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Structure analysis and degree of substitution of chitin, chitosan and dibutyrylchitin by FT-IR spectroscopy and solid state ^{13}C NMR

Kathleen Van de Velde*, Paul Kiekens

Department of Textiles, Ghent University, Technologiepark 907, B-9052 Zwijnaarde, Belgium

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Abstract

Chitin, an important constituent of the exoskeleton of many organisms such as crustacea and insects, and its derivates promote the ordered healing of tissues and are therefore very suitable for use in wound dressings. The degree of substitution (DS) is an important parameter when assessing the conversion of chitin into one of its derivates. The degree of acetylation of chitin and chitosan and the degree of butyrylation of dibutyrylchitin was evaluated. It is found that FT-IR spectroscopy is a relatively easy but indirect way of determining the DS. FT-IR spectroscopy proved to be very useful for comparing the degrees of conversion and -substitution, as well as for differentiating between different chitin types. Absolute DS determinations by FT-IR however are only reliable when a calibration, using a direct technique such as ^{13}C-NMR, is made.

Keywords: Chitin; Chitosan; Dibutyrylchitin; FT-IR spectroscopy; Degree of substitution; Solid state ^{13}C NMR

1. Introduction

Depending upon its crystalline structure, chitin exists in several forms with each their specific properties. Two of these crystalline polymorphic forms are α-chitin and β-chitin. α-Chitin is extracted from shrimp or crab shells and has an antiparallel structure with strong intermolecular hydrogen bridges. β-Chitin is extracted from squid or loligo pens and exhibits weaker intermolecular hydrogen bonding due to the parallel arrangement of the polymer chains (Kim, Kim, & Lee, 1996). These differences in structure become clear in techniques such as infrared spectroscopy and ^{13}C-NMR where certain bands have the tendency to merge or become less well separated when comparing β-chitin with α-chitin (Focher, Naggi, Torri, Cosani, & Terbojevich, 1992).

The degree of acetylation (DA) is the share of nitrogen sites occupied by acetyl groups (each nitrogen atom can react with one acetyl group). The DA is hardly ever 100% in commercially available chitin since chitin purification involves alkali treatment for protein removal, also resulting in a lower DA. Values of about 90% are typical for chitin (Ravi Kumar, 2000). An extra reaction is necessary to reach 100% DA. Reaction with acetic anhydride in a dry solvent (e.g. methanol) results in *N*-acetylation. Sometimes methanesulfonic acid or perchloric acid is used as catalyst, but this may result in a lower molecular mass. Undesired *O*-acetylation—occurring after *N*-acetylation—may be removed by diluted alkali treatment (East & Qin, 1993; Nishi, Noguchi, Tokura, & Shiota, 1979).

Chitosan is obtained by deacetylating chitin. This lower-DA derivative product results from reaction with concentrated alkali at elevated temperatures at prolonged exposures, and is soluble in weak acidic solutions. In the case of homogeneous deacetylation, a DA $<60\%$ is enough to result in chitosan. If heterogeneous conditions apply, the necessary DA is lower (Kurita, Kojima, Nishiyama, & Shimojoh, 2000; Ottøy, Vårum, & Smidsrød, 1996).

Chitin and derivates are (partially) biodegradable in the presence of human enzymes and are non-toxic and beneficial to the human body. When used to heal wounded tissues, the healing process is facilitated, allergies and undesirable reactions are absent, and desired reactions with

* Corresponding author. Tel.: +32-9-264-5741; fax: +32-9-264-5846.
E-mail address: kathleen.vandevelde@ugent.be (K. Van de Velde).

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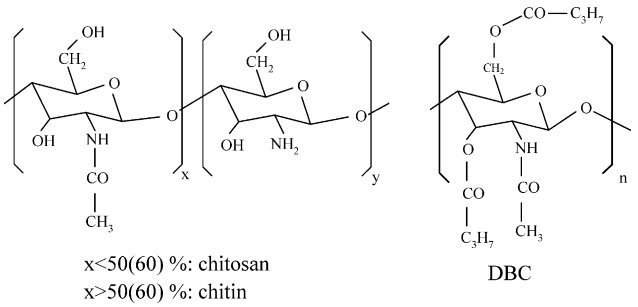

Fig. 1. Structural formulas of chitin, chitosan, and dibutyrylchitin (DBC).

antiseptic agents occur (Paluch et al., 2000; Szosland, Szumilewicz, & Struszczyk, 1996). However, chitin is insoluble in common organic solvents, and therefore direct industrial application is difficult. Recently dibutyrylchitin (DBC, an ester of chitin) synthesis was elaborated (Szosland & Janowski, 1996). DBC is easily soluble in common organic solvents and has film and fibre forming properties. First biological investigations showed its biocompatibility, bacteriostatic characteristics and sensibility to enzymatic degradation. This invention opens the way for production of a wide assortment of novel functional biomaterials made from DBC and regenerated chitin from DBC (Szosland et al.; Van de Velde, Szosland, & Krucińska, 2003).

