimicrobial activity of chitosan (**CH**) and chitosan derivatives **CHSr** and **CHSm** in concentration of 0.05% (A, D), 0.02% (B, E) against pathogenic strains of Escherichia coli (A, B) and Staphylococcus aureus (D, E). Anti- Escherichia coli activity in 1% acetic acid and chitosan (C)

Tabelul 5.4*. Evolution of the activity of chitosan (CH) and functionalized chitosan derivatives against pathogenic strains of Escherichia coli andStaphylococcus aureus*

Samples	Conc. %	Bacteria *E. coli*	*S. aureu*
Ch	0.05	11±0.51	8±0.32
	0.02	7±0.52	6±0.34
Ch-DPE	0.05	14±0.4	12±0.31
	0.02	10±0.21	ND
	0.01	ND	ND
Ch-DP	0.05	15±0.8	14±0.58
	0.02	11±0.58	ND
	0.01	ND	ND

Figure. 5.18. Antibacterial activity of chitosan and chitosan (**CH**) derivatives against *Bacillus cereus*

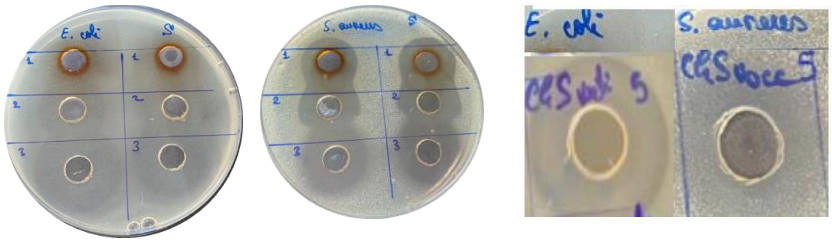


Figura 5.19. Antibacterial activity (diffusimetric method) of quaternary Sr salt against (a) *Escherichia coli*,(b) *Staphylococcus aureus* and c) for the **CHSr** derivative with *Escherichia coli* and *Staphylococcus aureus*

Preliminary studies, however, showed that chitosan and its derivatives show clear inhibition of the Gram-positive sporulated species Bacillus cereus (5 mm), in 0.01% acid medium **(Figure 5.18).** The minimum inhibitory concentrations (MIC) for the samples tested against *Escherichia coli* are 0.02% and 0.05% against *Staphylococcus aureus*, respectively. (**Figure 5.19).** No activity against *Listeria monocytogenes* was observed [Dediu Botezatu A.V. et al., 2023].

It is already known that chitosan shows antimicrobial activity only in acidic pH environment due to its polycationic amines that interact with negatively charged molecules existing on the surface of bacteria, changing the polarity and permeability of the membranes and subsequently inhibiting the growth of the microorganism. For functionalized chitosan derivatives no essential changes are improved, but the antimicrobial activity of chitosan is not lost.

The results obtained are in agreement with other similar studies [Chylinska M. et al., 2019; Kritchenkov A.S. et al., 2020] in which it is emphasized that chitosan has a stronger influence on Gram-negative bacteria than on Gram-positive ones. The explanation is related to the structure of the cell wall, considering that the outer membrane that has negatively charged molecules is absent in Gram-positive bacteria, and the peptidoglycan layer is thicker than in Gram-negative ones. It is believed that there are numerous factors that interfere in the

performance of the antimicrobial action of chitosan and its derivatives, including the degree of deacetylation, their concentration, pH and the presence of halogen [Chaudhary et al., 2020; Ibanez P. et al., 2020].

5.6. Conclusions

- ✓ Chitosan derivatives functionalized with organic compounds are considered interesting biomaterials for medical applications especially due to their potential antimicrobial activity, biocompatibility, biodegradability and nontoxicity.
- ✓ The chemical modification of chitosan soluble in acetic medium was achieved by introducing dipyridyl quaternary salts.
- ✓ Functionalized chitosan derivatives were synthesized using salts of *N,N'-diphenacyl)-4,4'-dipyridylium dibromide* **(S1)** and *N,N'diphenacyl-1,2-bis(4-pyridyl)ethane dibromide* **(S2),** salts that differ by an additional ethylene group to the compound marked **S_2**, process carried out in 2% acetic acid medium, in an ultrasonic bath, for 3 h. The functionalized chitosan polymer product, obtained after the reaction of synthesis was precipitated with 15% NaOH, dried, purified and analyzed.
- ✓ The synthesis of new chitosan derivatives proceeds in two stages: in the first stage functionalized chitosan is obtained on marginal sites and then the formation of heterocyclic bifunctional molecules occurs, through the connection with the amino group from the chitosan molecule and the ylide carbocation from the dipyridyl salt structure.
- ✓ The mass ratio between chitosan and N-heterocyclic salt was 10:1, and a synthesis yield of over 65% was obtained for the functionalized polymer samples, dried at 60°C.
- ✓ The elemental composition for the total content of C (%) and N (%) confirmed the synthesis of new chitosan derivatives, chemically modified with N-heterocyclic quaternary salts that favored their interaction in the polymer chain of chitosan, and thus obtaining new materials with specific properties.
- ✓ The new chitosan derivatives quaternized with the N-heterocyclic salts were characterized spectroelectrochemically, by UV-VIS spectroscopy, by dissolution in acetic acid medium and in solid state by infrared spectrometry (FTIR).
- ✓ Changes and differentiations in the structure of the polymer are confirmed, through the chemical interaction with the structure of the dipyridyl salt, used in the synthesis, having a different molecular mass.
- ✓ The characteristic adsorption bands of the functional groups of chitosan were identified in the FTIR spectra, which reflect the chemical structure, as well as the structural differences that can be induced by the type and amount of salt used in the synthesis process.
- ✓ Infrared spectral information can be considered a simple and fast analytical method in the development and exploitation of process steps for the selective functionalization of chitosan.
- ✓ The electrochemical evaluation performed by cyclic voltammetry (CV) indicates redox activity capacity, improved for the functionalized chitosan, antioxidant potential in the derivative marked **CHS_1** (with *N,N'-diphenacyl 4,4'-dipyridylium dibromide*).
- ✓ SEM images confirm changes in the morphology of chitosan after its reaction with dipyridyl salts, moving from a non-porous membrane structure to a polyphasic microporous structure.
- ✓ The new functionalized chitosan derivatives designated **CHS_1** (*N,N'-diphenacyl 4,4'-dipyridylium dibromide)* and **CHS_2** *(N,N'-diphenacyl-1,2-bis(4-pyridyl)ethane*

dibromide) were morphologically evaluated by scanning electron microscopy (SEM) and were shown to exhibit improved antimicrobial activity against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* compared to chitosan and N-heterocyclic salts.

