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ORIGINAL PAPER

A novel titration method based on fiber-optic refractive index sensing for the determination of deacetylation degree of chitosans

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Abstract We describe a novel method for determining the degree of deacetylation (DD) of chitosans from fiber-optic measurements of refractive index. Theory analyses and the experimental setup of the method are introduced and discussed. The analytical performance of the method is described for the determination of alkaline titration end-points. Experimental results reveal that the equivalence point can be directly identified from the titration curve. The proposed method is simple, low-cost, accurate, reliable, and easy to operate for industrial application. The DD values of four chitosan samples obtained with this new method show good agreement with those yielded from ^{1}H NMR. Such a mechanism of refractive-index monitor should open up a new application in the field of chitosanolytic enzymes, such as chitosanase, pectinase that are important in bioprocesses.

Keywords Chitosan · Degree of deacetylation · Refractive-index sensor · Fiber optic · Titration

Introduction

Chitosan is a biocompatible and biosorbable biopolymer which is currently receiving a great deal of interest for medical and pharmaceutical applications due to its interesting intrinsic properties. The degree of deacetylation (DD), i.e., the average number of—glucosamine units per 100 monomers, expressed as a percentage, influences chemical, physical and biological properties of chitosan [1, 2], such as the tensile strength of films, ability to chelate metal ions [3], and immunoadjuvant activity [4, 5]. Many methods have been used to determine the DD of chitosan. These include

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alkalimetry (acid–base titration, potentiometric titration [6, 7], conductometric titration [8], infrared spectroscopy [9, 10], UV-spectrophotometry [11, 12], colloidal titration [13], enzymatic determination [14], nuclear magnetic resonance (NMR) [15], ninhydrin test [16], and circular dichroism measurements [17], gel permeation chromatography (GPC) [18], first derivative ultraviolet spectrophotometry [19]. However, many of these methods are not suitable for routine purposes because of the cost of facilities and sophistication. The most common methods are IR and NMR spectroscopy and pH-potentiometric titration. The advantage of IR spectroscopy is that it is rapid, does not require aqueous solutions, is suitable for samples with any DD and that it is relatively insensitive to most inorganic and organic impurities. It suffers, however, from systematic errors associated with the arbitrary definition of the baseline and more importantly the need of extremely well-dried samples. ^{1}H NMR method is relatively accurate, and unlike other methods, ^{1}H NMR avoids precise weighting of samples. However, expensive equipments, complicated procedures, and high-qualified staff limit its applicability for serial measurements. Compared with other methods, potentiometric titration methods, with low reagent and equipment cost, seem more convenient and cost-saving. The major source of error by alkaline titration of acid solution of chitosan is the dissociation of protonated amino groups, what causes the additional consumption of titrant. To compensate for appropriate systematic error, the second equivalence point of the titration must be determined separately and the analytical concentration of the acid be corrected for it. Potentiometric titration also has the shortcomings of complex data processing.

A standard method used to determine the DD of chitosan that satisfies the producers and end users is essential, if the wider exploitation of chitosan is to be realised. A standard method has to be simple, rapid, cost effective, and reliable yet tolerate the presence of impurities.

The objective of our work was to research a novel alkalimetry method for determining the degree of deacetylation of chitosans with lower price, easy to operation, rapid, precise, and suitable for industrial application.

In this paper, we present a novel method to determine the DD of chitosan based on fiber-optic refractive index sensing to detect the end-point of alkaline titration. Theory analyses and the experimental setup of the method are introduced and discussed in detail. With our method, DD values of four kinds of chitosan samples were determined and compared with the results from ^{1}H NMR spectroscopy.

Principle of operation

During the alkaline titrations, the refractive index of the chitosan solutions was measured using a fiber sensor based on a two-channel Fresnel reflection technique. The experimental optical configuration consisted of a light source, three single-mode fused couplers, two photodetectors (PDs), and two optical fibers with sensing tips (Fig. 1). The light source was a diode laser operating at a wavelength of 1,550 nm. The split ratio of couplers 1 and 2 was 50:50, and the split ratio of coupler 3 was 10:90. The sensing ends of the Corning SMF-28 fibers were polished flat and perpendicular to the fiber axis and mounted in standard 2.5 mm-diameter

