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A new linear potentiometric titration method for the determination of deacetylation degree of chitosan

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Abstract

The degree of deacetylation (DD) is one of the most important properties of chitosan. Therefore, a simple, rapid and reliable method for the determination of DD of chitosan is essential. In this report, two new potentiometric titration functions are derived for the determination of DD of chitosan. The effects of the precipitation and the errors induced in pH measurement are discussed in detail. To make this method more simple and reliable, two universal pH regions for the accurate plotting of different DD chitosan samples are proposed for the new potentiometric titration functions. The DD values of three chitosan samples obtained with this new method show good agreement with those yielded from elemental analysis and ^{1}H-NMR.

Keywords: Chitosan; Deacetylation degree; Linear potentiometric titration

1. Introduction

Chitosan, the poly-(β-1 → 4)-2-amino-2-deoxy-D-glucopyranose (Muzzarelli, 1977), is a partially deacetylated derivative obtained by alkaline treatment of chitin. The degree of deacetylation (DD) influences chemical, physical and biological properties of chitosan (Miya, Iwamoto, & Yoshikawa, 1983). Therefore, it is important to have an accurate and rapid method for the determination of the DD. In the past 30 years, many methods have been developed, including infrared spectroscopy (Sanan, Kurita, Ogura, & Iwakura, 1978), UV-spectrophotometry (Tan, Khor, Tan, & Wong, 1998), nuclear magnetic resonance (Hiral, Odani, & Nakajima, 1991), colloidal titration (Terayama, 1952), and potentiometric titration (Broussignac, 1968; Ke & Chen, 1990). However, many of these methods are not suitable for routine purposes because of the cost of facilities and sophistication. Potentiometric titration proposed by Broussignac, 1968 is one of the simplest methods. Equipment and reagents required are readily available in a normal chemistry laboratory. In this method, chitosan is dissolved in a known excess of hydrochloric acid and the solution is then titrated potentiometrically with sodium hydroxide. This gives a titration curve (shown in Fig. 1) having two inflexion points. The first and second inflexion points, denoted as 1 and 2, are the equivalence points of the titration of excessive hydrochloric acid and the titration of protonated chitosan, respectively.

The difference between the two inflexion points along the abscissa corresponds to the amount of acid required to protonate the amine groups of chitosan. However, due to the precipitation of chitosan in the neutral pH range, the second inflection point does not coincide with the actual equivalence point (Domard & Rinaudo, 1983). Ke and Chen, 1990 also proposed a simple method for determining DD by acid–base potentiometric titration. In their method, the titration of excessive hydrochloric acid with sodium hydroxide was considered as the titration of strong acid with strong base, so the simplified form of the linear function derived by Ingman and Still, 1966 was used for linearizing the titration curves. The function is shown as follows:

$$V = V_e - \left(\frac{V_0 + V}{C_B}\right)([H^+] - [OH^-]),$$

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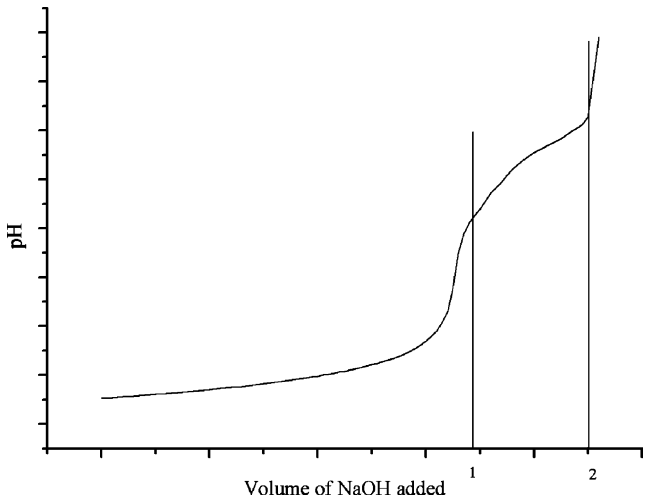

