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Application of FTNIR Spectroscopy for Evaluation of the Degree of Deacetylation of Chitosan Fibres

Abstract
Microporous dibutyrylchitin (DBC) fibres spun from DBC solution in ethyl alcohol were subjected to alkaline treatment in potassium hydroxide aqueous solutions. Alkaline treatment with a diluted KOH solution at room temperature causes a gradual decrease in the number of butyric groups, which leads to obtaining fibres from the regenerated chitin. Further treatment of the fibres with a saturated KOH solution at an elevated temperature produces chitosan fibres with various degrees of deacetylation (DD), values which depend on the duration of the deacetylation process. The course of the deacetylation process was evaluated on the basis of the results of the near infrared (NIR) spectroscopy measurements. The method suggested allows for the precise measuring of the level of conversion without additional mechanical treatment (rubbing, powdering). A prepared sample is placed in the measuring chamber of the spectroscope. The FTNIR spectra of the fibres examined have characteristic bands whose analysis allows for clear determination of the level of deacetylation during chemical treatment.

Key words: *FTNIR, deacetylation degree, dibutyrylchitin fibres, chitosan fibres.*

Introduction

Chitin poly-(1-4)-(2-acetamido-2-deoksy-D-glucopyranose) is a natural polymer formed in a biosynthesis process [1]. The interest in chitin and its derivatives mainly results from the fact that these materials possess specific properties, such as biocompatibility, bioactivity and biodegradability, which makes them useful for biomedical purposes. Such properties accelerate wound healing, reduce scars, prevent inflammatory states and osteoporosis as well as strengthen the immunity of organisms without any side reactions [2 - 5].

Chitin obtained from natural sources is, however, insoluble in popular organic solvents. Therefore, to obtain chitin fibres, the initial polymer should be transformed into one of its soluble derivatives, e.g. into dibutyrylchitin (DBC). An original method for the synthesis of di-O-butyrylchitin (DBC), a soluble derivative of chitin, was worked out at the Lodz University of Technology, Poland [6]. The method of preparation of DBC and several of its chemical and physical

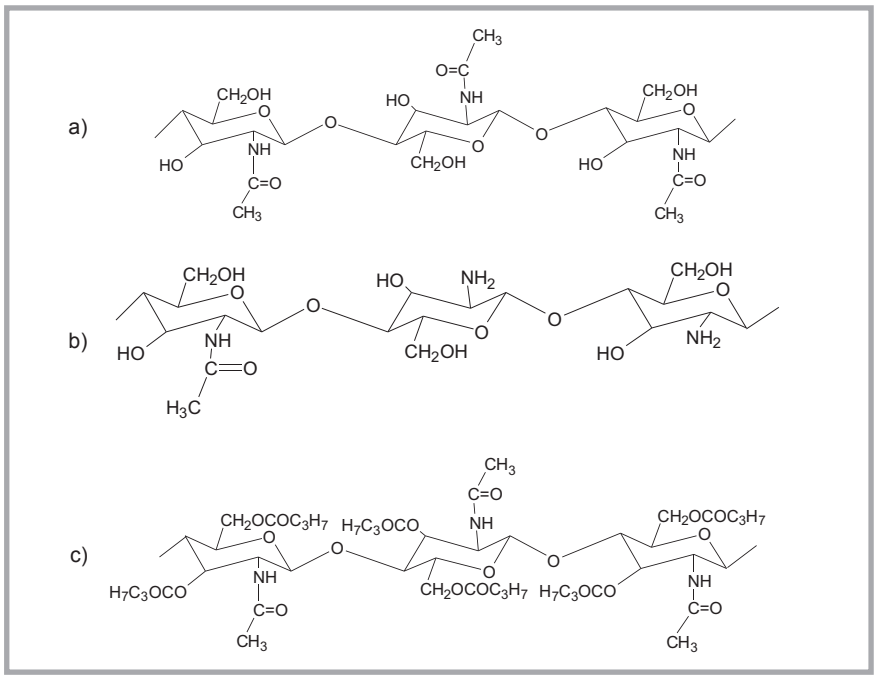

Figure 1. *Structure of a) chitin, b) chitosan, and c) dibutyrylchitin.*

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properties were described in our previous publications [6 - 9]. The easy solubility of DBC in organic solvents permits to make from it fibers, fleece, non-woven and knitting materials in forms useful for industrial applications. Dibutyrylchitin products are proposed for such as medical products as wound dressing materials or implants Investigations of the biological properties of DBC materials showed the good biocompatibility of the polymer [9 - 11].

The degree of deacetylation of chitosan has been determined by NMR spectroscopy [12, 13], IR spectroscopy [13, 14], UV spectroscopy [15], potentiometric titration [16] and CHN elemental analysis [17].

Experimental

Materials:

- krill chitin – $(C_8H_{13}O_5N)_n$ (Euphausia superba) according to technology developed at the Sea Fisheries Institute in Gdynia, Poland [18] (***Figure 1.a)***,
- chitosan – $(C_6H_{11}O_4N)_n$ a commercial product of Vanson supplied by Vanson Halo Source, USA, with a deacetylation degree of 90% and viscometric average molecular weight Mv = 346.0 kDa (***Figure 1.b)***,
- dibutyrylchitin (DBC) $(C_{14}H_{27}O_7N)_n$ - (synthesised at the Department of Physical Chemistry of Polymers, Lodz University of Technology, of Mw = 160 kDa. DBC in an esterification process for krill chitin using butyric anhydride ($(CH_3CH_2CH_2CO)_2O$) (Aldrich), and as the catalyst of the reaction - perchloric acid (70% $HCLO_4$) (Merck), [9 - 11] (***Figure 1.c)***,
- ethyl alcohol (C_2H_5OH) (POCH, Poland),
- potassium hydroxide (KOH) – (POCH, Poland).

Preparation of fibres

In this study, dibutyrylchitin prepared as described earlier [19, 20] was next dissolved in anhydrous ethyl alcohol and a concentrated solution formulated. DBC fibres were spun using a wet–dry method. The fibres, partly solidified, were then introduced into a water bath, taken up on a bobbin device, stretched twice and next dried in air. Fibres from the regenerated chitin were obtained by the reaction of the DBC fibres with 5% KOH solution at room temperature during 240 min [19, 20].