Several types of chitin, chitosan and DBC were studied with FT-IR and solid state ^{13}C NMR in order to establish differences in structure and degree of substitution (DS) (and thus degree of conversion). The structural formulas of the studied products are given in Fig. 1.

2. Experimental

2.1. Materials

Four types of chitin were available:

- 'α-chitin' from shrimp shells (60 mesh), from France Chitin;
- 'β-chitin' from squid bones (500 μm), from France Chitin;
- 'chitin S': shrimp chitin powder from France Chitin, treated with 1.5 M HCl (1 day), 0.5 M NaOH (3 days), 1 M NaOH (3 days), and acetone (12 h);
- 'Aldrich chitin' from crab shells (product no. 41795-5), from Sigma-Aldrich. The manufacturer reports a deacetylation of 0% for this α-chitin type. But it was unclear whether this implied a lack of deacetylation or a real DA of 100%.

For acetylation reactions acetic anhydride (Fluka p.a., ≥99%) and methanol (Riedel-deHaën, min. 99.8%) were used, while deacetylation involved 40% (w/w) NaOH.

Purified Aldrich chitin was obtained after extraction in a buffered acid solution with anhydrous sodium acetate (VEL, purity >98%), acetic acid (Chem Lab, p. 99–100%, dens. 1.05 g/cm^3) and hydrochloric acid (Riedel-deHaën, p.a., min. 37%, dens. 1.19 g/cm^3).

Two types of chitosan were available:

- 'Chitosan 2' (500 μm, DA = 2%) made from squid chitin, from France Chitin;
- 'Chitosan 20' (100 mesh, DA = 20%) made from shrimp chitin, from France Chitin.

The potassium bromide that was used for FT-IR measurements, was type 'Spectrosol' from BDH Laboratory Supplies.

2.2. Reactions

Acetylation was performed on dried α-chitin and β-chitin (0.5 g at 105 °C during 3 h) that was immediately suspended into a mixture of 19.4 ml acetic anhydride and 174 ml methanol. This suspension was continuously shaken and kept at 40 °C for 3 h. Afterwards, the acetylated chitin was recuperated on a G2 glass filter and washed with pure methanol. After drying at 50 °C (during 60 h), the chitin was re-suspended into 174 ml of 1 M NaOH in order to eliminate unwanted *O*-acetylation (during 8 h at room temperature). After neutralising with a little diluted acetic acid and washing with water, the sample was dried overnight at 105 °C. This procedure should provide complete acetylation and thus a DA of 100% (East et al., 1993).

Deacetylation was performed on 1 g of unconditioned α-chitin and β-chitin suspended into 100 ml of 40% (w/w) NaOH and kept at 117 °C during 3 h (suspension most of the time quietly boiling). This procedure was repeated twice. This procedure should provide complete deacetylation and thus chitosan with a DA of 0% (Kurita et al., 2000). Afterwards, the sample was abundantly washed with water until neutral and then dried at 50 °C (during 60 h).

A purification (extraction) step was performed on the Aldrich chitin sample. This step should remove chains with low DA (chitosan) thus resulting in a higher overall DA. A typical solvent for chitosan [buffered acid solution: 0.02 M CH_3COONa + 0.02 M CH_3COOH + 0.1 M HCl] was used for this purpose (Berth, Dautzenberg, & Peter, 1998). Three dry G2 glass filters with each approximately 1 g dry chitin (16 h at 105 °C) were put into a beaker with approximately 200 ml buffered acid solution for 30′. Then the beaker was put into an ultrasonic bath for 1′. Each glass filter was rinsed (using vacuum) with some of the acid from the beaker and the whole procedure was repeated two times on the same samples. Afterwards, each glass filter (+chitin) was rinsed with approximately 150 ml buffered acid solution, followed by 1×40 ml demineralised water, 1×40 ml diluted NH_4OH, and finally 7×40 ml demineralised water. After drying at 105 °C for 16 h, the amount of extracted matter could be calculated by comparing dry masses before and after extraction.

