

## THE STUDY OF INFRARED SPECTRUM OF CHITIN AND CHITOSAN EXTRACT AS POTENTIAL SOURCES OF BIOMASS

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The chitin and chitosan are polysaccharides and abundant resources of biomass, which draw attention through their distinctive biological and physical-chemical characteristics. The chitosan is a linear polysaccharide. Commercially, the chitosan is produced through the deacetylation of chitin. In order to explore the potential of these polysaccharide biopolymers we have identified the particularities in infrared of chitin (absorbance vs. wave number), with the purpose of emphasizing the analytical possibilities of characterizing them. In this purpose, the absorption spectrum of chitin was studied in ultraviolet and mid-infrared field. The record of absorption spectrums in these favourable conditions and the processing of results, allows the quantitative expression of the acetylation degree of the amines in the polysaccharide structure. This study showed that the integral intensities of the infrared spectrum of normalized overlapped images of "amide I" straps in the case of standard mixes show a linear dependence of acetylation degree with Regression lines  $y = 3.716499x + 0.444500$  and  $r$ -squared value  $R^2 = 0.995747$ ; and on the grounds of the standard line and following an analytical procedure similar to the phases of standard mixes preparation, the residual acetylation degree of the real samples of chitosan can be determined.

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*Keywords:* biomass, chitin, chitosan, absorption spectrum.

### 1. Introduction

The biomass is represented by organic matter, vegetal and animal metabolic residues, as well as microorganisms. The production of biomass represents a domain in expansion due to the increase of interest for the alternative sources of energy. The biomass sets the carbon dioxide (CO<sub>2</sub>) of the atmosphere; afterwards, the carbon is released under the form of a mix consisting of carbon dioxide and methane (CH<sub>4</sub>), depending on the utilization of material. The importance of chitin and chitosan (Figure 1) increased lately, on one hand due to the fact it represents sources of renewable and biodegradable materials, and, on the other hand, due to a better knowledge of their functionality through applications in fields such as biology, pharmacy, bio-technology, medicine and the chemistry of materials [1]. The chitin (C<sub>8</sub>H<sub>13</sub>NO<sub>5</sub>)<sub>n</sub> forms the exoskeleton of insects or of other arthropods; it can also be found in lichens or some species of fungus. The chitin can be described as cellulose with a group of hydroxyl for each monomer replaced with a group of acetyl-amine, thus the structure allows tight connections between the hydrogen atoms of the adjacent polymers, which results in a more resistant material [2]. From a chemical point of view, it is poly N acetyl D glucosamine β-(1,4)-2-Acetamido-2-dezoxi-D-glucoză or N-acetyl-D-glucosamine β-(1,4) N-acetyl-D-glucosamine [3]. The chitin is a natural material, spread in the world of

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exoskeleton crustaceans [4] and represents a polymer that was identified in 1811 year and it is on the 2<sup>nd</sup> place as spreading level, after cellulose [5].