Fig. 1. Titration curve in Broussignac's method.

where V_0 is the volume of chitosan solution before the titration commenced, V is the volume of strong base added, and C_B is the concentration of titrant solution. If the function is plotted against V, a straight line is obtained that intersects the V-axis at V_e, the equivalence volume. DD is calculated from the following formula (Tan et al., 1998):

$$\mathrm{DD}(\%) = \frac{d}{\left(\frac{W - 161d}{204} + d\right)} \times 100 \quad d = \frac{(C_1V_1 - C_BV_e)}{1000}.$$

Compared with Broussignac's method, V_e in this linear potentiometric titration method can be extrapolated with the data obtained within the pH range that chitosan does not precipitate. Hence, its accuracy should be better. However, Ke and Chen did not provide the results from other methods for comparison and thus the accuracy of their method could not be evaluated. Nevertheless, this method has already been used in chitosan industry for many years because of its low reagent and equipment cost.

Tan studied the DD values of three samples using Ke and Chen's method, but the results showed poor agreement with those obtained with ^{1}H-NMR spectroscopy and first derivative UV-spectrophotometry (Tan et al., 1998). The authors pointed out that the major source of error in this method was the reliability of pH measurement because the results were very dependent on the $[H^+]$ and $[OH^-]$ concentrations. However, it is also important to note that the chitosan solution for titration is actually the mixture of hydrochloric acid and protonated chitosan, a weak acid with $pK_a = 6.5$ (Domard, 1987), whereas Ingman and Still's function (Ingman and Still, 1966) is not applicable to the titration of mixed acids with a strong base. Therefore, both the errors in pH measurement the use of an inappropriate linear function may have resulted in the inaccuracy of Ke and Chen's method.

In this paper, two new potentiometric titration functions were derived based on the idea that chitosan/HCl solution is a mixture of a strong acid and a weak acid. More importantly, the indeterminate error in pH-measurement, one of the major errors in the acid–base potentiometric titration method, was minimized by restricting the pH region of titration. With our method, DD values of a series of chitosan samples were determined and compared with the results from ^{1}H NMR spectroscopy and elemental analysis.

2. Materials and methods

Chitosan samples of various DDs were gifts from Shandong Luyang Chemicals Ltd (China). All other chemicals were of reagent grade.

2.1. Purification of chitosan

Chitosan (5.0 g) was dissolved in 200 ml of 2-wt% acetic acid and filtered through 0.45 μm 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 of the filtrate was same as the distilled water. After several washings with acetone, the final product was dried overnight in a vacuum oven at 60 °C.

2.2. Titration of chitosan solution

Chitosan (0.20–0.23 g) was dissolved in 25 ml of 0.1042 M standard HCl aqueous solution. The solution was then topped up to 100 ml with distilled water and calculated amount of KCl was added to adjust the ionic strength to 0.1. The titrant was the solution of 0.1025 M NaOH containing 0.1 M KCl. A pHS-3B meter (REX, Shanghai) was used for pH measurements. Under continuous stirring, titrant was added until the pH value of the solution reached 2.0. The standard NaOH was then added stepwise and 0.5 ml was added each time. The volume of added NaOH and pH values of solution were recorded. The titration was terminated when the pH value of the solution reached a value of 6.0. Three replicates were performed for each sample.

2.3. Elemental analysis

The elemental composition of chitosan samples was determined using Perkin–Elmer PE 2400 CHN and CHNS elemental analyzer. The DD values of chitosan samples were calculated from the following formula (Kasaai, Arul, & Charlet, 2000):

$$\mathrm{DD} = \left(1 - \frac{\mathrm{C/N} - 5.145}{6.816 - 5.145}\right) \times 100,$$

where C/N is carbon/nitrogen ratio.