Two types of DBC (dibutyrylchitin), starting from α-chitin, were produced in Poland (Szosland et al., 1996, 2001). A first synthesis was done under heterogeneous conditions, a different procedure is used for the second modification and this DBC was additionally washed. Reagents were butyric anhydride and perchloric acid (catalyst). These samples will be called:

- 'DBC (TUL)'. Synthesis was carried out at +8 to +18 °C and was finished after 3 h 50′. It has an estimated weight average molecular mass of 172,550 g/mol.
- 'DBC (IDOP)'. It has an estimated weight average molecular mass of 161,670 g/mol.

2.3. FT-IR measurements

Measurements were carried out on a Perkin Elmer Spectrum GX (FT-IR System). Previous tests have pointed out (Van de Velde et al., 2003) that chitin rapidly absorbs rather large amounts of water which would interfere with IR measurements. Combining literature data (Duarte, Ferreira, Marvão, & Rocha, 2002; Khan, Peh, & Ch'ng, 2002; Sannan, Kurita, Ogura, & Iwakura, 1978) with own experience led to following procedure designed to avoid presence of moisture. A small amount of sample was dried at 105 °C for at least 1 h and consequently grinded for 5′. KBr that is permanently kept at 50 °C was first grinded manually in a mortar and per sample type three sample/KBr mixtures (approx. 1/100 w/w and 0.22 g in total) were made. These were put for at least 1 h in an oven at 105 °C, then grinded for 5′ and put back into the oven to stay overnight. The next day pellets (13 mm diameter) were pressed without any further grinding, as soon as possible after removal from the oven (force between 100 and 120 kN). Also one KBr pellet was made as a reference. The pellets were then put into a desiccator (containing silica gel) in an oven at 95 °C, for 3 h and FT-IR was done as soon as the pellets were taken out of the oven.

Absorbance values (A) were determined between 4000 and 400 cm^{-1}. These values were used since they are proportional to concentrations (Lambert–Beer law). This only applies in the proportional part of the concentration-absorbance relation (when A is sufficiently low; i.e. well below 1). Several authors report on the possibility of determining the DA of chitin and chitosan by comparing a peak that is proportional to the DA (measurement peak 'M') to one that is independent of the DA (reference peak 'R'). Some possible peaks are listed in Table 1. The limits of the baseline intervals were chosen in such a way that—in the majority of the cases—they coincided with minima in A-values. Once set, however, they were not adapted to eventual shifts in A-minima since this resulted in an increased variance on the obtained results. Equations as found in literature are listed in Table 2. The difference between II and III is due to the different baselines, respectively, 2a and 2b. The DA range in Table 2 corresponds to the range of DA values as they were determined in the reference in question. The actual valid DA range may however be larger.

No similar method for the degree of butyrylation (of DBC) is found in literature. However, comparing some relevant peaks (that are proportional to the butyrylation) might also give an idea of the degree of butyrylation. These peaks however first will have to be identified (see Section 3.1).

Table 1
Overview of useful absorbance peaks for DA determination

Wave number (cm^{-1})	Type	Explanation of band	Used baseline
3450	R	Hydroxyl stretching	1a
2878	R	C–H stretching	1b
1420	R	C–H deformations	1c
1550	M	C=O stretching in secondary amide (amide II)	2a
1661	M	C=O stretching in secondary amide (amide I)	2b
1655	M	C=O stretching in secondary amide (amide I)	2a or 2b
1625	M	C=O stretching in secondary amide (amide I)	2b
1320	M	C–N stretching in secondary amide (amide III)	2c
Used intervals (cm^{-1}) for baselines	1a: 1950–3840, 2a: 1242–1850	1b: 2670–3010, 2b: 1602–1750	1c: 1402–1478, 2c: 1276–1348

Table 2
FT-IR equations for DA determination as mentioned in literature

No	DA (%)	DA range	References
I	$35.461A_{1550}/A_{2878}$	10–95%	Duarte et al. (2002) and Sannan et al. (1978)
II	$75.188A_{1655}/A_{3450}$	40–60%	Khan et al. (2002)
III	$115A_{1655}/A_{3450}$	40–60; ~70%	Khan et al. (2002) and Khor (2001)
IV	$442.48A_{1320}/A_{3450}-13.92$	0–100%	Brugnerotto et al. (2001)
V	$31.918A_{1320}/A_{1420}-12.20$	0–100%	Monal, Desbrières, and Rinaudo (2000)
VI	$85.5[A_{1650}/A_{3450}+A_{1630}/A_{3450}-0.13]$[a]	70–90%	Huang, Moon, and Pal (2000)

[a] This formula was meant for films. When testing with KBr pellets, peaks shifted to 1661 and 1625 cm^{-1}. The latter were used instead of the originally proposed peaks at 1650 and 1630 cm^{-1}.

