# Charge Density Quantification of Polyelectrolyte Polysaccharides by Conductometric Titration: An Analytical Chemistry Experiment

**ARTICLE** *in* JOURNAL OF CHEMICAL EDUCATION · JANUARY 2012

Impact Factor: 1.11 · DOI: 10.1021/ed200261w



**4 AUTHORS**, INCLUDING:

Giorgio Capretti
Stazione Sperimentale Carta, Cartoni e Past…
**7** PUBLICATIONS **46** CITATIONS
SEE PROFILE

Luciano Piergiovanni
University of Milan
**100** PUBLICATIONS **739** CITATIONS
SEE PROFILE

JOURNAL OF CHEMICAL EDUCATION

LABORATORY EXPERIMENT

pubs.acs.org/jchemeduc

# Charge Density Quantification of Polyelectrolyte Polysaccharides by Conductometric Titration: An Analytical Chemistry Experiment

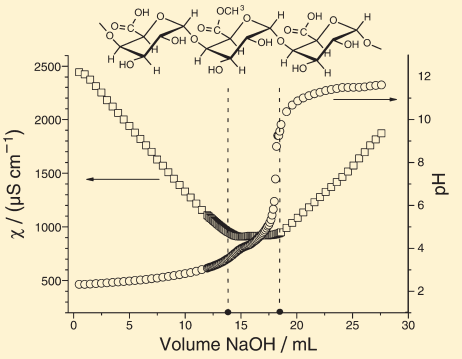
Stefano Farris,*[,†] Luigi Mora,[†] Giorgio Capretti,[‡] and Luciano Piergiovanni[†]

[†]DiSTAM, Department of Food Science and Microbiology, University of Milan, Via Celoria 2, 20133 Milan, Italy

[‡]SSCCP, Italian Pulp and Paper Research Institute, Piazza L. da Vinci 16, 20133 Milan, Italy

Supporting Information

**ABSTRACT:** An easy analytical method for determination of the charge density of polyelectrolytes, including polysaccharides and other biopolymers, is presented. The basic principles of conductometric titration, which is used in the pulp and paper industry as well as in colloid and interface science, were adapted to quantify the charge densities of a negatively charged polysaccharide (pectin) and a positively charged biopolymer (chitosan), two biomacromolecules commonly used in food and biomaterials applications. This novel conductometric titration method can be easily applied in most analytical chemistry teaching laboratories, due to its ease-of-use, safety, and educational benefits. This analytical technique can also be used in a wide-range of laboratory activities and has extensive research applications in areas of chemistry involving charged biopolymers, such as food science, materials science, and physical chemistry.




**KEYWORDS:** Upper-Division Undergraduate, Analytical Chemistry, Laboratory Instruction, Hands-On Learning/Manipulatives, Carbohydrates, Conductivity, Food Science, Materials Science, Titration/Volumetric Analysis

Titration is a commonly used analytical technique in many research and classroom laboratory activities and is defined as the addition of a solution with known concentration (the titrant) to a second solution with an unknown concentration (the analyte), with the goal of determining the concentration of the latter. Titration is complete when a specific end point is reached. There are several methods for end point determination: pH indicators (e.g., phenolphthalein), redox indicators, pH meters, conductometers, potentiometers, ζ-potential, isothermal calorimeters, spectrophotometers, and amperometric instruments.

This article focuses on conductometric titration, a titration technique based on measuring conductance changes during stepwise addition of a titrant to an analyte. The conductivity of a solution depends on several factors, including solute concentration, the degree of solute dissociation, the valence of the ion(s) present in the solution, temperature, and the mobility of the ions in the solution. Conductometric titration is a versatile technique, with a wide range of applications. It is a well-established analytical method for simple acid−base systems[1,2] and has recently been applied to analyze biological molecules for various purposes.[3−5] In addition, conductometric measurements are routinely conducted in the pulp and paper industry to assess the mechanical performance of paper by absorption of additives onto the fiber surface, the deposition of colloidal materials, such as small cellulose fragments and filler particles; or when stoichiometric neutralization of anionic trash is required.[6,7] The potential usefulness of conductometric titration as a routine laboratory technique has been proposed in textbooks over 20 years ago.[8] Moreover, the basic principles behind related conductometric titration apparatus have been described in this *Journal*.[9−14]