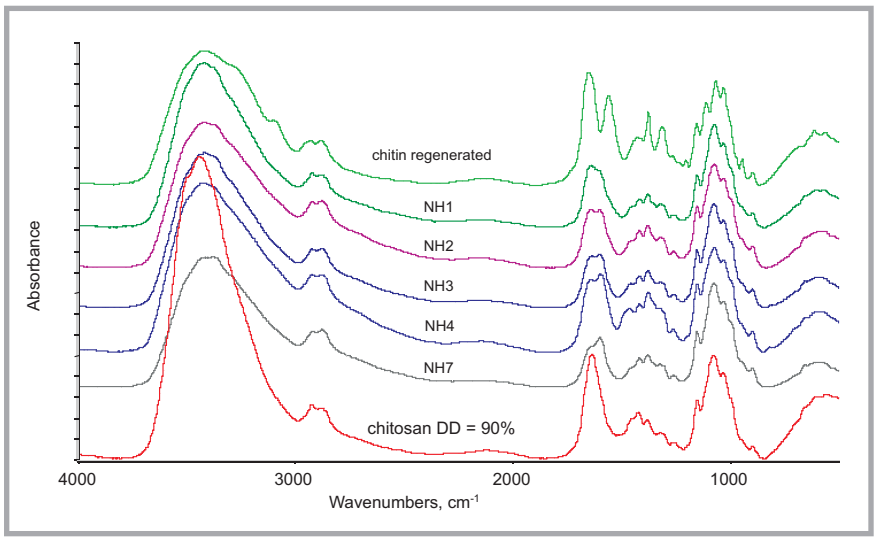

Figure 2. *FTIR spectrum within the range of 4000 - 400 cm^{-1} for chitosan (Vanson = DD 90%) and for fibres from regenerated chitin and the same fibres after alkaline treatment at 140 °C in saturated KOH solution, sample after: 10 min - NH1, 30 min - NH2, 60 min - NH3, 120 min - NH4, 240 min NH7 of deacetylationand.*

Table 1. *Wavenumbers from FTIR spectra of chitosan [21, 22].*

Sample	Wave number from FTIR, cm^{-1}	Oscillation bands
chitosan	9559	-N-H- (III overtone)
	8110	-N-H-
	6720	-O-H-
	6547	-N-H- (II overtone)
	6483	-N-H- (II overtone)
	6250	-O-H-
	5762	-C-H -

Alkaline treatment of DBC and chitin fibres

N-deacetylation was applied for fibres made from the regenerated chitin and for the DBC fibres. and N-deacetylation reactions were carried out in solutions of potassium hydroxide saturated at definite temperatures, i.e. at 70 °C – series NC (chitin fibres), at 70 °C – series ND (DBC fibres), at 100 °C – series NF (chitin fibres), at 120 °C – series NG (chitin fibres), and at 140 °C – series NH (chitin fibres).

When the reaction was completed, the fibres were rinsed several times with ethyl alcohol [19, 20].

Measurements and equipment

Spectroscopy determinations were performed in the medium IR range for DBC fibres which had undergone debutyrylation and in the near infrared range (NIR) for fibres that were deacetylated. All the measurements were carried out using a spectrometer of the MAGNA 860 type - a product of Thermo Nicolet.

Measurements in the medium IR range of 4000-400 cm^{-1}

The fibres were cut into 1 - 3 mm pieces and then blended with powdered sodium chloride (spectrally pure). The samples were then pressed at 200 MPa to obtain a tablet. The tablet was placed in the measuring chamber of the spectrometer, equipped with a mirror beam collimator (magnified 16×). As a reference, a tablet made of NaCl (without fibres) was used. The following measurement parameters were applied - range: 4000 - 400 cm^{-1}, resolution: 4 cm^{-1}, number of scans: 128, source of radiation: IR, detector: DTGS, and beamsplitter: KBr.

Measurements in the range of near infrared (NIR) 10000 - 5700 cm^{-1}

Measurements were carried out using FTNIR in the range of 10600 - 5600 cm^{-1} for fibres which were not mechanically processed. The fibres were placed in a measuring tube in such a manner as to ensure homogeneous exposure of the whole sample to the radiation. The following measuring parameters were used: range – 10600 - 5600 cm^{-1}, resolution

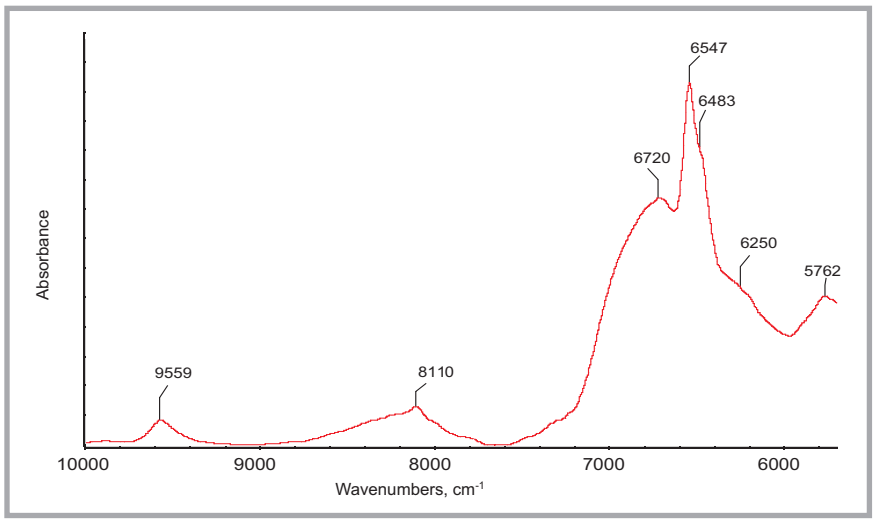

Figure 3. *FTNIR spectrum within the range of 10000 to 5700 cm^{-1} for chitosan (Vanson - DD = 90%) with the marked characteristic oscillation.*

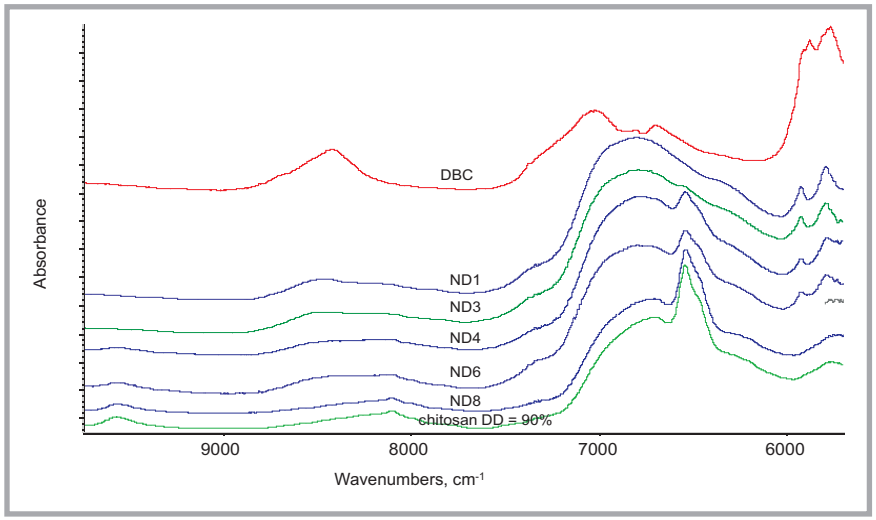

Figure 4. *FTNIR spectra ranging from 9750 cm^{-1} to 5700 cm^{-1} for DBC fibres and same fibres after alkaline treatment at 70 °C in saturated KOH solution, sample after: 10 min - ND1, 30 min - ND3, 60 min - ND4, 180 min - ND6, 1440 min - ND8 (24 h) of deacetylation and for chitosan (Vanson = DD 90%).*