2.4. Nuclear magnetic resonance

Chitosan sample was dissolved in 2% (w/w) CD_3COOD/D_2O solution. The ^{1}H-NMR spectra were obtained at 70 °C (Bruker 400 MHz; Model AV400). The DD values of chitosan were determined with Hiral's method (Hiral et al., 1991).

3. Results and discussion

3.1. The derivation of linear function for the determination of DD

Chitosan is dissolved in V_1 ml of HCl aqueous solution at a concentration of C_1, and then is titrated with V ml of NaOH. The excessive amount of HCl is denoted by V_e; the concentration of deacetylated amine groups of chitosan in solution is defined as C_0, and the volume of solution at the beginning of titration is V_0 ml.

Chitosan dissolved in HCl aqueous solution becomes a polyelectrolyte due to the protonation of the amine groups. The following equilibrium reaction described the state of ionization:

$$\text{R-NH}_2 + \text{H}^+ \Leftrightarrow \text{R-NH}_3^+.$$

The dissociation constant of chitosan is defined as:

$$K_a = \frac{[\text{R-NH}_2][\text{H}^+]}{[\text{R-NH}_3^+]}. \quad (1)$$

In our titration, the ionic strength of titrate was kept as 0.1 during the whole course of titration. Therefore, the concentration of hydrogen ions $[H^+]$ can be converted from the activity of hydrogen ions, α_H^+ ($\alpha_H^+ = 10^{-pH}$) according to the following relationship (Ingman and Still, 1966),

$$[\text{H}^+] = \alpha_H^+ \times 10^{0.08}. \quad (2)$$

Therefore, the concentration of R-NH_2 is

$$[\text{R-NH}_2] = K_a \frac{[\text{R-NH}_3^+]}{[\text{H}^+]}. \quad (3)$$

The total concentration of chitosan when V ml of NaOH has been added is

$$\frac{C_0V_0}{V_0+V} = [\text{R-NH}_3^+] + [\text{R-NH}_2]. \quad (4)$$

According to the definition of C_0 and V_0, the following equation can be derived

$$C_0V_0 = C_1V_1 - C_BV_e. \quad (5)$$

Substituting R-NH_2 in Eq. (4) with Eq. (3) gives:

$$\frac{C_0V_0}{V_0+V} = [\text{R-NH}_3^+] + \frac{K_a}{[\text{H}^+]}[\text{R-NH}_3^+]. \quad (6)$$

Rearrangement of Eq. (6) results in the equation below:

$$[\text{R-NH}_3^+] = \frac{C_0V_0}{V_0+V}\frac{[\text{H}^+]}{[\text{H}^+]+K_a}. \quad (7)$$

The charge balance equation is as following:

$$[\text{Cl}^-] + [\text{OH}^-] = [\text{H}^+] + [\text{Na}^+] + [\text{R-NH}_3^+]. \quad (8)$$

The concentrations of Cl^-, Na^+, and OH^- in solution are expressed by

$$[\text{Cl}^-] = \frac{C_1V_1}{V_0+V} \quad (9)$$

$$[\text{Na}^+] = \frac{C_BV}{V_0+V} \quad (10)$$

$$[\text{OH}^-] = \frac{K_w}{[\text{H}^+]}. \quad (11)$$

where K_w is the ion-product of water. Substituting Eqs. (9)–(11) for the concentrations of Cl^-, Na^+ and OH^- ions in charge balance Eq. (8) gives:

$$\frac{C_1V_1}{V_0+V} + \frac{K_w}{[\text{H}^+]} = [\text{H}^+] + \frac{C_BV}{V_0+V} + [\text{R-NH}_3^+]. \quad (12)$$

By combining Eqs. (5), (7), (12), we get the following equation:

$$V + \frac{V_0+V}{C_B}([\text{H}]-[\text{OH}]) = V_e + \left\{\frac{C_1V_1}{C_B\times 10^{-\text{pH}}} + \frac{K_w(V_0+V)}{C_B\times 10^{-2\text{pH}}} - \frac{V_0+V}{C_B} - \frac{V}{[\text{H}^+]}\right\}K_a. \quad (13)$$