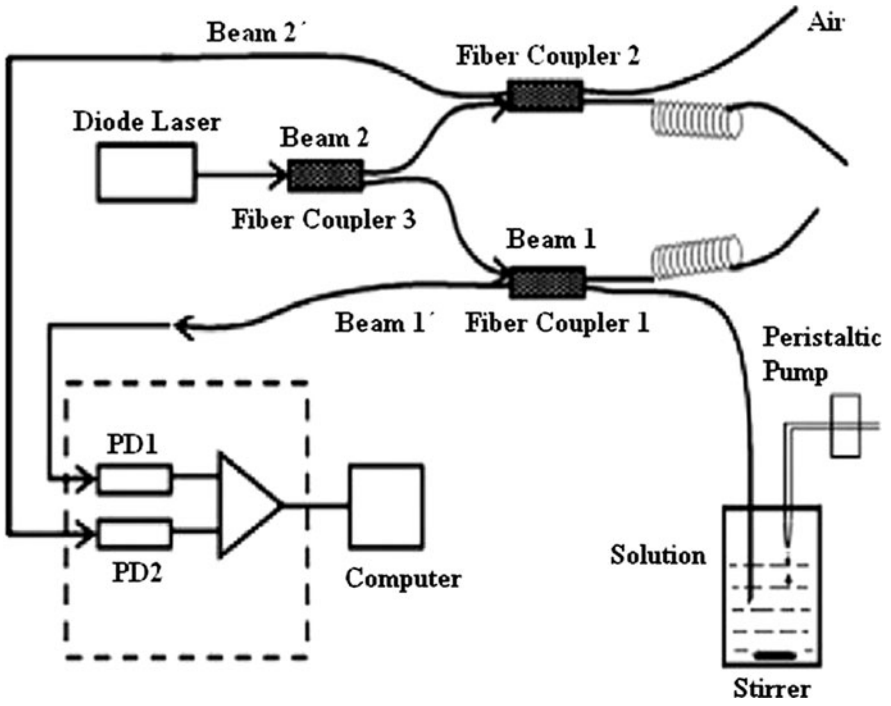

Fig. 1 Schematic diagram of refractive index titration system

telecommunications ferrules for protection and ease of cleaning. The light from the laser diode was initially split into two beams by coupler 3. The higher intensity beam 1 was passed through coupler 1 into a fiber immersed in the surfactant solution, where the beam was reflected at the sensor–solution interface. Beam 2 was directed through coupler 2 into a reference fiber whose sensing tip was exposed to air. The reflected beams $1'$ and $2'$ were detected using PD1 and PD2 after passing through crossed couplers 1 and 2. The intensity I_1 incident on PD1 could be obtained using the Fresnel formula

$$I_1 = k_1 k_1' k_3 I_0 \left(\frac{n_\mathrm{f} - n_\mathrm{x}}{n_\mathrm{f} + n_\mathrm{x}} \right)^2 \tag{1}$$

Likewise, the reflected intensity I_2 at PD2 is given by

$$I_2 = k_2 k_2' k_3' I_0 \left(\frac{n_f - n_\mathrm{air}}{n_f + n_\mathrm{air}} \right)^2. \tag{2}$$

In these equations I_0 is the output intensity of the light source, n_f is the effective refractive index of the single-mode fiber, n_x and n_air are the refractive indices of the solution and air, and the parameters k_1, k_1', k_2, k_2', k_3, and k_3' represent the bar-state and cross-state transmittances of couplers 1, 2, and 3. If insertion losses are neglected, the transmittances satisfy the formulas $k_1 + k_1' = 1$, $k_2 + k_2' = 1$, and $k_3 + k_3' = 1$. The relative reflective intensity R can be obtained by combining Eqs. (1) and (2):

$$R = \frac{I_1}{I_2} = \frac{k_1 k_1' k_3}{k_2 k_2' k_3'} \left[\left(\frac{n_f - n_x}{n_f + n_x} \cdot \frac{n_f + n_{air}}{n_f - n_{air}} \right)^2 \right]. \quad (3)$$

Equation (3) may be rewritten as:

$$R = K \left[\left(\frac{n_f - n_x}{n_f + n_x} \cdot \frac{n_f + n_{air}}{n_f - n_{air}} \right)^2 \right], \quad (4)$$

where $K = k_3 k_1' k_1 / k_3' k_2' k_2$ and is determined from the transmittances of the couplers.

When the probe fiber is exposed to air, n_x is equal to n_{air} and Eq. (4) becomes

$$K = I_1^{air} / I_2^{air}. \quad (5)$$

Therefore, the value of K can be obtained directly and conveniently in the apparatus, avoiding the trouble of transmittance measurement and calculation.

From Eqs. (3) and (4) one obtains

$$n_x = n_f \cdot \left(\frac{1 - \eta}{1 + \eta} \right). \quad (6)$$

$$\eta = \frac{n_f - n_{air}}{n_f + n_{air}} \cdot \sqrt{\frac{R}{K}} \quad (7)$$

The refractive index of air is 1.0003. The effective index n_f of the fiber mode can be calculated from the known fiber group index n_g and the dispersion relation. The reported value for n_f is 1.44961 at $\lambda = 1550$ nm.

Materials and methods

Apparatus

The structure of the sensor is shown in Fig. 1. Two Lightcomm OPM2012AA optical power meters connected to the computer via the RS-232C port was used for monitoring and collecting the reflective intensity–time (R–t) data. A program written in C++ collected R–t data in fixed intervals (can be adjusted), calculated the refractive index (RI) and saved it as the excel format for further processing.