In light of the rapidly increasing interest in the use of biomacromolecules for a broad spectrum of practical applications, in the present work an easy-to-use conductometric titration method to quantify the charge density of biomacromolecule polyelectrolytes is described. The charge density, defined as the amount of electric charge per mass unit, provides a quantitative measure of the charged groups along the molecular backbone of a biomacromolecule. These groups may be either positively charged or negatively charged. There are several methods for the determination of the charge density, among which electrophoretic and light scattering techniques, colloidal titation, and pH titration are the most widely exploited. In this article, the advantages of conductometric titration for determination of the charge density of polyelectrolyte biopolymers are illustrated. The educational goal of this work is to introduce students to the fascinating world of biomacromolecules and to teach students how to manipulate and investigate their versatile nature using a simple analytical technique. The student experiments have been successfully used for teaching purposes, in particular within lab activities of undergraduate students of the food science program. The entire activity includes three parts: (i) setup of the experiment (∼30 min), (ii) carrying out the conductometric titration experiments (∼150 min), and (iii) data analysis (∼30 min).

**Published:** October 18, 2011

**Figure 1.** Equipment required for conductometric titration.

## EXPERIMENTAL PROCEDURE

### Materials

All raw materials can be readily purchased from chemicals suppliers: 0.1 N hydrochloric acid (HCl), 0.1 N sodium hydroxide (NaOH), and low-methoxyl pectin from citrus with a degree of esterification (DE) = 28.5%, were purchased from Sigma-Aldrich (Milan, Italy). Medium molecular weight shellfish chitosan was provided by Giusto Faravelli Spa (Milan, Italy). The degree of acetylation, DA (%), of the chitosan was approximately 16.13%.

### Sample Preparation

Pectin, an anionic polyelectrolyte, and chitosan, a cationic polyelectrolyte, must be dispersed in water before use. A highly dilute dispersion (e.g., 0.1 wt %) can be prepared in approximately 30 min (see Supporting Information).

### Conductometric Titration Instrument Setup

To simultaneously monitor the pH and conductivity of the prepared dispersion, a pH meter and conductometer must be carefully fixed close to the beaker containing the water dispersion (the analyte). A magnetic stirrer is used to continuously mix the water dispersion throughout the experiment. Because both pH and conductivity are strongly influenced by temperature, a temperature-controlling device is used to ensure that this parameter is constant during analysis. In addition, because the titrant should be dispensed precisely, the use of an automatic microburet is recommended. However, for the purposes of the student laboratory exercise, a manual buret is sufficient. A typical instrument setup is illustrated in Figure 1. Setup will normally require approximately 30 min.

## ANALYSES

Conductometric titration of both biopolymers requires approximately 2.5 h.

### Conductometric Titration of Pectin

A pectin aqueous dispersion (0.1 wt %) was first treated with an excess (15 mL) of 0.1 N hydrochloric acid (HCl), to completely neutralize the negative charge distributed along the pectin backbone from the unprotonated carboxylic groups. Conductometric titration was performed by adding 0.1 N sodium hydroxide (NaOH) under gentle stirring (100 rpm). Ionic conductivity was evaluated after sequential injections of NaOH in three stages: (i) initially, 0.5 mL drops were dispensed at a flow rate of 0.40 $\mu$L $s^{-1}$; (ii) as the conductance decreased (approaching the first equivalence point), the dispensed volume was reduced to 0.1 mL at a flow rate of 0.15 $\mu$L $s^{-1}$; and (iii) beyond the "constant-conductivity" region, 0.5 mL drops were dispensed at a flow rate of 0.40 $\mu$L $s^{-1}$. The titrant was added approximately every 60 s, to allow sufficient time for equilibrium to be reached between readings. pH was continuously measured simultaneously.