If terms

$$\frac{C_1V_1}{C_B\times 10^{-\text{pH}}} + \frac{K_w(V_0+V)}{C_B\times 10^{-2\text{pH}}} - \frac{V_0+V}{C_B} - \frac{V}{[\text{H}^+]}$$

and

$$V + \frac{V_0+V}{C_B}([\text{H}]-[\text{OH}])$$

are denoted by X and Y, respectively, a linear function is obtained in following form:

$$Y = V_e + K_aX. \quad (14)$$

When Y is plotted against X, a straight line obtained should intersect Y-axis at V_e, the volume of excessive hydrochloric acid. Then DD can be calculated from V_e with the formula mentioned above.

3.2. Major errors in our titration method

In existing acid–base potentiometric methods, errors could be introduced in many ways. For example, errors due to the measurement of the weight of chitosan, the volume of titrant and the binding of water by the chitosan samples. However, these errors are avoidable (e.g. binding water of

chitosan was removed by vacuum drying overnight in our experiments) or negligible in comparison with the error caused by precipitation of chitosan and the indeterminate error in pH measurement, which are the major sources of errors in terms of acid–base potentiometric methods.

3.2.1. Error caused by precipitation of chitosan

Chitosan normally precipitates from solution when the pH > 6.0 (Domard & Rinaudo, 1983). The precipitation reduces the concentration of chitosan in solution, which results in a considerable error in our linear function. Furthermore, the precipitated chitosan may cover the surface of electrode and thus the electrode would lose its accuracy. For the reasons above, the titration should be terminated before the pH value exceeds 6.0.

3.2.2. Error induced in pH measurement

The pH meter we used has a measurement error of ±0.01 according to its specification and this measurement error applies to most of pH meters for routine laboratory use. If we denote the accurate activity and measured activity of hydrogen ions by α_H^+ and α'_H+. The following equation relates α_H^+ to α'_H+,

$$\alpha_H^+ = 10^{\mathrm{pH}\pm 0.01} = 10^{\pm 0.01}\alpha'_H+. \quad (15)$$

Therefore, α'_H+ ranges from $0.977\alpha_H^+$ to $1.023\alpha_H^+$, and thus the relative error of α_H^+ is ±2.3%. Similarly, $[H^+]$ should have the same relative error as $[H^+] = 10^{0.08}\alpha_H^+$ in our titration.

$[H^+]$ ranges from 0.977 $[H^+]'$ to 1.023 $[H^+]'$ and relative error of $[H^+]$ is ±2.3%.

Terms X and Y in the linear function have the dimension of volume and the overall errors of X and Y may contribute the error of V_e. For the term

$$X = \frac{C_1V_1}{C_B[H^+]} + \frac{K_w(V_0+V)}{C_B[H^+]^2} - \frac{V_0+V}{C_B} - \frac{V}{[H^+]}$$

in the linear function, the error of each term in X can be calculated separately. If the relative error in pH measurement is ±2.3%, the relative errors for the first, second and last parts are ±2.3, ±4.6 and ±2.3%. The errors of three parts are expressed as ΔX_1, ΔX_2 and ΔX_3, respectively, and the overall error of X is expressed as:

$$\Delta X = \Delta X_1 + \Delta X_2 - \Delta X_3. \quad (16)$$

As for the term

$$Y = V + \frac{V_0+V}{C_B}([H^+] - [OH^-]),$$

the error induced by $[OH^-]$ is negligible in the pH range in our titration. The error of Y induced in pH-measurement is

$$\Delta Y = \pm 0.023\frac{V_0+V}{C_B}[H^+]. \quad (17)$$

As already pointed out above, both $K_a\Delta X$ and ΔY are responsible for the error of V_e and ΔV_e. The relationship between ΔV_e and $K_a\Delta X$ and ΔY is shown as follows:

$$\Delta V_e = |K_a\Delta X| + |\Delta Y|. \quad (18)$$

It also can be deduced from the formula (1) that the error of V_e will be passed on to the DD value and a certain relative error in V_e will give almost the same relative error in the DD value. Thus, the relative error of DD could be minimized by reducing the relative error of V_e.