A flow-inject system for the addition of titrant.

Materials

Chitosan samples of various DDs were supplied by Zhejiang Golden-shell Biochemicals Ltd (China). All other analytical reagent grade chemicals and distilled water were used; these chemicals were used as received without further purification. All working solutions were prepared by standard procedures and appropriate dilution. Stock solutions of titrant were prepared. From one stock solution, solutions of different concentrations were prepared. An exact

concentration of those acid or base solutions was determined using a conventional titrimetric procedure. The masses of the samples and solvents were weighted with an analytical balance with an uncertainty of ±0.0001 g.

Methods

Chitosan (0.20 g) was dissolved in 20 mL of 0.10 M standard HCl aqueous solution. The solution was diluted with 30 mL of distilled water. The titrant was the solution of 0.10 M NaOH.

The sensing tip was dipped into the sample and sodium hydroxide solution was added from a peristaltic pump. The magnetic stirrer was switch on. At the same time of adding titrant by using a flow-inject system at a constant rate to the beaker, by pressing a computer key, the program is started and *RI–t* data are displayed in the program window timely and recorded in fixed intervals to a data file for processing. The cell is cleaned and dried after each use by distilled water.

The addition process required about 15 min. Seven replicates were performed for each sample.

All of the measurements were carried out at room temperature (298 K).

Purification of chitosan

Chitosan (5.0 g) was dissolved in 200 mL of 2-wt% acetic acid and filtered through 0.45 mm filter membranes. One molar NaOH was then added to the chitosan solution to precipitate the polymer. The precipitate was washed with distilled water until the pH 7.0. After several washings with acetone, the final product was dried overnight in an oven at 60 °C.

Results and discussion

From Eq. (4), the measured R has nothing to do with the incidence intensity I_0 by relative technique, which indicates that the reference signal received by PD2 is used to eliminate the influence of light source fluctuation. Furthermore, the undesirable effects or the errors resulted from the different losses of fibers and couplers and the influence of environment temperature can be also decreased. The long-term measurement stability is thus significantly improved. To prove the truth of the mentioned above, a simulated experiment of checking the influence of light fluctuation is done by altering the attenuation of an additional variable optical attenuation added after light source. Figure 2 displays the relative deviation changes with the attenuation of the light intensity. It can be seen that the relative deviations are less than ±2 % even though the drop of the light intensity exceeds 20 %.

In this method, chitosan is dissolved in a known excess of hydrochloric acid and the solution is then titrated with sodium hydroxide. The titration is based on the measurement of a solution refractive index. The variation of solution refractive

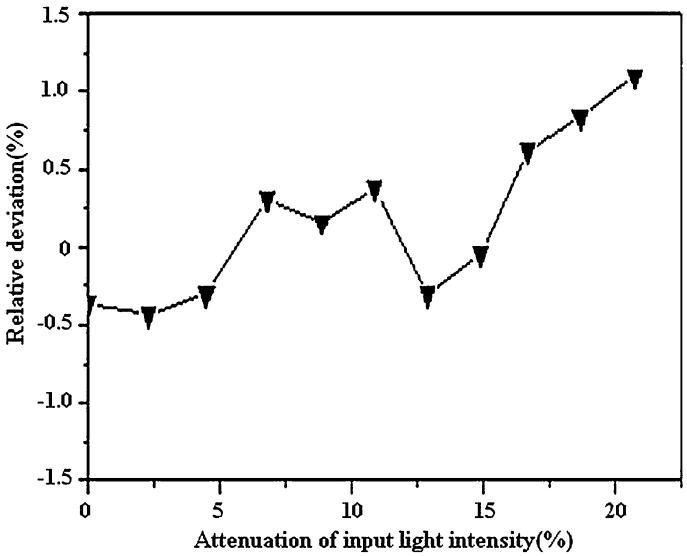

Fig. 2 Plot of change in relative deviation of salt solution (5 % NaCl) as a function of change in attenuation of incident light intensity. The attenuation changes of incident light intensity in experiment are performed by altering an additional variable optical attenuator after the light source

index is measured during the chemical reaction in titrations, the equivalent point shows when the excess of hydrochloric acid is neutralized by sodium hydroxide.

After the completion of the titration, the scale readings were plotted against the volume of the titrant. Because the peristaltic pump is operated at constant speed, the time-axis of the chart can be calibrated in volume unit. The values of refractive index with the corresponding titrant volumes were plotted in a graphic to find the linear variation before and after the equivalence point. The interception of the slopes with an acute angle with projection in abscise affords the corresponding volume of the equivalence point. Moisture content of chitosan samples was measured by a Rapid Moisture Meter (MX-50, A&D Company Ltd., Japan).