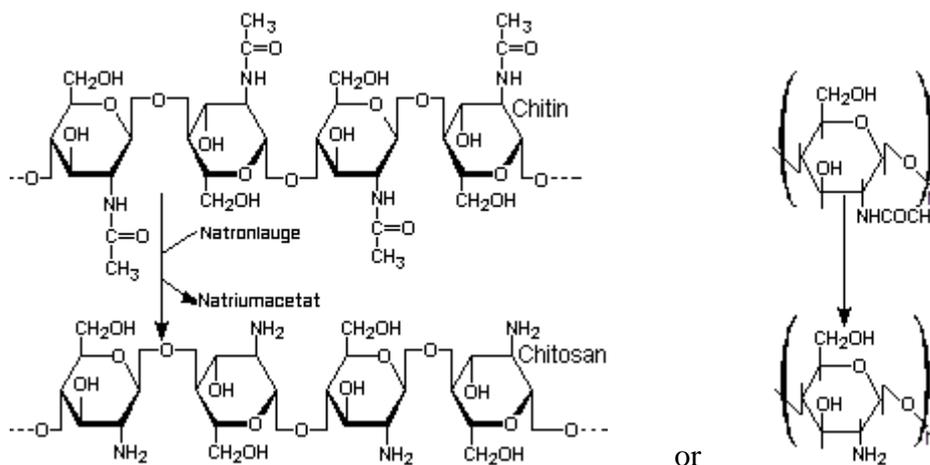


Fig 1. The chemical structure of chitin and chitosan

The chitin is nitrous derivate with a structure resembling to the structure of cellulose; it is insoluble in water and resistant to acids, bases or to a large number of organic solvents. In the case of crustaceans, the chitin is impregnated with calcium carbonate a shows a higher asperity [6]. Due to the insolubility, the chitin hasn't a large applicability and therefore it goes through a partial deacetylation process, thus the chitosan is obtained [4]. This is natural cellulose, having as main component the acetylated chitin, the chitosan being a product obtained after the removal of acyl. The chitosan is natural polymer with properties such as bio-compatibility and bio-absorbability. It is non-toxic and easily soluble in weak organic acids. The chitosan has several commercial and bio-medical utilizations. It can be used in agriculture for the treatment of seeds and as a bio-pesticide, protecting the plants against fungus infections; in wine production it is used in order to prevent the alteration of wine [7]. On industrial scale it is used in the process of water filtration, being considered a good styptic, the chitosan is useful in medicine, for the production of bandages for bleeding reduction (blood coagulation) and as an antibacterial agent [8], and it is also used in the cure of gum affections and bleedings [9]. Controversial to a certain extent, the chitosan plays a role in the reduction of fat absorption, which makes it useful in the case of diets, because there still aren't proofs that contradict this special property of the chitosan [10]. It was used for weight loss, due to its properties of fat absorption reduction in the bowels by fat binding, the complexation of heavy metal ions and styptic action [11]. It has a cationic structure that results from the partial deacetylation of chitin and contains units of glucosamine and N-acetyl-glucosamine [12, 13]. The structure is similar to natural glucosamine-glycans and this is why the biopolymer has a high bioactivity [14]. The chitosan can be metabolized by human enzymes, such as "lysozyme". As it can be seen from the chemical structures, the two substances differ through the presence (in the case of chitin) and subtle presence (in the case of chitosan) of the acetyl group grafted by the amidic function [6]. Following the process of obtaining the chitosan through the deacetylation (which usually is not complete) of chitin, we considered as significant the studies related to the analytical characterization of chitin and chitosan, and the studies concerning the degree of deacetylation performed in order to produce chitosan. These researches can be important for the obtaining of natural biomass quantities having the characteristics of biocompatibility and bio-absorbability.

## 2. Experimental

### *Materials*

The acetonitrile (used for the registration of UV spectrums) and the potassium bromide (needed for the inclusion of samples in the KBr tablet), used in the spectral study in the infrared field, were of spectroscopic purity, produced by Merck Company.

The references of chitin extract (stated acetylation degree of 100%) and chitosan (stated acetylation degree of 3%), came from the agency of Merck Company.

### *Methods*

#### *UV-Visible Spectrophotometric Method*

The UV spectrum of the chitin extract was recorded in acetonitrile solution, with the help of a spectrophotometer with double beam, type PG Instruments UV-VIS spectrophotometer, using UV WIN 5.05 soft. The investigated solution was inserted in quartz with an optical path of 1 cm. As standard it was used quartz cell identical with the mentioned one and filled with acetonitrile. The record speed of the absorption spectrum was of 10 nm/min; the sampling frequency was of 0.2 nm. In order to improve the signal/noise proportion, which is necessary especially to obtain a derived spectrum of appropriate quality, it was resorted to an algorithm of medium flattening of the original spectrum [0]. The infrared spectrum were recorded with a spectrophotometer with Fourier transformation with Jasco origin (680 plus model), which operates in the  $4000 - 400 \text{ cm}^{-1}$  spectral field. The registration of the spectrum was made with the sample included in a potassium bromide tablet (13 mm diameter). The used instrument allows the representation of spectrums, in the form transmittance vs. wave number, as well as in the form absorption vs. wave number. The UV spectrum of the chitin extract was recorded in acetonitrile solution in the spectral field 196–248 nm.