Take the titration of chitosan sample C_3 as an example, it can be seen from Table 1 that ΔV_e increases with the increase of pH from the beginning of the titration and reaches its minimum value around the equivalent point. However, after the equivalent point, it starts to increase again. Therefore, ΔV_e could be restricted within a certain range by limiting the pH region for plotting. In this manner, if 1% of relative error for DD is required (1% of relative error for DD is accurate enough for most applications), then only the pH region in which $\Delta V_e < 0.01V_e$ can be used for plotting.

However, it is impossible to know the accurate V_e before plotting and thus the relative errors of V_e resulting from X, Y cannot be calculated. Fortunately, this problem can be solved in an alternative way. Theoretically, the minimum V_e, denoted by V_{emin}, among all chitosan samples in our titration (suppose 0.23 g DD = 100% chitosan is titrated) is 11.48 ml. Therefore, $\Delta V_e < 0.01V_{emin}$ can be used as the standard for all samples in the selection of pH region. Based on this standard, Y terms in the pH region from 2.43 to 5.88 are plotted against X in Fig. 2 (denoted by Plot 1). As a contrast, Y terms in the whole titration pH region are also plotted against X (denoted by Plot 2). As is clear from Fig. 2, Plot 1 shows better linearity against Plot 2. Therefore, V_{e1} = 0.00149 l from Plot 1, was used for the calculation of DD value of sample C_3 and the result correlates well with the results obtained with elemental analysis and ^{1}H-NMR (shown in Table 3).

3.3. Simplification of the method

As is clear from the discussion above, the DD values of chitosan can be determined accurately by setting $\Delta V_e < 0.01V_{emin}$, as the standard in the selection of pH region for plotting. However, the linear function is still a little too complicated for rapid determination of DD. Besides, the pH region for accurate plotting may vary with the weight and DD of chitosan samples. Hence, a simpler function and a universal pH region are expected for rapid test.

3.3.1. Maximum errors of ΔX and ΔY induced in pH measurement

As discussed in Section 3.2, the pH region for accurate plotting can be determined by comparing the values of $K_a\Delta X + \Delta Y$ with $0.01V_{emin}$. However, with respect to the samples having different DD and weight, ΔX and ΔY at the same pH values may be different, and thus the pH region for each sample may also vary accordingly. Nevertheless, a

Table 1
Terms in the new linear function and the errors induced in pH measurement for the titration of chitosan sample C_3 (weight of $C_3 = 0.2255$ g) (pK_a is assumed to be 6.5 (Muzzarelli, 1977))

