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Determination of the degree of acetylation of chitosan by UV spectrophotometry using dual standards

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Abstract—Determination of the degree of acetylation of chitosan by UV spectrophotometry using dual standards is investigated. The UV absorbance of a pure chitosan solution is contributed additively by the *N*-acetylglucosamine and glucosamine residues; the absorbance divided by the total molar concentration of the residues (A/c_t) is linearly related to the degree of acetylation (DA). Using acetyl glucosamine and glucosamine hydrochloride as standards in 0.1 M hydrochloric acid solution, the equation obtained by linear regression is $A/c_t = 3.3615\mathrm{DA} + 0.0218$, $R^2 = 0.9958$. The DA of the analytical sample (m milligram of chitosan in V liters solution) can be calculated by

$$\mathrm{DA} = \frac{161.1 \cdot A \cdot V - 0.0218m}{3.3615m - 42.1 \cdot A \cdot V}.$$

Keywords: Degree of acetylation; Chitin; Chitosan; UV

Chitin is one of the most abundant, easily obtained, renewable natural polymers available, second only to its closely related chemical cousin, cellulose. Chitosans are the fully or partially N-deacetylated derivatives of the chitin polymers that are usually produced by treatment with alkali. They have been widely used in pharmaceutical, agricultural and industrial fields because of their extraordinary properties and have been regarded as a 'biomaterial in waiting' in the 21st century.[1]

Chitin and chitosan are poly[β-(1→4)-2-acetamido-2-deoxy-D-glucopyranose] and poly[β-(1→4)-2-amino-2-deoxy-D-glucopyranose], respectively. The chemical and biochemical properties of these polymers depend heavily on the degree of acetylation (DA), a parameter defined as the mole fraction of acetylated units in the polymer chain. In practice, the terms 'chitin' and 'chitosan' refer to a sample of polymers that vary in the DA, with 'chitin' referring to high (ideally 100%) DA polymers and 'chitosan' referring to low (ideally 0%) DA. When the DA is 50% or lower, the polymer becomes water-soluble due to the protonation of the $-NH_2$ groups of the glucosamine unit. Therefore, in practice, the term 'chitosan' is usually used to refer to polymers that are soluble in a dilute acid solution and 'chitin' to those polymers that are not. Many methods have been reported for determining the DA, including the following: UV,[2–4] IR,[5,6] circular dichroism,[7] NMR,[8–11] GPC,[3] dye absorption,[12] titration,[9,13] elemental analysis,[14] and analysis of the released acetic acid.[15] However, these methods frequently yield different values for DA.[16] Therefore, there is a need for a simple, reliable and precise method for determining the DA of chitosan.

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As noted above, chitosan is usually obtained by deacetylation of purified chitin by treatment with concentrated alkali at high temperature. The product obtained in this way generally has very low levels of impurities and only trace amounts of amino acids, so chitosan can be regarded as a pure compound. Chitosan is composed of two far UV chromophoric groups, *N*-acetylglucosamine (GlcNAc) and glucosamine (GlcN). Because these groups show no evidence of interacting within the polymer in a manner that would affect absorption of UV radiation, the monomer units contribute in a simple, additive way to the total absorbance of the material at a particular wavelength.[17] Therefore, the UV absorbance of chitosan solution can be expressed as

$$A = \varepsilon_a \cdot c_a + \varepsilon_g \cdot c_g \quad (1)$$

where ε_a and ε_g are the absorptivities of GlcNAc and GlcN and, c_a and c_g are the concentrations of GlcNAc and GlcN in mmol/L, respectively.