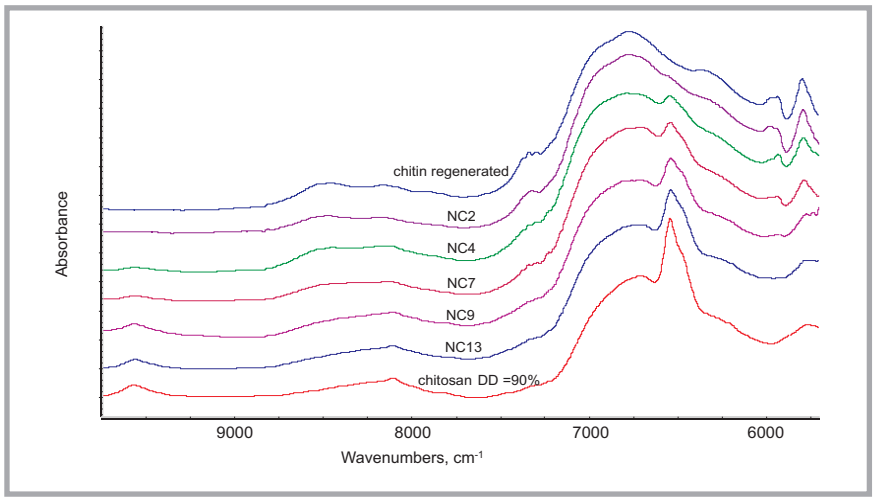

Figure 5. *FTNIR spectra ranging from 9750 cm^{-1} to 5700 cm^{-1} or fibres from regenerated chitin and same fibres after alkaline treatment at 70 °C in saturated KOH solution, sample after: 10 min - NC2, 30 min - NC4, 60 min - NC7, 120 min - NC9, 240 min - NC13 (24 h) of deacetylation and for chitosan (Vanson = DD 90%).*

– 8 cm^{-1}, number of scans – 512, source – halogene lamp detector – InGaAs, and beamsplitter – CaF_2.

After washing and drying, the fibres were placed in a measuring tube in such a manner as to ensure the homogeneous penetration of radiation through the whole sample.

The parameters of NMR spectroscopy measurements

^{13}C NMR spectra were prepared by means of a DSX 300 MHz Bruker spectrometer. The samples were placed in a 4 mm rotor made of zirconium oxide (ZrO_2) using a CP-MAS experimental, which enables the transfer of magnetisation from highly sensitive ^{1}H spins to the ^{13}C spins diluted with isotopes. The sequence modification RAMP was applied, which made the magnetisation transfer irrespective of the rotation speed.

The following measuring parameters were used: the number of accumulated points – 2048, the number of accumulations (scans) - 1024, the replication period, i.e. the break between accumulations – 6 s, mixing time – the transfer time for polarisation transfer 2.5 ms, and period - 6 dB.

Results and discussion

As a result of deacetylation, the amide group –CO-NH- in chitin gets decomposed and an amine group $–NH_2$ is formed in the polymer chain. When FTIR spectroscopy is applied (wave number ranging from 4000 - 400 cm^{-1}), only slight changes in the spectra can be observed in the course of deacetylation (***Figure 2***, see page 11). Bands of oscillators remaining in the macromolecules interfere with each other, which makes the analysis of the spectra difficult. The Amide II band mainly consists of a stretching band of a –C-N- oscillator. The C=O band interferes with the deformation oscillations of $–N<H_2$ and therefore only some deformations of the latter band can be noticed (***Figure 2***). For the above reasons the spectroscopy in the medium infrared makes the exact analysis of the course of the deacetylation process impossible.

The course of the deacetylation process expressed by changes in the degree of deacetylation values of the initial fibres and next of the chitosan fibres were es-

timated by means of near infrared spectroscopy (NIR). The method of evaluation applied enabled very precise measurement of the conversion without any additional mechanical treatment, such as grinding or powdering. The fibres were next placed in a measuring tube in such a manner as to ensure a homogeneous penetration of the radiation through the whole sample. Spectra within the range of the near infrared revealed characteristic bands, which created the basis for the degree of deacetylation determination (***Figure 3*** and ***Table 1***, see page 11) [21, 22].

For a particular series of the fibre samples, spectra from the near infrared range were made. A set of spectra for the NC, ND, NF, NH series is shown in ***Figures 4 - 8***.

Calculation of the degree of deacetylation (DD)

For all the samples examined as well as for standard commercial chitosan (products Vanson) with a definite degree of deacetylation (the value calculated from the data of potentiometric titration) from the near infrared range, spectra were obtained.

A set of spectra for series NC (as an example) is showed in ***Figure 9*** (see pages 14 - 16). As a result of the mathematical treatment of the spectra using "GRAMS" software, spectral coefficients for particular spectra were calculated from the formula:

$$F = \sum S_{-N\text{-}H} / S_w$$

where,
$S_{-N\text{-}H}$ – total area determined by the distribution of the –N-H, band for the following positions: 9559 cm^{-1}, 8110 cm^{-1}, 6547 cm^{-1} and 6483 cm^{-1};
S_w – total area spectrum.

Based on the value of the spectral coefficient calculated for standard commercial chitosan (products Vanson), the degree of deacetylation (DD) values for the samples examined were calculated from the formula:

$$DD = F \times 90\% / 0.20573 \text{ in } \%$$

where,
F – spectral coefficient for the adequate spectrum
0.20573 – the coefficient calculated for commercial chitosan (Vanson).

