ABSTRACT

LUO, ZHENHUA. A Comparison among Conductometric, Two-abrupt-change Potentiometric and Linear Potentiometric Titrations on Determining DD of Chitosan. (Under the direction of Dr. Sam M. Hudson).

Chitosan, considered as an abundant natural resource, has been introduced into miscellaneous fields, ranging from pharmaceutical purposes, cosmetics to water purification. The degree of deacetylation (DD) of chitosan is one of the most important parameters that influence the physiochemical, biological, and mechanical properties of chitosan and thus affect its applications. Conductometry and potentiometry as two major conventional methods of characterization of DD have many advantages over other methods. The objectives of the work are to statistically compare conductometric, two-abrupt-change potentiometric and linear potentiometric titrations and to unveil the principles behind these three analytical methods as to perform like a handbook for students. The work introduced the chitosan mass fraction and volume correction into the conductometry method and graphically obtained the DD values based on the conductometric titration curves. For two-abrupt-change potentiometry, the second derivative titration curve was adopted to determine the DD. And degrees of deacetylation were also determined graphically by linear potentiometry within restricted pH regions in order to confine the error induced in pH measurement. Several statistical models were then adopted to compare these three methods in terms of mean and precision. The results indicate that the three methods have the same degree of precision at the significance level of 0.05, while their means are significant different at this level. The incorporation of chitosan mass fraction and volume correction has no impact on the mean and variance of the DD. And the sources of error of each method were analysed in this work.

A Comparison among Conductometric, Two-abrupt-change Potentiometric and Linear Potentiometric Titrations on Determining DD of Chitosan

by
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DEDICATION

To my mother who offers me her perpetual love.

BIOGRAPHY

Zhenhua Luo was born in Wuhan, Hubei, China on November 15th, 1993. After graduating from Hongshan High School, Wuhan in June 2011, she got enrolled in Wuhan Textile University, where she was intrigued by the interdisciplinary nature and wide application of textile engineering. During her undergraduate study, she worked under the guidance of Dr. Guangming Cai on preparation and performance research of bacterial cellulose/alumina composite membrane. She continued her study in the program of textile engineering provided in North Carolina State University towards her master's degree in 2015 fall. After the first semester, Zhenhua joined the research group of Dr. Samuel Hudson and has been working on characterization of deacetylation degree of chitosan by different titration methods.

ACKNOWLEDGMENTS

I would like first express my profound gratitude to my advisor, Dr. Samuel Hudson, for his instructive guidance, continuous support and patience. He provides me with this precious opportunity to work on characterization of the deacetylation degree of chitosan and imparts extensive knowledge regarding chitosan to me. And the whole work would definitely be impossible without his careful revisions and insightful comments. I would like to extend my appreciation to my committee members Dr. Wendy Krause and Dr. Ashley Brown for their valuable support and precious time on reviewing my thesis.

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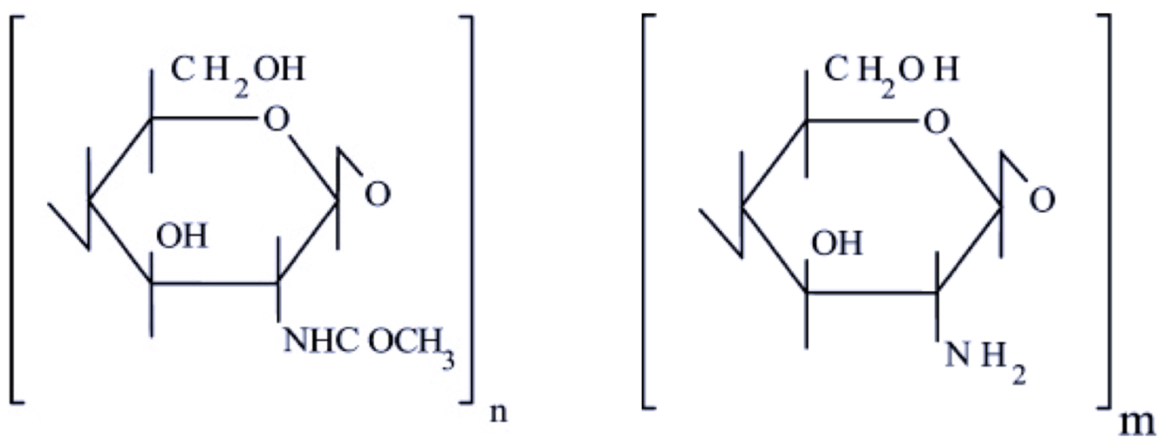
CHAPTER 1 INTRODUCTION

Chitin, considered as the second most abundant natural biomass on the Earth, was first identified in 1884 [1]. It is a naturally occurring polysaccharide composed of 2-acetamido-2-deoxy-D-glucose (N-acetyl glucosamine, GlcNAc) with covalent β-D-(1→4) linkages. When chitin is treated by deacetylation process, both its partially and completely N-acetylated derivatives are referred to as chitosan. The chemical structures of fully N-acetylated chitin and completely N-deacetylated chitosan are provided below.



Fully N-acetylated chitin Completely N-deacetylated chitosan

Figure 1-1: Chemical structures of fully N-acetylated chitin and completely N-deacetylated chitosan [2].

Existing ubiquitously in the biosphere, chitin can be found in the supportive structures of animals such as the exoskeletons of arthropods and crustaceans, and lower evolutionary organisms, including yeasts, fungi and diatoms. It is estimated that the annual worldwide production of chitin approximates to $10^{10}-10^{12}$ tons [3]. Not only has its easy accessibility and enormous annual production aroused intense scholarly interest, but also its positive attributes of excellent biodegradability with low toxicity of the its hydrolyses and versatile biological features such as acceleration of skin recovery and healing. Despite Chitin's easy availability and great futuristic potential in various fields, it still remains an under-utilized resource primarily because of its intractable structure. Chitin is sparingly soluble in general mild solvents mainly because of its highly rigid semi-crystalline structure through hydrogen bonding. Although it can be dissolved in strong solvents such as concentrated hydrochloric acid, concentrated sulfuric acid, phosphoric acid (78%-97%) and methane sulfonic acid, the molecular weight of chitin begins to be suppressed immediately after dissolution by hydrolysis [1].

On the other hand, chitosan, as a partial or completely deacetylated derivative of chitin, possesses high solubility in aqueous organic acid solution and hydrochloric acid aqueous solution. The predominant functions of chitosan seem to be slow degradation rate by lysozyme and healing-accelerating effects in animal body in addition to the transdermal absorption promoting characteristics with safe toxicity profile [4]. The high solubility enables miscellaneous applications of these predominant functions, ranging from pharmaceutical purposes, cosmetics, food additives, agricultural materials to water purification. Chitosan is considered to be readily soluble in dilute acidic solutions below pH 6.0. At low pH, the amines of chitosan are protonated and become positively charged which makes chitosan water soluble. When the pH gets higher than 6, these amines become deprotonated with charged state altered and chitosan becomes insoluble [5]. Therefore, the solubility of chitosan is highly dependent on the degree of deacetylation (DD) which is an important characterization index of chitosan reflecting the balance between its two monomers.

The DD of a chitosan is determined by the formula below.

$$\mathrm{DD}=100\frac{n_{D-GlcN}}{n_{D-GlcN}+n_{D-GlcNAc}} \tag{1-1}$$

where n_{D-GlcN} and $n_{D-GlcNAc}$ are the average number of D-GlcN and D-GlcNAc units within the macromolecule respectively. The DD influences various properties of chitosan including biological physicochemical, and mechanical properties. And knowledge of the DD is essential for understanding the structure-properties relationship of chitosan and predicting its possible industrial applications. Up to now, several methods have been developed to determine the DD for chitin and chitosan, including spectroscopy (^{1}H NMR, ^{13}C NMR, ^{15}N NMR, IR, near-IR, and UV); conventional (colloidal titration, conductometric titration, potentiometric titration, and ninhydrin assay) and destructive (elemental analysis, acidic or enzymatic hydrolysis followed by HPLC or colorimetry analysis and thermal analysis using DSC) methods [2].

The objective of this thesis is to compare conductometric, two-abrupt-change potentiometric and linear potentiometric titrations in determining the DD of chitosan and to unveil the principles of these three analytical methods as to perform like a handbook for students who are searching for employing these methods to acquire DD values with statistical confidence. The precision of the methods will also be evaluated.

CHAPTER 2 LITERATURE REVIEW

2.1 Degree of Deacetylation

The fully N-acetylated chitin is a linear β-(1→4)-Linked polymer of 2-acetamido-2-deoxy-D-glucose units, which can be considered as the derivative of cellulose where the 2-hydroxy group has been substituted with an acetamido group. After being treated with sodium hydroxide solutions at high temperatures for a period of time, some acetyl groups at the C-2 position in the 2-acetamido-2deoxy-D-glucopyranose units have undergone thermochemical alkaline deacetylation with resulting product -- chitosan. The chitin deacetylation reaction was shown in Figure 2-1. Considering that both commercial chitin and chitosan are copolymers composed of 2-acetamido-2-deoxy-D-glucose and 2-amino-2-deoxy-D-glucose, the degree of deacetylation, DD, has been employed to distinguish between chitin and chitosan. It is referred to chitosan when both the DD is great than a certain value (for example, DD>50%) and the sample is soluble [2].

The DD of chitin/chitosan is also one of the most important parameters that influence the physiochemical, biological, and mechanical properties of chitosan such as crystallinity, hydrophilicity, degradation and cell response [6]. Blair et al. [7] found that the molecular weight of the chitosan prepared from commercial chitin exhibited remarkable dependence on the severity of the deacetylation process. Tensile strength and elongation at the break of the chitosan films decreased with increase in deacetylation and so did their moisture adsorption. Urbanczyk and Lipp-Symonowicz observed that the degree of the crystallinity and the size of the crystallites of chitosan membranes were larger than those obtained from samples of low N-acetylation fraction [8]. Of the factors that determined the biological properties, DD again emerged to be critical. Huang et al. [9] evaluated the effects of molecular weight and degree of deacetylation on the cellular uptake and cytotoxicity of chitosan molecules and nanoparticles, and they have found that nanoparticle uptake is a saturable event for the chitosan samples, with the uptake capacity subsiding with decreasing polymer DD and molecular weight. The cytotoxicity of both chitosan molecules and nanoparticles, however, was attenuated by decreasing chitosan DD but had less correlation with the polymer molecular weight. The knowledge of the DD makes it possible to understand the structure-properties relationship of chitosan and to predict its industrial applications.

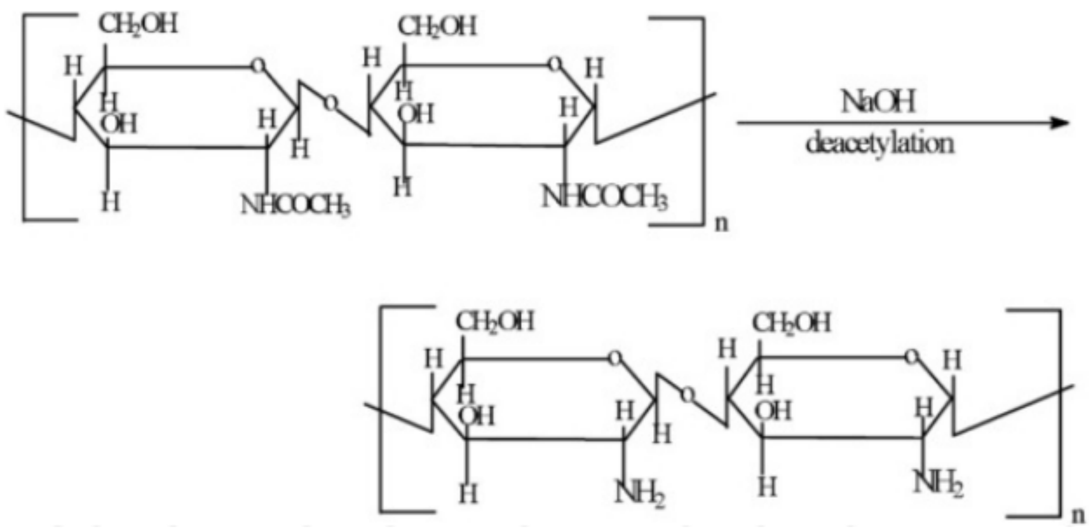


Figure 2-1: Chitin deacetylation [10].

2.2 Methods for Determination of the DD

Up to now, several characterization methods have been developed to determine the degree of deacetylation for chitosan. These techniques involve various nuclear magnetic resonance spectroscopy methods (^{1}H NMR, solid state cross polarization (CP)/magic angle spinning (MAS) ^{13}C NMR, CP/MAS ^{15}N NMR), infrared spectroscopy, near-infrared spectroscopy, ultraviolet spectroscopy, gel permeation chromatography-ultraviolet analysis, ninhydrin assay, colloidal titration, conductometric titration, potentiometric titration and many destructive methods including enzymatic or acid hydrolysis followed by colorimetry or HPLC analysis, elemental analysis, thermal analysis, and pyrolysis-gas chromatography (pyrolysis-GC) analysis [2]. It is well documented in the literature that the DD values determined for the same chitosan specimen are almost always method dependent and often large variations are found between the results obtained from different techniques [2], [11]–[15]. Thus, selecting the suitable characterization method is an essential issue for obtaining values with good accuracy and saving cost and time as well.

2.2.1 Spectroscopy methods

The spectroscopy methods generally study the interaction between matter and electromagnetic radiation and measure the DD of chitin/chitosan by determining the ratio of the intensity of a probe band and the intensity/intensities of the reference band(s). The intensity of a probe band should proportionate to the concentration of N-acetyl or amine content, while the intensity/intensities of the reference band(s) cannot change with the DD.

2.2.1.1 NMR spectroscopy

^{1}H NMR has been employed in the determination of the degree of deacetylation for chitin and chitosan for the sake of its superior sensitivity and providing information on the distribution of the co-units [16]. Dilute solutions of pre-dried chitosan samples in a deuterated aqueous acid (CD_3COOD/D_2O or DCl/D_2O) are required, and their ^{1}H NMR spectra are recorded from 0 to 100 ppm utilizing a proton NMR spectrometer [2]. Considering the fact that the solubility of chitosan in aqueous acidic solution depends on numerous parameters including the degree of crystallinity, degree of polymerization, degree of neutralization of amine, distribution of glucosamine and N-acetyl glucosamine residues in macromolecules, etc. [5], proton NMR needs solution preparations and cannot be used for the chitosan samples with DD less than 40% or block copolymers of chitin/chitosan [16]. Requiring sample preparation and limited application for a certain range of the DD become the greatest obstacles for ^{1}H NMR. Moreover, resonance peaks of the solvent also may obscure that of the chitin/chitosan, which effect the accuracy of proton NMR. Hirai et al. [16] observed that the resonance peak of CH_3 residue in N-acetyl group overlapped with signal of CD_2H residue in the case of using CD_3COOD as the solvent.

^{13}C NMR and ^{15}N NMR remedy the limitation of ^{1}H NMR regarding solution preparation and applicable range of the DD using the combined techniques of proton dipolar decoupling (DD), cross-polarization (CP) and magic-angle spinning (MAS). The degree of acetylation (1- DD) is evaluated from the relative integral of N-acetyl groups compared to the carbon integrals of the D-glucopyranosyl ring by using carbon NMR. As the chemical shifts of these groups are well separated in the ^{13}C NMR spectrum, the measurement of DD does not require any peak deconvolution [11]. The typical ^{13}C NMR spectra of chitin/chitosan are shown in Figure 2-2. However, chitin is frequently associated with other polysaccharides like (1→3)-β-D-glucans, which makes the evaluation of the acetyl content delicate and, in some cases, impossible [11]. The ^{15}N NMR appears very promising to evaluate the acetyl content for a composite or a blend of chitin/chitosan with other polysaccharides, as ^{15}N nucleus is only present in chitin and chitosan among polysaccharides. Only two unique peaks displayed at respectively ca. 110 ppm for the amide and ca. 10 ppm for the amine groups gives clear and well-resolved ^{15}N NMR spectra [11]. However, for a sample with an acetylation level lower than 10%, the line of amino peak exhibits weakness and broadness, which make difficult the quantitative analysis of the DD. Additionally, the relatively sparing natural abundance of ^{13}C and ^{15}N compared with ^{1}H directly results in low sensitivity of ^{13}C NMR and ^{15}N NMR.

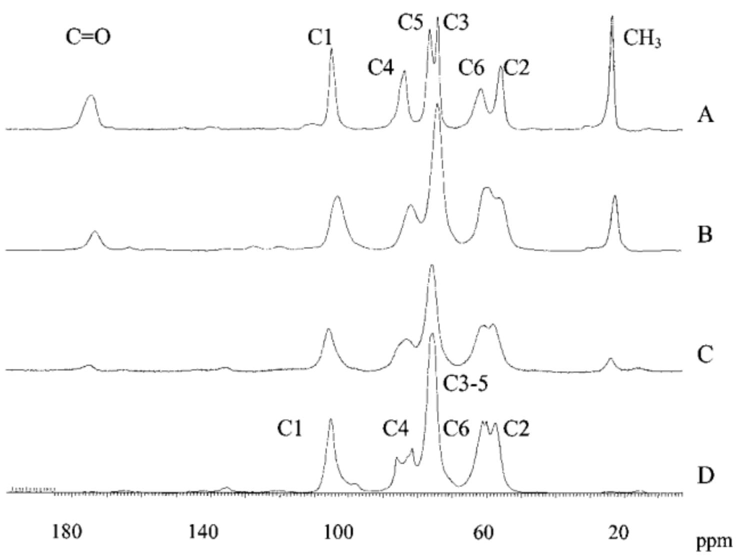


Figure 2-2: ^{13}C CP-MAS NMR spectra of samples (with decreasing DA from A to D) [11].

2.2.1.2 Infrared (IR) spectroscopy and near infrared (NIR) spectroscopy

Infrared (IR) spectroscopy and near infra-red (NIR) spectroscopy have also been employed to determine the DD. IR and NIR spectra are generally recorded in the range of 1200-4000 cm^{-1} and 9090-4000 cm^{-1} respectively [12]. The samples for DD measurement by IR were prepared as either a thin film made from the mixture of chitin/chitosan sample and KBr or a film cast from chitin/chitosan solution [2]. Various absorption band ratios of a probe band and the reference band(s) such as A_{1560}/A_{2875}, A_{1655}/A_{2875}, A_{1655}/A_{3450}, A_{1320}/A_{3450}, A_{1655}/A_{1070}, A_{1655}/A_{1030}, A_{1560}/A_{1160}, A_{1560}/A_{897}, and A_{1320}/A_{1420} have been already proposed to quantitatively determine the deacetylation levels [12]. Generally, the critical issues that determine the DD values obtained from IR spectroscopy are: choosing an appropriate measuring band, choosing an appropriate reference band, and drawing a good baseline. A FTIR spectrum of α-chitin was plotted with several probe and reference bands and corresponding baselines (see Figure 2-3). The complicated network of H-bonds induced by ubiquitous OH, C=O and NH groups within the polymer renders complexity to the IR spectra of chitin and derived chitosans. In addition, the typical broadness of the IR bands of natural polymers makes it more difficult to choose the correct baseline and to determine the absorbances of probe band and reference band(s). Duarte et al. [13] proposed the best absorbance ratios to accurately determine the DD by FTIR and a detailed method to obtain the level of deacetylation. However, the method requires the known standards of DD and the use of a calibration curve obtained using ^{13}C CP-MAS NMR. Besides, chitosan is very hygroscopic and it may contain residues such as proteins, organic pigments and minerals which are naturally occurring in chitin [17]. Because IR spectroscopy detects all signals of the chitin/chitosan and its impurities, the positions and intensities of some peaks are influenced by the presence of the impurities, which may induce interference peaks in the spectra as well.

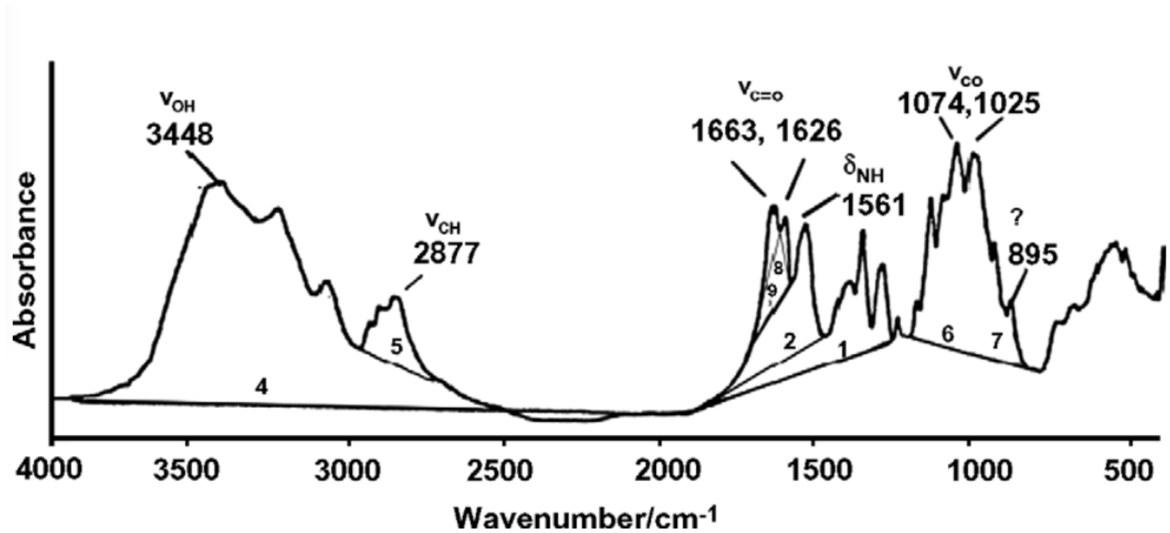


Figure 2-3: Probe and reference bands and corresponding baselines for FTIR spectrum of α-chitin [13].