The DA of chitosan is defined as the mole fraction of acetylated units in the polymer chain:

$$\mathrm{DA} = \frac{c_a}{c_a + c_g} \quad (2)$$

Let c_t equal the total concentration of the GlcNAc and GlcN:

$$c_t = c_a + c_g \quad (3)$$

Substituting Eqs. 2 and 3 into Eq. 1, followed by rearrangement, gives

$$\frac{A}{c_t} = (\varepsilon_a - \varepsilon_g)\mathrm{DA} + \varepsilon_g \quad (4)$$

Eq. 4 indicates that the absorbance at a particular wavelength divided by the total molar concentration of the residues (A/c_t) is linearly related to the DA, and it can be simplified as follows:

$$\frac{A}{c_t} = k\mathrm{DA} + C \quad (5)$$

In the current experiment, *N*-acetylglucosamine and glucosamine hydrochloride were used as standards to represent the GlcNAc and GlcN in chitosan, respectively. Figure 1 shows the UV spectra of a mixture of the standards and of a chitosan solution. It shows that the *N*-acetylglucosamine and glucosamine hydrochloride admixture has the same λ_{max} and a UV spectrum similar to that of chitosan. Specifically, the λ_{max} is 201 nm in a 0.1 M hydrochloric acid solution.

Therefore, solutions with varying concentrations of *N*-acetylglucosamine and glucosamine hydrochloride were prepared and their UV absorbance at $\lambda_{max} =$ 201 nm were measured (Table 1). The DA of the admixture solution is defined as the concentration of *N*-acetylglucosamine divided by the total concentration of *N*-acetylglucosamine and glucosamine hydrochloride.

The plot of A/c_t against DA is shown in Figure 2 revealing that the relation between A/c_t and DA is linear. The equation obtained by linear regression is $A/c_t = 3.3615\mathrm{DA} + 0.0218$, $R^2 = 0.9958$.

Table 1. The absorption and A/c_t of standard solutions

No.	c_a (mM)	c_g (mM)	c_t (mM)	DA	A	A/c_t
1	0.2400	0.1440	0.3840	0.6250	0.7918	2.0620
2	0.2400	0.3600	0.6000	0.4000	0.7931	1.3218
3	0.2400	1.2000	1.4400	0.1667	0.7933	0.5509
4	0.1800	0.1680	0.3480	0.5172	0.6034	1.7339
5	0.1800	0.4080	0.5880	0.3061	0.6098	1.0371
6	0.1800	1.6800	1.8600	0.0968	0.6292	0.338
7	0.1200	0.1200	0.2400	0.5000	0.4265	1.7771
8	0.1200	0.3120	0.4320	0.2778	0.4252	0.9843
9	0.1200	1.6800	1.8000	0.0667	0.4345	0.2414
10	0.0600	0.0720	0.1320	0.4545	0.2146	1.6258
11	0.0600	0.2160	0.2760	0.2174	0.2150	0.7790
12	0.0600	6.0000	6.0600	0.0099	0.3166	0.0522

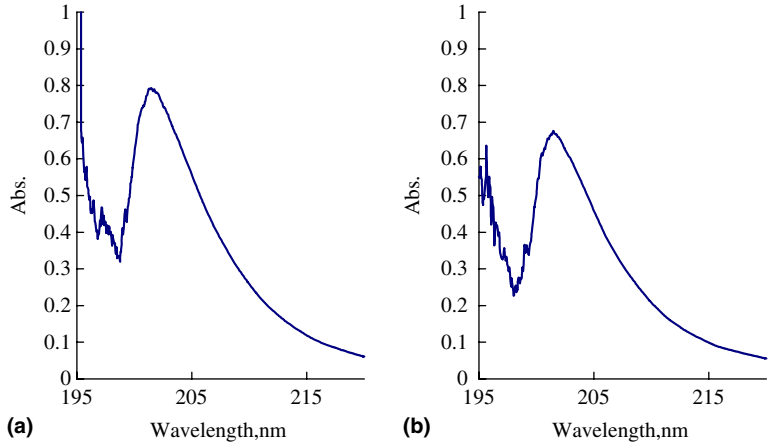

Figure 1. UV spectra: (a) *N*-acetylglucosamine and glucosamine hydrochloride admixture in 0.1 M hydrochloric acid solution; (b) chitosan in 0.1 M hydrochloric acid solution.