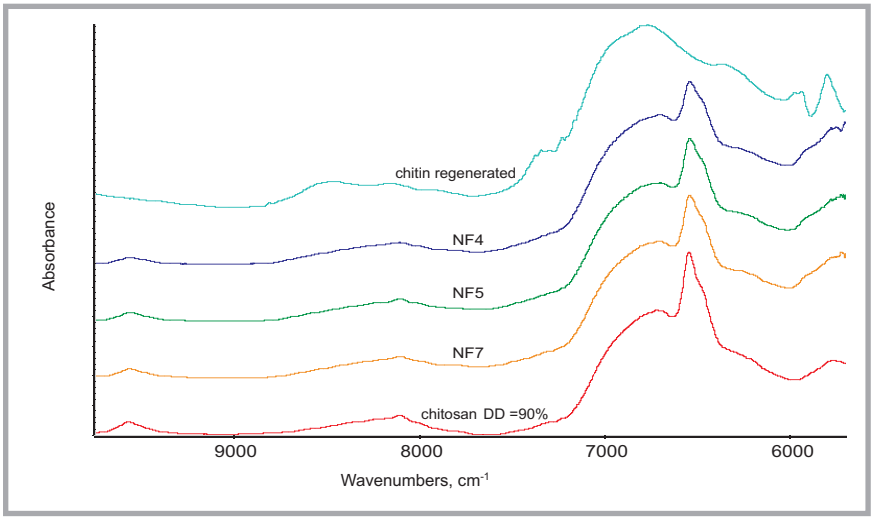

Figure 6. *FTNIR spectra ranging from 9750 cm^{-1} to 5700 cm^{-1} for fibres from regenerated chitin and selected samples of fibres after alkaline treatment at 100 °C in saturated KOH solution, sample after: 60 min - NF4, 120 min - NF5, 240 min - NF7 of deacetylation and for chitosan (Vanson = DD 90%).*

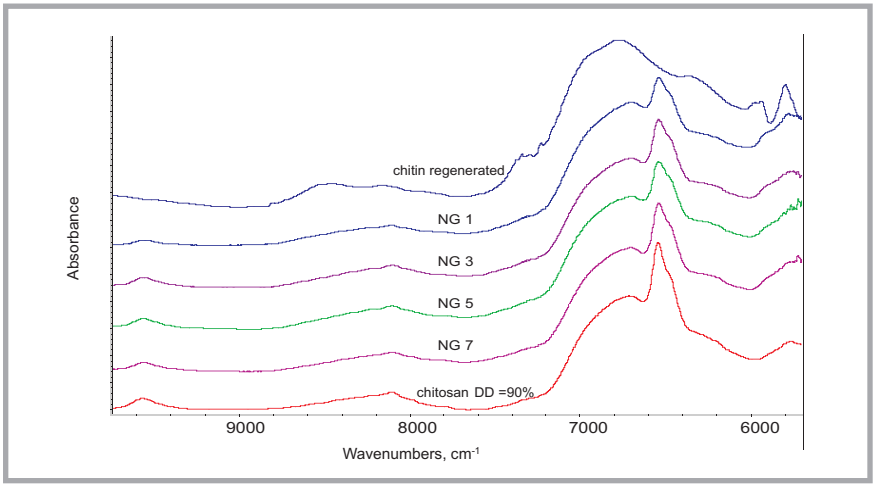

Figure 7. *FTNIR spectra ranging from 9750 cm^{-1} to 5700 cm^{-1} for fibres from regenerated chitin and selected samples of fibres after alkaline treatment at 120 °C in saturated KOH solution, sample after: 10 min - NG1, 60 min - NG3, 120 min - NG5, 240 min NG7 of deacetylation and for chitosan (Vanson = DD 90%).*

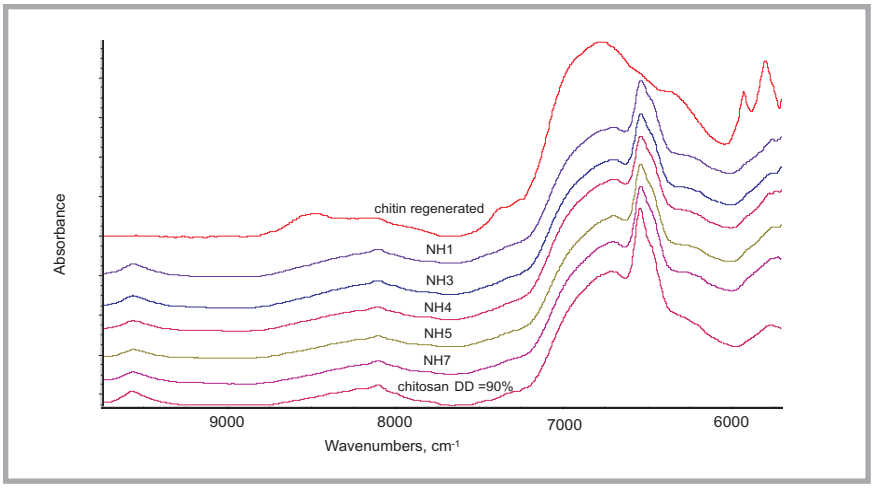

Figure 8. *FTNIR spectra ranging from 9750 cm^{-1} to 5700 cm^{-1} for chitosan (Vanson =DD 90%) and for fibres from regenerated chitin and same fibres after alkaline treatment at 140 °C in saturated KOH solution, sample after: 10 min - NH1, 30 min - NH3, 60 min - NH4, 120 min - NH5, 240 min - NH7 – sample after 240 min. of deacetylation.*