2.2.1.3 Ultraviolet spectroscopy

Ultraviolet/first derivative ultraviolet spectrometry was first employed to determine the DD of chitosan by Muzzarelli and Rocchetti [18]. They noticed that the first derivative spectra of acetic acid solutions in the range of 0.01 to 0.03 M shared a common point at 202 nm which was denoted as the zero-crossing point. The first derivative spectra for N-acetyl glucosamine and acetic acid were shown in Figure 2-4. As the zero-crossing point was close enough to the N-acetyl glucosamine maximum on the wavelength axis, the determination of N-acetyl glucosamine was independent on the acetic acid concentration in the usual concentration interval of dilute chitosan solutions. Moreover, the analysis revealed a linear correlation between N-acetylglucosamine absorbance readings at 199 nm and its concentration with significant level, which enabled the acquisition of DD values from reading the signal intensities at 199 nm of the UV spectra.

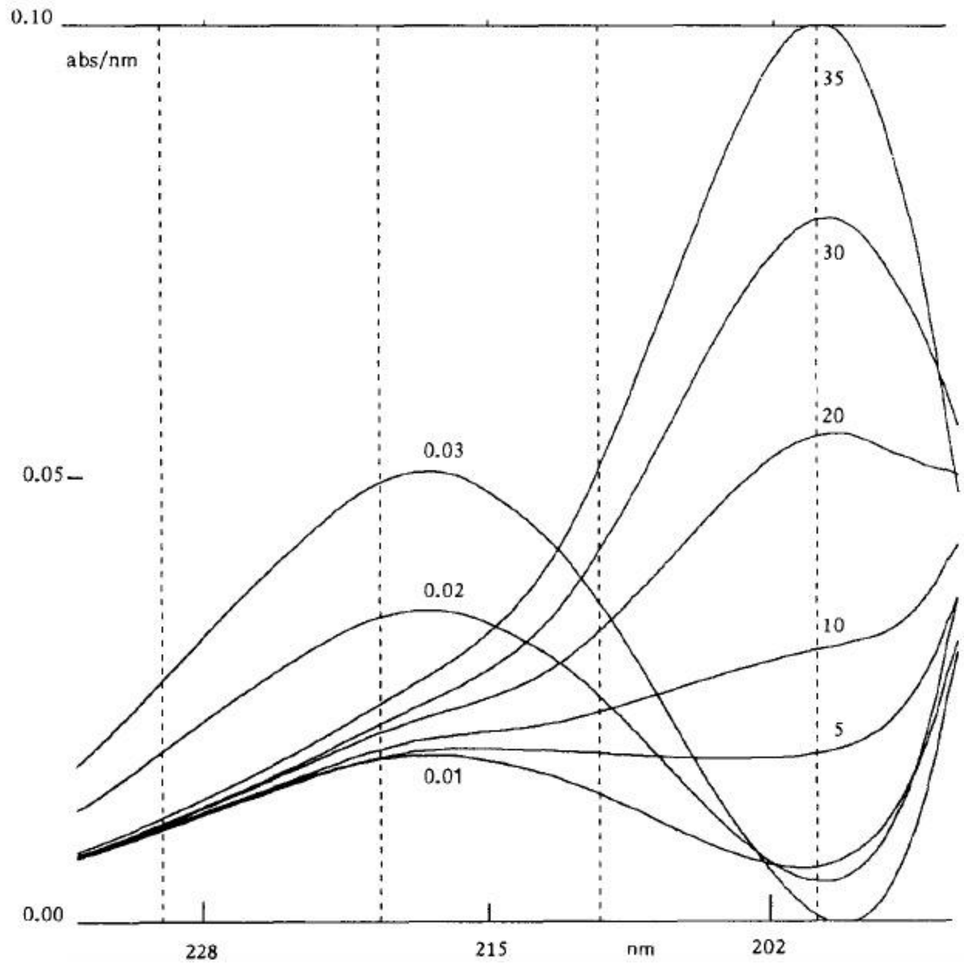


Figure 2-4: First derivative spectra of 0.01, 0.02 and 0.03 M acetic acid solutions and of N-acetyl glucosamine at various concentrations (mg litre^{-1}) in 0.01 M acetic acid [18].

Tan et al. [19] evaluated and refined the method proposed by Muzzarelli and Rocchetti and compared the 1DUVS method with NMR, LPT and ninhydrin test. All the DD values were calculated on a per mol basis other than on a per mass in the research for a better estimation of the deacetylation level. The 1DUVS method was highly recommended among the techniques mentioned above for its tolerance of high level of proteinaceous contaminants and sensitivity for GlcNAc. The maximum UV absorbance of proteins, which is typically at the wavelength of 280 nm, is far from 203 nm, which was the wavelength from which the vertical distance from zero crossing point to each GlcNAc solution spectrum was measured. And 1DUVS is so sensitive for GlcNAc detection that the detectable concentration of GlcNAc in 0.01 M acetic acid solution can be as low as 0.0005 mg mL^{-1} [19]. Wu et al. [20] further improved the 1DUVS method and expanded the detectable range of DD from 0-50% to the whole range by using concentrated phosphoric acid as the solvent in which even highly acetylated chitin can be dissolved. The UV spectrometry has been proposed to be a simple and accurate technique for quantitative analysis of DD in the whole range without expensive equipment [15]. However, the technique still requires sample preparation. Especially in the method using the conc. (85%) phosphoric acid as a solvent, heat treatment at 60 ºC for 40 min and incubation of diluted solutions at 60 ºC for 2 h are required [20]. Furthermore, high concentrations of chitosan solution for analyses are to be avoided as the dips of their first derivative spectra may be shifted beyond the valid range and add more experimental errors. Chitosan solutions of very low concentrations are also not advisable, as this may also reduce the accuracy [19].

2.2.2 Destruction methods

The destruction methods generally determine the degree of deacetylation by completely decomposing chitin/chitosan sample into various characteristic products whose contents are proportional to the deacetylation level and analyze the resulting products by the following techniques. For enzymatic hydrolysis/colorimetry or HPLC analysis, exo enzymes (β-D-glucosaminidase and β-D-acetylhexosaminidase) were employed to completely hydrolyze the chitin/chitosan sample into two monosaccharides $GlcNH_2$ and GlcNAc after 12 h at 40 ºC, and the resulting monosaccharides were quantitatively analyzed by colorimetric or HPLC technique. While acetic acid, released after digestion of chitin/chitosan sample by acid, was treated as characteristic product for acid hydrolysis/HPLC analysis. Acetic acid was then analyzed by HPLC technique. Elemental analysis is based on determination of C, H, N, and O. Elemental analysis of the chitin/chitosan sample was performed utilizing an analyzer of CHN with a carrier gas at a combustion temperature of 1000 ºC. The DD was calculated from the ration of C/N. Thermal analysis is based on decomposing the polymer sample into fragments bearing NH_2 and N-acetyl group. The differential scanning calorimetry (DSC) technique has been harnessed to analyze the decomposed species, whose resulting signals exhibits a linear relationship with the DD of the test samples under specific conditions. Pyrolysis-gas chromatography analysis is based on determination of the intensities of the characteristic products such as acetonitrile, acetic acid, and acetamide, which are liberated from N-acetyl groups of N-acetyl D-glucosamine units of chitin/chitosan by acid hydrolysis. Gas chromatography was then used for determination of target products [2]. Considering the methodology of destruction methods, at least two steps, including decomposition of chitin/chitosan and quantitative analysis of the decomposed species, are required for analysis. Destructive methods are also susceptible to the presence of impurities, such as other carbohydrates or polysaccharides due to the formation of additional characteristic products which can also be produced by the organic materials or polysaccharides other than chitin/chitosan. Besides, analyzing the DD by destruction techniques requires long times, implying that the methods are not suitable for routine purposes.

2.2.3 Conventional methods

A routine method used to determine the DD of chitosan that satisfies the students and end users is essential, if the better understanding the structure-properties relationship and wider exploitation of chitosan are to be realized. The method has to be simple, rapid, economical, and easy accessibility of the instrument, yet detect a deacetylation degree without specialist requirement. From our survey, two conventional methods, conductometric titration and potentiometric titration, yields reliable results with excellent precision, especially for the low acetyl samples due to amino group accessibility factors [21], [22]. Not only do the two methods appear to be the simplest among the feasible techniques, but the equipment and reagents required are also readily available in almost every normal chemistry laboratory. Therefore, this work is designed to compare and refine these two conventional methods hoping to advocate the use of one as the routine method for determining the DD of chitosan.

2.3 Potentiometry

2.3.1 Principle of potentiometry

Potentiometric techniques have been used for locating the end-points of titration since 1893 when the first potentiometric titration was carried out by Robert Behrend at Ostwald's Institute in Leipzig [23]. In more recent methods, ion concentrations are directly measured from the potential that appears across the ion-selective membrane. Perhaps no other type of chemical instrumental measurement can compare to the potentiometric measurement in applicability, considering the staggering number of potentiometric measurements made on the daily basis. Brewers use potentiometric titration for estimating the amounts of isohumulones in bitter hops, which are a key component of beer. Clinicians determine blood gases as significant indicators of disease states. Wastewater streams are monitored continuously to analysis pH and concentrations of pollutants. And these are but a few examples of the many thousands of applications of potentiometric methods.

Potentiometric techniques are based on measuring the potential of an electrochemical cell under static conditions without drawing appreciable current or altering the cell's composition. The potential difference between points A and B is a quantity that expresses the amount of work w_e performed by external forces when moving an electric test charge Q_t from A to B, divided by the magnitude of this charge [24]:

$$\Delta V^{(B,A)} = \frac{W_e}{Q_t} \tag{2-1}$$

Although it is not difficult to determine relative potentials, the concept of the potential of an individual (isolated) point is unable to be defined and becomes meaningful only when this potential is measured against the potential of another point chosen as the point of reference. As to oxidation/reduction reactions in electrochemical cells, an electrode potential is a measure of the extent to which the species concentrations in a half cell differ from their values in the equilibrium state. The Nernst equation which was proposed in 1889 revealed the quantitative relationship between concentration and electrode potential:

$$E = E^0 - \frac{RT}{nF} InQ \tag{2-2}$$

where E^0 is the standard electrode potential, which is defined as the electrode potential when all reactants and products of the half-reaction are at unit activity and is characteristic for each half-reaction. R is the ideal gas constant (8.314 J K^{-1} mol^{-1}) and T is temperature. And n, F and Q are number of moles of electron exchanged in the half-reaction for the electrode process, the

the faraday (96,484 C (coulombs) per mole of electrons) and reaction quotient, respectively. Although the standard electrode potential seems plausible as an absolute value, it is actually a relative quantity which is the potential difference measured against the standard hydrogen electrode, whose potential has been assigned a value of 0.000 V.

The Nernst equation provides a theoretical underpinning for direct potentiometric measurements, which serves as a rapid and convenient method for determining the activity or concentration of a variety of cations and anions. The concentration can be readily obtained by only a comparison between the potential developed by the indicator electrode in the analyte solution and its potential when immersed in the standard solution of known analyte concentration. One major application of this technique is the measurement of pH. The ubiquity of commercial glass electrodes, the utility of pH as a measure of the acidity/basicity of aqueous media, and proliferation of inexpensive solid-state pH meters have made the direct potentiometric measurement of pH perhaps the most common analytical technique [25]. Except for the direct measurement of ion activities, the potentiometric technique also serves as a powerful tool for indicating the end point of the titration. In a potentiometric titration, the potential of an indicator electrode is measured as a function of titrant volume. The potentiometric instrument signals the end point because a rapid change in potential will appear near the equivalent point and thus it behaves in an identical fashion to a chemical indicator. However, potentiometric titrations provide data that are more reliable than experimental data obtained from titrations using chemical indicators and are particularly useful for detecting the presence of unsuspected species and powerful with colored or turbid solutions [25]. Potentiometric titrations also embody additional advantages over the direct potentiometry.

Because the potentiometric measurement is dependent on the titrant volume which is indicated by the inflection point in the steeply rising portion of the titration curve, instead of the absolute values of E_{cell} which is of limited accuracy because of uncertainties in junction potentials and other influential factors. As useful instruments for analysing polyprotic acids, both the direct potentiometry and potentiometric titration techniques have been employed on the determination of the deacetylation degree of chitosan.

2.3.2 PH-titration

pH-Potentiometric titration, first proposed by Broussignac [26], is one of the simplest methods and has been utilized for routine determination of deacetylation degree by chitosan industry for many years because of the cost of facilities and sophistication. Chitosan, a derivative of chitin which is treated by the concentrated sodium hydroxide solution, is an alkaline polysaccharide whose alkalinity comes from amino groups exposed after deacetylation process. Therefore, chitosan can be volumetrically titrated with standard dilute acids with the pH of the solution being monitored with a calibrated pH-sensitive glass electrode. The abrupt change point of the numerical first derivative of the resulting titration curve corresponds to the equivalence point of the titration of the chitosan. However, pH-titration is found to be limited by the negative interference caused by residual acid or alkali impurities in the dry chitosan sample [27]. Due to the limitation, two other methods have displaced the pH-titration and become the dominant methods on determination of the DD.

2.3.3 Linear potentiometric titration

The linearization of potentiometric titration was initially proposed by Gran [28], [29]. The first method of Gran involves the plotting of either $\Delta V/\Delta E$ or $\Delta V/\Delta pH$, instead of the customary $\Delta E/\Delta V$ or $\Delta pH/\Delta V$, against the volume of added titrant, after several values of E or pH have been determined for values of V. The resulting curve consists of two straight lines in the vicinity of the equivalence point, whose common intersection point with the V-axis is the equivalence point [28]. For titration of weak electrolytes, however, the lines are more or less parabolic in shape, which interfere with reliability of the results [30]. In 1952, Gran developed a second method of transforming titration curves into straight lines by numerical manipulation. Gran improved an original idea of Sorensen, who plotted the antilogarithm of $-pH$ as a function of V, the volume of added titrant, by introducing a correction of the volume change during the process of the titration. The second method yields excellent results when titrating a moderately weak acid with a strong base with two straight lines intersecting each other and the V-axis at the equivalent point [29]. However, some deficiencies are induced by the simplifying assumptions made by Gran when deriving his equations. For example, the weak acids with stability constants of 10^7 to 10^8 do not suffice for obtaining straight lines but curved ones [30].

Gran's methods were then modified by Ingman [30] and Johansson [31]. Ke and Chen [32] also proposed a simple method for determining DD based on the linear function derived by Ingman under the assumption that the titration of excessive hydrochloric acid with sodium hydroxide can be considered as the titration of strong acid with strong base. Although their method has been employed in chitosan industry for many years, Ke and Chen did not evaluate the accuracy of their method by showing the agreement with other proven methods. Tan compared the DD values determined by Ke and Chen's method with nuclear magnetic resonance, first derivative uv-spectrophotometry and ninhydrin test, but the DD of linear potentiometric titration did not correlate well with other methods. It was pointed out by the author that the major source of error in this method was that the function proposed by Ingman was not applicable to the titration of mixed acids with a strong base, while it was noticeable that the chitosan solution for titration was actually a mixture of hydrochloric acid and protonated chitosan [19]. In 2003, Jiang et al. [22] derived two new potentiometric titration functions based on the fact that the chitosan solution is a mixture of a strong acid and a weak acid. They also proposed two universal pH regions to restrict the indeterminate error induced in pH measurement and the error caused by precipitation of chitosan. The DD values obtained with these new linear potentiometric titration functions showed good agreement with those obtained with elemental analysis and ^{1}H NMR.

According to Jiang's derivation of the linear function, the volume of added titrant V and the concentration of hydrogen ions $[H^+]$ obey the following equation:

$$V + \frac{V_0+V}{C_B}([\mathrm{H}] - [\mathrm{OH}]) = V_e + \{\frac{C_1V_1}{C_B\times10^{-pH}} + \frac{K_W(V_0+V)}{C_B\times10^{-2pH}} - \frac{V_0+V}{C_B} - \frac{V}{[H^+]}\}K_a \qquad (2\text{-}3)$$

where V is the volume of strong base added, V_1 is the volume of HCl aqueous solution where chitosan is dissolved in, and V_e is the excessive amount of HCl. C_1 and C_B are the concentrations of HCl aqueous solution and titrant solution, respectively. K_w is the ion-product of water, and K_a is the dissociation constant of chitosan. If terms $\frac{C_1V_1}{C_B\times10^{-pH}} + \frac{K_W(V_0+V)}{C_B\times10^{-2pH}} - \frac{V_0+V}{C_B} - \frac{V}{[H^+]}$ and $V + \frac{V_0+V}{C_B}([\mathrm{H}] - [\mathrm{OH}])$ are denoted as X and Y, respectively, a straight line can be obtained when Y is plotted against X. The line should intersect Y-axis at V_e, the volume of excessive HCl solution, from which DD can be easily calculated [22]. Plots of X, Y terms of a chitosan sample in different pH regions are shown in Figure 2-5.

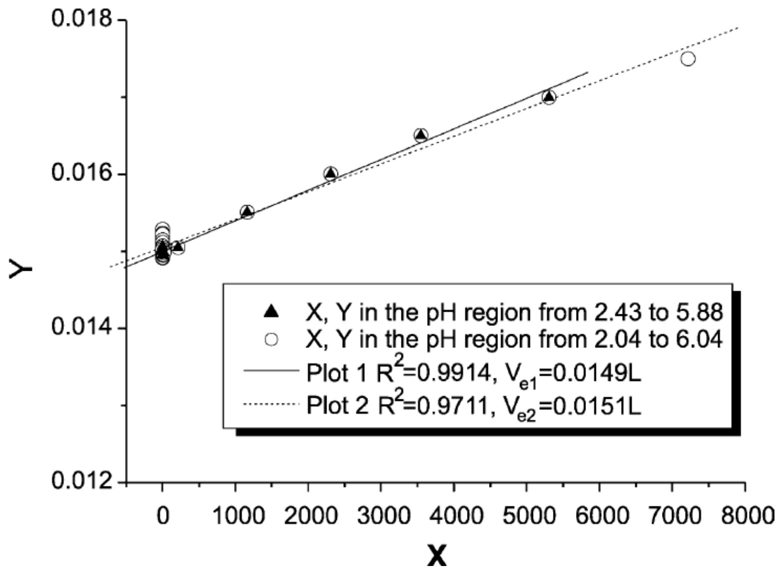


Figure 2-5: Plots of X, Y terms of the chitosan sample in different pH regions [22].

The linearization method provides an approximate estimation of the position of the end point before reaching the neutral pH range where the precipitation of the chitosan happens. Its accuracy should be better compared with the Broussignac's method, because the precipitation of chitosan at the vicinity of equivalent point would deviate the second inflection point from the actual equivalence point [33]. Moreover, only few titration points have to be collected for the method. This makes the Jiang's method easier and faster than other potentiometric titration methods which are more demanding and required to obtain titration points after the equivalence volume. The function values in the beginning of the titration can also be employed as the basis of a reliable way to predict the equivalence volume and to adapt the volume increments accordingly during the rest of the titration [34]. Nevertheless, the problem of residual acid or alkali in the dry samples is still remained and cause negative interference on the determination of the degree of deacetylation.

2.3.4 Two-abrupt-change potentiometric titration

The two-abrupt-change potentiometric titration method generally involves dissolving a precisely weighed quantity of the copolymer in a known excess of hydrochloric acid and then titrating the solution potentiometrically with sodium hydroxide, which results in a titration curve with two inflection points: the first one corresponds to the equivalence point of the titration of excessive hydrochloric acid, while the second to that of the protonated chitosan. The two inflection points can be located more precisely by plotting the first derivative data ($\Delta E/\Delta V$) as a function of the volume V, which produces a curve with two maximums that correspond to the points of inflection (shown in Figure 2-6). The difference between the two inflection points along the abscissa yields the amount of H^+ required for the protonation of the amine groups of chitosan and results in the moles of D-GlcN in the titrant solution. Supposing that the remaining of the sample is D-GlcNAc, the degree of deacetylation can be easily calculated using the following formula [35]:

$$DD = \frac{203.195 \times w(NH_2)}{16.02262 + 0.42037 \times w(NH_2)} \tag{2-4}$$

$$w(NH_2) = \frac{V \times c \times 100 \times 0.016}{W_{dry}} \tag{2-5}$$

where V, c and W_{dry} stand for volume of sodium hydroxide consumption between two abrupt changes of pH, concentration of NaOH titrant, and the dry weight of chitosan sample, respectively. Both DD and $w(NH_2)$are based on the percentage [35].

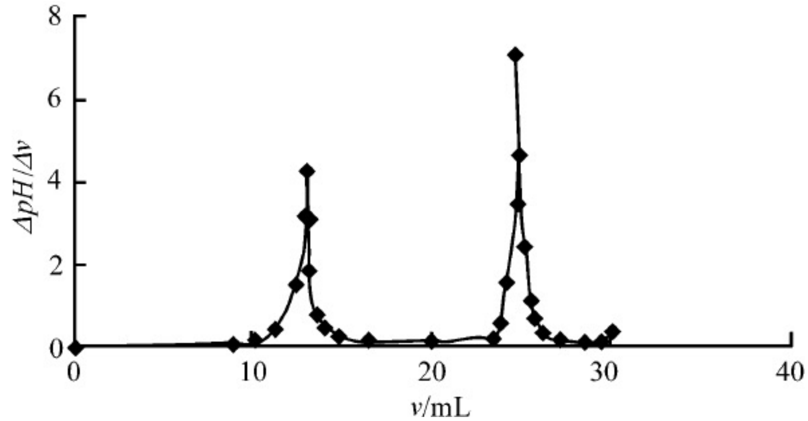


Figure 2-6: The first-derivative titration curve: titrating chitosan sample with sodium hydroxide [36].