- ✓ The synthesis of new chitosan derivatives chemically modified with heterocyclic N salts confirms the obtaining of new materials with specific biological and photochemical properties.
- ✓ Newly synthesized N-heterocyclic chitosan derivatives, through improved properties, can offer a new perspective in biomedical applications.

6. HYDROGELS WITH CHITOSAN AS BIOMATERIALS FOR MEDICAL APPLICATIONS

This chapter include a brief documentation on hydrogels and their medical importance respectively and exploration into the functionalization of chitosan as hydrogels with quaternary dipyridyl salts, by initiating such studies to evaluate their antimicrobial properties.

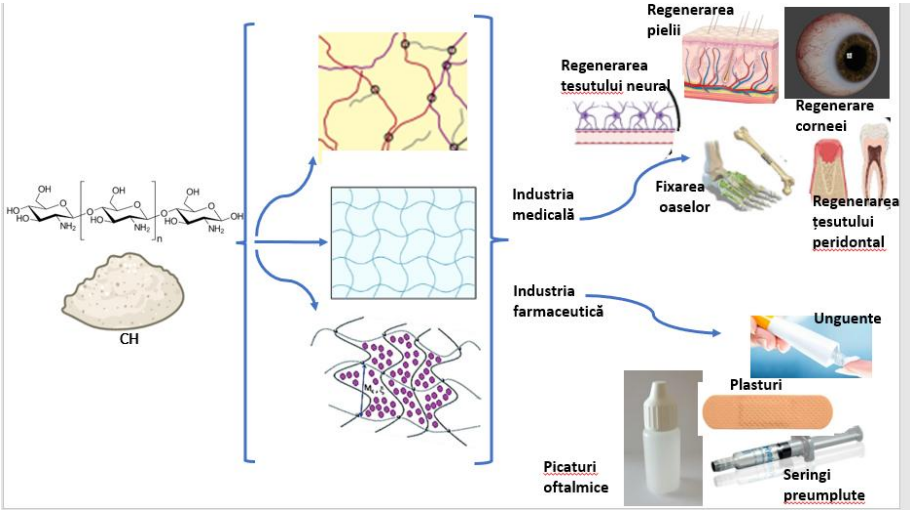
6.3. Biomedical applications for chitosan-based hydrogels

Most applications for hydrogels have been reported in medicine such those used in controlled drug release systems [Nazir I. et al., 2019; Tao G. et al., 2019].Chitosan is used for its special characteristics for biomedical applications **(Scheme 6.1).** The cationic form of chitosan allows it to interact with polymers or macromolecules that are anionic in nature and have certain polyanions. These interactions are also used to produce chitosan nanoparticles Wei H. et al., 2022]. The advanced development of chitosan-based hydrogels has led to new systems for incorporating and transporting drugs in different environments [Tao M. et al., 2019; Wei H. et al., 2022].

Hydrogels are studied as local drug delivery systems because they possess self-regulating properties[Nazir I. et al., 2019; Xu Y. et al., 2019], controlled biodegradation [Martău G.A. et al., 2019; Takei T. et al., 2020] and ability to protect termo-sernsitive drugs for delivery to the target site or organ [Zhou J. et al., 2019; Affes S. et al., 2021].

The development of polymeric drug delivery systems as an alternative to conventional drug formulations has been steadily increasing for several decades, with the greatest concern of trying to address inadequate local availability of drugs and the challenges associated with delivery sites [Xu Y. et al ., 2019; Affes S. et al., 2021].

Due to its specific properties, chitosan remains a versatile material with various applications in the field of biotechnology and biomedical (preparations with controlled release) [Nazir I. et al., 2019; Xu Y. et al., 2019]. The choice of an appropriate method for the detection and quantification of drugs released from hydrogel delivery systems depends primarily on the type of drug. Hydrogel drug delivery systems are based on the same analytical technologies, from the cheapest and most practical optical spectroscopy techniques [Tao G. et al., 2019; Takei T. et al., 2020], to the most selective, sensitive and expensive ones such as high performance liquid chromatography (HPLC) [Zhang H. et al, 2020], mass spectrometry [Tao M. et al ., 2019; Liu T. et al., 2020].] or polymerase chain reaction (PCR).



Scheme 6.1. Biomedical applications for chitosan-based hydrogels

Chitosan-based hydrogels have shown great potential and attracted considerable attention in recent years for biosensors and packaging materials as well. These materials act together to connect biology and electronics, and analytes can be recognized by bio-elements such as antibodies. Then, the interaction is transferred to optical/electronic signals, which are finally amplified into measurable results [Wang Y. et al., 2020]. The properties of chitosan-based hydrogels (excellent bioactivities, sensitivity to stimuli, incorporation capacity, swelling, biodegradability, nontoxicity and low cost) have allowed their expansion and use in biosensors. Changes in hydrogels caused by external factors (eg, pH and temperature) can be used as a signal to respond at a system level [Liu T. et al., 2020]. The response behavior of hydrogels can be modified by selecting raw materials and crosslinking methods [Pinelli F. et al., 2021; Rossi F. et al., 2020]. These characteristics have the suggested that hydrogels could be used as biosensors, which have been widely applied in various fields such as the food industry or tissue engineering.