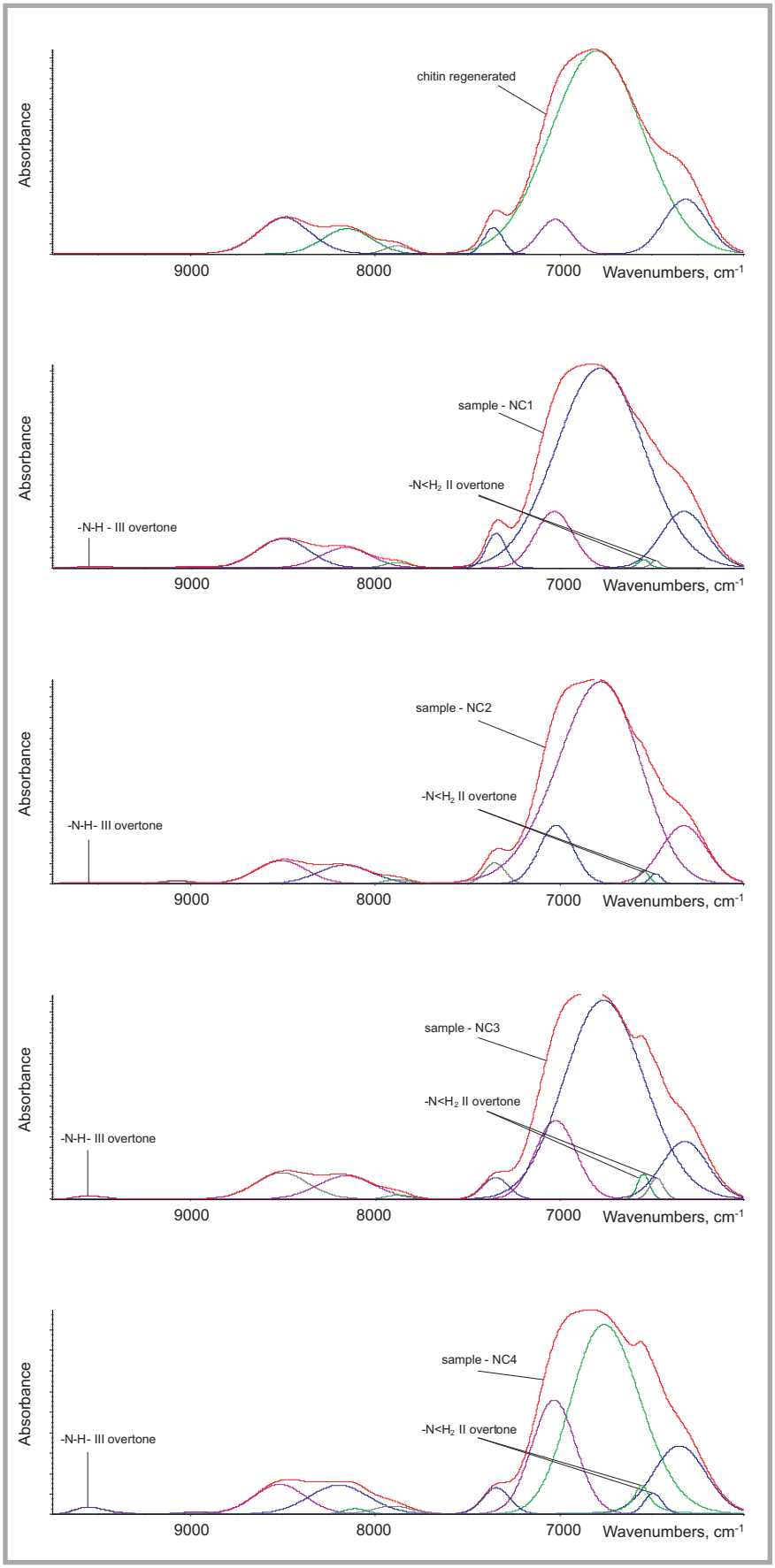

Figure 9a. *Distributions of FTNIR bands ranging from 9750 - 6000 cm^{-1} for the fibres 70 °C and 70 °C for N-deacetylated at in a saturated KOH solution as well as for chitosan (Vanson, DD = 90%) sample after: 5 min - NC1, 10 min - NC2, 20 min - NC4 of deacetylation. The diagrams were prepared by means of GRAMS software.*

Some examples of the degree of deacetylation values for the chitosan fibres (series NC, ND, NH - deacetylation) obtained from the dibutyrylchitin and regenerated chitin fibres deacetylated at 70 °C and 140 °C are given in ***Tables 2 – 5*** (see page 17).

As a result of deacetylation, the amide groups in chitin become decomposed and amide groups in the polymer chain are formed, shown in ***Figures 4 - 8*** (see page 12 - 13).

- Within the near infrared range, the overtones of the N-H group, i.e –N<H_2 appear in the form of an intensive duplet for overtone II at 6547 cm^{-1} and 6467 cm^{-1} as well as the bands for overtone III at 9558 cm^{-1} not separated from each other. However, if these bands are separated from the spectra, a quick and exact analysis of the changes occurring during the reaction is possible. The characteristic oscillation for the II and III overtones and their total of - NH bonds are shown in ***Figure 10*** (see page 18).
- At 70 °C, in a saturated KOH solution the deacetylation of fibres from the regenerated chitin runs slowly, making the quantitative control of their chemical composition possible. Under such conditions, already after 10 min of the reaction, the degree of deacetylation reaches 6%, whereas after 240 min the degree of deacetylation value is equal to 70%.

Comparison of the NC and ND series shows that the deacetylation process is slowed down. The diffusion of kalium butyrate into the fibre surface hinders the diffusion of K^+ and OH^- ions inside the fibre, which should affect the diffusion process. However, after completion of the first stage of deestrification, the number of amide groups stabilises. When DBC fibres are deacetylated for 240 min, chitosan fibres with a high degree of the deacetylation value (of about 67%) are obtained. Probably the skin structure of fibres from regenerated chitin is less porous than that of DBC fibres treated in a saturated KOH solution at 70 °C. A significant influence on the skin and its permeability is probably exerted by an aqueous medium in which the fibres get swollen.

The temperature of deacetylation significantly affects the rate of the process. At

140 °C, the deacetylation of chitin fibres runs quickly and already after 10 min chitosan fibres with a degree of the deacetylation value of about 78% are obtained. The longer the time of the reaction (240 min), the higher the degree of deacetylation values (93%) are obtained, as shown in ***Figures 11 - 12*** (see page 18).

The degree of deacetylation values for chitosan fibres (series NC - obtained from regenerated chitin deacetylated at 70 °C, series ND - obtained from DBC fibres deacetylated at 70 °C, series NH - obtained from regenerated chitin deacetylated at 140 °C, and for all series after 240 min. of deacetylation are given in ***Tables 2 – 5*** (see page 17) is shown in ***Figures 11 - 12*** (see page 18).

The results of the deacetylation process for both DBC fibres as well as those from regenerated chitin are affected by many factors. As it was found out, such factors as the temperature, time of the reaction and the origin of the initial material (i.e. DBC fibres or chitin fibre) play an important role. However, other factors such as the concentration of KOH solution, the diffusion of alkalia into the fibre structure etc. should also be considered.

Additionally the degree of deacetylation (DD) was calculated on the basis of the results from ^{13}C NMR spectroscopy. A few spectra for the NC series (deacetylation at 70 °C) were prepared. Using the intensity proportion of CH_3 to the C_1 group (C_1 - the first atom of glicoside ring), the DDA value was calculated from the following equation [14]:

$$\mathbf{DD = 1 - A_{CH_3}/A_{C_1} \times 100\%}$$

where,
$\mathbf{A_{CH_3}}$ - absorption intensity of CH_3 group,
$\mathbf{A_{C_1}}$ - absorption intensity of C_1 group.