### Conductometric Titration of Chitosan

Conductometric titration of chitosan aqueous dispersion (0.1 wt %) was performed without any prior neutralization step. Hydrochloric acid (0.1 N HCl) was added approximately every 2 min in two stages: (i) initially, 0.1 mL drops were dispensed at a flow rate of 0.15 $\mu$L $s^{-1}$ and (ii) as the conductance increased (after the breakpoint), the dispensed volume was increased to 0.5 mL, with a flow rate of 0.40 $\mu$L $s^{-1}$. Ionic conductivity was evaluated after each addition of titrant, and pH was continuously measured.

### Charge Density Determination

The charge density (as equivalent charge) of the polyelectrolytes can be determined by plotting the measured ionic conductivity versus total titrant. From the intersection points of the linear segments of the ionic conductivity plot before and after the equivalent point (or breakpoint), it is possible to graphically determine the volume (mL) of titrant required to fully deprotonate all carboxylic groups on pectin or fully protonate all amino groups on chitosan. By multiplying this value by its concentration (normality), and referring to the initial polyelectrolyte mass, the charge density of the polymer (mmol $g^{-1}$) can be calculated. A detailed example is reported in the Supporting Information. This step normally requires 30 min.

## HAZARDS

The conductometer, pH meter, and data logger are safe to use; however, high voltage power supplies must be used with caution. Concentrated sodium hydroxide solution is caustic; although high temperatures may result upon mixing sodium hydroxide with water, this was not observed during the stepwise sodium hydroxide titration described above. Both hydrochloric acid and sodium hydroxide solutions should only be handled (filling, closing, and shaking) while wearing a protective lab coat, gloves, and safety glasses. The pectin and chitosan used in these experiments are safe and are used as food ingredients. However, very low mesh powders may be irritating to the respiratory tract. Therefore, a protective mask is recommended during handling.

## RESULTS AND DISCUSSION

### Pectin

Typical plots of ionic conductivity ($\chi$) and pH versus the volume of titrant for dilute aqueous pectin dispersions are shown in Figure 2. Both curves clearly display three distinct zones, corresponding to three distinguishable physicochemical phenomena. In zone 1, the first descending part of the conductometric curve is due to neutralization of dissociated hydrogen ions from the previously added HCl (Figure 3A), as shown by

$$NaOH + H^+ + Cl^- \leftrightarrows H_2O + Na^+ + Cl^- \qquad (1)$$

Because the equivalent ionic conductance of $H^+$ (350 S $cm^2$ $mol^{-1}$) is approximately 7 times greater than the mobility of $Na^+$