2.4. Solid state ^{13}C-NMR measurements

Solid state cross-polarization/magic angle spinning CP/MAS ^{13}C NMR is a direct way of DA determination. Tests were carried out on a Varian at a recording frequency of 50.32 MHz. Spinning rate was 4000 Hz and 2048 scans were taken (Ottøy et al., 1996). The expected ^{13}C signals are: C1(δ104.5–104.6 ppm), C2(δ55.6–55.7 ppm), C3(δ74.0 ppm), C4(δ83.6–83.8 ppm), C5(δ76.1 ppm), C6(δ60.9–61.5 ppm), and CH_3(δ23.2–23.3 ppm). Worth mentioning is the fact that C3 and C5 tend to form one single resonance in β-chitin, whereas two separate resonances occur in α-chitin (Zhang, Haga, Sekiguchi, & Hirano, 2000). This is comparable to the effect in the 3500–3000 cm^{-1} interval in FT-IR testing.

The DA is calculated in the following way (Ottøy et al., 1996):

$$\mathrm{DA}\ (\%) = 100I[\mathrm{CH_3}]/(\{I[\mathrm{C1}] + I[\mathrm{C2}] + I[\mathrm{C3}] + I[\mathrm{C4}] + I[\mathrm{C5}] + I[\mathrm{C6}]\}/6)$$

with I the intensity of the resonance peak in question.

3. Results and discussion

3.1. IR spectrum analysis

In order to compare the obtained spectra, an overview is given in Fig. 2. Each spectrum is the average of three tests and all spectra are shifted upwards to prevent overlap.

A more detailed structure in the 3500–3000 cm^{-1} region was observed for α-chitin compared to β-chitin, where a broader band dominates. This is in accordance with literature (Focher et al., 1992) and thought to be caused by increased hydrogen bridge formation. If IR-measurements were to be done on cast films, this difference would not be found since dissolution and consequent precipitation would lead to the thermodynamically most stable structure, which is α-chitin (Saito, Okano, Gaill, Chanzy, & Putaux, 2000).

The observed tendencies (when comparing chitin derivates) were studied by comparing absorbance peaks as they are mentioned in several databases on IR spectra (Martin, 2003; Williams & Fleming, 1980; Vidrine, 1997).

Most absorbance peaks confirm that indeed the desired reactions have taken place.