pH	X	Y (l)	$K_a\Delta X \times 10^{-5}$ (l)	$K_aX \times 10^{-5}$ (l)	$\Delta Y \times 10^{-5}$ (l)	$\Delta V_e \times 10^{-5}$ (l)
2.04	0.9384	0.014916	0	0.030	25.1	25.1
2.06	0.9778	0.014926	0	0.031	24.0	24.0
2.08	1.0171	0.014959	0	0.032	22.9	22.9
2.1	1.056	0.015013	0	0.033	21.9	21.9
2.12	1.0946	0.015086	0	0.035	20.9	20.9
2.14	1.1327	0.015179	0	0.036	20.0	20.0
2.16	1.17	0.01529	0	0.037	19.1	19.1
2.19	1.2591	0.015239	0	0.040	17.8	17.8
2.22	1.3502	0.015225	0	0.043	16.6	16.6
2.26	1.5016	0.015091	0	0.047	15.2	15.2
2.29	1.5987	0.015153	0	0.051	14.2	14.2
2.33	1.7618	0.015114	0	0.056	12.9	12.9
2.38	2.0025	0.015006	0	0.063	11.5	11.5
2.43	2.2609	0.014964	0	0.071	10.3	10.3
2.47	2.4554	0.015073	0	0.078	9.37	9.37
2.53	2.8339	0.015049	0	0.090	8.16	8.16
2.6	3.3493	0.015023	0	0.106	6.95	6.95
2.69	4.1636	0.014959	0	0.132	5.66	5.66
2.79	5.2645	0.014955	0	0.166	4.50	4.55
2.92	7.1356	0.014951	0	0.226	3.34	3.35
3.10	10.84	0.014961	0.010	0.343	2.21	2.22
3.40	21.687	0.014983	0.016	0.686	1.11	1.13
4.40	216.47	0.015049	0.16	6.85	0.11	0.27
5.15	1163.7	0.015509	0.84	36.8	0.02	8.66
5.47	2309.9	0.016004	1.68	73.0	0.01	1.69
5.68	3547.8	0.016503	2.58	112.2	0.006	2.59
5.88	5308.1	0.017002	3.83	167.9	0.004	3.83
6.04	7217.1	0.017501	5.25	228.2	0.003	5.25

universal pH region can be achieved by estimating the maximum errors of ΔX and ΔY at different pH values.

As for

$$\Delta X = \pm\left\{0.023\frac{C_1V_1}{C_B[H^+]} + 0.046\times\frac{K_w(V_0+V)}{C_B[H^+]^2} - 0.023\frac{V}{[H^+]}\right\},$$

since V will not exceed 25.41 ml ($V_{max}=(C_1V_1)/(C_B)$) in our titration, ΔX_{max} is estimated as

$$0.023\frac{C_1V_1}{C_B[H^+]} + 0.046\frac{K_w(V_0+0.02541)}{C_B[H^+]^2}. \quad (19)$$

Similarly, with respect to

$$\Delta Y = \pm 0.023\times\frac{V_0+V}{C_B}[H^+],$$

ΔY_{max} is estimated as

$$0.023\frac{V_0+0.02541}{C_B}[H^+]. \quad (20)$$

The values of ΔX_{max} and ΔY_{max} in the pH range from 2.4 to 6.0 are calculated and listed in Table 2. It can be seen from Table 2 that when $\Delta V_e < 0.01V_{emin}$ is set as the standard, the pH region 2.50–5.80 can be used for accurate plotting of chitosan samples with weight <0.23 g regardless of their DD values.

3.3.2. Neglecting of term K_aX in the linear function

In Table 1, it is noteworthy that terms K_aX in a certain pH region are negligible in comparison with $0.01V_{emin}$. Therefore, term K_aX may be omitted within this pH region

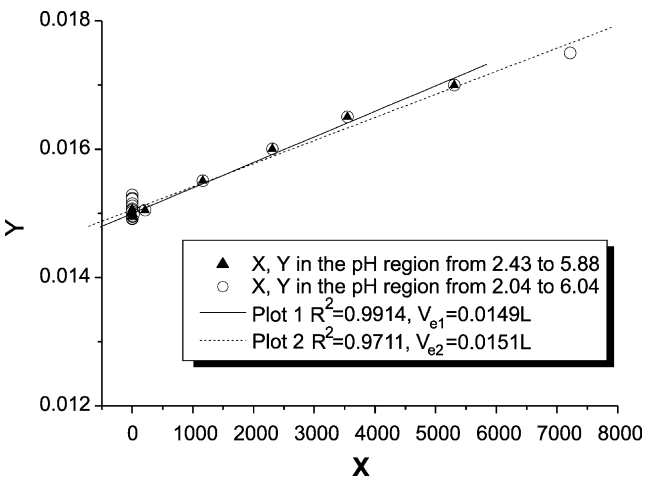

Fig. 2. Plots of X, Y terms of chitosan sample C_3 in different pH regions (method of least squares is used for regression).