Considerable attention in recent years has been paid to these chitosan-based materials because they provide real-time quality information about food, which can meet people's demands for high-quality and safe food [Yang J. et al., 2021] . Other studies have reported information on the applications of chitosan-based hydrogels as delivery systems in medicine due to the properties and behavior of the hydrogel with its subsequent application in biosensors [Liao H. et al., 2019; Fu S. et al., 2021].

6.4. Obtaining chitosan-based hydrogels with quaternary dipyridylium salts

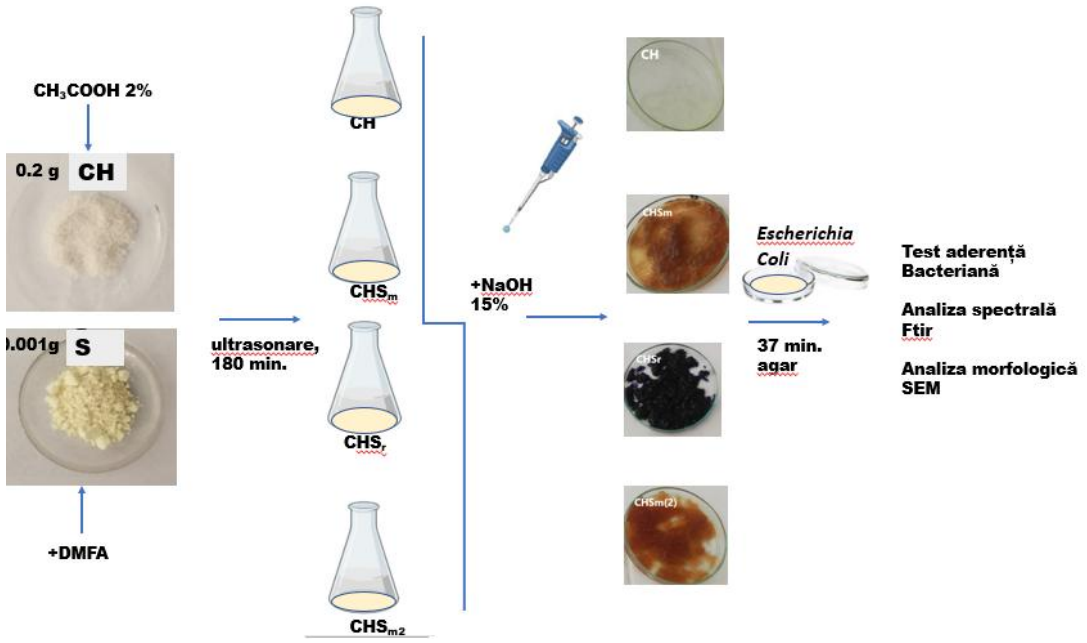
Chitosan exhibits antimicrobial and biocompatible properties and also serves as a good bio-adhesive [Li J. et al., 2022]. Thus, chitosan-based hydrogels show great potential to build a simple, biocompatible, and multifunctional interface between biology and electronics.

6.4.1. Methods of obtaining

Chitosan powder (**CH**), C12H24N2O9, deacetylated poly(D-glucosamine) chitin (M=340 $g \cdot mol^{-1}$; Sigma-Aldrich) and quaternary dipyridylium salts were used for the synthesis.

The synthesis procedure included 2 g of chitosan dissolved in 2% acetic acid and 0.010 g each of heterocyclic N salt, dissolved in DMFA. The salts used were *N,N'-diphenacyl-4,4'-dipyridylium dibromide* (**Sr**), *N,N'-diphenacyl-1,2- bis(4-pyridyl)ethane*

ibromide (**Sm**) and *dibromide of N,N' bis(p-nitrophenacyl)-1,2-bis(4-pyridyl)ethane* (**Sm2**). The samples were subjected to ultrasound for 180 min., at room temperature. Upon addition of 15% NaOH solution, samples with gel consistency and different colors were obtained, depending on the nature of the salt used. We believe that DMFA, a polar aprotic solvent has a stabilizing role in crosslinking in obtaining chitosan hydrogels [Heravi M.M. et al., 2018]. The stages of obtaining the synthesized hydrogels are shown in **Scheme 6.5**. The samples obtained after keeping for 24 hours in Petri dishes were investigated spectrally by FTIR and morphologically by SEM. The same equipment presented in the chapter was used in chapter 5 (**Figure 5.3**), the investigation conditions being similar.



Scheme 6.2. Work steps in obtaining chitosan hydrogels with quaternary dipyridyl salts

The bacterial species, Escherichia coli, was first kept overnight at 37°C on agar plates for antibacterial activity. For the bacterial adhesion test, against a Gram-negative bacterium (*Escherichia coli* ATCC 25922) 0.5 mL of *E. Coli* from the culture medium was incubated separately on different substrates of the hydrogel samples, for a period of 24 h, kept at 37°C. The samples were examined structurally by FTIR and morphologically by SEM.

6.4.2. Results and discussion

Images of the prepared hydrogel samples are shown in **Figure 6.1**, the salts being initially dissolved in DMFA. Chitosan samples with *N,N'-diphenacyl-4,4'-dipyridylium (Sr) dibromide* salt as hydrogel are deep purple in color. For the synthesis with *N,N'-diphenacyl-1,2-bis(4-pyridyl)ethane dibromide* salt **(Sm)** and the salt derivative, N,*N'-bis(p nitro-phenacyl) 1,2-dibromide bis(4-pyridyl)ethane* marked **Sm(2),** the hydrogels are more intense brown (brick) in color, compared to chitosan which appears as white gelatinous samples.