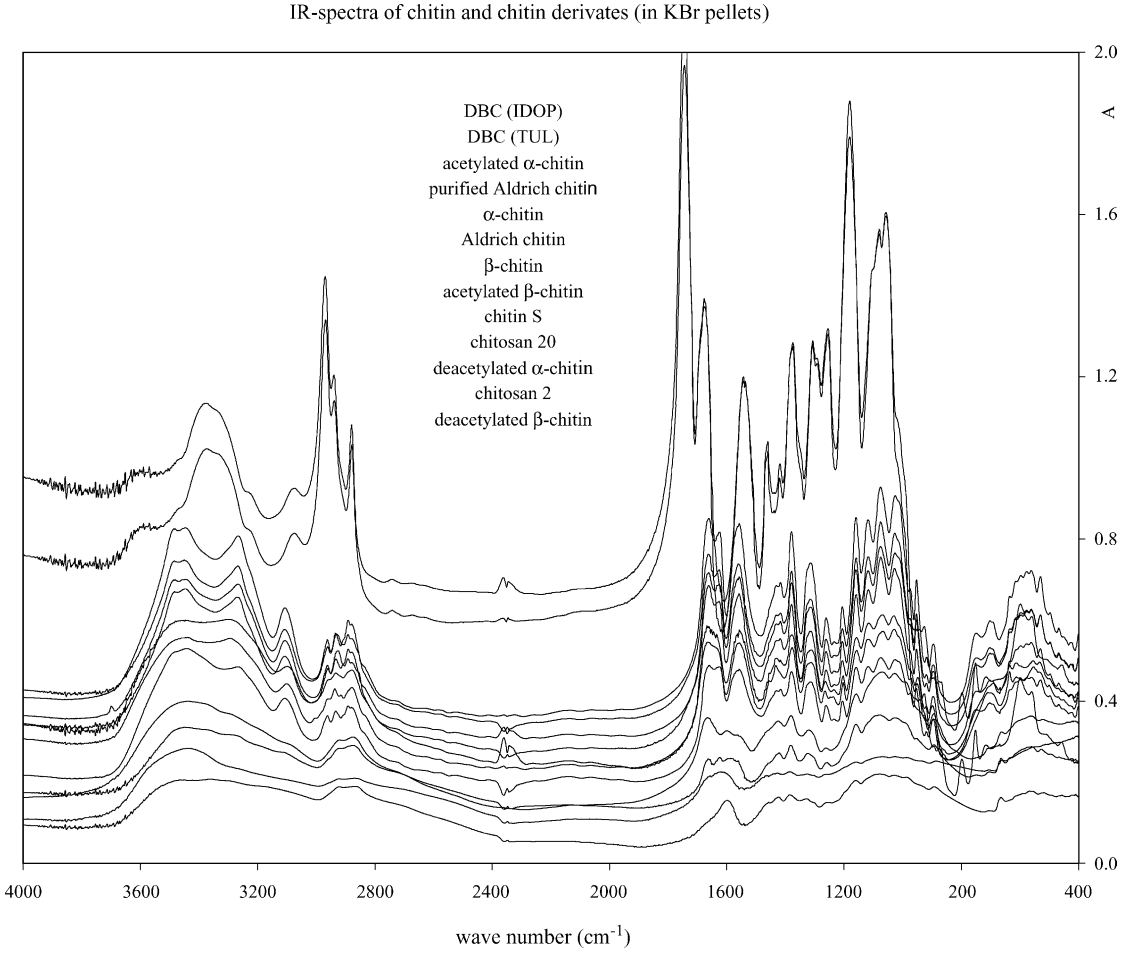

Fig. 2. FT-IR spectra of chitin and chitin derivates.

Conversion into chitosan or 'deacetylated chitin':

- Peaks that are related to secondary amides—and thus to chitin—are reduced or disappear: 3443–3265–3105–1662–1554–1315–1261 cm^{-1}.
- A peak that is related to primary amides, such as in chitosan, appears: 1596 cm^{-1}.
- Some peaks that are related to C–H bonds, and which are more present in chitin, are reduced or disappear: 2962–2934–2890–1380–1205 cm^{-1}.

These effects were generally stronger in the β-type, which was more deacetylated than the α-type. This seems to confirm the previously mentioned fact that β-chitin has a less dense structure than α-chitin and thus reactions proceed easier in the former type.

Conversion into dibutyrylchitin (DBC):

- Peaks that are related to esters appear: 1742–1179–1057 cm^{-1}.
- Some peaks that are related to alcohols are reduced or disappear: 3485–1117–1076–1026 cm^{-1}.
- Some peaks that are related to C–H bonds, which are more present in DBC, increase or appear: 2962–2934–2890–1460–1380 cm^{-1}.
- A peak that is related to primary amides disappears: 1596 cm^{-1}.

Acetylation or extraction did not seem to lead to any significant changes in the observed spectrum (compared to the original chitin). The effect of these and the other reactions however will be discussed more in detail in Section 3.2.

3.2. Quantitative analyses

As mentioned earlier, DA values of chitin and chitosan can be obtained by FT-IR measurements. Table 3 gives an overview of the obtained results. The high variation on some results was due to the combination of a small (wave number) interval and high slope of the spectrum at the applied interval limits. For deacetylated β-chitin no peaks around 1655 cm^{-1} could be detected, so the results from method III and VI were set to zero. The different equations (especially IV) clearly led to quite different DA values.

α-Chitin and chitin S were also tested with solid state ^{13}C NMR: (resulting DA by ^{13}C NMR, respectively, 89.8 and 69.7%). An example of such a spectrum is given in Fig. 3. The measured resonances were very close to the expected ones. Furthermore, the split in C3 and C5 resonances in these samples is in accordance with literature (Zhang et al., 2000).

The thus obtained DA for untreated chitin (89.8%) is very close to 90%, which is considered typical for chitin (Ravi Kumar, 2000). This value is furthermore in accordance with the observation that no peaks indicating primary amides could be detected in the unmodified and acetylated samples, which also would suggest a rather high DA value.

FT-IR results are clearly dependent upon the followed method and especially sample form and treatment. So great care should be taken when assessing the DA by FT-IR.

Together with the two chitosan samples of known DA (see Table 4), a quantitative calibration curve could now be proposed. Hereto the DA values of each of the samples with known DA were recalculated with following composed formula:

$$DA = C[a_I DA_I + a_{II} DA_{II} + a_{III} DA_{III} + a_{IV} DA_{IV} + a_V DA_V + a_{VI} DA_{VI}] + K$$

with

C, K: constants and $a_{I\ldots VI}$: multiplication factors ($\Sigma a_{I\ldots VI} = 1$)
$DA_{I\ldots VI}$: the DA values resulting from formulas found in literature.