#### *Infrared Spectroscopic Method*

The infrared spectrum was obtained with samples included in potassium bromide tablets. The quantity of sample included in one tablet (diameter of 13 mm) is of 1 mg, and the quantity of potassium bromide (having the role of binding agent) is of 200 mg. The sample of mix and the potassium bromide is homogenized until a fine granulation and a homogenous consistency is reached [15]. The pulverous material is then inserted into a special matrix, made of stainless steel, which undergoes through a pressure of 9 tons-force in a hydraulic press. Before the pressure is applied and while pressing, the inside of the matrix is vacuumed in order to avoid the inclusion of air micro-bubbles in the tablet. The presence of these micro-bubbles may disturb the optical behavior of the tablet on one hand, and on the other hand it may lead to the apparition of micro-fractures when the compression pressure is removed. Under the action of pressure, in approximately 5 minutes, the pulverous material of the matrix is sintered and thus a compact and transparent mass is obtained, which is homogenous from an optical point of view [16]. For a more advantageous signal/noise proportion the accumulation of several registrations is performed (in this case 64 registrations); in this way is accomplished an improvement of 8 times of the proportion between the useful signal and ambient noise compared to a single registration.

## 3. Results and discussion

### *UV-Visible Spectrophotometric*

For that purpose to its biocompatibility, biodegradability and cationic kind, the chitosan was researched for several applications in the field of tissue engineering. Figure 2 represents the absorption spectrum of the acetonitrile solution of the chitin extract. Even though the molecular structure of the chitin, which is predominantly saturated, does not allow a substantial absorption (it is not an efficient chromophore), the spectrum shows an absorption band between 260 nm and 260 nm, with a maximum absorption of 212.6 nm [17, 16].

This band can be caused by electronic transitions of  $n-\pi^*$  type, produced in the secondary amid fragment of the chitin. The very intense band, situated under 196 nm (outside Figure 2), is

the result of the transition of  $\pi-\pi^*$  type [18]. The band associated with the transition  $n-\pi^*$  would be appropriate for quantitative analytical determinations but it has two disadvantages: a relatively low intensity, on one hand, and a positioning on the descendant branch of the  $\pi-\pi^*$  band, on the other hand. In this situation, the  $\pi-\pi^*$  band would disturb the quantitative determinations based on this absorption band. The band associated with the electronic transition  $\pi-\pi^*$  would have the appropriate intensity for a reasonable analytical sensitivity, but the spectral field of the maximum (under 196 nm) is not accessible but for instruments able to function in the void ultraviolet field. The derived UV spectrum (Figure 3) has two extremes for values that are accessible from a technical point of view (210 nm and 214 nm), but even these signals are low, not being able to provide an appropriate sensitivity [19, 20].