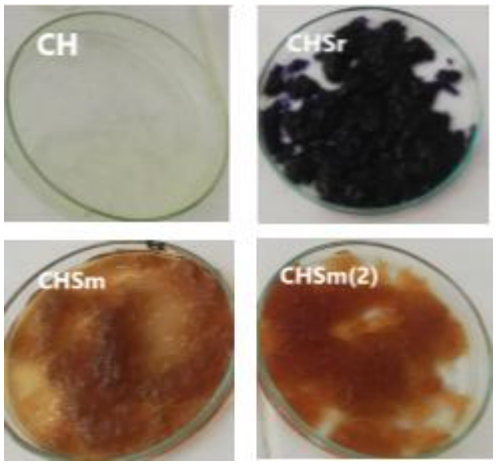


Figure 6.1. Images of hydrogels of chitosan with salts

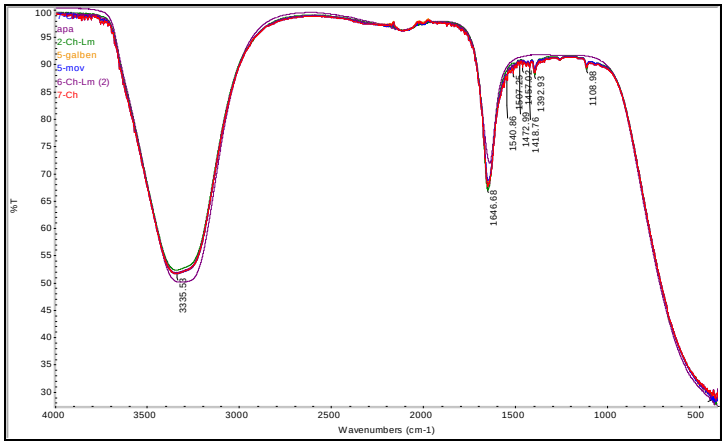


Figure 6.2. FITR spectra of hydrogel obtained from chitosan and salts

6.4.2.1. Investigating hydrogel structures using FTIR spectroscopy

FTIR spectroscopy was performed on wet samples to determine the mechanism by which quaternary dipyridylium salts cross-link in chitosan hydrogels (**Figure 6.3**). A large band is observed at 3600 – 3000 cm^{-1} in the spectra of chitosan and hydrogels with the dipyridyl salts, which is attributed to the group of O-H and N-H vibrations. Many other characteristic bands are not present in chitosan, the reference being the bands at 1647 cm^{-1} and 1418 cm^{-1}, which are also preserved in dipyridyl salt hydrogels.

6.4.2.2. Evaluation of morphological changes by SEM

All chitosan hydrogels with quaternary dipyridyl salts showed a surface morphology with a network-like structure with porous walls, with some interconnected pores **(Figure 6.3).** The SEM images indicate a polyphasic microporous structure, non-porous membrane phases, but smoother than in the hydrogel marked **CHSr** being dome-shaped holes, microfibrils and crystallites. For the hydrogels with *N,N'-diphenacyl 4,4'-dipyridylium dibromide salt* **(Sr)** images indicate a homogeneous composition without crowded or irregular areas, suggesting maximum compatibility between chitosan and salt. For hydrogels marked **CHSm**, with *N,N' bis-(p-bromophenacyl)-1,2- bis(4-pyridyl)ethane***(Sm)** salt, the morphological characteristics can be of real interest, particularly important in biomedical applications, being interconnected pores. In chitosan the structure is similar in appearance to that of the hydrogel marked **CHSm(2),** suggesting that the cross-linking is limited with this salt.

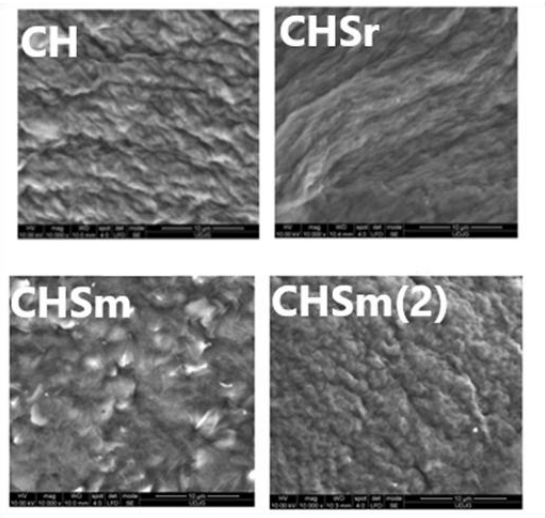


Figure 6.3. SEM images of chitosan hydrogel and dipyridylium quaternary salt chitosan hydrogels

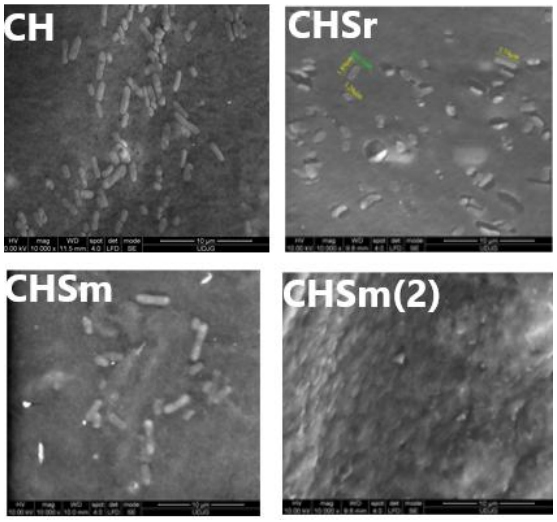


Figure 6.4. SEM images of chitosan hydrogel and dipyridylium quaternary salt chitosan hydrogels after treatment with *E. coli*

6.4.2.4. Antibacterial activity of hydrogels

The samples maintened to direct exposure with E coli, kept in conditions of 37°C for 24 h, were then examined regarding the morphology of the surfaces, their appearance after direct attack with pathogens. SEM images show that the surface formed on the hydrogels is now different to the samples **(Figure 6.4),** that it also has many microscale holes, similar to the size of bacteria, which will allow the bacteria to easily sink into the small pores and adhere to the chitosan [Shang B.B. et al., 2011]. After 24 h of contact with E. coli bacteria, the bacterial adhesion in the experiment was significantly high in the control chitosan. The amino groups in chitosan can be protonated in solution, making it positively charged and therefore very useful in adhering to the negatively charged *E. coli* bacteria. Chitosan hydrogels also have other functional groups, to which bacteria can adhere, but the number of bacteria is significantly reduced, voids also appear (in the hydrogel marked **CHSr**), the mechanism not being elucidated. The results indicated that chitosan hydrogels can have an effect on the bacterial adhesion of *E. coli*, and this benefit can be used for future applications for various purposes through further research.

