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Determination of the degree of acetylation (DA) of chitin and chitosan by an improved first derivative UV method

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Abstract

An economical and accurate determination of degree of acetylation (DA) for highly acetylated chitin has always been a challenge for researchers dealing with chitin and chitosan. A new protocol for the first derivative UV method using concentrated phosphoric acid as a solvent for highly acetylated chitin was developed in this study. The solvent was proposed based on thorough investigation of the effects of associated reactions including chain degradation, monomer dehydration, and oxazolinium ion formation. The reproducibility and performance of the new protocol was evaluated using commercial samples and the results showed the DA values of both chitin and chitosan could be determined accurately by a single analytical technique in less than 3 h.

Keywords: Degree of acetylation; Chitin; Chitosan; First derivative UV method; Method development

1. Introduction

Interest in application of chitin and its derivative chitosan in the food industry and biomedicine is constantly increasing (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). The degree of acetylation (DA) is a key parameter that influences the physico-chemical properties of chitin and chitosan, such as solubility, chain conformation (Berth & Dautzenberg, 2002), electrostatic properties (Sorlier, Denuziere, Viton, & Domard, 2001) and biological properties of chitosan films (Chatelet, Damour, & Domard, 2001). Numerous analytical methods, including Fourier transform infrared spectroscopy (FTIR) (Duarte, Ferreira, Marvao, & Rocha, 2002; Van de Velde & Kiekens, 2004), high performance liquid chromatography (HPLC) (Frederic, Nuria, Chornet, & Vidal, 1993), nuclear magnetic resonance (NMR) (Duarte, Ferreira, Marvao, & Rocha, 2001; Lavertu et al., 2003), titration (Jiang, Chen, & Zhong, 2003; Raymond, Morin, & Marchessault, 1993) and ultraviolet–visible (UV) adsorption spectroscopy (Muzzarelli & Rocchetti, 1985) have been proposed to precisely determine its value. However, all the methods have some limitations. For example, expensive instruments are needed in case of FTIR, NMR and HPLC methods, extensive sample preparation is required for HPLC, or accuracy is insufficient in titration and IR procedures. The first derivative UV method developed in late 1980s, offered a simple and fast measurement of DA value with good accuracy and precision (Muzzarelli & Rocchetti, 1985). Although zero order of UV spectra can be used for DA measurement as well (Hsiao, Tsai, Chen, Hsieh, & Chen, 2004), the first derivative of the spectra is less affected by the background noise and impurities and has been suggested as a standard method for routine determination of DA of chitosan (Tan, Khor, Tan, & Wong, 1998). Several modified first derivative UV methods have been proposed to improve the convenience and accuracy of the measurement (Liu, Wei, Yao, & Jiang, 2006; Pedroni, Gschaider, & Schulz, 2003). However, those methods use diluted acetic or hydrochloric acid to dissolve chitosan prior to analysis.

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This unavoidably limits the determination scope to highly deacetylated chitosan samples only, due to their solubility in diluted acids.

For highly acetylated chitin, concentrated phosphoric acid has been proposed as a good solvent and the UV determination appears to be valid in the whole range of DA (Hsiao et al., 2004). Additionally, the results obtained by this method are well correlated with a solid state ^{13}C NMR method, so far the best but the most expensive method for the DA analysis (Hsiao et al., 2004). However, polysaccharides generally undergo complex reactions under the conditions of concentrated acids and heat. For example, cellulose can be hydrolyzed into glucose and consequently converted into 5-hydroxymethylfurfural (HMF) and levulinic acid by acid catalyzed dehydration (Girisuta, Janssen, & Heeres, 2007). Compared to these well investigated reactions of cellulose, less information is available regarding the effects of hot concentrated acid on chitin and chitosan. One study has reported the formation of HMF from fully deacetylated chitosan after nitrous acid depolymerization (Tommeraas, Varum, Christensen, & Smidsrod, 2001). Apparently, the formation of HMF can introduce errors in the DA measurement because both acetyl-glucosamine and glucosamine can be converted to HMF.