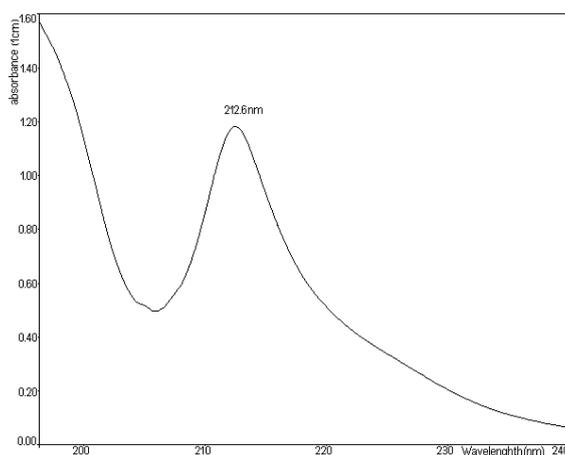


Fig. 2. The absorption spectrum of the chitin acetonitrile extract

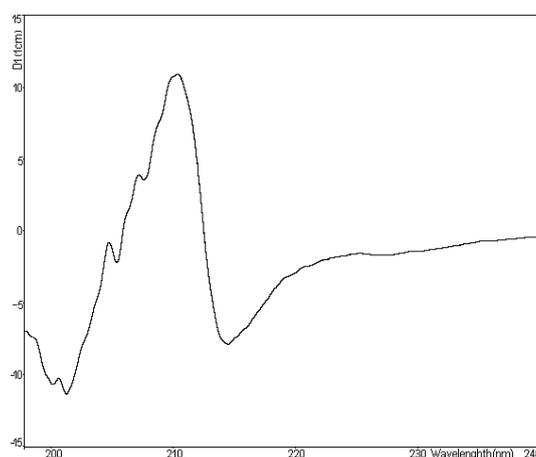


Fig. 3. The absorption spectrum D1 of the chitin acetonitrile extract

### ***Infrared Spectroscopic***

Due to the above mentioned reasons the analytical capitalization of infrared spectrums was started. Figures 4 and 5 shows the infrared spectrum of the chitin in the spectrum field  $4000 - 400 \text{ cm}^{-1}$  and  $2000-400 \text{ cm}^{-1}$ , in the form transmittance vs. wave number (vs-symmetric stretch; vas-asymmetric stretch;  $\delta$ -deformation;  $\omega$ -wagging). The bands are generally large due to the macromolecular character of the compound and because of the numerous intermolecular bindings of hydrogen, manifested even in the solid state of the sample [19, 20].

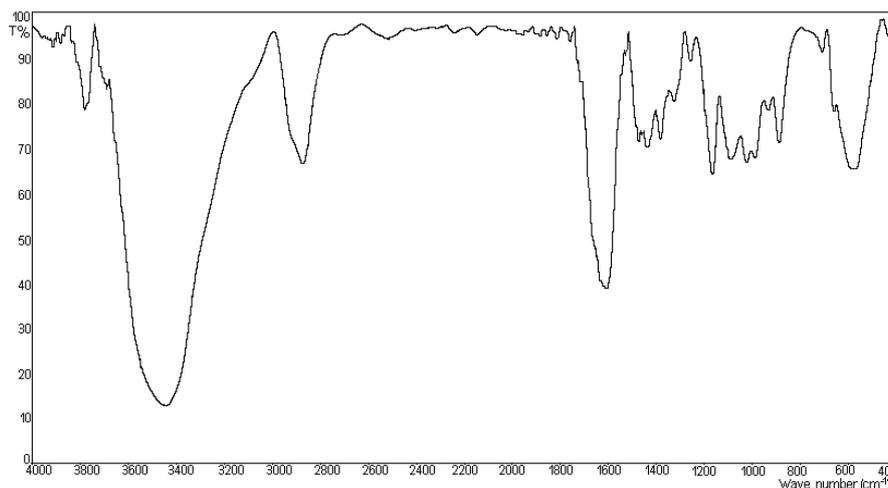


Fig. 4. Infrared spectrum of the chitin extract in the field  $4000\text{--}400\text{ cm}^{-1}$  (KBr tablet)

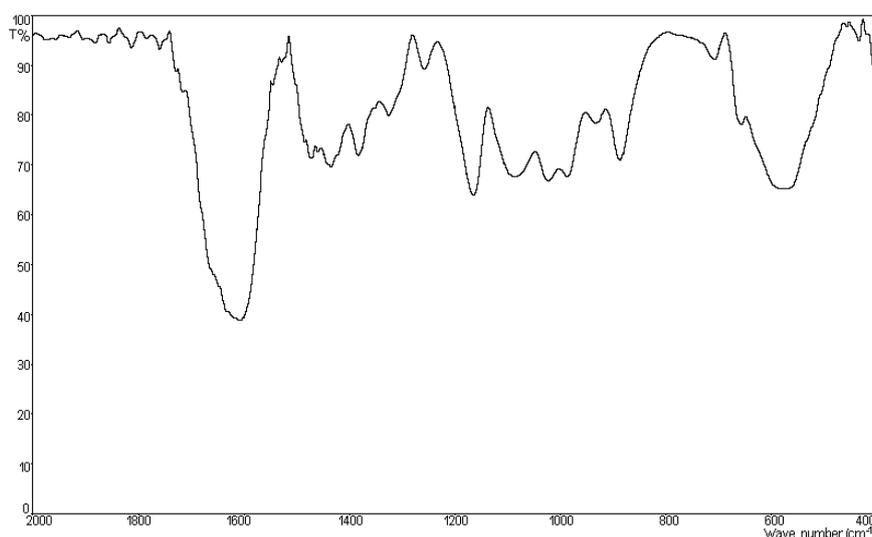


Fig. 5. Infrared spectrum of the chitin extract in the field  $2000\text{--}400\text{ cm}^{-1}$  (KBr tablet)