6.5. CONCLUSIONS

- ✓ New derivatives of chitosan can be considered biomaterials with an important role in medical applications due to their biocompatibility which is an essential characteristic that must be analyzed in the first place;
- ✓ Chitosan is a versatile polymer due to its specific properties and characteristics finds utility in various hydrogels, in many applications, such as the medical one, such as for the incorporation and transport of drugs.
- ✓ Hydrogels based on chitosan and other chemical compounds are materials of wide interest, with different structure and typologies and studies confirm great importance for applications in medicine, pharmacy, biotechnologies, food and environment.
- ✓ Obtaining new chitosan-based hydrogels for in vivo applications with minimal toxicity and natural excretion from the body remains a permanent challenge as biomaterials.

- ✓ The preparation of hydrogels based on chemically functionalized chitosan, with dipyridyl quaternary salts, under specific reaction conditions, in the presence of DMFA, a cross-linking agent, in an acetic acid environment, was initiated. The obtained hydrogels were characterized spectrally (FTIR) and morphologically (SEM).
- ✓ The hydrogels have a structure in the form of a network with porous walls, with some interconnected pores, for example in hydrogels prepared with the contribution of *N,N' bis-(p-bromophenacyl)-1,2- bis(4-pyridyl)ethane dibromide* salt, features particularly important in biomedical applications.
- ✓ The scanning electron microscopy images confirm the structure of the hydrogel and the morphology of the materials obtained, through the presence of interconnected pores, which are polydispersed and indicate the favorable action on the tested pathogens, as was the case for *E. coli*, compared to the chitosan hydrogel.
- ✓ The hydrogel with *N,N'-bis(diphenacyl) 4,4'-dipyridylium salt dibromide* showed improved properties for *E. coli* compared to the other hydrogels, but no relevant results are obtained and we will continue the research.
- ✓ Studies will continue to evaluate the physico-chemical characteristics of hydrogels (degree of hydration, deacetylation barrier), mechanical and antimicrobial, to explore new medical applications.

7. FINAL CONCLUSIONS AND ORIGINAL CONTRIBUTIONS

The doctoral thesis entitled "Studies regarding the functional characterization for new chitosan derivatives" was dedicated to investigations regarding the functionalization of chitosan for new effective properties for biomedical applications. Considering the new approach and complexity of the research, several major objectives were considered, such as:

(i) The use of chitosan which is a polymer with complex structure offers innovative solutions in obtaining new functional materials that meet the needs of humankind proved to be useful for obtaining new derivatives with solubility in aqueous medium and effective antimicrobial activity.

(ii) A class of chemical compounds of current wide interest for new materials, N-heterocyclic compounds (viologens) was researcehed during this thesis due to their electrochromicity, their reversible redox activity and their efficiency in current technologies, compounds that can interact with chitosan .

(iii) The functionalization of chitosan with quaternary dipyridyl salts, carefully selected, was carried out both in the aqueous medium and acetic acid medium.

(iv) Evaluation of the synthesis process was folloed by tracking the influencing factors and characterizing the functionalized chitosan derivatives by spectroelectrochemical, structural, electrochemical and antibacterial techniques.

(v) A secondary approach, regarding obtaining and characterizing absorption complexes of chitosan with some metal ions was followed.

The **general conclusions** are therefore the following:

(I) Research studies were carried out on the functionalization of chitosan with N-heterocyclic compounds, in aqueous solution, and the influencing factors on the synthesis, using in the alkylation process, quaternary dipyridyl salts (viologens) to improve the properties of chitosan and to increases the cationic capacity of the resulting biopolymer, to improve its biological properties. Initially, the synthesis, purification and structural characterization of N-heterocyclic chemical compounds,

with importance in the science of new materials, from the class of 4,4 dipyridyls (viologens), were presented in the literature as agents with remarkable antioxidant, antitumor and antibacterial properties when they are present in functional materials.

(II) The organic salts used were *N,N'-diphenacyl-4,4'-dipyridylium dibromide* and *N,N'-dinitrophenacyl-4,4'-dipyridylium dibromide* which has two additional nitro (-NO2) groups compared to the first salt. The salts of *N,N'-diphenacyl-1,2-bis(4-pyridyl)ethane* dibromide and *N,N'-dinitrophenacyl-1,2-bis(4-pyridyl)ethane dibromide* were also used, salts with an ethylene group, symmetrically positioned in the structure compared to the first salt, the last salt also having two nitro functional groups. The choice of salts is explained by their structure and reactivity, as well as their relationship with the functional properties required for certain applications, in new complex combinations and new functional materials reported in the literature. The synthesized salts characterized from a physical-chemical point of view were correlated with published results, reflecting their chemical structure and their purity, as well as the structural differences between them.,

(III) In order to obtain new chitosan derivatives, the target was to find compounds with improved solubility in the aqueous medium, at a pH >6.3, compounds compatible with physiological pH, and to present antibacterial and antipathogenic properties, for applications medical and pharmaceutical. Obtaining new chitosan derivatives functionalized with *N,N'-diphenacyl-4,4'-dipyridylium dibromide* salt was tested by testing samples of commercial chitosan with degree of deacetylation (DD of 85%) from Sigma Aldrich Chemie GmbH as chitosan pharmaceutical (DD of 95%). Although chitosan is not totally soluble in aqueous solution, in the presence of the N-heterocyclic salt, in the synthesis process, by mechanical agitation, between the compounds through the adsorption process, changes are observed in the polymer chain, color change in the sample solutions, pH variations and to the conductivity of the filtrate solutions and as a final result and the improvement of the biological properties. Structural differences appear (FTIR technique) in the composition of the samples, and the presence of excipients in the pharmaceutical chitosan influences the results and the study with this chitosan was abandoned.