Varying these constants and factors until a maximum correlation between calculated and known DA values was reached (see Fig. 4), led to: $C = 1.279$ and $K = -23.753$ and: $a_I = 0.294$, $a_V = 0.358$, $a_{VI} = 0.348$, $a_{II} = a_{III} = a_{IV} = 0$.

Table 3
Overview of obtained DA values by FT-IR measurements

Sample description	DA obtained by different equations (average of three tests)						Average
	DA_I (%)	DA_{II} (%)	DA_{III} (%)	DA_{IV} (%)	DA_V (%)	DA_{VI} (%)	DA (%)
Acetyl. α-chitin	75.8±0.9	69.5±1.4	75.8±1.4	175±5	102±1	96.0±1.7	98.9±39.2
Purif. Ald. Chitin	73.6±1.8	73.7±0.6	80.1±1.1	160±3	99.9±1.6	99.6±1.5	97.9±32.9
α-Chitin	73.6±0.1	70.7±0.3	73.7±1.2	169±1	97.6±0.9	92.5±1.1	96.2±37.3
Aldrich chitin	74.9±1.5	72.1±1.0	74.4±3.0	142±3	83.4±3.3	89.6±2.4	89.4±26.7
β-Chitin	71.3±1.5	68.9±2.8	69.7±3.8	114±5	83.1±3.2	81.2±3.0	81.4±17.2
Acetyl. β-chitin	65.3±1.0	66.9±1.1	70.7±1.1	118±8	71.4±2.8	81.5±1.8	78.9±19.8
Chitin S	62.7±1.4	53.5±3.2	49.8±3.4	99.5±9.1	96.9±2.0	57.1±4.3	69.9±22.3
Chitosan 20	42.0±0.5	48.6±0.6	33.3±0.7	41.5±0.3	42.4±1.8	20.3±0.3	38.0±10.0
Deacet. α-chitin	25.0±0.8	37.8±6.1	23.7±6.0	52.9±7.8	27.2±1.9	19.2±2.3	31.0±12.4
Chitosan 2	31.4±0.5	28.9±2.3	14.7±1.0	−8.5±2.9	21.4±2.4	8.5±0.6	16.1±14.8
Deacet. β-chitin	0.1±0.5	19.9±0.4	0	10.6±1.3	6.2±0.6	0	9.2±8.3

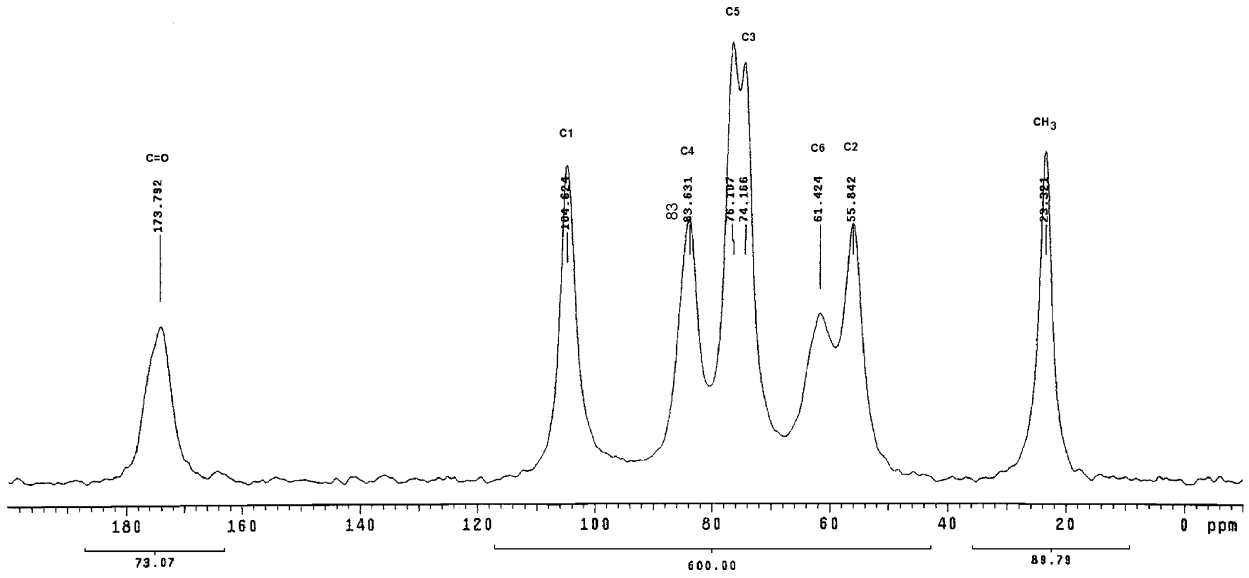

Fig. 3. Solid state ^{13}C NMR on unmodified α-chitin.