The bands  $3290\text{ cm}^{-1}$  (chitosan) and  $3285\text{ cm}^{-1}$  (chitosan oligomers) are determined by  $\nu(\text{OH})$  overlapped on  $\nu(\text{N-H})$ . The band from  $2867\text{ cm}^{-1}$  is determined by  $\nu(\text{C=O})$  of the amide group  $\text{CONHR}$  of the chitosan. The bands  $1568\text{ cm}^{-1}$  (chitosan) and  $1560\text{ cm}^{-1}$  (chitosan oligomers) are determined by  $\nu(\text{C=O})$  of the proton amide group, and  $\delta(\text{NH}_2)$  is determined by the proton amide group. The bands  $1417\text{ cm}^{-1}$  (chitosan) and  $1419\text{ cm}^{-1}$  (chitosan oligomers) are determined by  $\delta(\text{OH})$ . The bands  $1372\text{ cm}^{-1}$  (chitosan) and  $1375\text{ cm}^{-1}$  (chitosan oligomers) are determined by  $\delta(\text{CH}_3)$ . The bands  $1318\text{ cm}^{-1}$  (chitosan) and  $1317\text{ cm}^{-1}$  (chitosan oligomers) are determined by  $\nu(\text{CH}_3)$  third amide  $\omega(\text{CH}_2) + \text{OH}$  deformation in plane. The bands  $1152\text{ cm}^{-1}$  (chitosan) and  $1148\text{ cm}^{-1}$  (chitosan oligomers) are determined by  $\nu(\text{C=O})$  oxygen bridges resulting from the deacetylation of the chitosan. The bands  $1060\text{ cm}^{-1}$  (chitosan) and  $1081\text{ cm}^{-1}$  (chitosan oligomers) are determined by  $\nu(\text{C=O})$  by the bindings  $\text{C-O-H}$ ,  $\text{C-O-C}$  and  $\text{CH}_2\text{CO}$ . The bands  $892\text{ cm}^{-1}$  (chitosan) and  $892\text{ cm}^{-1}$  (chitosan oligomers) are determined by  $\omega(\text{C-H})$  from the polysaccharide's structure.

The structure of  $\alpha$ -chitin was studied rather comparatively with the forms  $\beta$ - and  $\gamma$ -, because this the most spread polymorph forms. There are few researches regarding the  $\gamma$ -chitin. It was suggested that  $\gamma$ -chitin is rather a modified form of  $\alpha$ - and even  $\beta$ -chitin, than a polymorph form [21]. In  $\alpha$ -chitin, the catenaries are arranged in layers (the catenaries of each end having the same direction or sense). In  $\beta$ -chitin, the adjacent layers are parallel and have the same direction,

while in  $\alpha$ -chitin the adjacent layers have different directions and are anti-parallel. In  $\gamma$ -chitin, every 3<sup>rd</sup> layer has an opposing direction towards the two precedent ones [22].

Figure 6 representing the absorption vs. wave number) shows in a different manner than the previous figures, the infrared spectrums in a different spectrum: absorption vs. wave number, recommended for quantitative determinations. The absorption bands can be easily assigned to some molecular fragments: the dominant band with a maximum of  $3450\text{ cm}^{-1}$  is caused by the valence's vibrations ("stretching",  $\nu\text{ O-H}$  and  $\nu\text{ N-H}$ ) of the bindings O-H and N-H intensely implicated in hydrogen bindings. The band with a maximum absorption at  $2870\text{ cm}^{-1}$  is owed to the valence's vibrations of the bindings C-H. The group of bands ranging from  $450\text{ cm}^{-1}$  and  $1750\text{ cm}^{-1}$  are characteristic to the amidic group (the bands "amide I", . . . "amide VI").