The superiority of the two-abrupt-change potentiometric titration over the two foregoing methods is that it eliminates the potential effect of residual acid or alkali in the sample, since the method is based on the deprotonation or protonation of amino group. The major flaw of the method is that the unavoidable flocculation of chitosan in the neutral pH range makes the solution viscous and may generate measurement error. Therefore, Zhang et al. [27] proposed a novel method to reduce the viscosity of chitosan solution by introducing an enzymatic pre-treatment preceding the two-abrupt-change potentiometric titration (EP-TPT). The Chitosan solution, which has experienced water-bath incubation for more than 6 h, was replenished with HCl solution and the pH was adjusted to about 2.5. With the monitoring of the pH meter, chitosan solution was titrated with NaOH and then, after two abrupt potential changes, re-titrated with HCl solution until the pH was back to <3 [27]. The acidic titration exhibited a much more rapid response than alkaline titration in a pH region of 6 or more, where the glass electrode became sluggish and required more time for obtaining stable values for alkaline titration while the protonation of amino groups of chitosan during the acidic titration caused a more rapid response of the pH meter. The titration curve of a chitosan sample is shown in Figure 2-7. The EP-TPT, which showed good agreement with other methods such as ^{1}H NMR, XRD, FT-IR, should be a promising method for industrial determination of chitosan's degree of deacetylation.

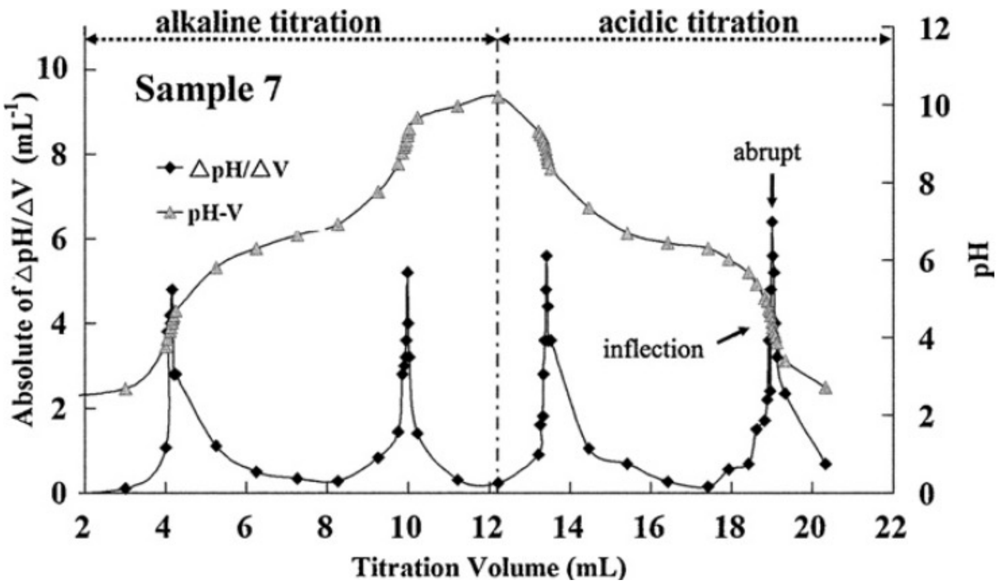


Figure 2-7: EP-TPT titration curve of the chitosan sample 7 [27].

2.4 Conductometry

2.4.1 Principle of conductometry

Compared with the theory of potentiometric titration which is similar to that of ordinary titrations, the principles underlying conductometry is entirely different. Conductometry is a nonselective analysis method. In other words, all mobile ions present contribute to the electrical conductivity of the solution (or other medium being examined), and the contribution of the individual types of ions cannot be distinguished by the measured values. Therefore, conductometry is primarily employed when determining the ionic concentrations in binary electrolyte solutions or when the composition of the solution is known and invariant in the case of multicomponent systems.

Conductometric analysis is also used to monitor the process of titration, which rests on the marked changes that occur at the vicinity of the titration endpoint in the relation between conductivity and the volume of titrant added [24]. In a conductometric titration, the conductivity is determined after the addition of successive amounts of reagent. The obtained points thus are plotted to give a graph which consists of two straight lines intersecting at the stoichiometric point. Since the point is found graphically, the values measured near the equivalence point have no special significance for conductometry, while the measurements are important for ordinary and potentiometric titration methods. Moreover, any of the two straight lines often does not pass through the points near the equivalence point, but the conductivity obtained is higher than the corresponding ones located on the straight lines. The graphically determined point makes the conductometric titration applicable where ordinary or potentiometric titration fail to yield results, because a marked dissociation, hydrolysis, or solubility of the reaction product does not influence the accuracy of the conductometric method very much [37].

Conductometry is the measurement of a solution electrical conductivity which is an important parameter that characterizes quantitatively the ability of an electrolyte solution to conduct electrical current, and for any conductor it depends on the concentrations and the mobilities of all types of ions but not on the geometry and size of the sample:

$$\sigma = F\Sigma z_j c_j u_j \tag{2-6}$$

where σstands for the electrical conductivity (units: S/cm), F is the Faraday constant (96,485 C/mol). And z_j , c_j , u_j stand for the charge number (the number of elementary charges associated with one ion), the volume concentration and the mobility of the ion (the velocity of migration corresponding to unit field strength), respectively [24].

As the ionic mobility characterizes the average velocity of the ionic migration, which is superimposed on the thermal motion of the ions, under the influence of potential of 1V, the mobilities $u_j$of ions in solutions are temperature dependent. According to the force analysis of charged particles in a stationary state under the electric field, four different forces are acting on a particle, which are an electric field force that acted as an external driving force, a friction force that are proportional to the radius of the particle, viscosity of the solvent and the electrophoretic velocity, a force exerted by the relaxation effect and the electrophoretic retardation force. Both of the latter two forces are due to the presence of oppositely charged particles. Therefore, the ionic mobility also depends on concentration and ion species [38]. Moreover, the volume of the solution and the ionic concentrations are always changing throughout a titration, the conductance should be corrected for this effect, otherwise non-linear titration curves result. Under the assumption that the conductivity is a linear function of dilution, the correction can be accomplished by multiplying the measured conductivity either by the total volume $(V + V')$ or by the factor $(V + V')/V$, where V corresponds to the initial volume of the solution and V' stands for the total volume of added reagent [39]. To simplify the principle of conductometry, the variation of solution electrical conductivity during the course of titration can be viewed as the substitution of ions with different conductivity and the equivalent point of the titration shows when the change is complete. Since the H^+ and OH^- distinguish themselves from other ions by their high mobility, conductometry, in a sense, is a function of the sum of conductivity of the hydrogen and hydroxyl ions in solutions in many cases [40].

2.4.2 Conductometric titration of chitosan

Conductometric titration, as an important analytical tool for quantifying acidic functional groups, is one of the most accurate techniques for analysing multifunctional polysaccharides and should provide reliable results characterizing glycosaminoglycans. Hence, we naturally think of using conductometric approach to determine the degree of deacetylation of chitosan. The process of conductometric titration generally behaves in the same fashion to the potentiometric titration, which involves preparing a given chitosan solution by the dissolution of a given mass of chitosan in hydrochloric acid and carrying out conductometric titration with sodium hydroxide using a conductivity meter. The values of conductance (mS cm^{-1}) are plotted with the corresponding titrant volumes. Figure 2-8 shows a typical curve of conductometric titration of a chitosan solution by NaOH obtained from the work.

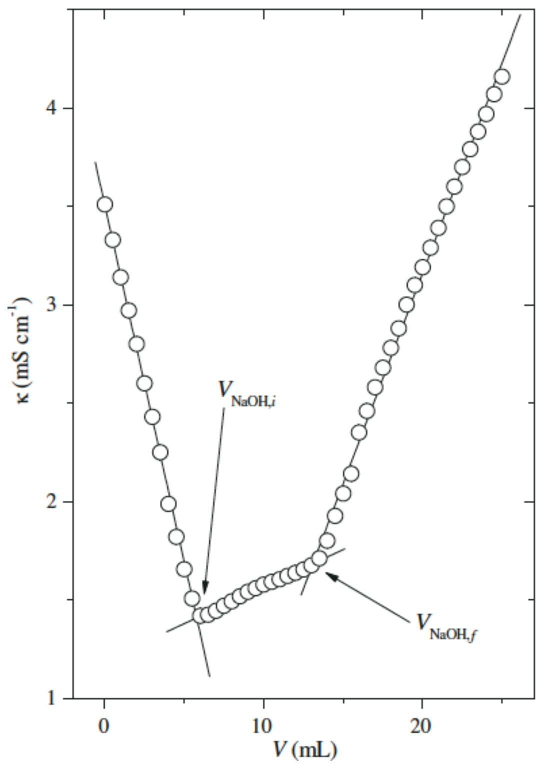


Figure 2-8: Typical curve of conductometric titration for the chitosan solution [41].

The curve can be divided into three regions: the first rapid descending branch is related to the neutralization of the hydrogen ions of the HCl solution added. The conductance falls dramatically due to the replacement of hydrogen ions by the sodium cation as hydrogen ions are the most conducting of all the ions, and hydrogen ions react with hydroxide ions to form undissociated water. The second region corresponds to the neutralization of the protonated amino groups present in chitosan. The second branch is ascending due to the higher mobility of sodium ions than protonated chitosan. The final upward region is related to the increase in conductance because of the addition of sodium and hydroxide ions to the solution as NaOH is added. And in most cases, a curvature occurs near the first inflection point $V_{\mathrm{Na},i}$ of the plot, which is attributed to the initial dissociation of the protonated amino acid groups. While in the vicinity of the second inflection point $V_{\mathrm{Na},f}$, a small deviation from linearity presents during the final phase of neutralization coinciding with the onset of the precipitation of chitosan [21]. Fortunately, the stoichiometric points can be determined graphically by plotting three linear branches which are intersecting at the equivalence points and the difference between the two intersection points gives the volume of base required to neutralize the amino groups of chitosan.

The feasibility and underlying theory of the conductometric titration on determining the degree of acetylation of chitosan was studied by de Alvarenga et al. [40] who employed conductometric analysis and ^{1}H NMR on D-glucosamine hydrochloride (DG-HCl), N-acetyl-D-glucosamine (NADG) and mixtures of NADG and DG-HCl of known concentrations. Two inflections points, with the first equivalence point associated with neutralization of the excess acid and the second one corresponding to neutralization of ammonium groups of the DG-HCl solution, were observed in the conductivity plot of DG-HCl (Figure 2-9), while only one inflection point was yielded by the neutralization of the HCl in excess in the conductivity graph of NADG (Figure 2-9).

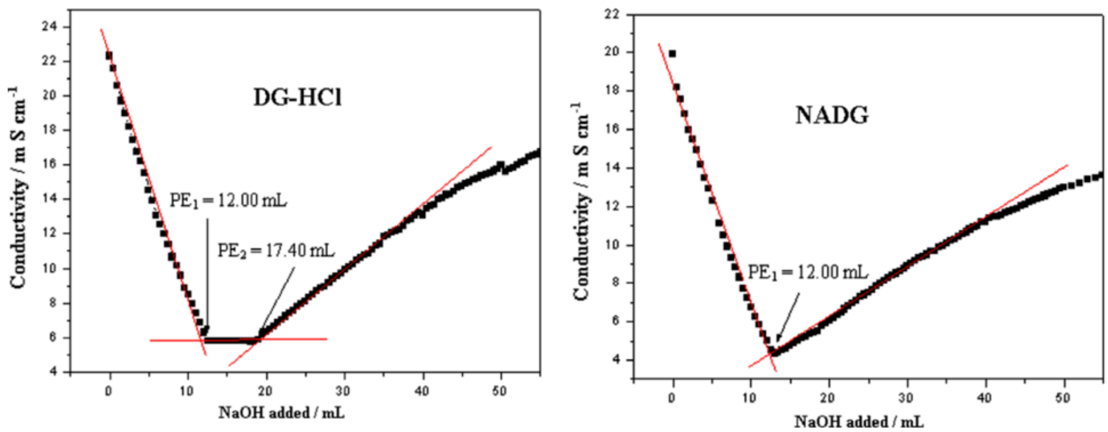


Figure 2-9: Conductometric titration of DG-HCl and NADG in HCl with NaOH at 25 ℃ [40].

The results indicated that the acetamide group of NADG was not protonated by hydrochloric acid and amide group had not been hydrolysed either, thus the second inflection point could not be seen in the conductivity graph of NADG. Besides, the DD values obtained by conductometric titrations and ^{1}H NMR for mixtures of monomers were statistically in agreement. The results determined by these two techniques were also in accord with the known proportions, which corroborated the conclusion that conductometric titration was a reliable technique measuring acetyl contents of chitosan.

Santos et al. [41] carried out a rigorous error analysis of conductometry and derived a mathematical expression relating data obtained from the conductometric titration and degree of deacetylation in conjunction with its associated error:

$$DD = 20319.25 \cdot \frac{\Delta V \cdot C_{\mathrm{NaOH}}}{42.0367 \cdot \Delta V \cdot C_{\mathrm{NaOH}} + W_{\mathrm{CHIT}} \cdot m_s} \tag{2-7}$$

$$\delta_{DD} \leq \frac{DD}{C_{\mathrm{NaOH}}\Delta V}(|1 - 0.00206881 \cdot DD|\delta_{C_{\mathrm{NaOH}}\Delta V} + 4.921381 \cdot 10^{-5} \cdot DD \cdot \delta_{W_{\mathrm{CHIT}}m_s} \tag{2-8}$$

where

$$\delta_{C_{\mathrm{NaOH}}\Delta V} = C_{\mathrm{NaOH}}\delta_{\Delta V} + \Delta V \delta_{C_{\mathrm{NaOH}}}, \tag{2-9}$$

and

$$\delta_{W_{\mathrm{CHIT}}m_s} = W_{\mathrm{CHIT}}\delta_{m_s} + m_s \delta_{W_{\mathrm{CHIT}}}. \tag{2-10}$$

ΔV corresponds to the volume difference between the two equivalence points, C_{NaOH} is the sodium hydroxide concentration, δ_{DD} is the propagated error of DD, and W_{CHIT} is chitosan mass fraction in the sample, which is determined by leaving a sample mass m_s in an oven at 105 ℃ until a constant mass m_{chit} has reached, and is defined as $\frac{m_{\mathrm{chit}}}{m_s}$. The chitosan deacetylation degrees calculated by this method were consistent with those obtained via the more established technique of CHN elemental analysis, which confirmed the accuracy of the mathematical expressions and proved conductometric analysis as an accurate method for the measurement of the degree of deacetylation of chitosan.

Despite conductometric titration being a very secure and economical method with excellent precision better than 1% at all levels [39], there are also few disadvantages with this technique, especially when compared with other analytical methods. Conductometric analysis often underestimates the degree of acetylation of crystalline or high acetyl contents, compared with CP/MAS [21], for the reason that only surface amino groups of the crystalline structure are detectable for titration while those within the crystallites are inaccessible. Additionally, one has to work very carefully with the total mass of pure chitosan (without moisture content) and the parameter W_{CHIT} must be taken into account with its associated error for conductometry, while CHN elemental analysis is relatively insusceptible to absorbed water since it rests on the ratio between the mass percentages of carbon and nitrogen in sample [41]. The presence of impurity also influences the results obtained by conductometric analysis, which doesn't affect the results of ^{1}H NMR [40]. Hence, the foregoing influential factors should be taken into consideration in our study and a series of corrections are needed in order to yield accurate DD values by conductometric titration.

CHAPTER 3 EXPERIMENTAL

3.1 Standardizing

3.1.1 Standardizing a sodium hydroxide solution

Two 0.10 N sodium hydroxide solutions (VWR Inc.) were used as titrants in this work. Since the major method of the thesis is titration, it is essential to know the exact concentrations of the titrant solutions in order to determine the accurate concentration of the tested solution. Therefore, the 0.1N NaOH solutions were standardized with potassium hydrogen phthalate (KHP, $KC_8H_4O_4H$ manufacturer) using both phenolphthalein and conductometer as the indicators.

The KHP was dried overnight at about 100°C in a heated vacuum desiccator (Precision Scientific Co.). Then the accurately weighed KHP was agitated to be fully-dissolved in distilled water on a magnetic stirrer. The volume of the added titrant till the first permanent appearance of pink and values of conductivity during the whole course of titration were recorded.

3.1.2 Standardizing a hydrochloric acid solution

A 0.10 N hydrochloric acid solution (VWR Inc.) was employed as a solvent of chitosan in this work. It is also critical to know the precise concentration of the HCl solution, because the excessive volume of the HCl aqueous solution and its predetermined concentration are quantitatively related with the DD values of chitosan samples.

Since the exact concentrations of the NaOH solutions were obtained, the HCl solution was standardized using the NaOH solutions as the titrant. The volume of the HCl solution being tested was measured and decanted into an Erlenmeyer flask. It was then dissolved in distilled water and titrated by the NaOH solutions under the monitoring of both phenolphthalein and conductometer. The volume of the added NaOH solutions to a persistent endpoint was obtained, and the conductivities during the titration were recorded as well.

3.2 Conductometric Titration

3.2.1 Determining the mass fraction of chitosan samples

While using conductometric titration for the determination of the degree of deacetylation, one has to work with the total mass of pure chitosan, namely without any absorbed water which cannot be eliminated even after complete cryodesiccation [41]. Therefore, a parameter, the mass fraction of chitosan samples W_{CHI}, must be introduced with its associated error in the calculations.

In order to determine the chitosan mass fraction for a given sample, W_{CHIT}, several weighing bottles have to be thoroughly cleaned and dried with their original weights being recorded. Then the chitosan samples (Primex ehf, Iceland) were transferred into the weighing bottles, and the bottles with their contents were weighed so as to determine the masses of original samples m_s by difference. Each weighing bottle with the chitosan sample was contained in a labelled beaker with a cover glass for the sake of protection the sample from accidental contamination [25], and was heated at about 100°C in the heated vacuum desiccator (Precision Scientific Co.) until a constant mass was reached. After being cooled down to the room temperature in a desiccator, the weighing bottles were weighed again along with the solid. The masses of oven-dried samples m_{chit} were determined by subtracting the weights of the bottles. And the mass fraction of chitosan samples was calculated as $\frac{m_{chit}}{m_s}$.

3.2.2 Preparation of the chitosan solutions

0.10 N sodium hydroxide solutions (VWR Inc.) and 0.10 N hydrochloric acid solution (VWR Inc.) were standardized and used as received. Chitosan samples were divided into two groups: sample A was oven-dried as reported in the foregoing step of the mass fraction determination, while sample B was the untreated chitosan with absorbed water. Both sample A and B (about 0.2 g) were dissolved in 20 mL of 0.1 N standard HCl aqueous solution. The solution was then mixed with 20 mL distilled water and was left under mechanical stirring for 18h at room temperature.

For the oven-dried group, a subset of chitosan was used to analyse the effect of volume changing on the determination of the DD values by conductometric titration. Therefore, the volume of solution at the beginning of titration should be determined. Desiccated chitosan (about 1 g) was dissolved in 125 mL of 0.1 M standard HCl aqueous solution under continuous stirring for 18h at room temperature. The solution was then directed by a stirring rod from the beaker into the funnel, which was inserted into the neck of a pre-weighed 500 mL volumetric flask. The stirring rod, the interior of the beaker, and the magnetic stirring bar were rinsed with distilled water several times with the washings transferred to the volumetric flask. The solution was then topped up to 500 mL with distilled water. The weights of the volumetric flask and its content were measured in order to obtain a certain volume of the viscous chitosan solution based on its density.

3.2.3 Titration

After the addition of 100 mL of distilled water to the chitosan solution prepared from either sample A or B, conductometric titration was carried out with the 0.1 N NaOH solution using a conductivity meter (Thermo Scientific Orion), model 162 (K=0.451 cm^{-1}). To analyse whether the introduction of m_{chit} influences the DD values or not, fifteen replicates were performed for each group. For the group of studying the volume changing effect, the prepared solution was poured into a pre-weighed beaker and the initial volume of the tested solution was calculated by dividing the net weight of the solution by its density. The titration process was carried out in the same fashion as described above without the addition of the distilled water. A graph of conductivity as a function of the volume of titrant was plotted and the equivalence points, corresponding to the inflection points in the graph, were employed to calculate the percentage of deacetylated amine groups in the chitosan sample with its associated error.

3.3 Two-abrupt-change Potentiometric Titration

Accurately weigh about 0.2 g chitosan (Primex ehf, Iceland) into 20 mL HCl (0.1 mol/L) of the standard solution to get protonation. After addition of 20 mL distilled water, the chitosan solution was mechanical stirred for 18 h at the room temperature and further mixed with 100 mL distilled water to get the solution for testing. Chitosan was titrated with NaOH standard solution added about 1 mL each time stepwise with the aid of the pH meter (Model 290, Thermo Scientific Orion). Both the pH value and the consumption of titrant V was recorded, and the first derivative and the second derivative of the titration curve, namely a graph of $\Delta pH/\Delta v$ as a function of the volume of the addition of NaOH V and that of $\Delta(\Delta pH)/\Delta v^2$, were plotted. The degree of deacetylation was calculated by volume of NaOH consumption between two abrupt changes of pH, namely the difference between the two main peaks along the abscissa of the first derivative curve or the distance between two zero crossing points on the x axis of the second derivative curve, using the following formula [35]:

$$DD = \frac{203.195 \times w(NH_2)}{16.02262 + 0.42037 \times w(NH_2)} \tag{3-1}$$

$$w(NH_2) = \frac{V \times c \times 100 \times 0.016}{W_{dry}} \tag{3-2}$$

where V, c and W_{dry} stand for the volume of sodium hydroxide consumption between two abrupt changes of pH, concentration of the standard titrant, and the dry weight of chitosan sample, respectively. To compare between conductometric titration and two-abrupt-change potentiometric titration, fifteen replicates were also conducted using potentiometry.