(IV) For samples of deacetylated chitosan (DD >75%) from Sigma Aldrich Chemie GmbH, at the mass ratio of chitosan to N-heterocyclic salt, *N,N'-diphenacyl-4,4'-dibromide dipyridyl* of 20: 1 and 10:1, in aqueous medium, products were obtained with reaction yields >60%, in derivatives of functionalized chitosan, other values to modify the mass ratio not having good results. The influencing factors in the synthesis process (contact time, synthesis temperature, mass ratio, agitation) were investigated. The physico-chemical properties of the new products were evaluated, the elemental analysis was carried out, as well as the structural (by XRD, FTIR,) and morphological (SEM) characterization, molecular mass and antioxidant capacity. The FTIR spectra of the functionalized chitosan compounds confirmed the presence of characteristic bands of the dipyridyl quaternary salt in the new structures of the derivatives, confirming the formation of covalent cross-linking bonds. The interactions with chitosan (DD 85 %) for the creation of new structures of chitosan derivatives in the aqueous medium are confirmed using other N-heterocyclic salts synthesized in the laboratory *(N,N'-bis(p-nitrophenacyl)-4 dibromide*, 4'*-dipyridyl; N,N'diphenacyl-1,2-bis(4-pyridyl)ethane dibromide* and *N,N'bis(p-nitrophenacyl)-1,2-bis(4-pyridyl) dibromide respectively ethane).*

(V) Research on the functionalization of chitosan with N-heterocyclic salts, *N,N'-diphenacyl-4,4'-dipyridyl dibromide* and *N,N'-diphenacyl-1,2-bis(4-pyridyl)ethane*

dibromide dissolved in DMFA, by synthesis in 2% acetic acid medium, the solubility of chitosan being optimal, reported functionalized chitosan with synthesis yield over 65%. The elemental analysis by the total content of C%, N% confirmed the synthesis of new functionalized chitosan derivatives, and the spectrophotometric characterizations also indicated changes and differentiations in the polymer structure. Spectral information, through the FTIR technique, can be considered a simple and fast analytical way in the development and exploitation of process stages for the selective functionalization of chitosan, to obtain new materials.

(VI) Electrochemical evaluation using the cyclic voltammetry (CV) method, in DMSO indicates sample-enhanced redox activity capacity, antioxidant potential in the chitosan derivative functionalized with *N,N'-diphenacyl 4,4'-dipyridylium dibromide*. Through the CV analysis of the new derivatives as well as the antibacterial analysis indicated favorable results for the development of new compounds with biological properties. The new chitosan derivatives functionalized with *N,N'-diphenacyl 4,4'-dipyridylium dibromide* and *N,N'-diphenacyl-1,2-bis(4-pyridyl)ethane dibromide* were morphologically evaluated by scanning electron microscopy (SEM-EDX). The SEM images obtained confirm morphological changes in the polymeric chitosan (and compositional (by EDX)) after the reaction with quaternary dipyridyl salts, changing to a polyphasic microporous structure. Functionalized chitosan derivatives have been shown to exhibit improved antimicrobial activity against strains of *Escherichia coli* and *Staphylococcus aureus* and for *Bacillus subtilis* compared to chitosan without N-heterocyclic salts.

(VII) Chitosan hydrogels could be considered biomaterials due to the relationship between the molecular structure of chitosan and all major biological properties in human wound healing (antimicrobial, antitumor, immunomodulatory, coagulation and wound healing and regeneration effects). Documentation was done for chitosan-based hydrogels, their characteristics and medical applications. In the final research, new materials were obtained, hydrogels by the synthesis between chitosan and quaternary dipyridyl salts, materials analyzed by structural (FTIR) and morphological (SEM) analysis and from the point of view of antibacterial activity, against *Escherichia coli*. Hydrogels prepared with *N,N'-diphenacyl 4,4'-dipyridyl dibromide* salt present a homogeneous composition, without agglomerated or irregular areas, indicating a maximum compatibility between chitosan and salt, compared to hydrogels with quaternary dipyridyl salts, in structure with a symmetrical ethylene bridge. The SEM images of chitosan hydrogels functionalized with dipyridyl quaternary salts showed a polyphasic microporous structure, non-porous membrane phases and confirm the hydrogel structure and the morphology of the obtained materials, through the presence of interconnected pores, which are polydispersed and indicate the favorable action on the tested pathogens, as was the case *for E. coli* compared to the chitosan hydrogel. The results indicate that functionalized chitosan hydrogels can have an effect on bacterial adhesion of *Escherichia coli* and this benefit can be used for future applications for various purposes by further research.

(VIII) Chitosan complexes with Cu(II) and Zn(II) ions were also studied in the first stage, proceeding experimentally to changes in concentration in solutions and variable contact time. The UV-Vis absorption spectra were evaluated, after chelation with metal ions of Zn and Cu respectively, individually and the quantitative value of the ions in the solution was evaluated, regarding the synergistic effect of the two metal ions, important in biological systems. Along with the increase in contact time, there are changes in the absorbance, electrical conductivity and pH of the filtered solutions.