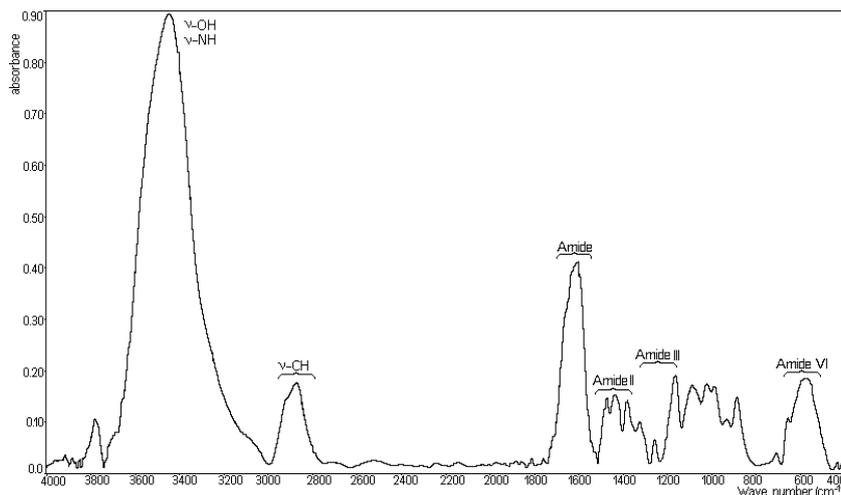


Fig. 6. The infrared spectrum of chitin (compressed in KBr)

The production of the band "amide I" is owed to a vibration mode of the amidic group in which the periodic extension of the binding C=O [23]. Due to the fact that this band is associated with the groups of acetyl of the molecule, it is justified to use it for stating the deacetylation degree of a chitosan sample (the more advanced is the acetylation degree, the more intense is this band). In order to be able to use the band's intensity "amide I", the spectrums that are obtained in different registrations must be rated. The rating can be performed by bringing (through mathematical processing) the maximum of intensity of the band  $\nu\text{ O-H}$  and  $\nu\text{ N-H}$  to 1. After this operation, the integral intensity of the band "amide I" (calculated between  $1750$  and  $1510\text{ cm}^{-1}$ ) is depending, to a significant extent, on the acetylation degree of the substance from the sample [24]. In other words, the absorbance measured to  $3450\text{ nm}$  is the internal standard. For the calibration of the connection between the integral intensity of the band "amide I" and the acetylation degree, five mixes were prepared with different proportions of chitin (stated acetylation degree 100%) and chitosan (stated acetylation degree 3 %) [25]. Table 1 shows the data of the method of preparation of the standard mixes. The degree of acetylation of the obtained mix is calculated with the formula (1).

$$\text{acetylation degree}(\%) = \frac{100 \cdot m_1 + 3 \cdot m_2}{m_1 + m_2} \quad (1)$$

where:  $m_1$ —chitin mass (in mg),  $m_2$ —chitosan mass in mg).

Figure 7 describes the stages of preparation for the standard samples and the stages of obtaining the potassium bromide tablets. The obtained tablet undergoes through the infrared spectrophotometer registration [26].