dx.doi.org/10.1021/ed200261w |J. Chem. Educ. 2012, 89, 121–124

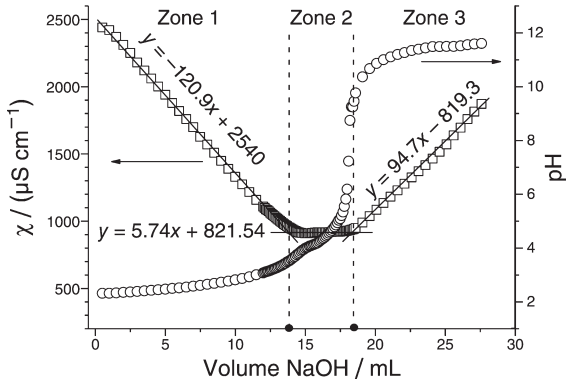



**Figure 2.** Mean conductometric and potentiometric titration curves for pectin (DE = 28.5%).

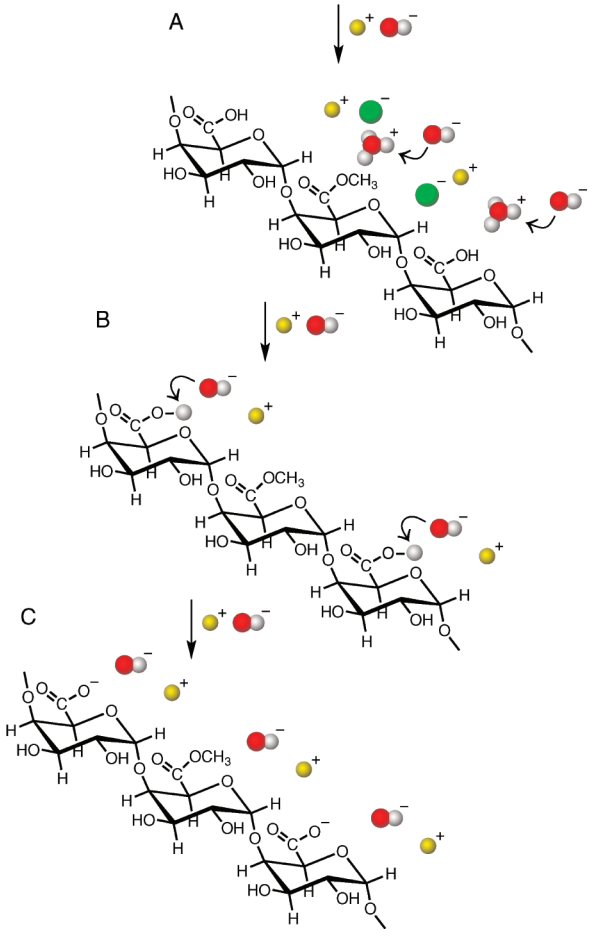



**Figure 3.** Schematic representation of chemical changes induced by NaOH titration of the pectin backbone: (A) neutralization of hydrogen ions (excess HCl) by NaOH; (B) early dissociation of carboxylic groups mediated by hydroxide ions; and (C) from pectic acid to pectate, dissociation of all carboxylic groups. Na = yellow, O = red, H = white, and Cl = green.

(50.9 S cm$^2$ mol$^{-1}$),[15,16] the net effect is a decrease in conductivity. At the same time, the pH increases moderately as the concentration of hydrogen ions decreases.

As the first equivalence point is approached, neutralization of the excess $H^+$ is complete and carboxylic acid groups began to dissociate (zone 2). In this zone, no changes in conductance values occur because of the neutralization of dissociated hydrogen ions from the pectic acid backbone by hydroxide ions ($OH^-$), which arise from the addition of titrant (Figure 3B) according to

$$-COOH + Na^+ + OH^- \rightleftharpoons -COO^- + Na^+ + H_2O \quad (2)$$

Simultaneously, the pH of the pectic acid dispersion progressively increases as deprotonation of the carboxylic groups proceeds, due to increasing quantities of free $OH^-$ ions in the dispersion.

Zone 3 begins beyond the second equivalence point. Because carboxylic groups have been titrated, the conductance increases in proportion to the $OH^-$ excess in the dispersion, arising from dissociation of the NaOH dispensed (the hydroxyl equivalent ionic conductivity is ~192 S cm$^2$ mol$^{-1}$) (Figure 3C). At this point, addition of even small volumes of titrant will yield a dramatic increase in pH, until NaOH dissociation becomes progressively hindered by higher concentrations.

According to the graphical method described in the previous section, the final anionic charge density of pectin is 2.471 ± 0.123 mmol g$^{-1}$ or approximately 0.492 mmol/0.2 g of pectin.