Thus:

$$DA = 13.334A_{1550}/A_{2878} + 14.614\ A_{1320}/A_{1420} + 38.054[A_{1661} + A_{1625}]/A_{3450} - 34.29$$

This calibration is only useable for the given combination of method and sample form. Using different measurement peaks has the extra advantage that an eventual anomaly in one of those peaks will only lead to a relatively small error in the resulting DA. Should one however only want to compare different chitin or chitosan types, FT-IR without calibration may already prove useful for detecting DA differences.

Table 5 lists the (from FT-IR) resulting recalculated DA values. Untreated β-chitin seems to be less acetylated than Aldrich chitin, and certainly less than α-chitin. β-Chitin and Aldrich chitin however show a rather large variation on the obtained results (due to large particle size, which causes disturbance in IR), making the difference between the two types not really significant. Apparently, the earlier reported 0% deacetylation for Aldrich chitin does not imply a DA of 100%, just a lack of deacetylation reaction.

Deacetylation of α-chitin seemed successful, while that of β-chitin led to impossible DA values. Latter chitin type in fact became darker during reaction, which could point to degradation. Since both DA values are well below 50–60% one may speak of chitosan. Because β-chitin possesses the ability to accept small molecules as intercalcates within its structure, it has a higher reactivity and thus its lower eventual DA and higher susceptibility to degradation can be explained.

Table 4
Samples of known DA

Sample	DA (%)
α-Chitin	89.8 (^{13}C NMR)
Chitin S	69.7 (^{13}C NMR)
Chitosan 20	20
Chitosan 2	2

Acetylation of α-chitin seemed to be successful, while acetylation of β-chitin led to a significant decrease in DA. This unexpected result may be caused by an ineffective treatment (no catalyst was used in order to prevent chain degradation) or simply by β-chitin's possible sensitivity to degradation by acetic anhydride.

Extraction (purification) of Aldrich chitin was done to remove (extract) that part of the sample that has a lower DA (is more chitosan-like) in order to obtain a higher residual DA (with reduced variation). In Table 5, one can see that the DA after extraction has significantly increased (while variation decreased). The average obtained mass loss due to extraction was 6.57%. This combination of mass loss and DA increase was most likely due to three effects: actual removal of low-DA material (chitosan), removal of impurities (calcium carbonate, proteins, etc.), and removal of low-molecular weight material (oligomers).

For DBC, no method for the assessment of the degree of butyrylation—based on IR tests—is found in literature.

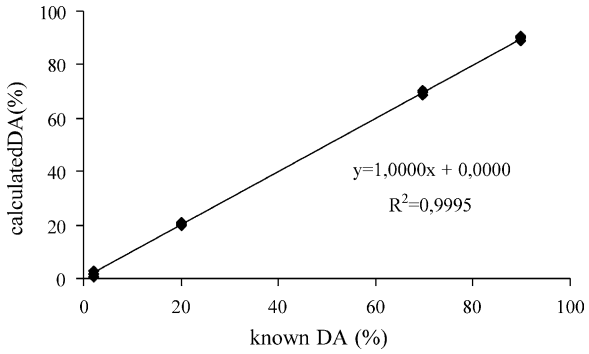

Fig. 4. Correlation between recalculated and known DA values.

Table 5
Overview of recalculated DA values by FT-IR, after calibration with samples of known DA

Sample	Recalculated DA values after calibration of FT-IR
Acetylated α-chitin	94.1 ± 1.5
Purified Aldrich Chitin	93.9 ± 1.6
α-Chitin	89.8 ± 0.9
Aldrich chitin	82.5 ± 2.0
β-chitin	77.3 ± 2.5
Acetylated β-chitin	69.8 ± 1.7
Chitin S	69.6 ± 0.8
Chitosan 20	20.5 ± 0.6
Deacetylated α-chitin	6.6 ± 0.4
Chitosan 2	1.6 ± 1.1
Deacetylated β-chitin	−20.9 ± 0.4

In other words: no formulas that use peak heights of reference and measurement peaks are known. Following reference peaks, however seem relevant:

- R1: carbonyl absorption in secondary amide (amide II) at 1542 cm^{-1}.
- R2: carbonyl absorption in secondary amide (amide I) at 1674 cm^{-1}.