(IX) New complexes of chitosan with ferric ions, Fe(III) were obtained in an acidic environment, less studied studies, the ferric ion being important for the environment but also for the medical field. The variation of the contact time between the compounds, chitosan and the Fe (III) ion solution, the stirring time, the amounts of chitosan and the concentration of metal ions in the samples as well as the amount of chitosan were varied. Interactions between the compounds were obtained, the studies providing information on the variation of pH and conductivity in solutions, changes in the complexation process between the metal ion and chitosan, at contact time and variable pH. It was found that the contact period influences the complexation process with chitosan and brings changes in the pH of the solutions and the absorbance in the analyzed spectra. The kinetic study carried out indicated that the equilibrium isotherm is more suitable with the Langmuir model, the chitosan complexation reaction took place, showing affinity for Fe(III) ions, at a pH between 3.0-3.2, reaching a balance between components of approx. 5 hours. Through the UV-VIS spectra recorded from the supernatant, after filtering the synthesis samples as before synthesis and after complexation, the solution was provided to evaluate the maximum complexation capacity with Fe(III) ions with chitosan (80 mg/L), and the morphological analysis of confirmed changes in the complexes when there are different amounts of chitosan, or when the synthesis conditions change.

ORIGINAL CONTRIBUTIONS

Obtaining new materials is a permanent concern for the research, and the complexity of chitosan as a biomaterial derived from chitin and commercially available in different degrees of deacetylation (molecular masses) for its controlled chemical modification is always a great challenge and a very difficult task due to its complex structure.

Discoveirng new solutions for obtaing new materials during research , based on chitosan, the experimental research carried out in this thesis brought some original results.

The main original scientific results obtained, through the established objectives of the thesis, can be as follows:

- Synthesis of new functionalized chitosan derivatives using N-heterocyclic salts, from the *4,4'-dipyridyl* class (viologens) not reported in the literature until now. In this way, the uniqueness of the chemical structures was demonstrated for new applications, and two ISI scientific articles were published.
- The original synthesis scheme of the new derivatives was developed, through selective stages using organic solvents (DMFA, DMSO, ethanol, acetic acid) and their mixtures with water in different ratios. It is considered that the most important solvent for N-heterocyclic salts, quaternary dipyridyl salts is DMFA, the salts being totally soluble.
- The individual solubility of chitosan was determined by structural factors, especially the content of free OH groups, capable of forming hydrogen bonds, and the synthesis of new chitosan derivatives functionalized with salts of the 4,4'-dipyridyl class had good results in acetic acid medium, but limited in aqueous medium, although the challenges for new applications are to obtain derivatives with aqueous solubility and at physiological pH.
- It was established that, among the dipyridyl quaternary salts used in the synthesis, for the functionalization of chitosan, the *N,N'-diphenacyl-4,4'-dipyridylium dibromide* salt had good alkylating activity, keeping an antioxidant efficiency, thus obtaining a more efficient water solubilization and improved biological activity against pathogens such as *Escherichia coli* and *Staphylococcus aureus*, as well as against *Bacillus subtilis.*

- A simple and fast method was used to support the obtaining of new functionalized chitosan derivatives, through the content and position of the essential functional groups in the biopolymer, based on FTIR analysis, in conditions where NMR analysis is much more expensive and laborious, and through the analysis X-ray diffraction (XRD) requires a more sensitive equipment (below 10 Θ), to confirm the structure, in the samples obtained, the mass ratio between chitosan and salt being 20:1.
- FTIR analysis was also useful for the rapid confirmation of hydrogel structures when the main structural changes that occurred were determined and how the presence of dipyridyl salts influences the synthesis process. A very important scientific result was the demonstration of the fact that in the action of *E.coli*, functional chitosan derivatives indicate effective antimicrobial activity.
- We can consider that an important path has been opened for new derivatives of functionalized chitosan, with the use of viologens, salts from the 4,4'-dipyridyl class, to obtain new materials, with possible applications in a number of fields in several main directions , such as: medical, food or materials engineering.
- Also of interest are the contributions regarding obtaining chitosan complexes with metal ions, especially for Fe(III) in acidic medium, data not reported in the literature, and our results are in the process of being published, as for complexes with Cu(II) ions) and Zn(II).
- The original contributions of the thesis were presented in two articles published in international journals with scientific visibility (Carbohydrate Research - 2023, Journal Physics Conference - 2021), a work published in a journal indexed in databases, contributions in 11 communications at international conferences of prestige from abroad and from the country, presentations during the Scientific Conference of the SCDS-UDJG Doctoral Schools, edition IX - XI.

PROSPECTS FOR CONTINUING RESEARCH

- -Extending the antibacterial analysis of the effectiveness of hydrogels on several bacterial species, against Gram-positive and Gram-negative bacteria, as well as yeast strains.
- -Evaluation of the antimicrobial and antiviral activity of the hydrogels obtained at the level of bacteria from hospital institutions, and the new synthesized compounds to be subjected to studies of antimicrobial activity in the framework of long-term, well-established collaborations, which respect the established protocol and ethical issues.
- -Carrying out research on the effectiveness of the antibacterial activity of hydrogels compared to other new materials that can be obtained: membranes, films, using samples of synthesized functionalized chitosan derivatives.
- -In silico investigation of the antimicrobial activity mechanism that will be based on the combination of docking and molecular dynamics (MD) simulations. in order to confirm that chitosan derivatives with N-heterocyclic salts can exhibit effective antimicrobial activity.
- -Evaluation of the antitumor activity of the obtained hydrogels
- -Research on the synergistic action of the categories of bioactive compounds through in vitro and in vivo tests.

The development of new research directions can provide important information to drug manufacturers, for food supplements, which constitute starting points in the foundation of new pharmaceutical forms long awaited for the improvement of human health.

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DISSEMINATION OF RESULTS

Publications in ISI journals

1. Andreea Veronica Dediu Botezatu, Roxana-Mihaela Apetrei, **Iuliana Florina Costea (Nour)**, Vasilica Barbu, Leontina Grigore-Gurgu, Florina Botez, Rodica Mihaela Dinica, Bianca Furdui, Geta Cârâc, Synthesis and characterization of novel chitosan derivatives (containing dipyridinium quaternary salts) with antimicrobial potential, Carbohydrate Research (f.i.=2.97), **2023**, 108964, doi.org/10.1016/j.carres.2023.108964.