Table 2
$K_a\Delta X_{max}$, ΔY_{max}, ΔV_{emax} and $\Delta V'_{emax}$ for the titration of chitosan samples in the pH region from 2.40 to 6.00

pH	$K_a\Delta X_{max} \times 10^{-5}$ (l)	$\Delta Y_{max}(\Delta Y') \times 10^{-5}$ (l)	$K_aX_{max} \times 10^{-5}$ (l)	$\Delta V_{emax} \times 10^{-5}$ (l)	$\Delta(V'_{emax} = \times 10^{-5}$ (l) (after neglecting K_aX_{max})
2.40	0	13.5	0.43	13.5	13.9
2.50	0	10.7	0.57	10.7	11.3
2.60	0	8.50	0.74	8.50	9.24
2.70	0	6.75	0.96	6.76	7.71
2.80	0	5.36	1.24	5.37	6.60
3.00	0.01	3.38	2.02	3.40	5.40
3.20	0.02	2.13	3.25	2.16	5.38
3.40	0.04	1.35	5.21	1.39	6.56
3.60	0.06	0.85	8.32	0.91	9.17
4.00	0.15	0.34	21	0.49	21.3
4.40	0.39	0.14	53	0.52	53.1
4.80	0.97	0.05	133	1.02	133
5.20	2.44	0.02	335	2.46	335
5.60	6.12	0.01	842	6.13	842
5.80	9.70	0	1334	9.71	1334
5.90	12.2	0	1679	12.2	1679
6.00	15.4	0	2114	15.4	2114

and the linear function could be simplified to the following form:

$$V = V_e + Y'$$

$$Y' = \left(\frac{V_0 + V}{C_B}\right)([H^+] - [OH^-]). \quad (21)$$

Since terms K_aX for different chitosan samples may also vary with the DD and weight, a universal pH region is essential to simplify the calculation. In order to obtain the universal pH region, the maximum error caused by neglecting term K_aX is estimated as:

$$K_a\left\{\frac{C_1V_1}{C_B[H^+]} + \frac{K_w(V_0 + 25.41)}{C_B[H^+]^2} - \frac{V_0}{C_B}\right\}. \quad (22)$$

Besides, in the simplified function, the error of Y' is actually equivalent to ΔY and it should also be taken into account before plotting. The maximum error of V_e thus is expressed as:

$$\Delta V'_{emax} = |K_aX_{max}| + |\Delta Y'_{max}|. \quad (23)$$

As can be seen from Table 2, $\Delta V'_{emax}$ in the pH region from 2.40 to 3.60 can be neglected when $0.01V_{emin}$ is taken as standard. In other words, when the simplified function is applied, this pH region can be used as the universal pH region for the accurate plotting of different DD chitosan samples with weight <0.23 g.

For instance, in the titration of chitosan sample C_3, the V, Y' terms in the derived universal pH region (denoted by Plot 1) and the whole titration pH region (denoted by Plot 2) are plotted in Fig. 3. With Plot 1 and Plot 2, the V_e values were obtained and the DD values were calculated from V_e to be 80.2 and 75.1%, respectively. Apparently, the DD value calculated from V_{e1} shows much better consistency with the results from the other two methods (shown in Table 3).

Interestingly, the simplified function has the same form as the linear function in Ke and Chen's method. However, our simplified function was based on the idea that chitosan solution for titration is the mixture of a weak acid and a strong acid. More importantly, Ke and Chen's method did not provide a universal pH region to minimize the pH-dependent errors, which could cause considerable errors to the DD values as demonstrated in the above case.