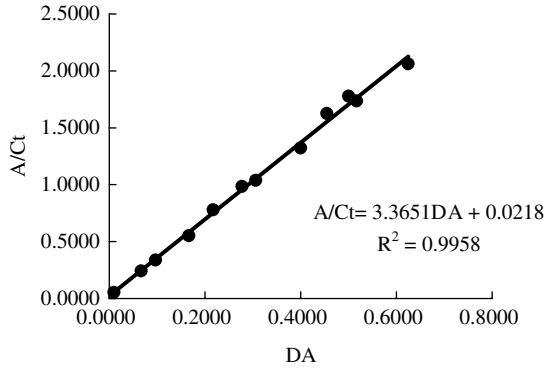

Figure 2. A/c_t versus DA of standards admixture solution.

For the samples undergoing analysis the total concentration of the GlcNAc and GlcN residues can be expressed

$$c_t = \frac{m}{[203.2\mathrm{DA} + 161.1(1 - \mathrm{DA})] \cdot V} \tag{6}$$

where m is the weight of chitosan in milligrams; 203.2 is the molecular weight of GlcNAc residue; 161.1 is the molecular weight of GlcN residue; and V is the volume of solution in liters.

Substituting into Eq. 5, followed by rearrangement, we obtain

$$\mathrm{DA} = \frac{161.1 \cdot A \cdot V - C \cdot m}{k \cdot m - 42.1 \cdot A \cdot V} = \frac{161.1 \cdot A \cdot V - 0.0218m}{3.3615m - 42.1 \cdot A \cdot V} \tag{7}$$

Reading the absorption of the chitosan solution at the $\lambda_{max} = 201$ nm, the DA can be calculated by Eq. (7).

The present method was used to determine the DA of four chitosan samples (Table 2) The results are comparable to the ones obtained by elemental analysis.

In summary, the UV spectra of mixtures of *N*-acetylglucosamine and glucosamine hydrochloride are quite similar to the UV spectra of chitosan. Further, the DA values of samples reported here are quite close to the values determined by elemental analysis. Determination of the degree of acetylation of chitosan by UV spectrophotometry using dual standards is a simple, convenient and accurate method to determine the DA of chitosan.

Table 2. The DA of chitosans determined by the present method and elemental analysis

No.	A	m (mg)	V (L)	DA values	
				Present method	Elemental analysis
1	0.9615	6.1	0.05	0.4114	0.4078
2	0.7975	6.8	0.05	0.2960	0.2916
3	0.6764	8.6	0.05	0.1912	0.1861
4	0.4775	8.3	0.05	0.1361	0.1302

1. Experimental

1.1. Materials

Food grade chitin from crab shell, purchased from Zhejiang Panan Chitosan Factory (China), was ground (grain size 100 mesh). Four chitosan samples were prepared after deacetylation of chitin with 40% NaOH at 80 °C for 1, 2, 4, and 6 h, respectively. The product was dissolved in 0.1 M HCl. After the solution was filtered, chitosan was precipitated from the filtrate by adding 1 M NaOH to the solution at room temperature, intensively washed with deionized water until neutrality was reached and freeze dried. *N*-Acetylglucosamine and glucosamine hydrochloride (purity > 99.9%) were supplied by Zhejiang Aoxing Biotechnology Co., Ltd (China) and were purified by re-crystallization twice. Other reagents used were analytical reagent grade chemicals.

1.2. UV spectra

UV spectra of standard and sample solutions were recorded on a Shimadzu UV 2501PC spectrophotometer. The standards and samples were dissolved in 0.1 M hydrochloric acid.

1.3. Elemental analysis

The elemental analysis was carried out on the PE2400II CHNS/O Analyzer (Perkin–Elmer Instruments), and the DA were calculated as follow:

$$\mathrm{DA} = \frac{1}{2} \times \left(\frac{14 \cdot \mathrm{C}\%}{12 \cdot \mathrm{N}\%} - 6 \right) \tag{8}$$

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