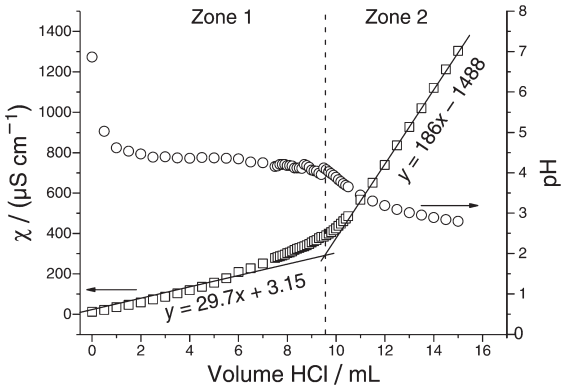



**Figure 4.** Mean conductometric and potentiometric titration curves for chitosan (DD = 83.87%).

## Chitosan

The degree of acetylation, DA (%), of the chitosan used in this work was approximately 16.13%, corresponding to a degree of deacetylation (DD) of ~83.87%. A typical evolution of ionic conductivity ($\chi$) and pH versus volume of titrant for dilute aqueous chitosan dispersions is shown in Figure 4. Both curves clearly display two distinct zones. Initially, the pH of the chitosan dispersion is above the p$K_a$ of chitosan (~6.5), with the absence of any predominant charge along the polysaccharide molecule; thus, all amino groups ($-NH_2$) are in their unprotonated form. The absence of positive charge greatly influences the physical properties of chitosan dispersions by affecting its solubility in water. In support of this, chitosan dispersions prepared in this work initially appeared very cloudy (i.e., high turbidity) due to the presence of insoluble particles of solute dispersed in the solvent. The lack of solubility of native chitosan in water has been ascribed to its inherent physical structure. Specifically, its $\beta$-1,4-configuration results in a rigid and unbranched structure, whereas the abundance of hydroxyl groups (one primary hydroxyl and one secondary hydroxyl) and a highly reactive amino group explain the tendency for intra- and intermolecular hydrogen


dx.doi.org/10.1021/ed200261w | J. Chem. Educ. 2012, 89, 121–124

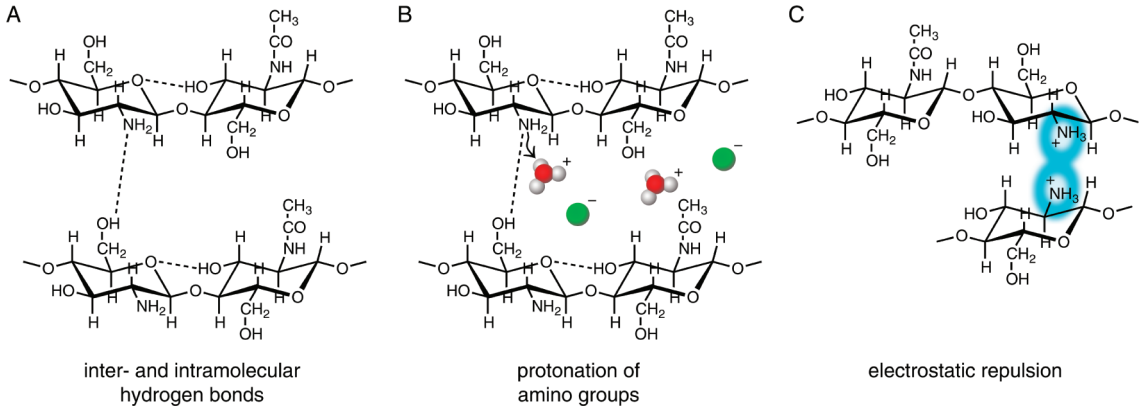



**Figure 5.** Schematic representation of chemical changes induced by HCl titration of the chitosan backbone: (A) undissolved chitosan with unprotonated amino groups (dashed lines indicate the intra- and intermolecular hydrogen bonds); (B) partial protonation of amino groups is promoted by the addition of HCl (figure shows the hydronium ion $H_3O^+$ and the chloride ion $Cl^-$); (C) full protonation of amino groups leads to complete solubility of chitosan because of increased polarity and electrostatic repulsion.

bond formation (Figure 5A). Increasing volumes of titrant (HCl) gradually lead to an increase in the solubility of chitosan, with a simultaneous increase in the transparency of the chitosan dispersion. This is due to protonation of amino groups ($-NH_3^+$), which promotes unfolding of the chitosan molecules by electrostatic repulsion (Figure 5B). Simultaneously (with the exception of an initial decrease in pH, presumably because of system stabilization), the pH values of the chitosan dispersion remain steady up to the equivalence point, due to continuous protonation of $-NH_2$ groups, while conductivity values increase slightly, because of the release of free chloride anions ($Cl^-$). The end of the first zone is assumed to correspond to the total protonation of amino groups, and maximum transparency of the chitosan dispersion is attained at this point (no chitosan particles can be seen by visual inspection). Further addition of hydrochloric acid prompts a steep increase in conductivity, due to the larger conductivity of hydrogen cations (350 S $cm^2$ $mol^{-1}$) compared to chloride anions ions (75.5 S $cm^2$ $mol^{-1}$) (Figure 5C).