3.4 Linear Potentiometric Titration

Oven-dried chitosan (about 1 g) was dissolved in 125 mL of 0.1 M standard HCl aqueous solution. The solution was then topped up to 500 mL with distilled water and calculated amount of KCl was added in order to adjust the ionic strength to 0.1. The titrant was the solution of 0.1 M sodium hydroxide containing 0.1 M KCl for the sake of maintaining the ionic strength of titrate as 0.1 during the whole course of titration. A 100mL protonated chitosan solution was tested each time, which was obtained by weighing a certain net weight of the solution calculated from its density. Under continuous stirring, the potentiometric titration was carried out with the standard NaOH added about 0.5 mL stepwise each time. The titrant was added until the pH value of the solution reached 6.0 under the monitoring of pH meter (Model 290, Thermo Scientific Orion). The volume of NaOH consumption and pH values of the solution were recorded to calculate their corresponding terms [22]:

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

and

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

which are denoted as X and Y, respectively. V is the volume of strong base added at the concentration of C_B, V_1 is the volume of HCl aqueous solution at concentration of C_1 where chitosan is dissolved in, and V_e is the excessive amount of HCl. K_w and K_a are the ion-product of water and the dissociation constant of chitosan, respectively. A straight line can be obtained when Y is plotted against X, which should intersect Y-axis at V_e, the volume of excessive HCl solution, from which DD can be easily determined. Ten replicates were performed for the Primex samples.

CHAPTER 4 NUMERIC RESULTS AND DISCUSSION

4.1 Comparison between Phenolphthalein and Conductometer as Indicators for Titration

The standardization of sodium hydroxide and hydrochloric acid solutions was carried out under the monitor of both phenolphthalein and conductometry. The volumes of titrant added and conductivities during the titrations for the sodium hydroxide solutions of batch Ⅰ and Ⅱ are listed in Table 1 and Table 2 (Appendix A), respectively. And Table 1 and Table 2 (Appendix B) contain the volumes of the added NaOH solutions and the conductivities for the standardization of hydrochloric acid solution of batch Ⅰ and Ⅱ, respectively. The experimental records, which are corresponding to the endpoints when the solutions were light pink, are presented in bold style in the tables. Figure 4-1 demonstrates a typical conductometric titration curve of standardizing NaOH based on the experimental results of subsample 2 in Table 1 (Appendix A), while Figure 4-2 illustrates a representative conductometric titration curve of standardizing HCl according to the data of subsample 1 in Table 1 (Appendix B), with V_{NaOH} indicating the volume of titrant added at the endpoint determined by the phenolphthalein color changing and V'_{NaOH} corresponding to the volume at the inflection point based on the conductometric curve. In addition, the experimental data and the calculated concentrations of the sodium hydroxide solutions and that of hydrochloric acid solutions are shown in Table 4.1 and Table 4.2, respectively.

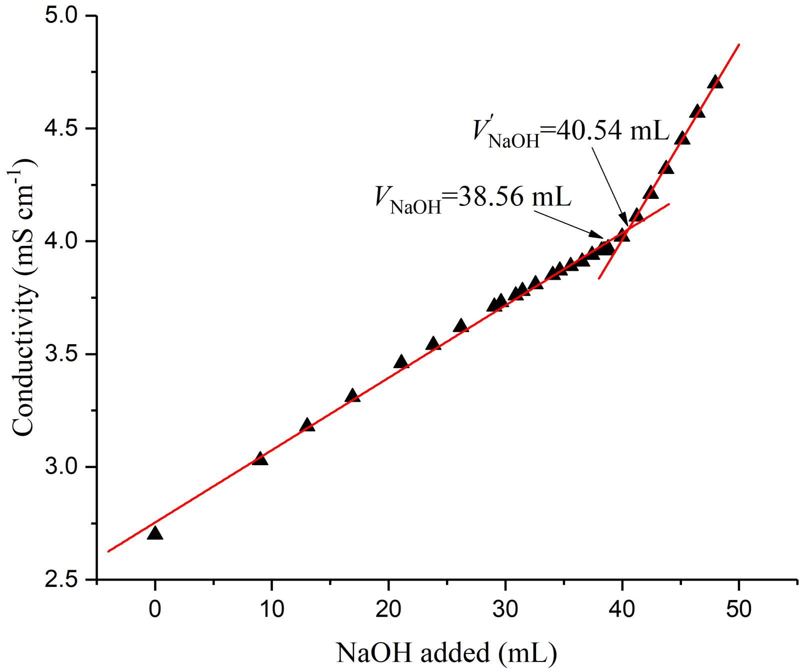


Figure 4-1: Typical conductometric titration curve for the NaOH solution subsample 2 from batch I (V_{NaOH} and V'_{NaOH} are indicated by arrows).

The principle of conductometric titration is based on the fact that ion replacement happens during the titration and the difference of ionic conductivities of these two ions results in conductivity variation of the solution during the course of titration. For the case of potassium hydrogen phthalate (KHP, $KC_8H_4O_4H$) reacting with the strong base, which is an acidic salt compound, the conductance increases on adding NaOH as NaOH neutralizes the un-fully dissociated $C_8H_4O_4H^-$ to $C_8H_4O_4Na_2$ which is the strong electrolyte. However, the replacement of H^+ by Na^+ at some degree weakens the increase tendency since the mobility of Na^+ is much less than that of H^+. The graph near the equivalence point is curved, which is probably due to the hydrolysis of salt $C_8H_4O_4Na_2$ [39]. Beyond the equivalence point, conductance ascends more rapidly with the addition of NaOH due to the highly mobile OH^- ions. And the stoichiometric point was estimated graphically by plotting two linear fitting lines that are intersecting at the equivalence point.

Compared with the conductometer, phenolphthalein, as an acid-base indicator, is commonly used to determine the end of the titration based on its color change and to serve as an indicator for standardizing the sodium hydroxide solution. From Figure 4-1, it is obvious to find that the estimated inflection points of the conductometric curve do not coincide with the equivalence point indicated by the phenolphthalein, but the estimated point is located at the right of the indicator determined point, which is not an exception. Actually, the experimental data obtained from six trials of NaOH standardization shown in Table 4.3 exhibits that the volume at the inflection point based on the conductometric curve, V'_{NaOH}, is generally larger than V_{NaOH}, which is the volume of titrant added at the endpoint determined by the phenolphthalein color changing. The corresponding calculated concentration of NaOH solution C'_{NaOH} is consequently smaller than C_{NaOH}.

For the case of conductometric titration, the angle between the two branches of the titration curve of KHP is obtuse at some degree as illustrated in Figure 4-1, which increases the influence of errors in the titration. Moreover, the titration of a slightly ionized salt generally does not give good results, since the conductivity increases continuously from the commencement. KHP as a salt present in the cell is not virtually completely dissociated, which also compromises the accuracy of the conductometry. As the selection of experimental data for linear fitting is arbitrary, the curvature near the equivalence point due to the hydrolysis of salt $C_8H_4O_4Na_2$ also brings about errors. A small error in the fitting lines can cause a large deviation, because the inflection points are determined graphically from the extrapolation of two linear trendlines. Besides, the always-increasing volume of the solution throughout a titration is a source of systematic error that results in non-linear titration curves. Without the correction counteracting the effect of increasing solution volume, the inflection point of the conductometric titration curve may not represent the actual stoichiometric point.

For an acid-base indicator, it is a weak acid or base that can react with some of the titrant, and the color of the indicator may not change at the exact pH of the equivalent point. Therefore, a small error is introduced in the titration using phenolphthalein as the indicator. However, this error can be minimized by using a small amount of the indicator. Generally, only 4 or 5 drops of phenolphthalein indicator are required for one trial of standardization. In addition, since phenolphthalein has a pKa of 9.2 [42], one would expect to observe a color change in a solution with a pH of about 9 which is reasonable for this experiment. Besides, C_{NaOH} is closer to the concentrations listed on the labels of the solutions compared with C'_{NaOH}. Therefore, the arithmetic mean value of C_{NaOH} is adopted as the molar concentration of the standardized NaOH solution in the article instead of using that of C'_{NaOH}.

Table 4-1 Estimated concentrations of NaOH solutions according to V_{NaOH} and V'_{NaOH}

Batch	**Subsample**	**m_{khp} (g)**	**V'_{NaOH} (mL)**	**V_{NaOH} (mL)**	**C'_{NaOH} (mol/L)**	**C_{NaOH} (mol/L)**
I	1	0.7096	36.52	34.89	0.09514	0.09958
	2	0.7837	40.51	38.56	0.09473	0.09952
	3	0.7028	36.58	34.70	0.09407	0.09917
				Mean	0.09465	0.09942
II	1	0.7673	39.67	37.91	0.09471	0.09910
	2	0.7260	37.31	35.78	0.09527	0.09935
	3	0.7410	38.18	36.62	0.09504	0.09908
				Mean	0.09501	0.09918

Considering the standardization of hydrochloric acids where a strong acid reacts with a strong base, the conductance is high initially due to the high mobility of hydrogen ions presenting in the cell. When the base is added, the conductance descends due to the replacement of hydrogen ions by the added sodium cations as H^+ ions react with OH^- ions to form undissociated water. The decrease in conductivity continues till the equivalence point where the solution only contains NaCl. After the equivalence point, the curve extends upward due to the increase in conductance because of the addition of sodium and hydroxide ions to the solution. Based on the Figure 4-2, it is not hard to find that V_{NaOH} is larger than $V'_{\text{NaOH},}$ and the experimental results from other replicates suggest a similar magnitude relation. We still employ the arithmetic averages of C_{HCl} determined by V_{NaOH} as the concentrations of standardized hydrochloric acids for the following study.

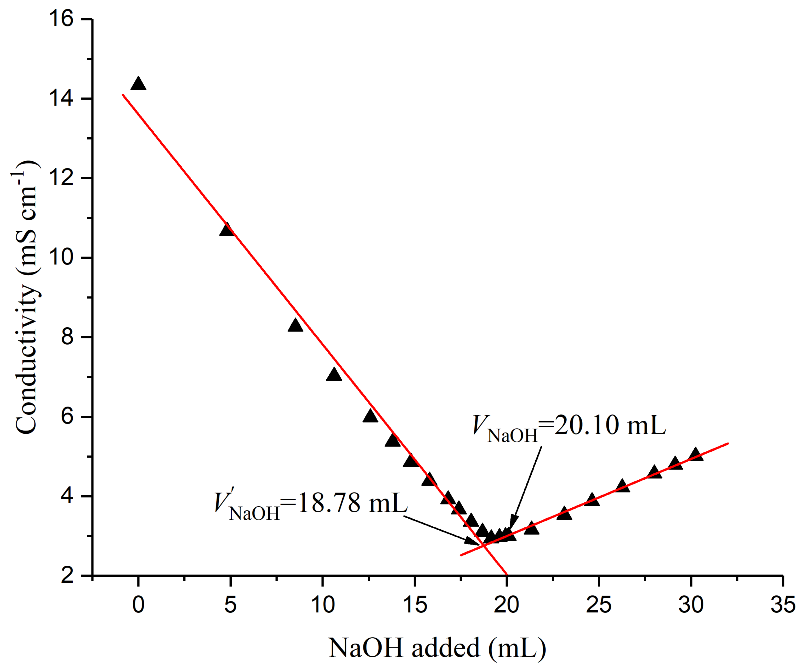


Figure 4-2: Typical conductometric titration curve for the HCl solution subsample 1 from batch Ⅰ (V_{NaOH} and V'_{NaOH} are indicated by arrows).

Table 4-2 Estimated concentrations of HCl solutions according to V_{NaOH} and V'_{NaOH}

Batch	Subsample	V_{HCl} (mL)	V'_{NaOH} (mL)	V_{NaOH} (mL)	C'_{HCl} (mol/L)	C_{HCl} (mol/L)
Ⅰ	1	20	18.78	20.10	0.09338	0.09992
	2	20	18.82	20.16	0.09356	0.1002
	3	20	18.90	20.10	0.09393	0.09992
				Mean	0.09362	0.1000
Ⅱ	1	10	9.10	9.79	0.09044	0.09734
	2	10	9.24	9.88	0.09190	0.09823
	3	10	9.20	9.75	0.09147	0.09694
				Mean	0.09127	0.09750

4.2 Conductometric Titration

A typical curve of conductometric titration of an acidic chitosan solution by standardized NaOH as obtained in this work is shown in Figure 4-3. The curve is constituted by three regions, with the first region related with the neutralization of excess HCl present in the cell, the second region associated with the neutralization of the protonated amino groups in the chitosan, and the third region connected to the addition of Na^+ and OH^- to the solution. The regions are linearly fitted and described as three linear regression lines. The intercept between the first and second regression lines gives the volume in which protonated amino groups start to be neutralized, $V_{\mathrm{NaOH},i}$ and the intercept between the second and third regression lines gives the volume in which the amino groups are completely neutralized, $V_{\mathrm{NaOH},f}$. Therefore, the number of moles of deacetylated units can be determined by the difference between $V_{\mathrm{NaOH},f}$ and $V_{\mathrm{NaOH},I}$ multiply the sodium hydroxide concentration.

The experimental data of conductometric titration is listed in Appendix C, including the volume of standardized NaOH solution added and conductivity of conductometric titration for oven-dried (shown in Table 1) and undried chitosan (shown in Table 2), and the volume of added titrant, conductivity and corrected conductivity of conductometric titration analysing the effect of ever increasing total volume of the solution on the DD value determined by conductometry (shown in Table 3).

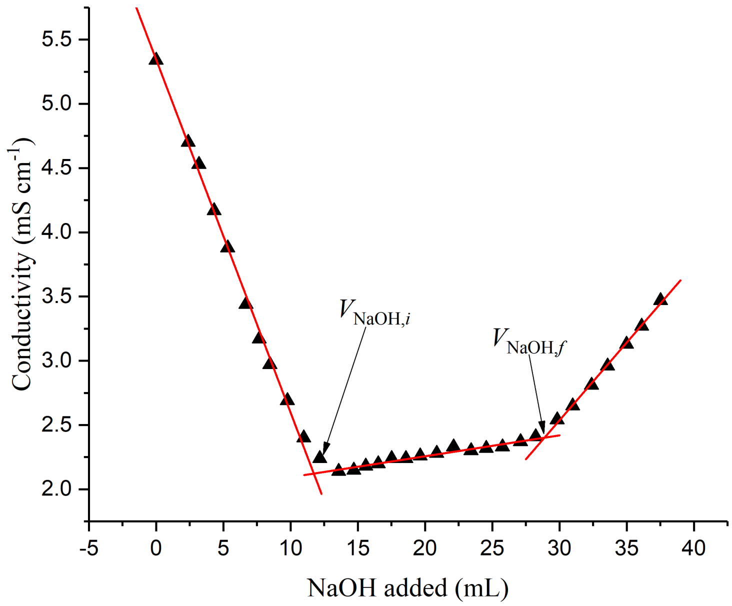


Figure 4-3: Typical conductometric titration curve for the chitosan used in this work. ($V_{\mathrm{NaOH},i}$ and $V_{\mathrm{NaOH},j}$ are indicated by arrows).

4.2.1 Effect of introducing the chitosan mass fraction

Chitosan is hygroscopic in nature with a great capability to form hydrogen bonding with water by hydroxyl and amino groups. Its hygroscopicity would be problematic for quantitative measurement of the degree of deacetylation, since the total mass of pure chitosan (without absorbed water) is always a key parameter for conventional methods of determining deacetylation degree, and water content may influence the accuracy of some spectroscopic methods adopting reference bands associated with H or O elements. Therefore, we are supposed to either make an attempt to completely eliminate the moisture content in the chitosan samples or introduce a parameter to exclude the influence of water. According to the study conducted by Balázs, N. and Sipos, P. [43], the sample contained a significant amount of moisture after 24 h of storing in vacuum storage over P_2O_5, and TG measurements still indicated about 0.8% by weight of water after six days of such treatment. And dried samples can obtain moisture content when they are exposed to the air only for a very short period of time. Therefore, the ordinary drying may not be suitable for the complete elimination of moisture contend. The other solution might be determining the chitosan mass fraction W_{CHIT} before the measurement of DD and enter it in the calculus to obtain the value of pure chitosan mass. In the work, graphically determined results $V_{\mathrm{NaOH},i}$ and $V_{\mathrm{NaOH},f}$ are recorded with their corresponding masses of the chitosan samples in order to calculate the degree of deacetylation using the following formula [35]:

$$\mathrm{DD} = \frac{20319.25 \times \mathrm{C_{NaOH}} \times \Delta \mathrm{V}}{42.0367 \times \mathrm{C_{NaOH}} \times \Delta \mathrm{V} + \mathrm{m_{Chitosan}}} \tag{4-1}$$

Where m_{Chitosan} is the mass of the chitosan used in the analysis (in a dried basis). For oven-dried chitosan sample, m_{Chitosan} is the mass measured after drying treatment, while it is taken as the mass of pristine chitosan without treatment, m_{Sample}, multiplying the chitosan mass fraction, W_{CHIT}, for undried chitosan. The results related to conductometric analysis of oven-dried and undried chitosan are listed in Table 4.3 and Table 4.5, respectively. And the records associated with chitosan mass fraction are shown in Table 4.4, where m_{Bottle} is the weight of the weighing bottle, m'_{Bottle} indicates the weight of the weighing bottle together with chitosan sample after the oven drying, and the mass of pure chitosan mass, m_{Chitosan}, is the difference between m'_{Bottle} and m_{Bottle}. W_{CHIT} is defined as $\frac{m_{Chitosan}}{m_{Sample}}$ and the average number of W_{CHIT} is employed to calculate the values of m_{Chitosan} in Table 4.5.

Table 4-3 Results related to conductometric analysis of oven-dried chitosan

Run	$V_{\mathrm{NaOH},i}$ **(mL)**	$V_{\mathrm{NaOH},f}$ **(mL)**	ΔV **(mL)**	$\mathbf{m}_{\mathbf{Chitosan}}$ **(g)**	**DD**
1	57.21	74.37	17.16	0.3076	91.39
2	56.86	68.63	11.77	0.2138	90.41
3	64.52	75.51	10.99	0.1976	91.15
4	63.58	75.25	11.67	0.2092	91.39
5	73.47	85.09	11.62	0.2016	93.82
6	73.45	85.45	12.00	0.2069	94.28
7	63.62	74.56	10.94	0.1904	93.58
8	63.09	75.05	11.96	0.2159	90.84
9	66.58	78.30	11.73	0.2074	92.40
10	66.89	78.37	11.48	0.2086	90.40
11	71.74	83.07	11.33	0.2033	91.30
12	74.65	85.68	11.02	0.1959	92.03
13	72.65	81.92	9.27	0.1595	94.45
14	61.44	73.95	12.50	0.2270	90.45
15	61.41	75.85	14.44	0.2697	88.36

Table 4-4 Results related to chitosan mass fraction

Run	$\mathbf{m}_{\mathbf{Bottle}}$ **(g)**	$\mathbf{m}'_{\mathbf{Bottle}}$ **(g)**	$\mathbf{m}_{\mathbf{Sample}}$ **(g)**	$\mathbf{m}_{\mathbf{Chitosan}}$ **(g)**	$\boldsymbol{w}_{\mathbf{CHIT}}$
1	76.1296	76.7490	0.7018	0.6194	0.8826
2	78.8562	79.4382	0.6649	0.5820	0.8753
3	73.8117	74.3836	0.6454	0.5719	0.8861
4	66.0768	66.6199	0.6278	0.5431	0.8651
5	32.5888	33.4618	0.9971	0.873	0.8755
6	75.4431	76.3304	1.0166	0.8873	0.8728
7	75.442	75.8754	0.4953	0.4334	0.8750
8	67.5835	68.0311	0.5080	0.4476	0.8811
9	32.5877	33.0358	0.5095	0.4481	0.8795
10	74.3242	74.7639	0.5004	0.4397	0.8787
11	77.6733	78.3929	0.8282	0.7196	0.8689
12	79.1682	79.6016	0.4947	0.4334	0.8761
13	66.0789	66.5357	0.5126	0.4568	0.8911
14	75.4392	75.8831	0.5035	0.4439	0.8816
15	75.8205	76.2536	0.4917	0.4331	0.8808
16	79.157	79.5801	0.4814	0.4231	0.8789
17	73.8105	74.2261	0.4696	0.4156	0.8850
				Mean	0.8785

Table 4-5 Results related to conductometric analysis of undried chitosan

Run	$V_{NaOH,i}$ (mL)	$V_{NaOH,f}$ (mL)	ΔV (mL)	$m_{Chitosan}$ (g)	$m'_{Chitosan}$ (g)	DD
1	61.90	75.62	13.72	0.2847	0.2501	90.18
2	65.27	77.82	12.54	0.254	0.2231	91.96
3	70.45	83.36	12.91	0.261	0.2293	92.09
4	72.75	84.96	12.21	0.2433	0.2137	93.17
5	67.90	82.71	14.81	0.3014	0.2648	91.59
6	72.69	86.20	13.51	0.2790	0.2451	90.49
7	66.63	80.23	13.59	0.2739	0.2406	92.33
8	62.22	76.01	13.79	0.2756	0.2421	92.94
9	70.30	83.75	13.45	0.2801	0.2461	89.90
10	68.46	80.75	12.29	0.2466	0.2166	92.65
11	67.76	80.27	12.51	0.2459	0.2160	94.20
12	74.15	86.17	12.01	0.2302	0.2022	96.14
13	72.46	83.65	11.19	0.2209	0.1941	93.86
14	65.98	77.80	11.82	0.2284	0.2006	95.48
15	68.14	80.52	12.37	0.2418	0.2124	94.64

As a summary of the data, boxplots (Figure 4-4), was plotted with a central rectangle spanning the first quartile to the third quartile, a segment inside the rectangle indicating the median and whiskers above and below the box showing the minimum and maximum of the experimental data, show that the data obtained from oven-dried samples are a little skewed to the right. Another view of these data provided by normal probability plots (Figure 4-5), however, indicate the DD values obtained by oven-dried and undried samples follow the normal distribution, since the result for each of them is approximately a straight line.