The results are presented in ***Table 6***. As it can be seen from ***Table 6***, the results of the degree of deacetylation obtained by means of the ^{13}C NMR method remain in good correlation with those obtained using the FTNIR method presented in ***Tables 2 - 5***. Thus the FTNIR measurements can serve as a tool to obtain reliable results for the degree of deacetylation.

Conclusions

Deep hydrolysis by saturated KOH solutions at higher temperatures (70, 100,

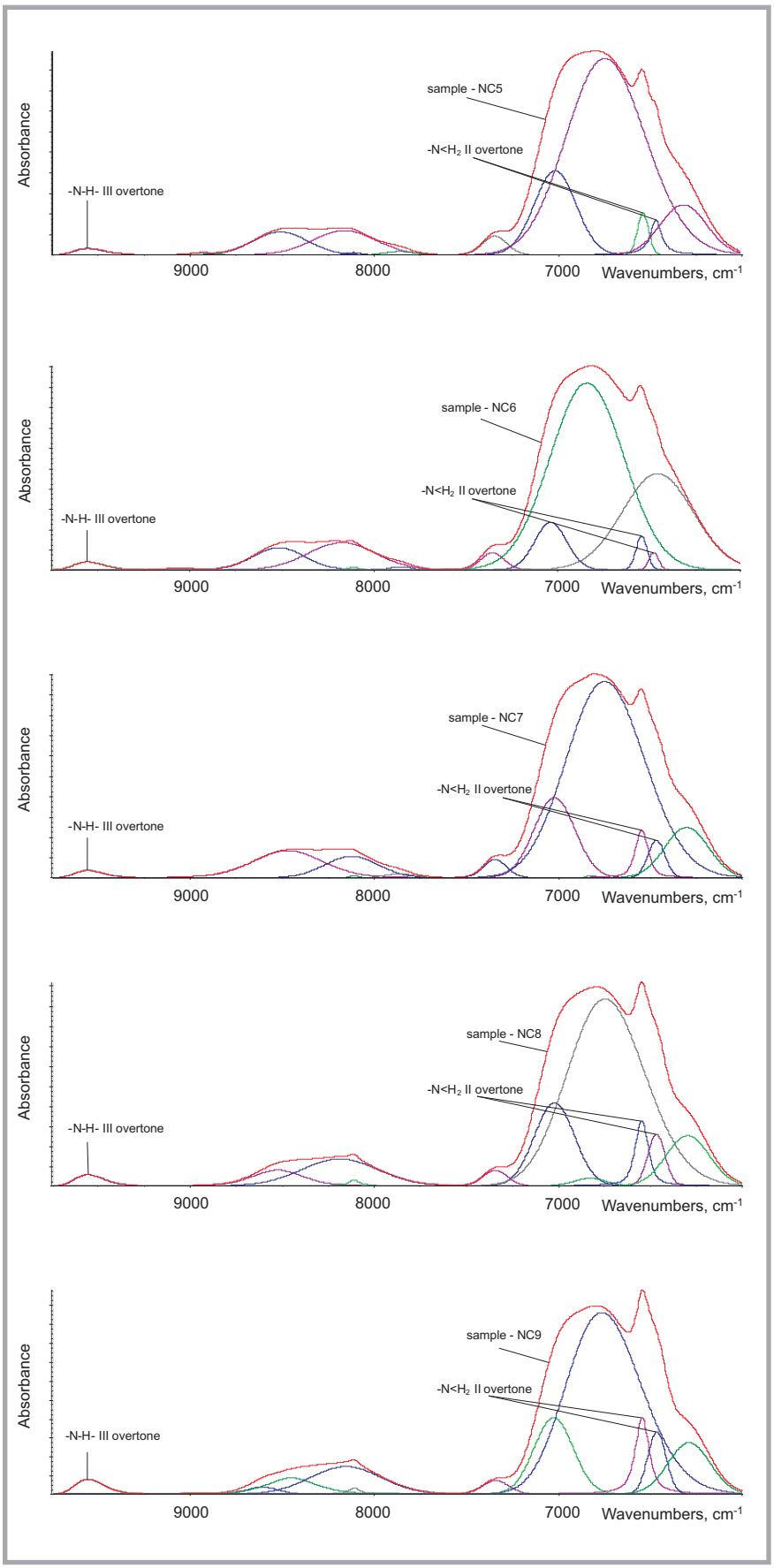

Figure 9b. *Distributions of FTNIR bands ranging from 9750 - 6000 cm^{-1} for the fibres 70 °C and 70 °C for N-deacetylated at in a saturated KOH solution as well as for chitosan (Vanson, DD = 90%) sample after: 40 min - NC5, 50 min - NC6, 60 min - NC7, 90 min - NC8, 120 min - NC9 of deacetylation. The diagrams were prepared by means of GRAMS software.*