These reference peaks are based upon the assumption that the secondary amide functions remain intact upon butyrylation. This implies that the DA of the sample must not be influenced by butyrylation. However, since both DBC types originated from the same chitin sample (hence the DA is anyway a constant) this should pose no problem. No other reference peaks could be used since introduction of the butyryl group ruled out this possibility. In other words: the amount of CH_x and CO functions is no longer independent of degree of butyrylation. The only possible choice (other than the already proposed R1 and R2) would be an ether related peak. This peak however is not useable because it is masked by another peak (1157 cm^{-1} is masked by 1179 cm^{-1}).

Following measurement peaks are proposed:

- M1: 3376 cm^{-1}. Thought to be OH (and water) related, but also possible NH stretching in secondary amides.
- M2: 1304 cm^{-1}. Thought to be (partly) due to OH bending.
- M3: 1742 cm^{-1}. Caused by carbonyl absorption in saturated esters.
- M4: 1179 cm^{-1}. Caused by CO stretching in esters.
- M5: 2878 cm^{-1}. Caused by CH stretching.
- M6: 2968 cm^{-1}. Caused by CH stretching.

Other peaks were not used because they were not relevant, too small or subjected to interference from nearby peaks. Combining the proposed measurement and reference peaks led to the results that are reported in Table 6. M1 is subject to large variations and therefore not useable. M2 should not be really, relevant, if butyrylation is nearly complete, since this peak may also be due to the presence of water. Most relevant should be M3 and M4 since they are completely dependent upon the degree of butyrylation. M5 and M6 are only partly related to the degree of butyrylation since they also depend upon quantities that also are present in non-reacted chitin. Further considering the most relevant M3/R2 and M4/R2 combinations, one may conclude that DBC (IDOP) is slightly more butyrylated, although M5/R2 and M6/R2 seem to contradict this. So, using only FT-IR, no significant difference in degree of butyrylation between the two DBC samples could be detected.

Testing a series of samples with different degrees of butyrylation with a direct technique such as ^{13}C NMR as done for chitin and chitosan would yield a calibration curve, which would indicate which M/R combinations are really

Table 6
Overview of the obtained M/R ratios for two DBC samples

Results with respect to R1						
DBC (TUL)	M1/R1	M2/R1	M3/R1	M4/R1	M5/R1	M6/R1
Average	0.677	0.405	1.881	1.407	0.449	1.019
C.V. (%)	11.8	8.6	16.3	15.3	1.5	4.3
DBC (IDOP)	M1/R1	M2/R1	M3/R1	M4/R1	M5/R1	M6/R1
Average	0.521	0.410	1.953	1.556	0.385	0.932
C.V. (%)	1.1	0.6	1.2	0.6	1.1	0.4
Results with respect to R2						
DBC (TUL)	M1/R2	M2/R2	M3/R2	M4/R2	M5/R2	M6/R2
Average	0.785	0.465	2.148	1.607	0.518	1.171
C.V. (%)	19.8	0.4	7.9	7.1	9.5	4.3
DBC (IDOP)	M1/R2	M2/R2	M3/R2	M4/R2	M5/R2	M6/R2
Average	0.592	0.466	2.217	1.767	0.437	1.059
C.V. (%)	0.1	0.3	0.3	0.3	2.1	1.3

relevant with respect to the degree of butyrylation. This might be the subject of further study.

4. Conclusion

Several types of chitin, chitosan and DBC were studied with FT-IR in order to establish differences in structure and degree of substitution (and thus degree of conversion). FT-IR peak analysis confirmed that indeed the desired reactions had taken place but FT-IR done as suggested in literature did not provide accurate absolute DA values. FT-IR DA determinations are clearly dependent upon the followed method and especially sample form and treatment. So great care should be taken when assessing the DA value of chitin by FT-IR measurements, and a calibration with respect to a direct technique such as ^{13}C NMR should be performed. This calibration is then only useable for one certain combination of FT-IR method and sample form. A formula for the quantitative determination of the degree of acetylation by FT-IR is proposed in this article. Should one however want to compare different chitin or chitosan types qualitatively, FT-IR without calibration may prove to be a very useful tool for determining differences in DA and crystalline structure.

For DBC no method for the assessment of the degree of butyrylation—based on FT-IR measurements—is found in the literature. Several measurement peaks and reference peaks were investigated but the results were not conclusive enough to differentiate between the two studied DBC types. Comparing with a direct technique such as ^{13}C NMR would yield a series of calibration curves, thus resulting in relevant FT-IR measurement and reference peaks. This might be the subject of further study.

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