3.3.3. Comparison of DD values determined with the new linear functions, elemental analysis and ^1H NMR methods

In order to examine the accuracy of our method, three chitosan samples having different DD values were titrated and the new linear function and its simplified form were used for plotting in combination with their universal pH regions. As can be seen from Table 3, the results obtained

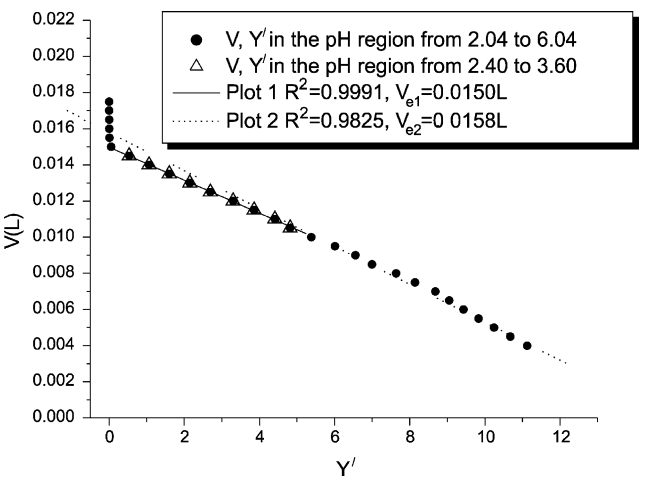

Fig. 3. Plots of V, Y' terms of chitosan sample C_3 in different pH regions (method of least squares is used for regression).

Table 3
DD values of chitosan samples determined by two linear functions, elemental analysis and ^{1}H NMR

Sample name		C_1	C_2	C_3
Degree of deacetylation (%)	Linear function	93.0 ± 0.5	88.2 ± 0.5	80.6 ± 0.3
	Simplified linear function	93.7 ± 0.3	89.0 ± 0.4	80.8 ± 0.2
	Elemental analysis	97.4	90.5	82.7
	^{1}H NMR	95	91	82

with the new linear function correlate well with the results obtained from its simplified form. The good consistency between the results from our method and the other two methods suggests that our linear function and its simplified form may be used as a simple and accurate method in industrial application.

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References

Broussignac, P. (1968). *Chemistry Industry Genie Chimica*, *99*, 1241.

Domard, A. (1987). pH and c.d. measurements on a fully deacetylated chitosan: Application to Cu^{II}–polymer interactions. *International Journal of Biological Macromolecules*, *9*, 98–104.

Domard, A., & Rinaudo, M. (1983). Preparation and characterization of fully deacetylated chitosan. *International Journal of Biological Macromolecules*, *5*, 49–52.

Hiral, A., Odani, H., & Nakajima, A. (1991). Determination of degree of deacetylation of chitosan by ^{1}H NMR spectroscopy. *Polymer Bulletin*, *26*, 87–94.

Ingman, L. F., & Still, E. (1966). Graphic method for the determination of titration end-points. *Talanta*, *13*, 1431–1442.

Kasaai, M. R., Arul, J., & Charlet, G. (2000). Intrinsic viscosity–molecular weight relationship for chitosan. *Journal of Polymer Science and Polymer Physics*, *38*, 2591–2598.

Ke, H., & Chen, Q. (1990). Potentiomtric titration of chitosan by linear method. *Huaxue Tongbao*, *10*, 44–46.

Mima, S., Miya, M., Iwamoto, R., & Yoshikawa, S. (1983). Highly deacetylated chitosan and its properties. *Journal of Applied Polymer Science*, *28*, 1909–1917.

Muzzarelli, R. A. A. (1977). *Chitin*. Oxford: Pergamon Press.

Sanan, T., Kurita, K., Ogura, K., & Iwakura, Y. (1978). Studies on chitin: 7. IR spectroscopy determination of degree of deacetylation. *Polymer*, *19*, 458–459.

Tan, S. C., Khor, E., Tan, T. K., & Wong, S. M. (1998). The degree of deacetylation of chitosan: Advocating the first derivative UV-spectrophotometry method of determination. *Tlanta*, *45*, 713–719.

Terayama, H. (1952). Method of colloid titration (a new titration between polymer ions). *Journal of Polymer Science*, *8*, 243–253.