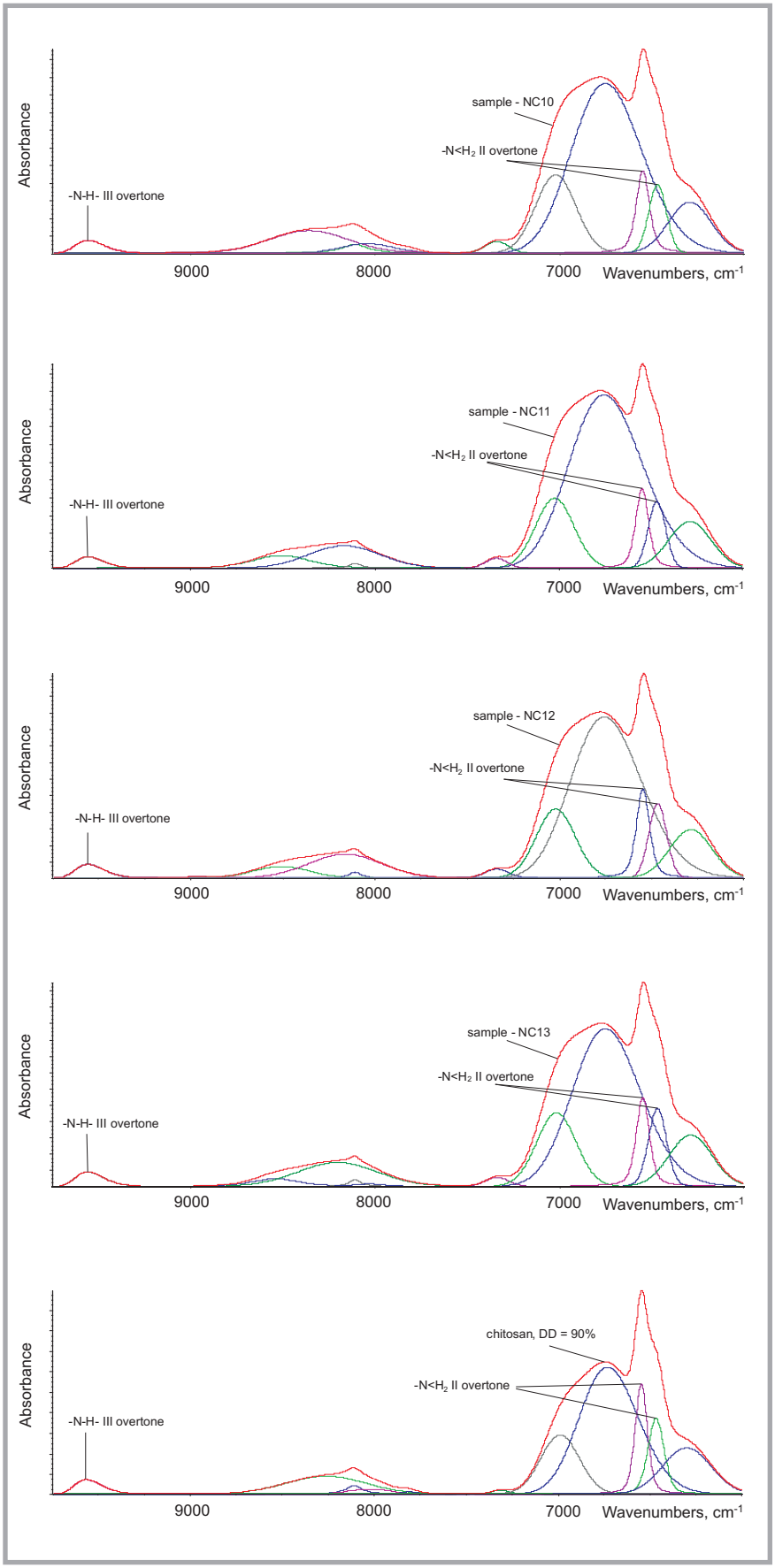

Figure 9c. *Distributions of FTNIR bands ranging from 9750 - 6000 cm^{-1} for the fibres 70 °C and 70 °C for N-deacetylated at in a saturated KOH solution as well as for chitosan (Vanson, DD = 90%) sample after: 150 min - NC10, 180 min - NC11, 210 min - NC12, 240 min - NC13 of deacetylation. The diagrams were prepared by means of GRAMS software.*

120, and 140 °C) leads to chitin deacetylation and obtaining microporous chitosan fibres with various degrees of deacetylation values.

The degree of deacetylation values depends on the concentration of KOH solution as well as on the time and temperature of the treatment. At high temperatures the deacetylation runs quickly and chitosan fibres with a high degree of deacetylation values can be obtained. This tendency is illustrated well in ***Table 5***.

A new method of calculating the degree of deacetylation based on the analysis of FTNIR spectra and adequate software was elaborated. Using the FTNIR method to determine the degree of deacetylation values for chitin or chitosan fibres is easy and quick. Besides it requires no special preparation of the samples.

The application of FTNIR analysis to determine the degree of deacetylation by means of GRAMS software correlates well with the results obtained from the NMR method. Additionally, the FTNIR method to determine the degree of deacetylation values for chitosan fibres is the most sensitive method.

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Table 2. *Degree of deacetylaction (DD) values for chitosan fibres (series NC) obtained from regenerated chitin deacetylated at 70 °C.*

Sample	Alkaline treatment, min	Total area spectrum ± 0.01	Area of peak –N-H at ± 0.01, cm^{-1}				Spectral coefficient, F ± 0.00001	Degree of deacetylaction (DD), %
			9559	8110	6547	6483		
N C1	5	815.00	1.52	-	2.98	2.54	0.00869	3.8
N C2	10	774.97	2.15	-	4.63	3.56	0.01343	5.9
N C3	20	810.35	2.94	-	9.58	9.58	0.02743	12.0
N C4	30	865.78	6.50	-	12.64	5.39	0.31970	14.0
N C5	40	823.95	6.17	-	15.27	12.10	0.04739	20.7
N C6	50	836.26	7.70	0.79	22.26	18.37	0.04859	21.3
N C7	60	848.66	6.76	0.82	24.95	21.40	0.06387	27.9
N C8	90	853.36	10.96	3.21	36.09	30.26	0.09483	41.5
N C9	120	871.67	15.12	3.44	50.25	40.75	0.12627	55.2
N C10	150	861.72	14.48	3.33	46.63	44.45	0.12723	55.7
N C11	180	840.95	12.12	3.26	51.48	46.81	0.13541	59.2
N C12	210	879.03	15.96	4.12	57.33	54.21	0.15048	65.8
N C13	240	882.22	16.87	4.76	58.02	59.21	0.15891	69.5
chitosan Vanson	-	566.00	13.41	6.70	51.40	44.93	0.20573	90.0

Table 3. *Degree of deacetylaction (DD) values for chitosan fibres (series ND) obtained from DBC fibres deacetylated at 70 °C.*

Sample	Alkaline treatment, min	Total area spectrum ± 0.01	Area of peak –N-H at ± 0.01, cm^{-1}				Spectral coefficient, F ± 0.00001	Degree of deacetylaction (DD), %
			9559	8110	6547	6483		
N D3	30	836.51	1.227	-	4.069	3.139	0.01014	4.4
N D4	60	817.48	5.530	0.883	13.053	8.435	0.03442	15.1
N D5	120	859.31	5.526	2.640	33.890	20.375	0.07031	31.9
N D6	180	891.56	10.780	3.998	50.090	36.917	0.11468	50.2
N D7	240	689.26	11.496	3.063	54.269	36.072	0.15301	66.9
N D8	1440	583.65	10.406	2.589	56.526	39.672	0.18812	82.3
chitosan Vanson	-	566.00	13.413	6.700	51.400	44.930	0.20573	90.0

Table 4. *Degree of deacetylaction (DD) values for chitosan fibres (series NH) obtained from regenerated chitin deacetylated at 140 °C.*