2. **Costea (Nour) Iuliana Florina**, Melinte Rodica Gabriela, Noapteș Silvia Nicuta, Cudălbeanu Mihaela, Dediu (Botezatu) Andreea Veronica, Dinică Rodica Mihaela, Cârâc Geta, Factors of influence for functionalised of chitosan with nheterocyclic salt in aqueous medium, Journal of Physics: Conference Series, 1, **2021**, 1960, 012001, doi:10.1088/1742-6596/1960/1/012001.

Publications in international databases

1. **Costea (Nour) Iuliana Florina**, Sorcaru Florentina, Cârâc Geta, Investigation of Structural Characteristics by FTIR Spectroscopy of Chitosan Derivative with N Heterocyclic Compound, The Annals of "Dunarea de Jos" University of Galati Fascicle IX Metallurgy and Materials Science 45, **2022,** 4, 46-52, doi:10.35219/mms.2022.4.08.

Papers and posters presented at international and national congresses

1. **Iuliana Florina (Costea) Nour**, Gabriela Melinte, Ana Cazanevscaia Busuioc, Andreea Veronica Dediu Botezatu, Geta Cârâc, Rodica Mihaela Dinică, "Synthesis and characterization of novel heterocyclic chitosan derivatives (containing N,N'-Diphenacyl-4,4' -Dipyridinium Dibromide) with antimicrobial potential", Euroinvent International Conference on Innovative Research, the 14th edition, Iași, **2022,** Oral Presentation, Section 3 - Materials Application, O.P. 75. http://www.euroinvent.org/conference/doc/Program_ICIR_2022.pdf
2. **Iuliana Florina (Costea) Nour**, Rodica Melinte, Silvia Nicuta Noapteș, Mihaela Cudălbeanu, Andrea Dediu Botezatu, Geta Cârâc, Rodica Mihaela Dinică, "Factors of influence for functionalised of Chitosan with N-heterocyclic Salt in Aqueous Medium", International Conference on innovative research EUROINVENT, the 13th edition, Iasi, **2021**, Poster presentation, Section 2- Procedures and Technologies for Materials Engineering, P.52 (Best poster Award), www.euroinvent.org/conference/doc/Program_ICIR_2021.pdf
3. **Iuliana Florina (Costea) Nour**, Gabriela Melinte, Silvia Nicușa Noapteș, Geta Cârâc, "Chitosan Derivates with N-Heterocyclic Pyridinium salts:Synthesis and Characterization", International Conference on Materials Science and Technologies ROMAT, 8th edition (International Conference on Materials Science and Technologies RoMat, the 8th edition), Bucharest, **2020**, Poster Presentation, Section 2 - Nanomaterials, Advanced Materials and Nanotechnologies for Innovation Ecosystem Development, p.57. https://docs.upb.ro/wp-content/uploads/2021/05/ROMAT-2020

4. **Iuliana Florina (Costea) Nour**, Geta Cârâc, Rodica Mihaela Dinică, "Natural products in Drug Discovery and Human Health - Chitosan - metal ions complexes having antimicrobial potential", Natural products In Human, Lisbon, **2019**, Poster presentation, Section 1, p.p 32, http://www2.ff.ul.pt/pselisbonmeeting2019/files/2017/11/Book-of-Abstracts_26-de-Julho2.pdf

Papers and posters presented at the Scientific Conference of the SCDS-UDJG Doctoral Schools

1. **1.Iuliana Florina (Costea) Nour**, Geta Cârâc, Chitosan hydrogels as biomaterials for biomedical applications, SCDS-UDJG Doctoral Schools Scientific Conference, XIth Edition, Galați, 2023, Oral Presentation, S6-Future of Eco-Nanotechnologies, Functional Materials and Coatings, O.P.6.4 https://cssdudjg.ugal.ro/images/2023/08/Program%20CSSD%202023.pdf
2. **Iuliana Florina (Costea) Nour**, Gabriela Melinte, Rodica Mihaela Dinică, Viorica Barbu, Geta Cârâc, Chitosan Based Hydrogels Modified with N-Heterocyclic Scaffolds for Antibacterial Applications, SCDS-UDJG Doctoral School Scientific Conference, Xth Edition, Galați, 2022, Oral Presentation, S6-Future of Eco-Nanotechnologies, Functional Materials and Coatings, O.P.6.3 (Mention).
3. **Iuliana Florina (Costea) Nour**, Gabriela Melinte, Geta Cârâc, Rodica Mihaela Dinică, Structural modification of chitosan derivatives as potential antifungals, SCDS-UDJG Doctoral School Scientific Conference, IXth Edition, Galați, 2021, Oral presentation, S6- Future of Eco-nanotechnologies, Functional Materials and Coatings, O.P.6.9.
4. **Iuliana Florina (Costea) Nour**, Mihaela Cudălbeanu, Geta Cârâc, Rodica Mihaela Dinićă Galati, Synthesis and characterization of novel heterocyclic chitosan derivatives, for potential antifungal and antioxidant applications, Scientific Conference of Doctoral Schools SCDS-UDJG, Edition a–VIII, Galați, 2020, Oral Presentation, S6- Future of Eco-Nanotechnologies, Functional Materials and Coatings, O.P.1.3 (3rd Prize).
5. **Iuliana Florina (Costea) Nour**, Geta Cârâc, Complex of absorption on chitosan with zinc and copper ions, SCDS-UDJG Doctoral Schools Scientific Conference, VIIth Edition, Galați, 2019, Poster S5-Future of Eco-Nanotechogies, Functional Materials and Coatings, P.P.1.3.

Other contributions

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