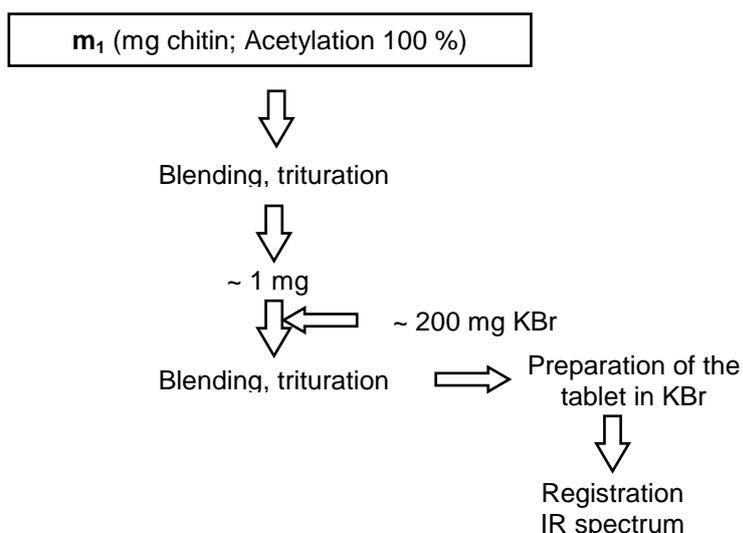


Fig. 7. The stages of preparation for the standard samples

Figure 8 represents the normalized overlapped images of “amide I” bands in the case of standard mixes. The numbers corresponding to the individual spectrums relate to the acetylation degree (Table 1).

Table 1. The preparation of standard mixes

$m_1$ (mg chitin)	$m_2$ (mg chitosan)	Acetylation degree (%)	Integral Intensity
10.3	989.7	3.999 $\approx$ 4	15.8044
10.4	191.2	8.004 $\approx$ 8	31.1149
18.6	181.5	12.016 $\approx$ 12	43.4620
13.4	86.6	15.998 $\approx$ 16	58.2787
70.1	329.8	20.003 $\approx$ 20	76.5525

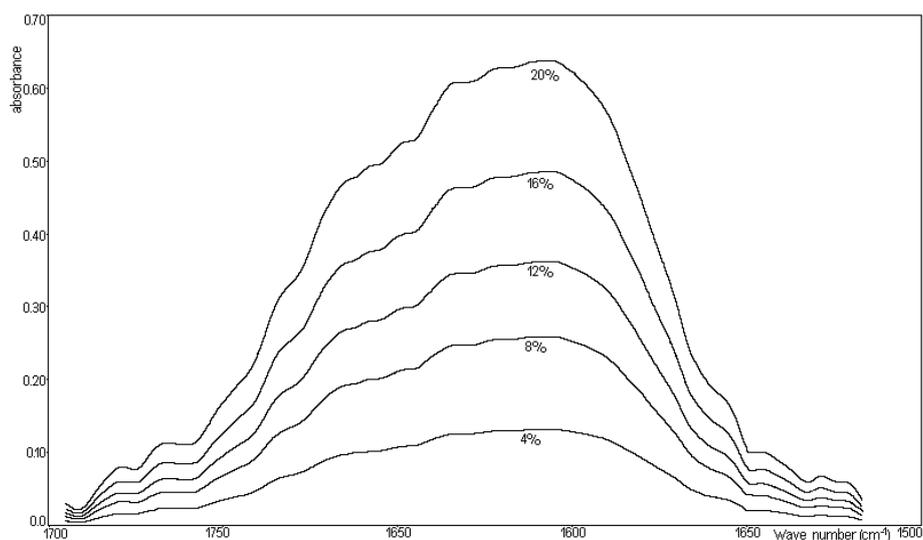


Fig. 8. Infrared spectrum of the overlapped normalized images of “amide I” bands in the case of standard mixes

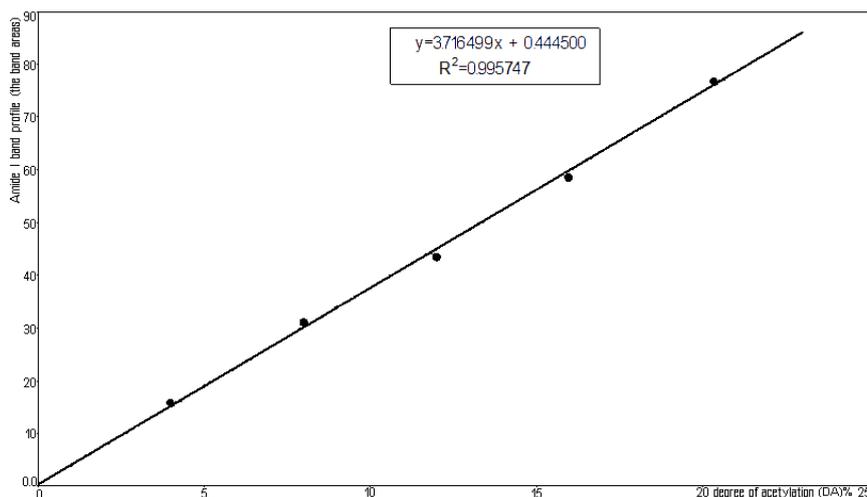


Fig. 9. Standard line (the surface of Amide I band vs. Acetylation degree)