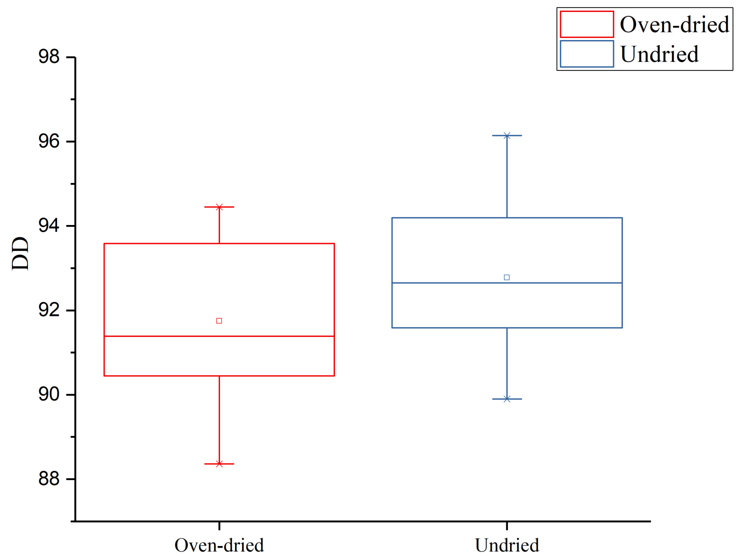


Figure 4-4: Boxplots of measurements of deacetylation degree obtained from oven-dried and undried chitosan samples.

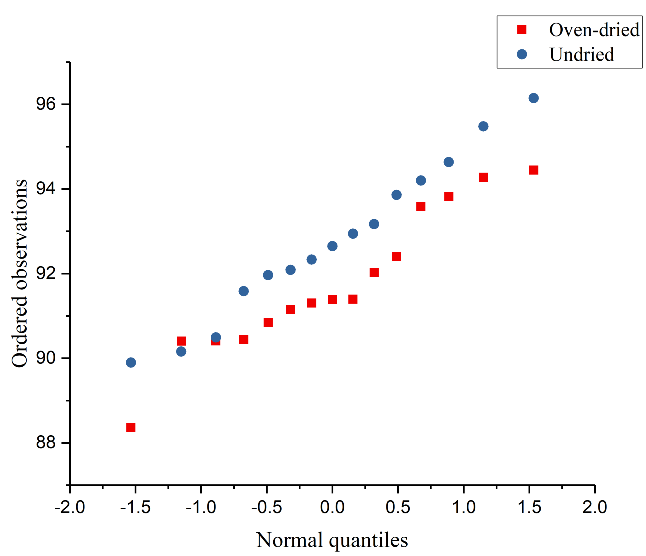


Figure 4-5: Normal probability plots of conductometry data.

Therefore, we can use F-test to verify if the variance of two populations are equal, since the DD values are tested to follow the normal distribution. Based on Table 4.3 and Table 4.5, the mean and standard deviation of the oven-dried group are 91.75 and 1.695; for the undried group, the mean is 92.77 and the standard deviation is 1.864. The statistic *F*, which is defined as the ratio of the two sample variances ($F = s_1^2/s_2^2$), is equals to 1.209554. The statistic *F* is compared with the upper and the lower critical values from the *F* distribution with 14 (for numerator) and 14 (for denominator) degrees of freedom and a significance level of 0.05, which are 2.978595 and 0.33572876, respectively. Since, *F* is larger than 0.33572876 and less than 2.978595, we cannot reject the null hypothesis that the variance of the oven-dried group is equal to that of the undried group and conclude that there is no difference in precision.

Then, we compare whether there is any difference in sample means of the two groups. The null hypothesis to be tested is the mean of the oven-dried group is equal to that of the undried group. The test statistic that be used to decide whether to reject the null hypothesis is:

$$t = \frac{\bar{X}-\bar{Y}}{S_{\bar{X}-\bar{Y}}} \tag{4-2}$$

where $S_{\bar{X}-\bar{Y}}$ is equal to $S_P\sqrt{\frac{1}{n}+\frac{1}{m}} = \sqrt{\frac{(n-1)S_X^2+(m-1)S_Y^2}{m+n-2}} \times \sqrt{\frac{1}{n}+\frac{1}{m}}$.

$\bar{X}$, S_X^2 and n indicate the mean, variance and sample size of the standard method, respectively, and $\bar{Y}$, S_Y^2 and m are the mean, variance and sample size of the modified method, respectively. Hence, t can be calculated, and it is equal to -1.5724857. From the handbook of *t* distribution, the .975 quantile of the *t* distribution with 28 degrees of freedom is 2.048. Because $|t|$=1.5724857<2.048, the two-sided test would thus not reject at the level α=0.05. So, it is obvious that there is no difference between the means of the two groups.

Based on the analysis above, the introduction of chitosan mass fraction seems to have no impact on the measurement of deacetylation degrees. On one hand, the impact from the absorbance of moisture during the time in transferring the chitosan samples from the desiccator to the analytical balance is low at some degree that it might be at the same scale with the within-group variation. On another hand, there exist some random errors in experimental data of chitosan mass fraction. The ununiformity of the size of the chitosan flakes induces the variation of the content of moisture within chitosan samples, since the substance with a larger specific surface area generally absorbs more moisture than the smaller one of the same weight. From the practical perspective, however, the method incorporated with the chitosan mass fraction is easier to operate compared with the conventional method. For the conventional one, the mass of the oven-dried chitosan sample for each trial is determined by the difference of the weight of the weighing bottle. The contents of the bottles should be poured into its receiving vessel quickly and the bottle should be capped immediately due to the hygroscopic nature of chitosan. The weight of pure chitosan is the initial weight of the bottle with the chitosan minus the weight of bottle along with any solid that does not get transferred. While for the method involving the chitosan mass fraction, the chitosan sample is weighed conventionally, and its pure chitosan content is determined by multiplying the chitosan mass fraction. As the introduction of fraction of the chitosan mass does not influence the variance and the mean of DD values, it is recommended to incorporate W_{CHIT} into the measurement of deacetylation degree.

4.2.2 Effect of introducing the volume correction

Although the mechanism of conductometric titration is regarded as the ion replacement during titration, which is actually a simplified model under the assumption that the total volume of the solution is kept constant throughout a titration. In a real case, ions with equivalent total charge number get replaced, while the volume of the solution during the titration is always increasing. Considering that a solution electrical conductivity depends on the concentrations and mobilities of all types of ions presenting in the solution, the conductance is supposed to be corrected for the effect by multiplying the observed conductivity by the factor $(V+V')/V$, where V is the initial volume of solution and V' indicates the total volume of the titrant added. Otherwise, non-linear titration curves may result. The records are listed in Table 4.6 containing the slope, A, the intercept, B, and the R-squared value of the fitted linear regression lines based on the uncorrected conductivities. And the volume of base required to neutralize the amino groups of chitosan, ΔV, and the corresponding deacetylation degree DD are calculated for each chitosan sample. The counterpart values obtained from the corrected conductivities are shown in the Table 4.7.

The effect of introducing the volume correction is analysed in the same fashion as the influence of chitosan mass fraction is tested in the section 4.2.1. The DD values obtained by conventional method and volume corrected method are assumed to follow the normal distribution. Based on Table 4.6 and Table 4.7, the mean and standard deviation of the unmodified group are 90.41 and 1.731; for the corrected group, the mean is 90.16 and the standard deviation is 1.487. Therefore, the statistic F is 1.355, which is smaller than the upper critical value $F_{0.975,4,4}$, 6.39, and larger than the lower critical value $F_{0.025,4,4}$, 0.1565. So the null hypothesis that the variance of the uncorrected group is equal to that of the corrected group is verified and there is no difference in precision. Moreover, the t-statistic is 0.2514 based on the experimental results of Table 4.6 and Table 4.7, which is less than the .975 quantile of the t distribution with 8 degrees of freedom, 2.306. So there is no difference between the means of the two groups. Therefore, the volume correction is not necessary since introducing the volume correction by multiplying the conductance with the factor $(V+V')/V$ exerts no influence on the variance and mean of the results. The volume correction assumes that the conductivity is a linear function of dilution, this is true only to a first approximation. The conductivity of a solution σ is equal to $F\Sigma z_j c_j u_j$, the volume correction takes the variation of concentration into account, but it ignores the fact that the ionic mobility, u_j, also depends on concentration. So there is little effect of volume correction on the determination of DD. Nevertheless, the difference between the R-squared values of the uncorrected group and their corresponding R-squared values of the corrected group does suggest that the three branches constituting the titration curve of the corrected group may have better linearities than the uncorrected one, since most of the values of $R'^2 - R^2$ in Table 4.7 are positive and R-squared is a statistic measuring how close the data are to the fitted regression line.

Table 4-6 Experimental data and slope, intercept and R-squared value of fitted linear regression lines based on uncorrected conductivities

Sample	m (g)	A	B	R^2	ΔV (mL)	DD
		-0.1663	2.3316	0.99803		
1	0.2031	0.0207	0.8125	0.99944	11.2987209	91.18304548
		0.0998	-0.7238	0.9995		
		-0.1455	1.3965	0.99936		
2	0.3075	0.0208	0.9084	0.99576	16.48719032	88.48568421
		0.1089	-0.8027	0.99941		
		-0.2567	3.3496	0.9989		
3	0.2042	0.0251	1.1726	0.99017	11.63946581	92.99522456
		0.1342	-0.9401	0.99798		
		-0.2524	3.5264	0.99671		
4	0.1987	0.0253	1.2095	0.99022	10.82879843	89.67037722
		0.1352	-0.8975	0.99945		
		-0.276	3.7453	0.99511		
5	0.2033	0.0267	1.3231	0.99369	11.08963409	89.73723332
		0.1424	-0.8858	0.99957		

Table 4-7 Experimental data and slope, intercept and R-squared value of fitted linear regression lines based on uncorrected conductivities

Sample	m (g)	A’	B’	R'^2	$R'^2 - R^2$	$\Delta V'$ (mL)	DD’
		-0.1605	2.345	0.99864	0.00061		
1	0.2031	0.0292	0.7891	0.99948	4.00E-05	11.32659968	91.36550632
		0.1257	-1.0954	0.99951	1.00E-05		
		-0.1383	1.3969	0.9995	0.00014		
2	0.3075	0.0301	0.8923	0.99661	0.00085	16.47060101	88.41293561
		0.1378	-1.2043	0.99925	-0.00016		
		-0.24	3.3592	0.99856	-0.00034		
3	0.2042	0.0416	1.132	0.99432	0.00415	11.488289	92.01730922
		0.179	-1.5332	0.99852	0.00054		
		-0.2396	3.5484	0.9979	0.00119		
4	0.1987	0.0429	1.1667	0.99413	0.00391	10.81322604	89.56532021
		0.1851	-1.5698	0.99924	-0.00021		
		-0.2603	3.7689	0.99681	0.0017		
5	0.2033	0.0474	1.2751	0.99828	0.00459	11.04272104	89.42784654
		0.1994	-1.6353	0.99932	-0.00025		

4.3 Two-abrupt-change Potentiometric Titration

A potentiometric titration curve of chitosan as shown in Figure 4-6 is obtained by dissolving a chitosan sample in a known excess of hydrochloric acid and titrating the solution potentiometrically with NaOH. One can easily discover that the curve shows two sudden changes of pH with the curve moving upward sharply. The difference between the two inflection points where the abrupt changes happen indicates the amount of OH^- required for the neutralization of the protonated amine groups of chitosan. With a sigmoid-shaped titration curve like Figure 4-6, the inflection point is the steepest part of the titration curve where the change of pH with respect to volume is a maximum. These inflection points can be estimated visually from the plot or we can use calculus to obtain the first and second derivatives of the titration curve. The first and second derivative of the potentiometric titration curve based on the experimental data of Run 1 in Table 1 of Appendix D are illustrated in Figure 4-7 and Figure 4-8, respectively.

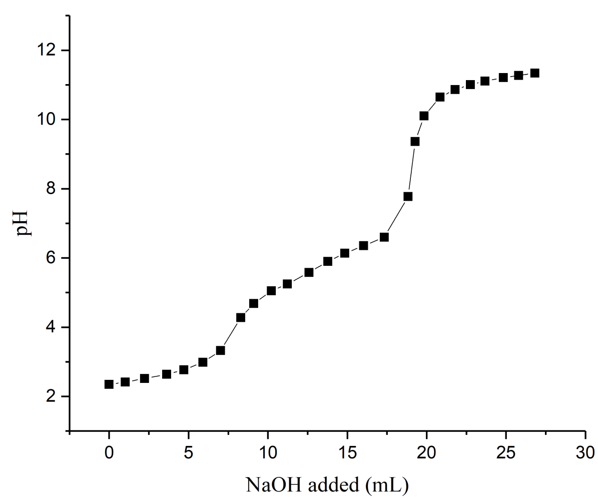


Figure 4-6: Typical potentiometric titration curve for the chitosan used in this work.

The first derivative, which is $\Delta pH/\Delta V$, is the slope of the titration curve. It goes from nearly zero before the inflection points to a maximum at the equivalence point and back to zero far beyond the end point. Therefore, the two inflection points can be located more precisely by plotting the first derivative against the volume of added titrant, which produce a curve with two peaks that indicate to the equivalence points. Nevertheless, the precise locations of the maximums are still hard to be determined due to the possible obtuse or asymmetric shapes of the peaks.

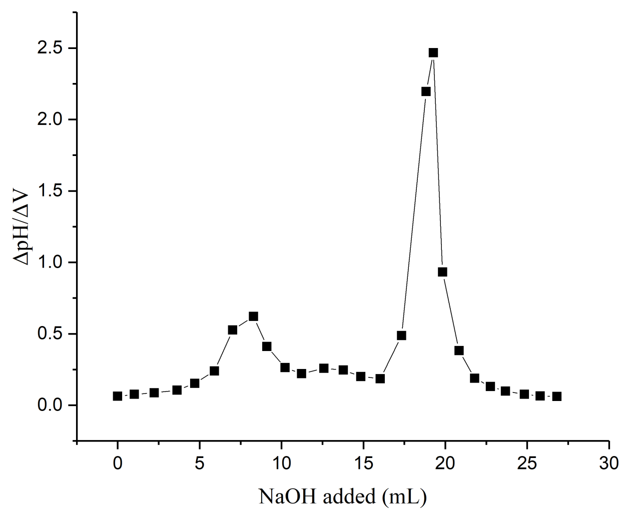


Figure 4-7: The first derivative potentiometric titration curve based on the experimental data of Run 1 in Table 1 of Appendix D.

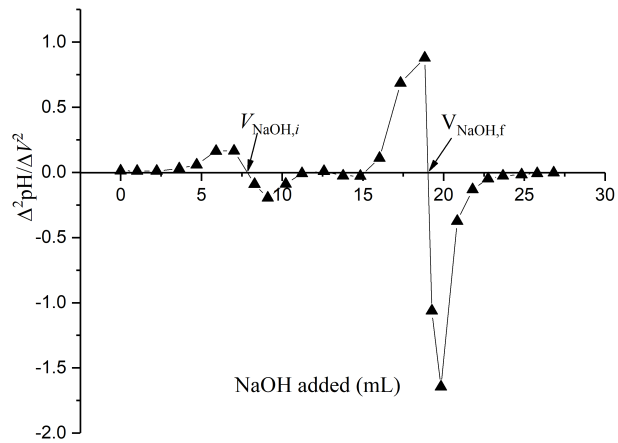


Figure 4-8: The second derivative potentiometric titration curve based on the experimental data of Run 1 in Table 1 of Appendix D. ($V_{NaOH,i}$ and $V_{NaOH,j}$ are indicated by arrows).

Hence, we locate the inflection points by the second derivative potentiometric titration curve in this work. The estimated second derivative, $\Delta^2 pH/\Delta V^2$, is plotted as a function of the added base volume, and it is zero at the inflection point as $V_{NaOH,i}$ and $V_{NaOH,j}$ shown in Figure 4-8 based on mathematics. The experimental records related to the two-abrupt-change potentiometric titration are listed in the Table 1 of Appendix D. The results of fifteen replicates using Primex chitosan are determined by their second derivative potentiometric titration curves and are listed in Table 4-8, adopting the following equations [35]:

$$DD = \frac{203.195 \times w(NH_2)}{16.02262 + 0.42037 \times w(NH_2)} \tag{4-3}$$

$$w(NH_2) = \frac{\Delta V \times C_{NaOH} \times 100 \times 0.016}{m_{Chitosan}} \tag{4-4}$$

where ΔV , C_{NaOH} , $m_{Chitosan}$ and $w(NH_2)$ stand for volume of sodium hydroxide consumption between two abrupt changes of pH, concentration of NaOH titrant, the dry weight of chitosan sample and the amino group weight fraction of the chitosan sample, respectively.

Table 4-8 Results determined by second derivative potentiometric titration curve

Run	$V_{NaOH,i}$ (mL)	$V_{NaOH,f}$ (mL)	ΔV (mL)	$m_{Chitosan}$ (g)	C_{NaOH} (mol/L)	$w(NH_2)$	DD
1	66.21	77.40	11.19	0.2059	0.09942	8.6465	89.38
2	65.35	76.87	11.52	0.2131	0.09942	8.5966	88.96
3	62.96	73.74	10.79	0.1965	0.09942	8.7321	90.10
4	64.05	73.95	9.90	0.1983	0.09942	7.9454	83.38
5	77.81	87.46	9.65	0.1744	0.09942	8.8064	90.72
6	77.51	86.67	9.16	0.1726	0.09942	8.4392	87.62
7	77.37	88.45	11.08	0.2071	0.09942	8.5112	88.23
8	67.93	76.09	8.16	0.1541	0.09942	8.4209	87.47
9	71.19	81.93	10.74	0.2140	0.09942	7.9823	83.70
10	72.23	81.38	9.14	0.1628	0.09942	8.9323	91.77
11	64.33	75.00	10.66	0.1858	0.09918	9.1072	93.22
12	60.12	72.33	12.21	0.2278	0.09918	8.5040	88.17
13	73.94	86.92	12.97	0.2407	0.09942	8.5741	88.77
14	63.80	76.45	12.65	0.2328	0.09942	8.6420	89.34
15	61.58	74.27	12.68	0.2334	0.09942	8.6442	89.36

4.4 Linear Potentiometric Titration

The linear function for the determination of DD was derived based on the ionization equilibrium of amine groups, the law of conservation of mass, and charge balance of the solution during a titration. Jiang et al. derived their linear function, in which the volume of added titrant V and the concentration of hydrogen ions [H$^+$] satisfy the following equation:

$$V + \frac{V_0+V}{C_B}([\mathrm{H}] - [\mathrm{OH}]) = V_e + \{\frac{C_1V_1}{C_B\times[\mathrm{H}]} + \frac{K_W(V_0+V)}{C_B\times[\mathrm{H}]^2} - \frac{V_0+V}{C_B} - \frac{V}{[H^+]}\}K_a \tag{4-5}$$

where V is the volume of strong base added, V_1 is the volume of HCl aqueous solution where chitosan is dissolved in, V_0 is the initial volume of the solution and V_e is the excessive amount of HCl. The concentration of hydrogen ions [H] is converted from the activity of hydrogen ions, α_{H}^{+} ($\alpha_{\mathrm{H}}^{+} = 10^{-pH}$), by multiplying the activity with $10^{0.08}$ [30]. C_1 and C_B are the concentrations of HCl aqueous solution and titrant solution, respectively. Ka indicates the dissociation constant of chitosan, while K_w is the ion-product of water [22]. If terms $\frac{C_1V_1}{C_B\times[\mathrm{H}]}+\frac{K_w(V_0+V)}{C_B\times[\mathrm{H}]^2}-\frac{V_0+V}{C_B}-\frac{V}{[H^+]}$ and $V+\frac{V_0+V}{C_B}$([H] - [OH]) are denoted by X and Y, respectively, and Y is plotted as a function of X, a straight line can be obtained with a constant slope, K_a, and a intercept, V_e, which is the volume of excessive hydrochloride acid. In our study, the terms in the linear potentiometric function are determined by the foregoing function, and the experimental data and calculated results related to the linear potentiometry are listed in the Table 1 of Appendix E. Taken the titration of Trial 1 as an example, Table 4.9 shows the terms in the linear function and errors induced in pH measurement for the titration of Run 1 of the chitosan sample.