Sample	Alkaline treatment, min	Total area spectrum ± 0.01	Area of peak –N-H at ± 0.001, cm^{-1}				Spectral coefficient, F ± 0.00001	Degree of deacetylaction (DD), %
			9559	8110	6547	6483		
N H2	20	658.161	13.235	3.079	60.231	42.321	0.18115	79.2
N H3	30	622.162	13.112	3.120	52.235	45.653	0.18342	80.2
N H4	60	644.408	12.785	3.113	61.332	46.651	0.19224	84.1
N H5	120	611.121	13.561	3.253	63.251	45.221	0.20501	89.7
N H6	180	604.004	13.782	3.412	65.324	44.323	0.21000	91.8
N H7	240	625.527	15.112	3.521	67.221	46.320	0.21130	92.9
chitosan Vanson	-	566.000	13.413	6.700	51.400	44.930	0.20573	90.0

Table 5. *Degree of deacetylaction (DD) values for chitosan fibres obtained from DBC fibres (series ND) and regenerated chitin (series NC, NF, NG, NH) treated for 240 min at different temperatures using various KOH concentrations and chitosan – Vanson=90% DD.*

Sample	Alkaline treatment, min	Total area spectrum ± 0.001	Area of peak –N-H at ± 0.001, cm^{-1}				Spectral coefficient, F ± 0.00001	Degree of deacetylaction (DD), %
			9559	8110	6547	6483		
NC13	240	882.220	16.870	4.760	58.020	59.210	0.15891	69.5
NF7	240	701.734	15.322	5.231	60.221	46.324	0.18112	79.2
NG7	240	634.814	15.651	5.336	62.652	45.698	0.20374	89.1
NH7	240	625.527	15.112	3.521	67.221	46.320	0.21130	92.9
chitosan Vanson	-	566.000	13.413	6.700	51.400	44.930	0.20573	90.0

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Table 6. *Degree of deacetylaction (DD) values obtained by the ^{13} CNMR method.*

Sample	Absorption			Degree of deacetylaction (DD), %
	$-CH_3 \pm 0.01$	$-C_1 \pm 0.01$	$-CH_3/C_1 \pm 0.001$	
NC7	19.60	24.65	0.795	20.5
NC9	10.46	24.86	0.421	57.9
NC13	7.54	24.62	0.306	69.4

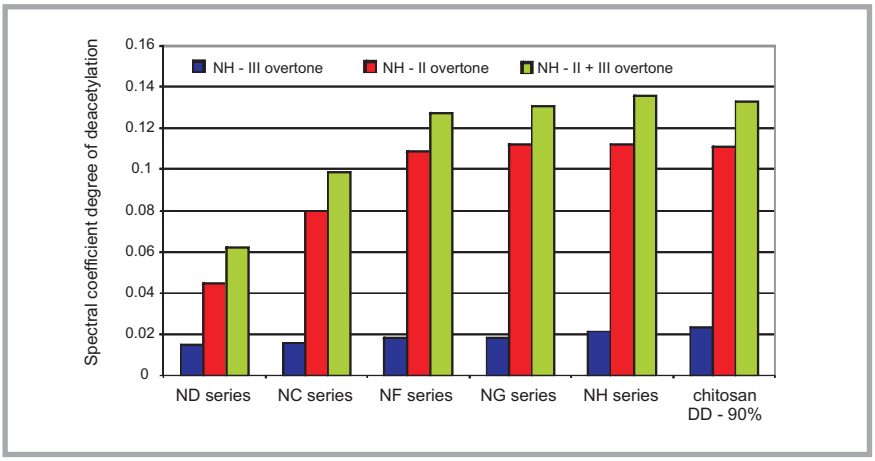

Figure 10. *Characteristic overtones of oscillations II and III and its total of - NH bonds.*

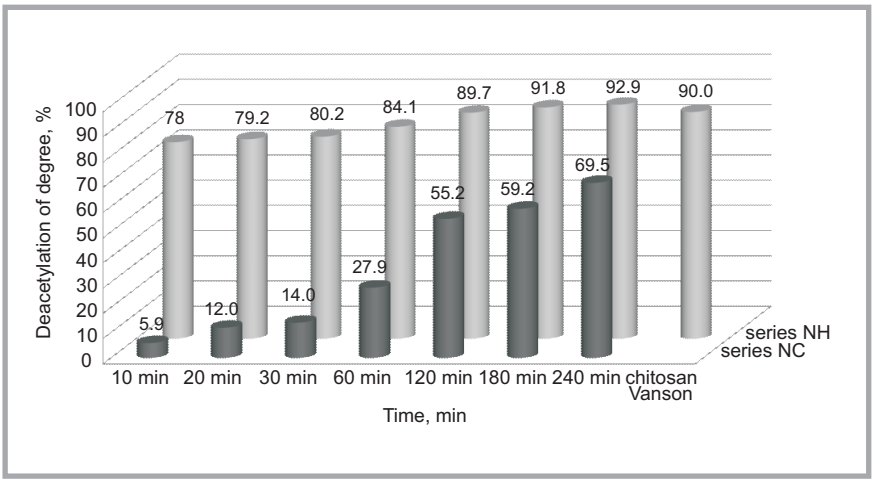

Figure 11. *Deacetylation of degree values for chitosan fibres determined from spectral coefficients in relation to Vanson chitosan for samples from series NC (deacetylation of chitin fibres at 70 °C in saturated KOH solution) and NH (deacetylation of chitin fibres at 140 °C in saturated KOH solution).*

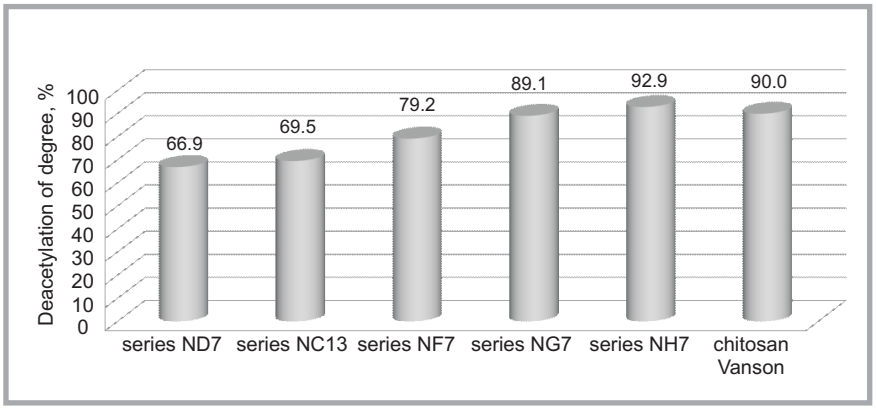

Figure 12. *Deacetylation of degree values of chitosan fibres determined from spectral coefficients in relation to Vanson chitosan. All samples treated for 240 minuntes at different temperatures using various KOH concentrations.*

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