The percent of ($-NH_2$) of the chitosan sample was then calculated by the empirical equation:

$$(-\mathrm{NH_2})\% = \frac{(C_1V_1 - C_2V_2) \times 0.016}{G(100 - W)} \times 100\,\%$$

where C_1 and C_2 is the concentration of the HCl and NaOH solution (mol L^{-1}), respectively, V_1 is the volume of HCl (mL), V_2 is the volume of NaOH used in the titration before equivalence point, 0.016 is the mass of ($-NH_2$) matching the mass of 1 mL 1MHCl(g), G is the weight of the sample (g), and W is the moisture content of chitosan samples (%).

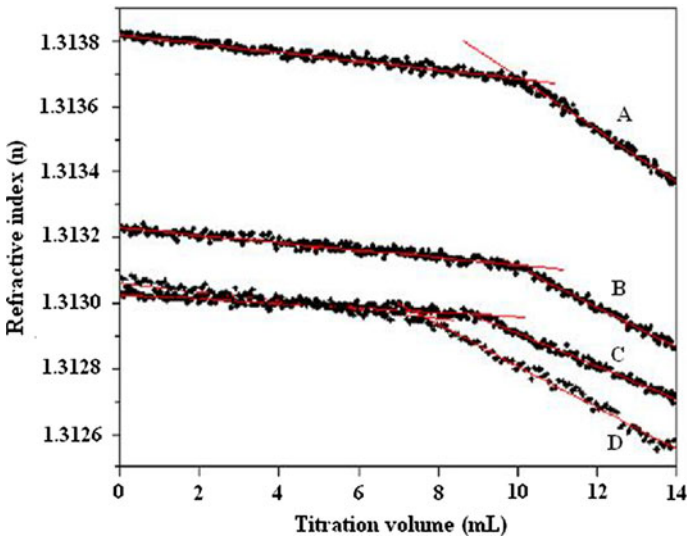

Fig. 3 Refractive-index titration curves of chitosans with different DD value. Samples *a–d* are described in Table 1. The addition rate of the titrant is 1.2 mL/min, the speed of data collection is 1 s^{-1}

Table 1 Percentage of deacetylation by our method and ^{1}H NMR

Samples	Our method[a]	^{1}H NMR
A	95.86 ± 0.63	95.12
B	92.21 ± 0.56	91.08
C	86.53 ± 0.58	87.04
D	78.92 ± 0.66	79.85

[a] All values are the means of three determinations ± standard deviation

DD is calculated from the following formula:

$$\mathrm{DD}\,\% = \frac{(-\mathrm{NH_2})\%}{9.94\%} \times 100\,\%$$

Refractive index titrations were carried out for four kinds of chitosans (with different deacetylation degree) and are shown in Fig. 3. This gives a titration curve having one inflexion points and two line segments are observed. The stoichiometry point is found by intersection of the two lines. The first segment corresponds to neutralization of HCl in excess, the second rapid descending branch refers to neutralization of the ammonium group. From this figure we know that as DD value increase, the abrupt changes of refractive index suffer almost no change except for the volume of NaOH solution. It means our method is suitable for chitosans with both low and high degree of deacetylation. One equivalent point can be obtained corresponding to neutralization of the acid (in excess). The volumes of sodium hydroxide of the equivalent points were determined from the intersection of the lines above and below the breakpoint. The observed transition is quite clear in the curve. The degree of deacetylation of these chitosans by our method and ^{1}H NMR are presented in Table 1, and the results are statistically in agreement. The results

show that the proposed method is not only simple and inexpensive, but also accurate and reliable in the determination of chitosan's DD.

A better sensitivity can be obtained by this technique, the sensitivity of this method is relation of the samples. Generally speaking, it can reach to 10^{-3}. To small changes in DD, this method yielded results which were superior to those of any of the titration methods, such as, colloidal titration, acid–base titration with indicator, but inferior to the well-known accurate techniques, for example, IR and NMR.

Conclusion

A novel method for the determination of DD values of chitosans was described in which a fiber-optic refractive-index sensor was employed to monitor refractive-index changes in alkaline titration solutions. Compared to the conventional titrations using electrodes, such as conductometric titrations and potentiometric titration, the sensing unit does not require a reference cell and subject to electrical interferences. Furthermore, the unreacting electrolyte has an insignificant effect on the results.

The experimental results illustrate that this approach is capable of providing DD measurements that are in good agreement with the data from the ^{1}H NMR method. The method is simple, easy to use, and cheap to construct, with good accuracy and reproducibility. This method can be used for routine determination of the DD of chitosan due to its minimal interference of results from contaminants and easy to be performed.

This technique opens a new way to determining the DD of chitosans. It is possible for other chitosanolytic enzymes, such as chitosanase, pectinase that are important in bioprocesses.

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