Table 4-9 Terms in the linear function and errors induced in pH measurement for the titration of Run 1 of the chitosan sample (weight = 0.277118 g) (pK_a is assumed to be 6.5 [17])

NaOH added (L)	pH	X	Y (L)	$K_a\Delta X \times 10^{-5}$ (L)	$\Delta Y\times 10^{-5}$ (L)	$\Delta V_e\times 10^{-5}$ (L)
0.0008	2.314	3.150842939	0.006898632	0.00325	14.027	14.030
0.00142	2.355	3.443246052	0.007002413	0.00366	12.840	12.843
0.00219	2.401	3.776444899	0.007248441	0.00418	11.634	11.639
0.00294	2.445	4.110683108	0.007543689	0.00476	10.588	10.593
0.00382	2.509	4.687576067	0.007825926	0.00568	9.214	9.219
0.00442	2.554	5.132023253	0.008051938	0.00643	8.353	8.360
0.00503	2.609	5.758583741	0.008248108	0.00745	7.402	7.409
0.00582	2.683	6.705801848	0.008553813	0.00907	6.288	6.297
0.00642	2.771	8.157594889	0.008664706	0.0113	5.163	5.174
0.00718	2.886	10.47091818	0.008914479	0.0151	3.989	4.004
0.00782	3.024	14.23349333	0.00908966	0.0212	2.920	2.941
0.00843	3.209	21.55832331	0.009263824	0.0330	1.918	1.951
0.00932	3.717	68.06612831	0.009580935	0.109	0.600	0.709
0.01001	4.679	605.4556107	0.010038654	1.0195	0.0659	1.085
0.01064	5.227	2052.865445	0.010648157	3.665	0.0188	3.684
0.01117	5.445	3269.174443	0.011174959	6.144	0.0114	6.155
0.01174	5.666	5219.02122	0.011742993	10.380	0.00689	10.387

Considering that a measurement error of ± 0.01 exists for most of pH meters for routine laboratory use, the relative error of ΔV_e, which will be directly passed on to the DD, should be restricted within a certain range. The error of *X* induced in pH-measurement is given by the following equation:

$$\Delta X = \pm(0.023\frac{C_1V_1}{C_B \times [H]} + 0.046\frac{K_w(V_0 + V)}{C_B \times [H]^2} - 0.023\frac{V}{[H^+]}) \quad (4\text{-}6)$$

The error of *Y* generated by pH-measurement is:

$$\Delta Y = \pm 0.023\frac{V_0+V}{C_B}[H^+] \quad (4\text{-}7)$$

As both ΔX and ΔY are responsible for the error of V_e, the relationship between ΔV_e and ΔX and ΔY is given as follows:

$$\Delta V_e = |K_a\Delta X| + |\Delta Y| \quad (4\text{-}8)$$

Based on the Table 4.9, it can be seen that ΔV_e decreases along with the increase of pH and reaches its minimum value around the equivalence point. After that point, ΔV_e starts to increase with the increase of pH again. Therefore, ΔV_e can be confined within an acceptable range by limiting the pH region for plotting. In our study, the pH region in which $\Delta V_e < 0.01V_{emin}$ can be adopted for plotting, where V_{emin} is the excess volume of hydrochloric acid supposing that the chitosan used for titration is completely deacetylated. For chitosan sample examined in Trial 1, the V_{emin} is 7.9275 mL which indicates the *Y* terms in the pH region from 2.609 to 5.666 are supposed to be plotted against *X* (the corresponding plot is shown in Figure 4-9). And the experimental data and calculated records including the minimum volume of excess HCl, V_{emin} and DD of the ten trials are listed in the Table 4-10.

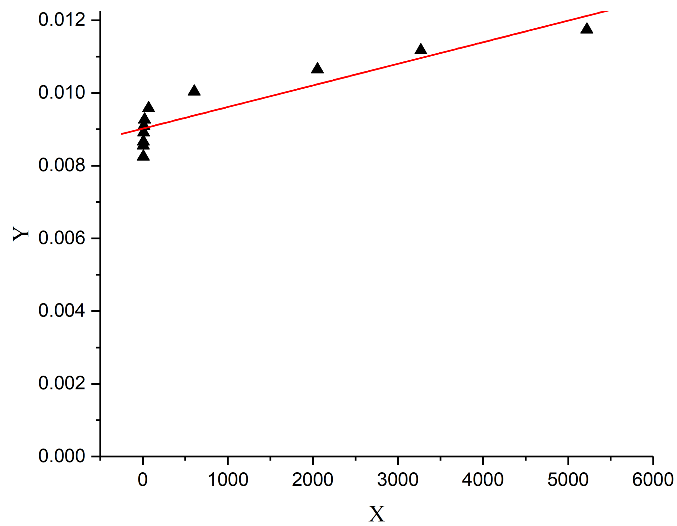


Figure 4-9: Plot of X, Y terms of the potentiometric titration of Run 1 in the pH region from 2.609 to 5.666.

Table 4-10 Results determined by plots of X, Y terms in restricted pH regions

Run	$m_{Chitosan}$ (g)	V_1 (mL)	V_{emin} (mL)	V_e (mL)	DD
1	0.27712	25.7177	7.927	9	95.06
2	0.27115	25.025	7.620	8.5	95.87
3	0.27088	25	7.613	8.4	96.30
4	0.27088	25	7.613	8.5	95.83
5	0.27088	25	7.613	8.9	93.91
6	0.20396	25	11.803	13	92.46
7	0.20396	25	11.803	13.7	87.90
8	0.20459	25.573	12.328	12.5	98.93
9	0.20000	25	12.051	13.2	92.62
10	0.12921	16.15155	7.786	8.5	92.90

4.5 Comparison among Conductometric, Two-Abrupt-Change and Linear Potentiometric Titrations

To test the distribution of the DD values determined by two-abrupt-change and linear potentiometric titrations, the normal probability plots for two methods are plotted in Figure 4-10 and Figure 4-11, respectively. As the probability plots for the two methods approximate to straight lines, the DD values measured by two-abrupt-change and linear potentiometric titrations are considered to follow the normal distribution. Under the provision that the populations follow the Gaussian distribution, the *F* test is employed to judge whether the null hypothesis that the two population variances under consideration are equal is true or false. Based on Table 4-3, Table 4-8 and Table 4-10, the mean and standard deviation of the conductometry group are 91.75003 and 1.695; the mean and standard deviation of the two-abrupt-change potentiometry group are 88.66561 and 2.5881; for the linear potentiometry group, the mean is 94.17837 and the standard deviation is 2.97992. All these results are calculated from the experimental data of titrations on oven-dried chitosan samples. At the significance level of 0.05, the test statistics *F* comparing the DD variances of conductometry and two-abrupt-change titration is 2.33141897, which is lower than the critical value $F_{(0.025, 14, 14)}$, 2.978595, and larger than $F_{(0.975, 14, 14)}$, 0.33572876. The *F* test indicates that there is not enough evidence to reject the null hypotheses that the two variances are equal at the 0.05 significance level. The *F* tests are conducted pair-wisely to compare the degrees of deacetylation obtained from conductometric, two-abrupt-change and linear potentiometric titrations, and find out that the variance obtained from any titration method is not significantly different from the ones generated from other methods

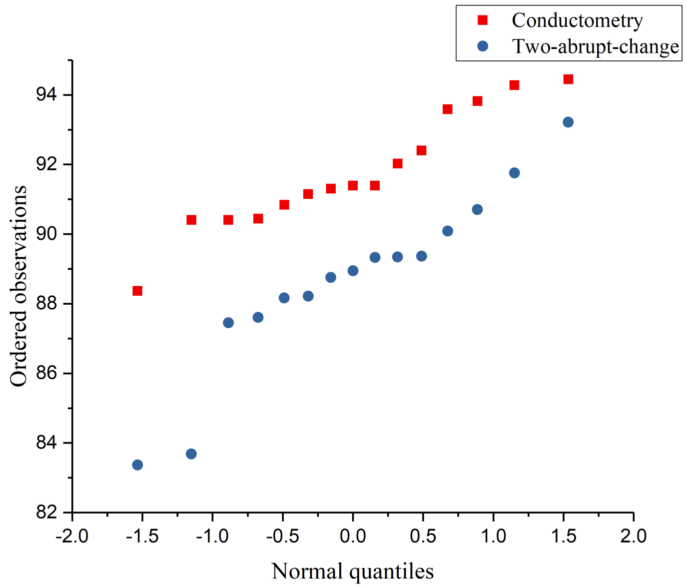


Figure 4-10: Normal probability plots of conductometric and two-abrupt-change potentiometric titration data.

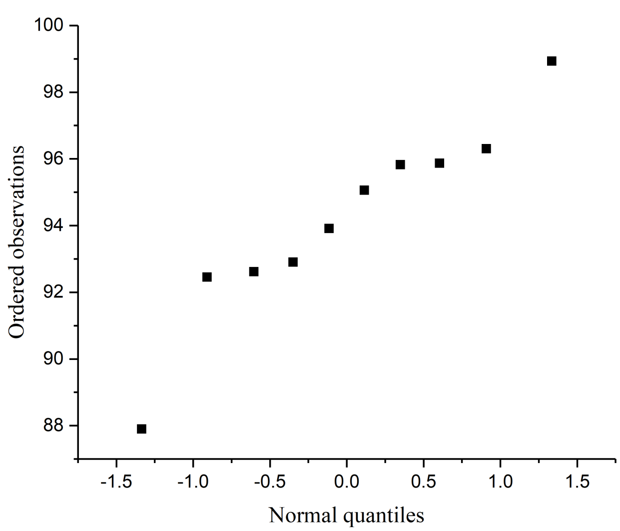


Figure 4-11: Normal probability plot of linear potentiometric titration data.

Since the variances of the populations are assumed to be identical, the ANOVA method is applied to determine whether there is or is not a difference among the population means and the results are listed in the ANOVA table 4.11.

Table 4-11 The ANOVA table determining the existence of a population mean difference among the DD values obtained by three methods

Source of Variation	**Sum of Squares**	**Degrees of Freedom**	**Mean Square**	**F Value**
Factor effect	189.59213	2	94.79606	16.39632
Error	213.91717	37	5.78155	
Total	403.5093	39		

From the *F* table, the critical value of *F* at the 95% confidence level for 2 and 27 degrees of freedom is 3.354131. Since *F* exceeds 3.354131, we reject the null hypothesis at the 95% confidence level and conclude that there is significant difference among the analysts. Therefore, the determination of the difference among the population means require pairwise comparisons as is done with the t test. And the results are listed in the Table 4.12. Since the test statistics are larger than the corresponding critical *t* values, we can conclude that there is a difference between any two means of three titration methods.

Table 4-12 The results of t tests examining the equality of population means at the significance level of 0.05

Method	**Number of measurements**	**Mean**	**Variance**	**Compared method**	***t* statistic**	**Critical *t* statistic**
Conductometry	15	91.75003	2.8730306	Two-abrupt-change potentiometry	3.86132	2.048
				Linear potentiometry	-2.60256	2.069
Two-abrupt-change potentiometry	15	88.66561	6.6982380	Linear potentiometry	-4.91378	2.069
Linear potentiometry	10	94.17837	8.879934			

Compared with the conductometry, the two-abrupt-change potentiometric titration has the superiority over the conductometry since it eliminates the potential effects of residual acid or alkali in the sample [36]. There might be some residual acetic acid remained in the chitosan specimen, which is often employed during the purification of the product during manufacturing and induces the higher DD values obtained by conductometric titration. In addition, the inflection points of conductometric titration curve may not coincide with the actual equivalence points based on the analysis of section 4.1, where the estimated inflection points overshoot the indicator determined points during the titration of KHP, which can be treated as a weak acid. For the titration of hydrochloric acid, the estimated inflection points are located at the left of the equivalence points determined by the indicator. Since chitosan/HCl solution is a mixture of a strong acid and a weak acid and the added titrant is considered to react with the excess HCl present in the cell first and then start to neutralize the protonated amino groups in the chitosan, the actual ΔV should be less than the ΔV determined graphically if the equivalence points indicated by the indicator is more accurate than by the conductometer and if the neutralization of protonated amino groups behaves similarly with that of KHP.

For the two-abrupt-change potentiometric titration, because chitosan was titrated with NaOH standard solution added about 1 mL each time stepwise, while the potentiometric titration curves generally rise steeply at the equivalence points, the data points are discrete with low density in the abrupt change regions, which are the steepest sections of the $pH=f(V_{NaOH})$ function. Therefore, the estimation of the inflection points based on the second derivative titration curves may have some errors. Moreover, based on the study of Balázs, N. and Sipos, P. [43], the chitosan may have already partly dissociated around the first equivalence point and thus the method based on the second derivative overshoots this equivalence point and gives the lower degree of deacetylation compared with other methods.

For the linear potentiometric titration, the discrepancy between the mean measured by linear potentiometry and the means determined by other two methods may be induced by the inaccuracy of chitosan mass in the solution, evaporation of hydrochloric acid and variation of the solution ionic strength, which are hard to avoid under the existing experiment condition. The initial volume of the chitosan solution is a key parameter that should be determined to conduct the linear potentiometry. However, the high viscosity of the chitosan solution makes it hard to acquire its volume by conventional volumetric measurement. Therefore, the initial volume of the chitosan solution was determined by measuring the weight of solution and dividing it by its density obtained in advance. So, the chitosan mass in the solution of each trial is an estimate value. In addition, the full-dissolution of chitosan in hydrochloric acid requires about 18 hours under the magnetic stirring. It is inevitable to prevent the hydrochloric acid from evaporating. While the amount of KCl is calculated based on the volume of hydrochloric acid added to keep the ionic strength of the solution constant during titration, the evaporation of hydrochloric acid may yield the difference between the mean of DD determined by linear potentiometry and that by other methods. Besides, the error of the amount of KCl can also lead to the variation of the solution ionic strength.

For the linear potentiometric method, only the error induced by pH-measurement is taken into consideration. Although omitting the experimental results beyond the pH regions, which is determined to restrict the error induced by pH-measurement, can improve the linearity based on the study of Jiang et al. [22], the data points in the Figure 4-9 still exhibit some degree of dispersity along the linear trendline. While some data points are clustered at x = 0 initially, they are then approximately linear distributed as it is shown in Figure 4-7. Further investigation of the criterion for defining the suitable pH region may be required for a better fitting precision may be needed, and the criterion used in our test may also induce the difference of means of the three methods.

CHAPTER 5 CONCLUSIONS AND FUTURE WORK

5.1 Conclusion

With the goal to compare the three conventional titration methods of determining the degree of deacetylation and to unveil the principles of these analytical methods as to perform like a handbook for students who are searching for employing these methods to acquire DD values with statistical confidence, the following conclusions can be generated based on this study:

1. The measurement of acetylation degree of chitosan can be accomplished by conductometric, two-abrupt-change and linear potentiometric titrations with the same degree of precision at the significance level of 0.05. And the test methods are elaborated in detail in the study.
2. Phenolphthalein as the indicator monitoring the standardization of NaOH and HCl solutions gives better indication of the equivalence point compared with the conductometer.
3. For conductometric titration, incorporating the chitosan mass fraction has no impact on the mean and variance of the results of DD values, compared with the results obtained by the control group using the oven-dried chitosan sample. From the practical perspective, the chitosan mass fraction is recommended to be involved in the method of conductometry.
4. Introducing the volume correction also has no effect on the mean and variance of the DD values. But it does improve the fitness between the observed outcomes and the values predicted by linear regression fitted line.
5. The means of DD values determined by these three methods are different with each other based on pairwise *t* tests. And the possible factors inducing the discrepancy of the mean values are listed below:

a) The results generated from conductometry may be affected by the residual acetic acid and the inflection points estimated by conductometry titration curves are likely to deviate from the exact equivalence points.
b) The data points are somewhat discrete with low density in the abrupt change regions, which may lead to the error of two-abrupt-change potentiometry. The partial dissociation of chitosan prior to the first equivalence point is possible to induce a lower mean of deacetylation degree compared with other methods.
c) For the linear potentiometric method, the possible sources of errors include the inaccuracy of chitosan mass in the solution, evaporation of hydrochloric acid, variation of the solution ionic strength and the criterion for defining the pH region adopted in the test.

5.2 Future Work

Although the precision of the three methods are tested to be equal at the significance level of 0.05, an experiment is needed that can give a relative accurate value of the DD of the chitosan sample, which can be treated as a good estimate of the true value of deacetylation degree. Based on the literature review of current methods for determination of DD, ^{13}C NMR can be a good choice to give the accurate DD value. The means of these methods then can be compared pairwise to determine the accuracy of each method.

Moreover, the possible sources of errors haven't been tested yet. Take the possible presence of acetic acid for example, a conductometry experiment using purified chitosan specimen is required as a control group to test whether the residual acetic acid is present in the chitosan sample or not. Besides, the investigation of the criterion for defining the suitable pH region needs further study to get a better fitting precision for linear potentiometric titration.

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APPENDICES

Appendix A. *Standardization of Sodium Hydroxide Solutions*

Table 1. Volumes of NaOH added and conductivities for the standardization of the batch I sodium hydroxide solutions

Subsample 1		**Subsample 2**		**Subsample 3**	
NaOH added(mL)	**Conductivity (mS cm^{-1})**	**NaOH added(mL)**	**Conductivity (mS cm^{-1})**	**NaOH added(mL)**	**Conductivity (mS cm^{-1})**
0	2.23	0	2.7	0	2.99
1.49	2.29	9	3.03	5.05	3.2
3.3	2.35	13.03	3.18	10.99	3.47
4.27	2.39	16.91	3.31	16.47	3.7
5.56	2.43	21.1	3.46	20.13	3.84
6.66	2.47	23.83	3.54	22.4	3.93
7.85	2.52	26.19	3.62	25.08	4.03
9.04	2.57	29.05	3.71	28.61	4.14
10.15	2.61	29.63	3.73	30.1	4.19
11.61	2.67	30.89	3.76	31.4	4.23
12.97	2.72	31.46	3.78	32.4	4.27
14.25	2.77	32.58	3.81	34.03	4.32
15.73	2.82	34.04	3.85	34.59	4.33
17.28	2.88	34.66	3.87	**34.7**	**4.33**
19.28	2.95	35.59	3.89	36.32	4.42
21.7	3.04	36.57	3.91	37.53	4.52
23.44	3.1	37.42	3.94	38.99	4.68
25.67	3.18	38.23	3.96	40.6	4.86
28.39	3.26	38.43	3.96	42.4	5.05
30.86	3.34	**38.56**	**3.96**	44.4	5.25
31.67	3.36	38.77	3.97	46.4	5.46
32.89	3.41	39.97	4.02		
33.39	3.41	41.25	4.11		
34.27	3.44	42.44	4.21		
34.77	3.45	43.75	4.32		
34.81	3.45	45.15	4.45		
34.89	**3.46**	46.43	4.57		
35.88	3.5	47.96	4.7		
36.98	3.57				
38.07	3.65				
39.07	3.74				
40.07	3.84				
41.43	3.96				

Table 2. Volumes of NaOH added and conductivities for the standardization of the batch Ⅱ sodium hydroxide solutions

Subsample 1		Subsample 2		Subsample 3	
NaOH added(mL)	**Conductivity (mS cm^{-1})**	**NaOH added(mL)**	**Conductivity (mS cm^{-1})**	**NaOH added(mL)**	**Conductivity (mS cm^{-1})**
0	2.24	0	3.07	0	2.4
1.34	2.27	9.72	3.43	18.13	3.05
20.09	2.89	16.46	3.68	22.59	3.2
28.57	3.13	30.4	4.13	27.53	3.35
34.67	3.3	32.24	4.18	28.58	3.38
37.17	3.36	35.58	4.26	30.13	3.42
37.74	3.37	**35.78**	**4.27**	34.02	3.53
37.91	**3.38**	36.58	4.3	36.06	3.58
38.23	3.39	38.23	4.4	**36.62**	**3.59**
38.84	3.41	40.21	4.59	37.86	3.64
40.21	3.48	42.19	4.79	39.55	3.76
43.15	3.69	44.17	4.99	41.23	3.89
44.37	3.78			43.09	4.05
				44.79	4.19

Appendix B. *Standardization of Hydrochloric Acid Solutions*

Table 1. Volumes of NaOH added and conductivities for the standardization of the batch I hydrochloric acid solutions

Subsample 1		**Subsample 2**		**Subsample 3**	
NaOH added(mL)	**Conductivity (mS cm^{-1})**	**NaOH added(mL)**	**Conductivity (mS cm^{-1})**	**NaOH added(mL)**	**Conductivity (mS cm^{-1})**
0	14.34	0	13.5	0	12.52
4.82	10.67	5.06	9.98	5.38	9.1
8.53	8.26	9.35	7.44	9.5	6.88
10.62	7.03	12.36	5.85	11.73	5.76
12.6	5.98	14.36	4.86	14.45	4.52
13.8	5.37	15.42	4.38	16.31	3.73
14.79	4.86	16.52	3.9	17.35	3.29
15.8	4.39	17.57	3.43	18.4	2.87
16.82	3.92	18.58	3.01	19.11	2.67
17.4	3.67	19.2	2.82	19.33	2.68
18.06	3.36	19.55	2.83	19.53	2.69
18.67	3.1	19.68	2.84	19.71	2.7
19.15	2.94	19.76	2.85	19.94	2.72
19.6	2.97	19.96	2.86	**20.1**	**2.72**
19.93	2.99	20.03	2.87	20.3	2.75
20.1	**3**	**20.16**	**2.87**	21.33	2.88
21.35	3.16	20.26	2.88	22.46	3.09
23.12	3.53	21.24	3	23.8	3.37
24.63	3.87	22.42	3.23	25.23	3.66
26.27	4.22	23.55	3.47	26.47	3.89
28	4.57	24.8	3.74	28.16	4.22
29.13	4.79	26.23	4.03		
30.24	5.01	27.44	4.27		

Table 2. Volumes of NaOH added and conductivities for the standardization of the batch Ⅱ hydrochloric acid solutions

Subsample 1		Subsample 2		Subsample 3	
NaOH added(mL)	**Conductivity (mS cm^{-1})**	**NaOH added(mL)**	**Conductivity (mS cm^{-1})**	**NaOH added(mL)**	**Conductivity (mS cm^{-1})**
0	5.26	0	4.33	0	6.08
1.92	4.34	5.04	2.45	1.75	5.11
3.8	3.46	7.12	1.775	2.78	4.51
5.06	2.92	8.14	1.42	4.15	3.81
6.18	2.47	8.84	1.215	5.58	3.08
7.2	2.06	9.65	1.129	6.85	2.5
8.93	1.399	**9.88**	**1.143**	7.85	2.07
9.2	1.307	9.98	1.16	8.75	1.68
9.4	1.319	11.28	1.369	9.15	1.5
9.6	1.334	12.55	1.624	9.43	1.49
9.72	1.343	13.94	1.879	9.69	1.51
9.79	**1.348**	15.22	2.12	**9.75**	**1.521**
11.19	1.607			9.95	1.535
12.4	2.11			10.15	1.578
15.6	2.56			11.15	1.79
17.7	2.98			12.33	2.07
19.29	3.29				
21.06	3.62				