An intermediate glucofuranosyl oxazolinium ion of acetyl-glucosamine and similar products have been found in chitin solutions in concentrated phosphoric acid and anhydrous hydrogen fluoride (Bosso, Defaye, Domard, Gadelle, & Pedersen, 1986; Vincendon, 1997). The intermediate ion is not stable and can be hydrolyzed into monosaccharide phosphate in diluted acids (Bosso et al., 1986; Vincendon, 1997). Apparently, the formation and hydrolysis of such intermediate ions affect the DA measurements due to blocking or liberating acetyl group (Fig. 1). In addition, chitin, chitosan, and acetyl-glucosamine may undergo acid deacetylation what would result in underestimated DA (Gizatulina, Chebotok, Novikov, & Konovalova, 2005).

Therefore, without thorough investigation of these possible reactions, DA determination method employing concentrated phosphoric acid as a solvent for chitin and chitosan should be used with caution. The objectives of this study were (1) to evaluate the effects of chemical reactions associated with utilization of phosphoric acid as a solvent on the DA determination by first derivative UV method, and (2) to improve the method so it can be used for quick and accurate determination of whole range of DA values.

2. Experimental details

2.1. Materials and instruments

Acetyl-glucosamine (GlcNAC), D-glucosamine hydrochloride (GlcN) and 85% phosphoric acid were purchased from Sigma (St. Louis, MO). Chitin and chitosan samples were provided by Primex (Primex, Iceland). A Shimadzu 2010 (Shimadzu, Columbia, MD) double beam UV–vis spectrophotometer was used to collect the UV spectra of standards and samples under scan mode in the range of 400–190 nm. Sampling interval and slit width were both set at 1.0 nm. Far UV cuvettes with 10 mm pathway length were used for all samples. UV Probe software (Shimadzu) was applied to calculate the first derivative spectra in the range of 190–220 nm.

2.2. Standard preparation and formation of standard curve

Standard solutions of GlcNAc and GlcN were prepared in 0.85% phosphoric acid at concentrations of 0, 10, 20, 30, 40 and 50 μg/ml. The calibration curve was made by plotting the first derivative UV values at 203 nm (H_{203}) as a function of GlcNAc and GlcN concentration.

2.3. Sample preparation and the DA determination

Chitin and chitosan samples were ground using Thomas Wiley Mini-Mill with sieve #40 (Thomas Scientific, Swedesboro, NJ) and stored in desiccators at room temperature until analysis. A 3-step procedure was used to prepare a sample for the DA determination (Fig. 2). Aliquots of 100 ± 10 mg chitin or chitosan were heated in 20 ml 85% phosphoric acid for 40 min at 60 °C with constant stirring. After 40 min, when chitin/chitosan was completely dissolved, 1 ml clear solution was taken and diluted to 100 ml with deionized water. The dilution was necessary to get the chitin/chitosan concentration to the range detectable by a spectrophotometer. The diluted solutions were incubated at 60 °C for 2 h prior the UV measurement. This was considered as a standard method. These parameters were applied in all experiments if not otherwise explained.

2.4. DA calculation method

The degree of acetylation of chitin and chitosan samples was calculated as:

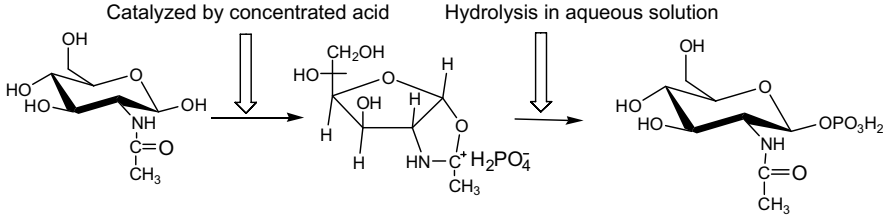

Fig. 1. Formation and hydrolysis of glucofuranosyl oxazolinium ion from acetyl-glucosamine. *Adapted from (Vincendon, 1997).