The integral intensities (the surfaces limited by the standard curves of Figure 8 show a linear dependence on the acetylation degree) with Regression lines  $y = 3.716499x + 0.444500$  and  $r$ -squared value  $R^2 = 0.995747$  (Figure 9). On the grounds of the standard line and following an analytical procedure similar to the one presented in Figure 7, the residual acetylation degree can be determined for real samples of chitosan. Table 2 represents the results obtained within the analysis of a commercial product.

Table 2. Integral intensity and acetylation degree of a chitosan based commercial product

Integral Intensity (The amide I band)	Acetylation degree (%)
11.3338	2.93
12.0028	3.11
11.9285	3.09
11.7427	3.04
11.1480	2.88
11.2967	2.92
11.8541	3.07
12.0771	3.13
11.1480	2.88
11.7055	3.03
average (%)	<b>3.008</b>
standard deviation (%)	<b>0.096</b>

The unmodified chitosan can be dissolved only in acid solution because of the intermolecular hydrogen bindings, which limits its usages as hydrogel [27]. The chitosan based hydrogels, reticulated with molybdate polyoxianions, have a transparent structure, thermally resistant and are able to increase their dimensions several times in proportion to the initial ones, following the swelling. The chitosan shows a crystalline structure, its polymorphism depending on its physical status. Its varied structures contain an anhydrous form, a hydrated form and several salts, recently analyzed through X-ray diffraction. There are 4 polymeric chains of the hydrated forms, which pass through the unit-cell. The two adjacent chains, along the “b” axis, are independent crystallographically, arranged in anti-parallel way and tied through two connection lines of hydrogen N2....O6. For the anhydrous chitosan, the two adjacent polymeric chains can be found along the “a” axis. The chitosan represents an alternative in obtaining synthetic polycations, used as flocculation agents. The treatment of waters offers possibilities ranging from removing the humic acid from potable water up to waste water treatment techniques or mud dehydration. For that purpose to their properties of oxygen barrier, the chitosan films could be used in the content of

food or pharmaceutical products packaging. The chitosan manifests an anti-microbial activity against a range of microorganisms (fungus, yeasts, and bacteria). The anti-microbial properties of the chitosan are an advantage against other biomaterials with possible applications in the packaging industry.

#### 4. Conclusions

The importance of chitin and chitosan increased lately, on one hand due to the fact that they represent sources of renewable and biodegradable biomaterials, and on the other hand for that purpose to a better knowledge of their functionality through applications in domains such as biology, pharmacy, biotechnology, medicine and the chemistry of materials. The biodegradability, biocompatibility and bioactivity represent a unique set of biological properties that confer the chitosan a potential for applications in the medical, pharmaceutical and food industry. The biological properties of the chitosan are strictly tied to its cationic character; the determinant parameter in this case is the deacetylation degree. In some situations, though, the predominant role might be held by the molecular mass, the solubility or the conformation of the molecular chains. The proportion between the glucosaminic units and their sum with the N-acetyl-glucosamine units is known as deacetylation degree. The molecular mass, the deacetylation degree and the crystallinity of the chitosan are the main structural parameters that influence the chitosan based solubility, mechanical resistance and degradability of materials. Due to the polycationic chemical structure, the chitosan is insoluble in neutral or alkaline solvents. It is soluble in acid solutions (pH < 6.2) because of the transformation through the stoichiometric protonation of the free amine groups on the macromolecular chains in soluble form, which mean the formation of some polyelectrolyte solutions. If the amino groups of the chitosan (NH<sup>3+</sup>) are neutralized by using some double base polyol complexes and if the temperature is increased, then gels are formed following a process of phases' separation.

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