Appendix C. *Experimental Data of Conductometric Titration*

Table 1. Volume of standardized NaOH solution added and conductivity of conductometric titration for oven-dried chitosan

Run 1		Run 2		Run 3	
NaOH added(mL)	**Conductivity ($mS\ cm^{-1}$)**	**NaOH added(mL)**	**Conductivity ($mS\ cm^{-1}$)**	**NaOH added(mL)**	**Conductivity ($mS\ cm^{-1}$)**
0	5.34	0	3.7	0	5.34
2.39	4.7	1.06	3.36	1.17	4.76
3.18	4.53	2.2	3.03	2.31	4.35
4.32	4.17	3.46	2.66	3.41	3.87
5.33	3.88	4.56	2.37	4.54	3.42
6.69	3.44	5.56	2.11	5.73	2.99
7.67	3.17	6.66	1.81	6.93	2.58
8.46	2.97	7.99	1.6	8.11	2.28
9.76	2.69	9.25	1.586	9.41	2.05
10.99	2.4	10.49	1.625	10.44	2.07
12.18	2.24	11.6	1.681	11.54	2.1
13.58	2.14	12.79	1.697	12.64	2.13
14.71	2.15	13.79	1.724	13.87	2.18
15.59	2.18	14.89	1.744	15.32	2.21
16.51	2.2	15.86	1.767	16.54	2.25
17.52	2.24	16.93	1.805	17.54	2.29
18.57	2.24	18.01	1.824	19.01	2.31
19.64	2.26	19.09	1.883	20.01	2.42
20.87	2.28	20.05	1.986	21.2	2.63
22.12	2.33	21.26	2.16	22.32	2.82
23.42	2.3	22.24	2.3	23.68	3.08
24.56	2.32	23.26	2.44	25.14	3.35
25.76	2.33	24.46	2.62	26.67	3.61
27.1	2.37	25.64	2.78	28.14	3.87
28.22	2.41	26.85	2.96	29.41	4.09
29.83	2.54	27.86	3.11	30.75	4.3
30.99	2.65				
32.38	2.81				
33.57	2.96				
34.99	3.13				
36.12	3.27				
37.52	3.47				

Table 1. Continued

Run 4		Run 5		Run 6	
NaOH added(mL)	Conductivity (mS cm^{-1})	NaOH added(mL)	Conductivity (mS cm^{-1})	NaOH added(mL)	Conductivity (mS cm^{-1})
0	3.57	0	4	0	3.55
1.22	3.21	1.13	3.65	1.37	3.2
2.28	2.91	2.28	3.26	2.48	2.86
3.78	2.53	3.45	2.9	3.45	2.58
4.97	2.2	5.04	2.43	4.51	2.33
6.21	1.855	6.19	2.11	5.38	2.06
7.43	1.593	7.52	1.798	6.53	1.781
8.63	1.5	8.5	1.653	7.58	1.602
9.94	1.565	9.4	1.65	8.75	1.499
10.91	1.557	10.69	1.682	9.7	1.509
11.89	1.574	11.57	1.703	10.84	1.536
13.23	1.617	12.5	1.734	12.05	1.574
14.23	1.637	13.63	1.767	13.01	1.6
15.6	1.671	14.6	1.796	14.18	1.63
16.7	1.698	15.65	1.819	15.32	1.658
18.03	1.728	16.69	1.838	16.6	1.693
19.09	1.774	17.55	1.864	17.79	1.717
20.23	1.898	18.58	1.899	19.18	1.773
21.42	2.05	19.66	1.971	20.37	1.871
22.32	2.18	20.63	2.1	21.38	2.01
23.3	2.33	21.49	2.23	22.38	2.16
24.45	2.48	22.62	2.4	23.37	2.29
25.51	2.62	23.6	2.54	24.37	2.43
26.47	2.76	24.77	2.72	25.48	2.58
27.6	2.91	25.89	2.88	26.52	2.71
		27.05	3.05		

Table 1. Continued

Run 7		Run 8		Run 9	
NaOH added(mL)	Conductivity (mS cm^{-1})	NaOH added(mL)	Conductivity (mS cm^{-1})	NaOH added(mL)	Conductivity (mS cm^{-1})
0	3.77	0	3.51	0	3.22
1.6	3.32	1.21	3.15	1.2	2.86
3.13	2.88	2.37	2.8	2.23	2.62
4.44	2.53	3.61	2.46	3.59	2.28
6.08	2.11	4.54	2.27	4.99	1.954
7.46	1.74	6.01	1.837	6.03	1.733
9.2	1.475	7.21	1.573	7.2	1.494
10.6	1.465	8.61	1.477	8.4	1.358
11.97	1.499	9.8	1.5	9.79	1.357
13.4	1.541	11	1.543	10.93	1.401
14.8	1.58	12.01	1.605	12.27	1.428
16.2	1.615	13.22	1.605	13.89	1.464
17.6	1.643	14.36	1.635	15.37	1.503
19	1.686	15.58	1.659	16.64	1.535
20	1.763	17.04	1.689	17.99	1.555
21.01	1.891	18.21	1.73	19.34	1.616
22.05	2.03	19.41	1.793	20.05	1.68
23.2	2.19	20.71	1.937	21.24	1.832
24.3	2.33	21.95	2.1	22.27	1.964
25.41	2.49	23.18	2.29	23.32	2.11
26.64	2.65	24.21	2.42	24.4	2.26
27.88	2.81	25.25	2.57	25.79	2.44
		26.26	2.71	27	2.58
		27.3	2.85	28.43	2.76

Table 1. Continued

Run 10		**Run 11**		**Run 12**	
NaOH added(mL)	**Conductivity (mS cm^{-1})**	**NaOH added(mL)**	**Conductivity (mS cm^{-1})**	**NaOH added(mL)**	**Conductivity (mS cm^{-1})**
0	3.58	0	3.68	0	3.49
1.1	3.28	1.15	3.31	0.69	3.31
2.2	2.96	2.32	2.97	1.69	3
3.69	2.54	3.34	2.66	2.69	2.76
4.9	2.27	4.54	2.34	3.86	2.44
6.09	1.876	5.74	2.05	4.88	2.19
7.48	1.573	7.04	1.697	5.96	1.889
8.49	1.483	8.21	1.488	6.89	1.656
9.69	1.504	9.33	1.478	7.99	1.443
11.1	1.542	10.67	1.514	9.1	1.377
12.29	1.581	12.14	1.561	10.36	1.401
13.5	1.617	13.33	1.599	11.42	1.433
14.9	1.658	14.43	1.62	12.57	1.466
16.3	1.68	15.49	1.648	13.75	1.499
17.4	1.72	17.96	1.699	14.89	1.52
18.89	1.758	19.1	1.763	16.04	1.559
20.29	1.901	20.14	1.87	17.69	1.597
21.49	2.07	21.18	2.01	18.77	1.624
22.72	2.26	22.33	2.18	20.06	1.734
23.91	2.42	23.31	2.32	21.08	1.872
25.36	2.61	24.36	2.45	22.22	2.03
27.05	2.84	25.53	2.61	23.36	2.17
28.32	3.02	27.13	2.84	24.48	2.33
				25.39	2.43
				26.41	2.57
				27.69	2.73

Table 1. Continued

Run 13		Run 14		Run 15	
NaOH added(mL)	Conductivity (mS cm^{-1})	NaOH added(mL)	Conductivity (mS cm^{-1})	NaOH added(mL)	Conductivity (mS cm^{-1})
0	3.87	0	3.36	0	2.29
1.2	3.54	1.23	3	1.24	1.996
2.59	3.17	3.17	2.46	2.19	1.79
4.2	2.76	4.51	2.06	3.28	1.553
5.8	2.39	6.22	1.681	4.39	1.353
7	2.1	7.46	1.516	5.36	1.266
8.63	1.675	9.41	1.572	6.5	1.277
9.84	1.452	11.25	1.635	7.77	1.308
11.12	1.393	12.87	1.662	8.87	1.343
12.8	1.44	14.92	1.706	10.2	1.371
14.4	1.481	16.07	1.738	11.47	1.404
15.76	1.511	17.42	1.77	13.05	1.443
17.4	1.546	18.72	1.813	14.43	1.472
18.78	1.582	20.06	1.929	15.61	1.496
20.34	1.731	21.47	2.13	16.7	1.519
21.56	1.892	22.68	2.32	17.83	1.542
22.93	2.07	24.54	2.58	19	1.591
24.27	2.25	25.95	2.78	20.23	1.693
25.59	2.42	27.39	2.98	21.66	1.872
26.6	2.53	28.49	3.15	23	2.05
27.79	2.68			24.4	2.24
29	2.83			25.6	2.4
30.3	2.99			26.79	2.52
				27.97	2.67
				29.1	2.81

Table 2. Volume of standardized NaOH solution added and conductivity of conductometric titration for undried chitosan

Run 1		Run 2		Run 3	
NaOH added(mL)	**Conductivity (mS cm^{-1})**	**NaOH added(mL)**	**Conductivity (mS cm^{-1})**	**NaOH added(mL)**	**Conductivity (mS cm^{-1})**
0	2.92	0	3.25	0	3.71
1.4	2.53	0.73	3.03	0.88	3.35
2.79	2.21	1.83	2.72	2.08	3
3.93	1.891	3.39	2.31	3.2	2.67
5.08	1.665	4.59	2.04	4.38	2.34
6.87	1.505	5.74	1.68	5.45	2.06
8.12	1.541	6.79	1.505	6.53	1.814
9.49	1.588	7.8	1.461	7.76	1.69
10.8	1.612	9.2	1.487	9	1.716
12.22	1.637	10.4	1.527	10	1.739
13.9	1.692	11.65	1.562	11.2	1.777
15.18	1.72	13.2	1.602	12.4	1.799
16.68	1.752	14.05	1.62	13.5	1.847
18.16	1.786	14.96	1.648	14.68	1.886
19.43	1.855	16.02	1.668	16.1	1.905
20.8	2.02	17	1.692	17.48	1.942
22.01	2.19	17.93	1.714	18.77	1.986
23.7	2.43	19	1.759	19.9	2.07
25	2.62	20.2	1.872	21.13	2.24
26.19	2.79	21	1.979	22.39	2.42
27.4	2.97	22.1	2.13	23.6	2.62
28.19	3.08	23.33	2.31	24.81	2.82
		24.39	2.44	25.8	2.98
		25.7	2.63	26.74	3.12
		26.99	2.82		
		28.02	2.96		

Table 2. Continued

Run 4		**Run 5**		**Run 6**	
NaOH added(mL)	**Conductivity (mS cm^{-1})**	**NaOH added(mL)**	**Conductivity (mS cm^{-1})**	**NaOH added(mL)**	**Conductivity (mS cm^{-1})**
0	3.72	0	2.62	0	3.09
0.92	3.44	0.98	2.39	0.58	2.92
2.04	3.06	2.18	2.12	1.58	2.67
3.23	2.7	3.26	1.703	2.58	2.32
4.42	2.38	4.4	1.5	3.53	2.14
5.57	2.07	5.6	1.436	4.79	1.818
6.77	1.735	6.76	1.462	5.84	1.597
7.67	1.62	8	1.501	6.89	1.528
8.94	1.611	8.94	1.525	8.11	1.55
10.1	1.653	10.28	1.552	9.03	1.577
11.08	1.675	11.27	1.583	10.08	1.599
12.17	1.706	12.26	1.617	11.19	1.634
13.61	1.739	13.4	1.638	12.17	1.653
14.64	1.767	14.48	1.654	13.16	1.681
15.74	1.795	15.38	1.681	13.99	1.717
16.78	1.816	16.39	1.702	14.88	1.728
17.83	1.842	17.45	1.725	15.78	1.745
19.18	1.895	18.6	1.772	16.42	1.763
20.36	2.02	19.83	1.867	17.21	1.782
21.49	2.2	20.92	1.988	18.17	1.802
22.79	2.4	21.8	2.13	19.1	1.845
24.03	2.57	22.83	2.27	19.78	1.895
25.04	2.72	23.93	2.41	20.52	1.974
26.03	2.86	25.01	2.57	21.53	2.12
		26.06	2.72	22.56	2.28
		26.99	2.85	23.41	2.39
				24.51	2.55
				25.4	2.69
				26.34	2.81

Table 2. Continued

Run 7		Run 8		Run 9	
NaOH added(mL)	**Conductivity (mS cm^{-1})**	**NaOH added(mL)**	**Conductivity (mS cm^{-1})**	**NaOH added(mL)**	**Conductivity (mS cm^{-1})**
0	2.86	0	2.73	0	2.68
1.26	2.57	1.1	2.47	1.22	2.31
2.33	2.29	2.5	2.13	2.66	2.08
3.18	2.09	3.68	1.836	4.28	1.652
4.3	1.818	4.76	1.55	5.49	1.413
5.39	1.506	5.93	1.396	6.78	1.356
6.46	1.432	7.27	1.363	8.48	1.392
7.49	1.41	8.49	1.39	9.88	1.451
8.59	1.439	9.5	1.421	11.27	1.466
9.51	1.473	10.7	1.455	12.68	1.497
10.52	1.49	11.91	1.484	14.07	1.532
11.64	1.513	13.32	1.519	15.43	1.565
12.72	1.557	14.72	1.548	17.05	1.602
13.81	1.564	16.3	1.585	18.31	1.633
14.77	1.593	17.9	1.621	19.3	1.684
15.93	1.62	19.15	1.668	20.32	1.777
16.94	1.643	20.67	1.813	21.69	1.961
17.82	1.662	21.9	1.984	22.87	2.12
18.93	1.702	23.16	2.15	23.88	2.26
19.94	1.78	24.51	2.34	24.78	2.38
20.93	1.894	25.84	2.5	25.84	2.5
21.82	2.02	27.03	2.65	26.87	2.64
22.82	2.16	28.1	2.8	27.88	2.77
23.93	2.33	29.32	2.95	28.68	2.87
24.98	2.46				
26.13	2.61				
27.04	2.74				

Table 2. Continued

Run 10		Run 11		Run 12	
NaOH added(mL)	**Conductivity (mS cm^{-1})**	**NaOH added(mL)**	**Conductivity (mS cm^{-1})**	**NaOH added(mL)**	**Conductivity (mS cm^{-1})**
0	4.04	0	3.13	0	4.28
1.41	3.5	1.33	2.76	1.12	3.83
2.78	3.1	2.66	2.37	2.39	3.53
4.41	2.65	4.2	2.03	3.55	3.07
5.8	2.24	5.88	1.633	4.82	2.72
7.36	1.766	7.32	1.393	5.92	2.39
8.42	1.741	8.65	1.376	7.05	2.11
9.74	1.774	10.5	1.445	8.51	1.779
11.01	1.805	11.94	1.474	9.58	1.715
12.17	1.842	13.53	1.514	10.92	1.748
13.24	1.887	15.69	1.559	12	1.779
14.41	1.903	17.33	1.593	13.15	1.805
15.55	1.941	18.54	1.63	14.51	1.85
16.8	1.968	19.94	1.718	15.5	1.867
18.41	2.02	21.4	1.901	16.5	1.893
19.43	2.08	22.74	2.09	17.52	1.915
20.69	2.22	23.87	2.24	18.6	1.944
21.88	2.4	24.93	2.39	19.71	1.987
23.21	2.62	26.25	2.53	20.74	2.08
24.57	2.83	27.32	2.68	21.6	2.19
26.08	3.08	28.26	2.79	22.72	2.37
				23.72	2.49
				24.91	2.67
				25.92	2.83

Table 2. Continued

Run 13		Run 14		Run 15	
NaOH added(mL)	Conductivity (mS cm^{-1})	NaOH added(mL)	Conductivity (mS cm^{-1})	NaOH added(mL)	Conductivity (mS cm^{-1})
0	3.99	0	4.63	0	3.94
1.19	3.5	1.31	4.14	1.05	3.59
2.48	3.3	2.65	3.62	2.21	3.19
3.7	2.95	3.98	3.22	3.66	2.74
4.89	2.62	5.37	2.82	5.33	2.26
6.04	2.25	6.67	2.42	6.94	1.805
7.18	2.02	7.81	2.11	8.08	1.629
8.3	1.735	9.36	1.816	9.63	1.633
9.52	1.568	10.68	1.797	11.31	1.671
10.78	1.572	12.17	1.837	12.82	1.726
11.87	1.593	13.51	1.881	14.61	1.776
13.11	1.626	14.65	1.916	16.01	1.804
14.43	1.675	15.98	1.952	17.41	1.837
15.84	1.699	17.13	1.975	18.76	1.87
17.1	1.73	18.38	2.01	19.85	1.934
18.3	1.759	19.41	2.04	21.34	2.12
19.4	1.789	20.53	2.1	22.65	2.31
20.5	1.872	21.98	2.3	24.31	2.55
21.6	1.996	23.19	2.48	25.42	2.73
22.72	2.16	24.32	2.65	26.79	2.92
24.11	2.36	25.74	2.88	28.43	3.17
25	2.46	26.98	3.08		
26.03	2.6	28.38	3.29		
27.09	2.74				
28.1	2.89				

Table 3. Volume of standardized NaOH solution added and conductivity and corrected conductivity of titration for oven-dried chitosan

Run 1			Run 2		
NaOH added(mL)	**Conductivity (mS cm^{-1})**	**Corrected conductivity (mS cm-1)**	**NaOH added(mL)**	**Conductivity (mS cm^{-1})**	**Corrected conductivity (mS cm-1)**
1.32	2.13	2.147081409	1.01	1.245	1.253383
2.45	1.923	1.951623026	2.17	1.083	1.0986674
3.62	1.712	1.749651519	3.22	1.013	1.034745733
4.78	1.521	1.565169988	4.21	1.001	1.029094733
5.87	1.352	1.40021531	5.22	1.017	1.0523916
7.02	1.183	1.233453584	6.2	1.041	1.084028
8.34	1.024	1.075884326	7.2	1.051	1.101448
9.4	1.008	1.065565006	8.29	1.081	1.140743267
10.59	1.031	1.09733226	9.34	1.099	1.167431067
12.02	1.061	1.138480073	10.43	1.132	1.210711733
13.38	1.092	1.180766464	11.22	1.142	1.2274216
14.6	1.116	1.214989064	12.14	1.158	1.2517208
15.8	1.137	1.246140948	13.07	1.177	1.279555933
17.05	1.166	1.286779465	14.2	1.209	1.323452
18.6	1.199	1.334488457	15.2	1.225	1.349133333
19.8	1.262	1.413808019	16.33	1.243	1.378321267
20.99	1.361	1.53455644	17.21	1.256	1.400105067
21.99	1.459	1.653917436	18.11	1.282	1.436780133
23	1.569	1.788240583	19.03	1.321	1.488590867
24.2	1.695	1.944204131	20.11	1.391	1.577486733
25.8	1.856	2.14691616	21.24	1.51	1.723816
27.13	1.989	2.316834569	22.59	1.643	1.8904358
28.77	2.15	2.525792831	23.63	1.777	2.056936733
30.2	2.28	2.698323208	24.94	1.909	2.226403067
			25.91	2.02	2.368921333
			27.21	2.16	2.551824

Table 3. Continued

Run 3			**Run 4**		
NaOH added(mL)	**Conductivity (mS cm^{-1})**	**Corrected conductivity (mS cm-1)**	**NaOH added(mL)**	**Conductivity (mS cm^{-1})**	**Corrected conductivity (mS cm-1)**
0.99	3.11	3.137079156	1.34	3.19	3.229252525
1.93	2.84	2.888207564	2.77	2.81	2.881475666
3	2.56	2.627546174	4.16	2.44	2.533208448
4.39	2.25	2.336873351	5.84	2.03	2.138863177
5.62	1.898	1.991814952	7.18	1.667	1.776908724
7.8	1.475	1.576187335	8.57	1.441	1.55440101
8.82	1.404	1.512911873	10.1	1.454	1.588852158
9.95	1.41	1.533390501	11.76	1.504	1.666415427
10.86	1.45	1.588496042	12.67	1.527	1.704659229
12	1.476	1.631778364	13.98	1.565	1.765906336
13.2	1.507	1.681955145	15.56	1.603	1.832042057
14.33	1.53	1.722831135	16.67	1.625	1.873748852
15.52	1.553	1.764983817	17.79	1.652	1.921872176
16.6	1.588	1.819845207	19.16	1.708	2.008507622
17.83	1.612	1.864787687	20.17	1.818	2.154722314
18.99	1.664	1.941918734	21.17	1.955	2.335049128
20	1.749	2.056651715	22.36	2.12	2.555291093
20.97	1.859	2.201860422	23.03	2.22	2.689482094
21.02	2.01	2.381593668	24.17	2.37	2.896013774
23.06	2.16	2.598079156	25.19	2.52	3.102909091
24.04	2.3	2.786297274	26.45	2.68	3.330927456
25.14	2.42	2.955081794	27.76	2.85	3.576501377

Table 3. Continued

Run 5		
NaOH added(mL)	**Conductivity (mS cm^{-1})**	**Corrected conductivity (mS cm-1)**
0.97	3.48	3.51409697
2.03	3.17	3.23500101
3.28	2.78	2.872105051
4.42	2.48	2.590723232
5.59	2.19	2.313657576
6.94	1.792	1.91762101
8.12	1.597	1.727986263
9.6	1.574	1.726630303
10.73	1.606	1.780064444
11.88	1.642	1.83904
13.19	1.683	1.90723
14.36	1.707	1.954601212
15.35	1.742	2.01209798
16.38	1.755	2.045372727
17.92	1.795	2.119913131
19.34	1.877	2.243678586
20.64	2.04	2.465309091
21.77	2.21	2.695976768
23.1	2.41	2.972333333
24.24	2.56	3.186812121
25.44	2.74	3.44409697
26.53	2.89	3.664461616