This is also consistent with slope values from the two distinct linear segments of the conductivity curve. The slope of the first linear segment of the conductivity curve is approximately 6-fold greater than the slope of the second linear segment (~30 vs ~185 $\mu$S mL $cm^{-1}$), in agreement with the smaller equivalent conductivity of $Cl^-$ ions (75.5 S $cm^2$ $mol^{-1}$) compared to the $H^+$ ions (350 S $cm^2$ $mol^{-1}$). Accordingly, pH values start to decrease from this point on, and throughout the second zone, due to increasing quantities of free $H^+$ ions in the medium. On the basis of these observations, the equivalence point is located at the intersection of the two linear segments of the curve, from which the corresponding volume of titrant used can be extrapolated. The calculated anionic charge of chitosan was 4.660 ± 0.056 mmol $g^{-1}$, or 0.932 mmol/0.2 g of sample.

## CONCLUSIONS

The results demonstrate that this conductometric technique is a valid method for quantifying the charge density of dilute solutions of polyelectrolyte polymers, such as polysaccharides. This tool could be especially relevant when assembly of biopolymers governed by electrostatic forces is required. In addition, because this technique is relatively rapid, safe, and easy-to-use, it can be successfully adapted for teaching laboratories for undergraduate student courses.

## ASSOCIATED CONTENT

### Supporting Information

Teacher and student guide to conductometric titrations. Full details of the operative conditions. This material is available via the Internet at http://pubs.acs.org.

## AUTHOR INFORMATION

### Corresponding Author

*E-mail: stefano.farris@unimi.it.

## REFERENCES

(1) Smith, K. C.; Edionwe, E.; Michel, B. *J. Chem. Educ.* **2010**, *87*, 1217–1221.
(2) Nyasulu, F.; Stevanov, K.; Barlag, R. *J. Chem. Educ.* **2010**, *87*, 1364–1366.
(3) Li, Q.; Song, B.; Yang, Z.; Fan, H. *Carbohydr. Polym.* **2006**, *63*, 272–282.
(4) Weinbreck, F.; Nieuwenhuijse, H.; Robijn, G. W.; de Kruif, C. G. *J. Agric. Food Chem.* **2004**, *52*, 3550–3555.
(5) Mascheroni, E.; Capretti, G.; Marengo, M.; Iametti, S.; Mora, L.; Piergiovanni, L.; Bonomi, F. *Packag. Technol. Sci.* **2010**, *23*, 47–57.
(6) Fardim, P.; Holmbom, B.; Ivaska, A.; Karhu, J. *Nord. Pulp Pap. Res. J.* **2002**, *17*, 346–351.
(7) Laine, J.; Stenius, P. *Pap. Puu* **1997**, *79*, 257–266.
(8) Vogel, A. I. *Textbook of Quantitative Chemical Analysis*, 5th ed.; Longman Scientific & Technical: Harlow, U.K., 1989; pp 519−528.
(9) Katz, D. A.; Willis, C. *J. Chem. Educ.* **1994**, *71*, 330–332.
(10) Zawacky, S. K. S. *J. Chem. Educ.* **1995**, *72*, 728–729.
(11) Da Rocha, R. T.; Gutz, I. G. R.; do Lago, C. L. *J. Chem. Educ.* **1997**, *74*, 572–574.
(12) Haworth, D. T.; Bartelt, M. R.; Kenney, M. J.; Howald, R. *J. Chem. Educ.* **1999**, *76*, 625–627.
(13) Ganong, B. R. *J. Chem. Educ.* **2000**, *77*, 1606–1608.
(14) Papadopoulos, N.; Limniou, M. *J. Chem. Educ.* **2001**, *78*, 245–246.
(15) Rouessac, F.; Rouessac, A. *Chemical Analysis: Modern Instrumentation Methods and Techniques*, 2nd ed.; Wiley: Hoboken, NY, 2007; p 101.
(16) Ötleş, S. *Handbook of Food Analysis Instruments*; CRC Press: Boca Raton, FL, 2008; p 173.