Appendix D. *Experimental Data of Two-abrupt-change Potentiometric Titration*

Table 1. Volume of standardized NaOH solution added and pH of potentiometric titration for oven-dried chitosan

Run 1		**Run 2**		**Run 3**	
NaOH added(mL)	**pH**	**NaOH added(mL)**	**pH**	**NaOH added(mL)**	**pH**
0	2.347	0	2.357	0	2.283
1.02	2.411	1.7	2.479	1.2	2.372
2.23	2.518	2.98	2.595	2.21	2.443
3.63	2.638	4.2	2.727	3.36	2.534
4.7	2.77	5.35	2.888	4.6	2.656
5.9	2.987	6.69	3.271	5.6	2.778
7.02	3.322	7.63	3.934	6.62	2.994
8.29	4.277	8.62	4.492	7.8	3.419
9.11	4.68	9.45	4.805	8.7	4.172
10.22	5.048	10.44	5.04	9.58	4.59
11.23	5.244	11.43	5.281	10.4	4.886
12.59	5.58	12.52	5.511	11.41	5.193
13.77	5.898	13.43	5.695	12.63	5.525
14.85	6.138	14.61	5.941	13.78	5.844
16.03	6.35	15.63	6.169	14.79	5.976
17.33	6.598	16.77	6.412	15.99	6.306
18.83	7.773	17.83	6.676	17.03	6.554
19.27	9.36	18.63	7.208	18	6.841
19.83	10.104	19.38	9.341	19	8.055
20.84	10.645	20.04	10.269	19.2	8.922
21.8	10.863	20.83	10.624	19.43	9.553
22.75	11.005	21.63	10.839	19.72	9.962
23.68	11.108	22.43	10.962	20	10.244
24.83	11.209	23.35	11.072	20.4	10.538
25.8	11.273	24.23	11.162	21.44	10.917
26.82	11.336	25.36	11.236	22.59	11.116
		26.43	11.301	23.68	11.245
		27.29	11.344	24.78	11.338
		28.23	11.387	25.9	11.414
				26.92	11.476

Table 1. Continued

Run 4		Run 5		Run 6	
NaOH added(mL)	**pH**	**NaOH added(mL)**	**pH**	**NaOH added(mL)**	**pH**
0	2.332	0	2.278	0	2.208
1.14	2.408	0.87	2.322	1.26	2.279
2.33	2.506	1.84	2.382	2.4	2.343
3.73	2.687	2.65	2.43	3.39	2.411
5	2.758	3.4	2.482	4.4	2.48
5.93	2.906	4.39	2.567	5.41	2.578
7.13	3.167	5.6	2.693	6.26	2.666
8.25	3.765	6.21	2.769	7.05	2.808
9.47	4.529	7.16	2.893	7.53	2.908
9.76	4.818	8.12	3.135	8.02	3.053
10.93	5.092	9	3.535	9.17	3.594
11.93	5.313	9.8	4.313	10.22	4.512
12.95	5.496	10.17	4.597	11.2	5.048
14.1	5.873	10.78	4.838	12.35	5.381
15	6.142	11.44	5.024	13.4	5.646
15.92	6.344	12.28	5.334	14.39	5.883
16.93	6.502	13.08	5.525	15.3	6.104
18.12	6.852	13.94	5.778	16.4	6.373
19.13	9.221	14.8	5.956	17.34	6.574
19.44	9.825	15.67	6.179	18.25	7.016
19.93	10.295	16.59	6.38	18.64	7.639
20.16	10.452	17.42	6.596	19.14	9.554
20.8	10.721	18.41	7.183	19.4	9.987
21.59	10.912	18.88	7.979	19.7	10.285
22.72	11.088	19.27	9.384	20.06	10.521
23.69	11.181	19.6	9.977	20.8	10.795
24.8	11.272	19.87	10.204	21.68	10.981
25.93	11.344	20.19	10.408	22.73	11.133
		20.76	10.65	23.59	11.223
		21.58	10.881	24.35	11.28
		22.45	11.039		
		23.27	11.133		
		24.22	11.219		
		25.18	11.292		

Table 1. Continued

Run 7		Run 8		Run 9	
NaOH added(mL)	**pH**	**NaOH added(mL)**	**pH**	**NaOH added(mL)**	**pH**
0	2.323	0	2.291	0	2.453
1.47	2.411	1.27	2.349	0.93	2.518
2.56	2.497	2.47	2.413	1.96	2.599
3.5	2.595	3.64	2.492	3.08	2.71
4.6	2.736	5	2.597	4.31	2.862
5.44	2.861	5.98	2.685	5.68	3.108
6.6	3.136	6.98	2.79	6.88	3.589
7.6	3.697	8	2.947	8.01	4.326
7.98	4.106	9.19	3.248	9.08	4.83
8.39	4.36	9.8	3.637	9.48	5.036
9.21	4.774	11.05	4.708	9.81	5.161
9.98	5.043	12.23	5.261	10.33	5.339
10.47	5.21	13.39	5.621	10.92	5.506
11.27	5.366	14.48	5.924	11.91	5.64
12.46	5.591	15.8	6.29	12.98	5.883
13.43	5.856	17.19	6.585	13.94	6.098
14.46	6.052	18.41	7.332	14.94	6.285
15.6	6.255	18.99	9.433	15.89	6.444
16.74	6.483	19.4	10.153	16.98	6.683
17.8	6.718	19.8	10.494	17.82	6.933
18.46	7.3	20.4	10.76	18.89	9.171
19.09	9.254	20.99	10.934	19.2	9.806
19.39	9.805	21.56	11.054	19.58	10.244
19.77	10.214	22.59	11.189	19.88	10.437
20.19	10.479	23.8	11.312	20.18	10.614
20.53	10.641	24.8	11.395	20.98	10.88
21.4	10.893	25.83	11.461	21.99	11.071
22.2	11.028	26.96	11.512	22.98	11.196
23.11	11.142			23.88	11.275
24	11.219			24.96	11.355
24.78	11.279			26.08	11.417
25.61	11.344			27.08	11.475
				28.08	11.515

Table 1. Continued

Run 10		**Run 11**		**Run 12**	
NaOH added(mL)	**pH**	**NaOH added(mL)**	**pH**	**NaOH added(mL)**	**pH**
0	2.36	0	2.294	0	2.445
0.86	2.401	1.07	2.337	1.43	2.557
1.89	2.458	2.27	2.396	2.85	2.719
3.12	2.536	3.64	2.514	4.05	2.891
4.11	2.602	4.98	2.634	5.5	3.256
5.4	2.702	6.37	2.829	6.94	4.225
6.95	2.869	7.99	3.297	8.49	4.832
7.88	3.015	9.35	4.412	9.68	5.193
9.08	3.446	10.86	5.122	11.16	5.518
9.39	3.652	12.2	5.469	12.28	5.751
9.76	4.052	13.89	5.913	13.47	6.018
10.22	4.489	15.12	6.125	14.68	6.206
11.28	5.036	15.98	6.378	15.68	6.395
12.47	5.403	16.6	6.378	16.63	6.563
13.51	5.773	17.8	6.625	17.88	6.911
14.54	6.017	18.14	6.713	18.78	8.598
15.6	6.274	18.6	6.887	19.08	9.56
16.68	6.495	19	7.327	19.41	9.953
17.7	6.791	19.4	7.859	19.91	10.444
18.35	7.229	19.8	9.45	20.28	10.631
18.68	7.849	20.06	9.969	20.87	10.799
18.93	8.916	20.43	10.347	22	11.057
19.28	9.816	20.79	10.55	23.01	11.182
19.55	10.18	21.2	10.722	24.13	11.282
19.84	10.386	21.9	10.941		
20.27	10.613	22.82	11.238		
20.96	10.859	24.84	11.328		
21.88	11.052	26.06	11.417		
22.95	11.192	27.31	11.49		
24.01	11.294				
24.88	11.351				
25.78	11.414				

Table 1. Continued

Run 13		**Run 14**		**Run 15**	
NaOH added(mL)	**pH**	**NaOH added(mL)**	**pH**	**NaOH added(mL)**	**pH**
0	2.502	0	2.419	0	2.475
1.55	2.597	1.22	2.533	1.4	2.594
3.21	2.788	2.47	2.643	3.1	2.751
4.57	3.071	4.14	2.866	4.18	2.918
5.8	3.583	5.55	3.271	5.41	3.295
7.28	4.573	6.93	4.215	6.77	4.142
8.4	4.93	7.95	4.698	8	4.715
9.59	5.127	9.14	4.899	9.1	4.985
10.66	5.379	10.35	5.252	10.05	5.237
11.68	5.614	11.8	5.442	11.2	5.427
12.79	5.809	13.15	5.717	12.56	5.673
13.81	5.961	14.41	5.932	13.8	5.905
15	6.126	15.74	6.236	15.04	6.16
16.13	6.345	16.77	6.461	16.03	6.343
17.21	6.627	18.05	6.814	17	6.514
18.2	7.031	19.45	9.439	18.23	6.872
19.2	8.554	20.75	10.584	19.4	9.311
20.23	10.332	21.92	10.903	20.54	10.484
21.19	10.819	22.88	11.042	21.1	10.687
22	11.013	23.96	11.165	22.04	10.905
23	11.159	25.07	11.248	23	11.04
23.99	11.265	25.96	11.311	23.99	11.137
24.81	11.328	27.15	11.371	24.98	11.209
25.79	11.388	28.16	11.419	26	11.279
26.74	11.475			27	11.333
				28.01	11.385

Appendix E. *Experimental Data of Linear Potentiometric Titration*

Table 1. Terms in the linear function induced in pH measurement for the titration of chitosan

Run 1				**Run 2**			
NaOH added (L)	**pH**	***X***	***Y***	**NaOH added (L)**	**pH**	***X***	***Y***
0.0008	2.314	3.150842939	0.006898632	0.00058	2.309	3.054862444	0.006571273
0.00142	2.355	3.443246052	0.007002413	0.00119	2.36	3.439619464	0.006549725
0.00219	2.401	3.776444899	0.007248441	0.00178	2.407	3.81825505	0.006617989
0.00294	2.445	4.110683108	0.007543689	0.00239	2.426	3.893485486	0.007048621
0.00382	2.509	4.687576067	0.007825926	0.00299	2.478	4.364099108	0.007147119
0.00442	2.554	5.132023253	0.008051938	0.00357	2.521	4.760528791	0.007356422
0.00503	2.609	5.758583741	0.008248108	0.00419	2.585	5.477775611	0.007477143
0.00582	2.683	6.705801848	0.008553813	0.00471	2.625	5.920076546	0.007722858
0.00642	2.771	8.157594889	0.008664706	0.00536	2.69	6.775127099	0.007970139
0.00718	2.886	10.47091818	0.008914479	0.00591	2.764	7.960037645	0.008122701
0.00782	3.024	14.23349333	0.00908966	0.00646	2.823	8.963971749	0.008401646
0.00843	3.209	21.55832331	0.009263824	0.00713	2.945	11.72208812	0.00860534
0.00932	3.717	68.06612831	0.009580935	0.00776	3.09	16.14586613	0.008822763
0.01001	4.679	605.4556107	0.010038654	0.00852	3.423	34.33030925	0.009017148
0.01064	5.227	2052.865445	0.010648157	0.00898	3.792	79.38458446	0.009193462
0.01117	5.445	3269.174443	0.011174959	0.00932	4.47	374.0033836	0.009364944
0.01174	5.666	5219.02122	0.011742993	0.00959	5.034	1349.1393	0.009602294
				0.01018	5.296	2370.253955	0.01018676
				0.01077	5.521	3817.066943	0.010774046

	0.0110 9	5.67 8	5353.03442 2	0.01109282 5

Table 1. Continued

Run 3				Run 4			
NaOH added (L)	**pH**	***X***	***Y***	**NaOH added (L)**	**pH**	***X***	***Y***
0.00056	2.331	3.266740778	0.006248545	0.00058	2.343	3.382802598	0.006114616
0.00114	2.374	3.592280071	0.006322017	0.00123	2.379	3.626861501	0.006357258
0.00176	2.412	3.874590005	0.006536975	0.00179	2.411	3.856587778	0.006579399
0.00234	2.458	4.27780187	0.006661369	0.00237	2.455	4.233833805	0.006722598
0.00289	2.507	4.759415971	0.006771048	0.00299	2.512	4.798492163	0.006830351
0.00344	2.548	5.166219066	0.006990295	0.00352	2.568	5.433432904	0.006913127
0.00404	2.602	5.782670259	0.007193379	0.00432	2.614	5.875491027	0.007395696
0.0048	2.669	6.619601749	0.007522307	0.00489	2.624	5.831633073	0.007912108
0.00536	2.722	7.364726277	0.007782432	0.00551	2.693	6.757344471	0.0081034
0.006	2.815	9.022940876	0.007967351	0.00601	2.764	7.899859857	0.008222701
0.00679	2.936	11.69033875	0.008290053	0.00652	2.843	9.388463354	0.008373562
0.00738	3.011	13.58768164	0.00864911	0.00709	2.943	11.67592485	0.008570215
0.008	3.209	21.220762	0.008809095	0.00757	3.07	15.53481804	0.008679857
0.00868	3.538	44.53965719	0.009061703	0.00802	3.21	21.24501024	0.008827384
0.0093	4.228	213.6932025	0.009378378	0.00879	3.623	54.0202376	0.00910417
0.00968	4.835	846.2875019	0.00969944	0.00953	4.814	814.4188084	0.009550375
0.01026	5.24	2068.256343	0.01026769	0.01012	5.217	1980.708903	0.010128098
0.0108	5.489	3531.852066	0.010804353	0.01072	5.475	3439.644557	0.010724493
0.01121	5.594	4364.22515	0.01121343	0.01132	5.666	5108.983723	0.011322907
0.01166	5.761	6195.367054	0.011662341	0.01184	5.803	6729.432997	0.011842128

0.012	5.82	6910.190077	0.012002049

Table 1. Continued

Run 5				Run 6			
NaOH added (L)	**pH**	***X***	***Y***	**NaOH added (L)**	**pH**	***X***	***Y***
0.00047	2.257	2.610546664	0.007209261	0.00079	2.05	1.2036548	0.011679134
0.00109	2.299	2.869570768	0.007245794	0.00147	2.068	1.224591718	0.011987525
0.00167	2.323	2.983185266	0.007528261	0.00206	2.083	1.238222351	0.012279543
0.00247	2.38	3.377660119	0.007648126	0.0028	2.113	1.313072005	0.012406585
0.00324	2.433	3.768828028	0.007857681	0.00347	2.155	1.465260809	0.012247894
0.00386	2.482	4.180588956	0.008009772	0.00407	2.175	1.502763019	0.012501434
0.00437	2.534	4.695339069	0.008069573	0.00486	2.2	1.541874917	0.01288021
0.00497	2.598	5.40412319	0.008181006	0.00559	2.248	1.73078022	0.012821005
0.00559	2.666	6.254265484	0.008351841	0.0062	2.29	1.909543391	0.012802378
0.00686	2.838	9.070521686	0.00874101	0.00682	2.34	2.154125179	0.012738729
0.00807	3.148	18.2147389	0.009001711	0.0074	2.391	2.432201651	0.012691517
0.00877	3.482	38.79077548	0.009204595	0.00806	2.444	2.729193995	0.012772386
0.00945	4.451	354.3143124	0.009496967	0.0088	2.505	3.100704853	0.012922919
0.01007	5.053	1362.102505	0.010081809	0.00938	2.573	3.625884983	0.012924164
0.01067	5.332	2483.26837	0.010676244	0.01	2.656	4.381938721	0.012944209
0.0114	5.528	3695.465029	0.011404001	0.01063	2.759	5.544428499	0.012965871
0.01207	5.813	6761.832163	0.012072083	0.0112	2.865	7.032293299	0.013039419
				0.01196	3.063	11.00335117	0.013133921
				0.01253	3.321	19.84812073	0.013181395
				0.01305	3.896	74.31697254	0.013224119

	0.0135	4.781	555.2767969	0.01352278
	0.014	5.22	1458.818011	0.014008325
	0.01447	5.474	2502.659966	0.014474656
	0.0152	5.701	3916.638437	0.015202775

Table 1. Continued

Run 7				Run 8			
NaOH added (L)	**pH**	***X***	***Y***	**NaOH added (L)**	**pH**	***X***	***Y***
0.00055	1.921	0.652248153	0.015170357	0.0004	2.108	1.603319322	0.010107709
0.00115	1.961	0.76131757	0.01456349	0.00099	2.157	1.842090736	0.009711772
0.00175	1.99	0.829497333	0.014371481	0.00153	2.186	1.966835374	0.00973105
0.00235	2.017	0.890561236	0.01428064	0.002	2.221	2.149995177	0.009600306
0.00295	2.033	0.902815157	0.014516508	0.00248	2.237	2.19640102	0.00983911
0.00354	2.063	0.978937841	0.014396356	0.003	2.272	2.38324473	0.009823
0.00409	2.065	0.929596844	0.014953879	0.00354	2.272	2.29377794	0.010397992
0.00455	2.102	1.052570362	0.014570743	0.00401	2.308	2.500052606	0.010350469
0.00517	2.146	1.198769142	0.014278939	0.00479	2.34	2.623375369	0.010723302
0.0058	2.173	1.259282493	0.014411158	0.0052	2.367	2.777373537	0.01079701
0.00647	2.214	1.39160084	0.014355026	0.00576	2.394	2.904000396	0.011047042
0.00701	2.248	1.507394442	0.01433825	0.00631	2.434	3.159468159	0.011156383
0.00757	2.26	1.489448436	0.014735839	0.0068	2.47	3.401942758	0.011280978
0.00815	2.308	1.686365828	0.014600634	0.0074	2.5	3.560049284	0.011604892
0.00875	2.361	1.926143289	0.014491233	0.00799	2.544	3.879879939	0.011810186
0.00951	2.44	2.347387992	0.014329807	0.0085	2.583	4.181357712	0.012008231
0.01	2.512	2.832337778	0.014101744	0.009	2.629	4.591322433	0.012169887
0.01059	2.603	3.548434346	0.013934196	0.00959	2.686	5.148533568	0.012384731
0.01117	2.719	4.717810616	0.013743733	0.01015	2.747	5.829274521	0.012590666
0.01175	2.866	6.709525441	0.013594265	0.01067	2.825	6.904865847	0.012718859

0.01225	3.067	10.83123723	0.013416171	0.0112	2.914	8.36736626	0.012877046
0.01265	3.319	19.54244747	0.013305099	0.0118	3.041	11.04374454	0.013058447
0.01308	3.934	81.00211816	0.013239574	0.01215	3.127	13.32059523	0.013185536
0.0136	4.867	671.0027437	0.013618704	0.0126	3.305	19.8934737	0.013290032
0.01408	5.228	1474.711649	0.014088179	0.01315	3.624	40.79382655	0.013482616
0.01486	5.592	3157.562801	0.014863559	0.0138	4.61	383.072711	0.013834544
0.0152	5.717	4063.667342	0.015202674	0.01419	5.087	1111.599843	0.014201556
0.01562	5.788	4571.189363	0.015622278	0.01452	5.313	1814.828304	0.014526886
0.01604	5.946	6269.034172	0.016041584	0.01489	5.509	2751.249467	0.014894397
				0.0152	5.629	3517.487235	0.015203342
				0.01573	5.807	5017.399583	0.015732225
				0.016	5.901	6051.353183	0.016001793

Table 1. Continued

Run 9				Run 10			
NaOH added (L)	**pH**	***X***	***Y***	**NaOH added (L)**	**pH**	***X***	***Y***
0.00055	2.025	1.103041231	0.01205689	0.00068	2.032	0.702514123	0.008031838
0.00122	2.061	1.215104304	0.011882087	0.00141	2.093	0.825140504	0.00786989
0.00182	2.061	1.151623053	0.012545289	0.00216	2.125	0.848381557	0.008229197
0.00242	2.102	1.298114345	0.01223659	0.00279	2.18	0.968144446	0.008187716
0.00299	2.111	1.279955198	0.012658764	0.00361	2.265	1.190532095	0.008102232
0.00348	2.147	1.418207605	0.012421952	0.00429	2.326	1.347114707	0.008232484
0.00402	2.174	1.503605823	0.01246681	0.00503	2.422	1.682112352	0.00822455
0.00493	2.23	1.717170377	0.012419892	0.0058	2.503	1.959266961	0.008480313
0.00545	2.253	1.785412174	0.012588755	0.00641	2.62	2.566859356	0.008475057
0.00604	2.288	1.923293012	0.012662861	0.00717	2.815	4.006908151	0.008502156
0.00669	2.319	2.025452382	0.012894397	0.00791	3.194	9.628544413	0.008472354
0.00742	2.361	2.193563734	0.013091018	0.00876	4.869	437.1407719	0.008772024
0.00812	2.425	2.55184626	0.013045859	0.0093	5.377	1302.695778	0.009303759
0.00869	2.467	2.7769486	0.01318537	0.00967	5.55	1831.35079	0.009672535
0.00926	2.526	3.175574068	0.01320491	0.01001	5.663	2245.649256	0.010011962
0.00982	2.598	3.756615098	0.013179366	0.0106	5.943	3849.293702	0.010601034
0.01047	2.696	4.712832524	0.013166623				
0.01099	2.782	5.721748457	0.013212592				
0.01162	2.916	7.756118566	0.013261791				
0.0122	3.102	11.88835863	0.013275397				

0.01262	3.354	21.3345921	0.013224215
0.01323	4.288	182.0319794	0.013300719
0.01362	4.875	682.2512401	0.013638366
0.01401	5.196	1379.019734	0.014018799
0.01442	5.404	2140.480838	0.014425469
0.01497	5.607	3231.549761	0.014973441
0.01573	5.818	4838.028396	0.015732127