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Method for Rapid and Accurate Measurement of Chitosan Viscosity

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Abstract

A simple and rapid method to estimate the viscosity of chitosan using laboratory pipettes was developed. The viscosities of nine different chitosan samples, prepared in 1% acetic acid at a 1% concentration, were measured with a standard viscometer. Prior to measurement of flow time of 1% chitosan solution with a pipette, twelve pipettes were assorted into three groups with flow times of 4, 5, and 6 sec after measuring passage of 9 ml of 1% acetic acid through a 10 ml pipette. With each group of pipettes, flow time of 1% chitosan solution was determined by measuring the delivery time of 5 ml of the 10 ml solution through a 10 ml pipette. Results of regression analyses revealed high linear relationships (R^2=0.9812, 0.9663, and 0.9754) between viscosities calculated with a viscometer and flow times measured with 4, 5, or 6 sec group pipettes. The viscosity of chitosan could be readily and accurately estimated from these linear regression equations by measuring flow times based on pipette delivery.

Key words: viscosity, chitosan, flow time, pipette

INTRODUCTION

Conversion of chitin to chitosan is generally achieved by treatment of the chitin with concentrated sodium hydroxide solution (40~50%), usually at 100°C or higher, to remove some or all of the acetyl groups from the polymer (1). During deacetylation, conditions are required that in a reasonable time will sufficiently deacetylate the chitin to yield a chitosan product soluble in dilute acetic acid without significant degradation. Several studies (2-4) have demonstrated that specific characteristics of chitosan, i.e., molecular weight, degree of deacetylation and viscosity, vary notably according to process conditions. Higher temperature and alkali concentrations, together with longer reaction time, increase the percentage of deacetylation but reduce molecular size and viscosity (4,5).

The physicochemical characteristics of chitosan influence its functional properties, which differ according to the particular preparation methods. To effectively utilize chitosan products for a variety of applications, the physicochemical properties of chitosan must be constantly monitored. A number of techniques have been developed and proposed over the years to determine the molecular weight of chitosan (6,7) and degree of deacetylation (8-11). In the main, the viscosity of chitosan has been determined by a commercial viscometer. Since such instruments are relatively expensive and not always available in the laboratory or production plant, a simple method to rapidly estimate chitosan viscosity has been needed especially for product quality control purposes.

The present research notes the development of a relatively simple and accurate method to estimate the viscosity of chitosan using standard laboratory pipettes.

MATERIALS AND METHODS

Materials

Nine chitosans were used for establishment of linear relationships between viscosity and flow time. Four chitosans (designated C1 to C4) were commercially available products from Primex (Avaldsnes, Norway), Keumho Chemical Products Co. (Seoul, Korea), Sigma Chemical Co. (St. Louis, MO), and Pronova Biopolymer (Raymond, WA). Five chitosans (designated L1 to L5) were prepared in our laboratory by reaction of chitin (Keumho Chemical Products Co., 20~40 mesh particle size) with 50% NaOH (w/w) solution at 100°C for 0.5, 1, 2, 4, and 6 hr following procedures described by No and Meyers (12).

To confirm the linear relationships established with the aforementioned nine chitosans, two chitosans (designated K1 and K2) from Keumho Chemical Products Co. with different viscosities from the nine chitosans were additionally used.

Determination of viscosity

Viscosities of chitosan samples were determined with a Brookfield viscometer, model RVT (Brookfield Engineering Laboratories, Inc., Stoughton, MA). Chitosan solution (500 ml) was prepared in 1% acetic acid at a 1% concentration on a dry basis. Measurements were made in duplicate using a spindle #2 at various speeds (2.5, 5, 10, 20, and 50 rpm) on solutions at 23±0.3°C and average values reported in centipoise (cP) units. All chitosan solutions were retained for subsequent flow time measurements.

Assortment of pipettes

Prior to measuring flow time of 1% chitosan solution with a pipette, twelve pipettes were assorted by calculating flow

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time of 9 ml of 1% acetic acid in a 10 ml pipette (Superior Class B Mohr pipette calibrated "to deliver/blow out," W. Germany). Measurements were made in duplicate at 23±0.3°C with average values reported.

Measurement of flow time

Flow time of 1% chitosan solution was determined by calculation of delivery time of 5 ml of the 10 ml solution in a 10 ml pipette. Measurements were in duplicate at 23±0.3°C, with average values reported.

Analyses

A regression analysis was carried out to determine the relationship between viscosity and flow time. Moisture content was determined using a Halogen Moisture Analyzer (HG53, Mettler Toledo, Switzerland).

RESULTS AND DISCUSSION

Assortment of pipettes

To evaluate possible difference in flow time among pipettes, twelve were tested by measuring flow time of 9 ml of 1% acetic acid in a 10 ml pipette. Results showed differences in flow time in seconds depending on the particular pipettes as follows; one pipette with 2 sec, three with 4 and 5 sec, respectively, and five with 6 sec. In subsequent flow time measurements with 1% chitosan solution, three groups of pipettes with 4, 5 and 6 sec were used.

Viscosity

Viscosities of chitosan samples measured with a viscometer (Table 1) ranged from 62 to 1025 cP for four commercial chitosans (C1-C4) and from 147 to 817 cP for five laboratory-prepared chitosans (L1-L5). These viscosity types were considered to be representative of ranges generally useful in various commercial applications (13). Differences recorded in viscosity with samples are probably due to dissimilar preparation methods (2-4).

Table 1. Viscosity and flow time of various chitosan samples

Chitosan sample	Viscosity[1)] (cP)	Flow time (sec)[2)] of 9 ml of 1% acetic acid: 4	5	6
		Flow time (sec)[2)] of 5 ml of 1% chitosan solution		
C1	62	3.3±0.6[3)]	4.7±0.6[3)]	6.2±0.4[4)]
C2	125	4.8±0.3	7.5±0.9	10.0±0.7
C3	543	17.8±0.8	26.0±2.6	35.5±3.0
C4	1,025	31.7±2.5	46.0±5.2	60.5±4.5
L1	817	24.0±2.0	34.7±4.0	46.4±3.9
L2	817	24.0±2.0	35.7±4.0	47.4±4.3
L3	475	15.7±1.5	23.5±2.6	31.7±2.7
L4	147	5.7±0.6	9.0±0.9	11.9±0.9
L5	174	7.2±0.8	11.3±1.1	15.0±1.4

[1)]Viscosity was measured with 1% chitosan solution in 1% acetic acid using a Brookfield viscometer.
[2)]Flow time of 1% acetic acid and 1% chitosan solution was measured with 10 ml pipettes (Superior Class B Mohr pipettes calibrated "To Deliver/Blow out," W. Germany).
[3,4)]Flow time in the column indicate mean±standard deviation of three and five pipettes, respectively.

Flow time

Flow time of 1% chitosan solution was determined by measuring delivery time of 5 ml of the 10 ml solution in a 10 ml pipette. Results are noted in Table 1. Differences in flow time among pipettes in each group were noted, although they had similar flow times when measured with 1% acetic acid. The variation was generally wider with higher viscosity as evident in the standard deviations. This can probably attribute to one or two pipettes showing dissimilar flow time according to different tip sizes among three or five pipettes in each group. It was also observed that measurement with above 5 ml of chitosan solution increased the flow time and the variation (data not shown). Measurement of flow time with 5 ml was considered to be an appropriate volume.

Linear relationship

Results of linear relationships between flow time and viscosity of 1% chitosan solution are shown in Fig. 1. Regres-

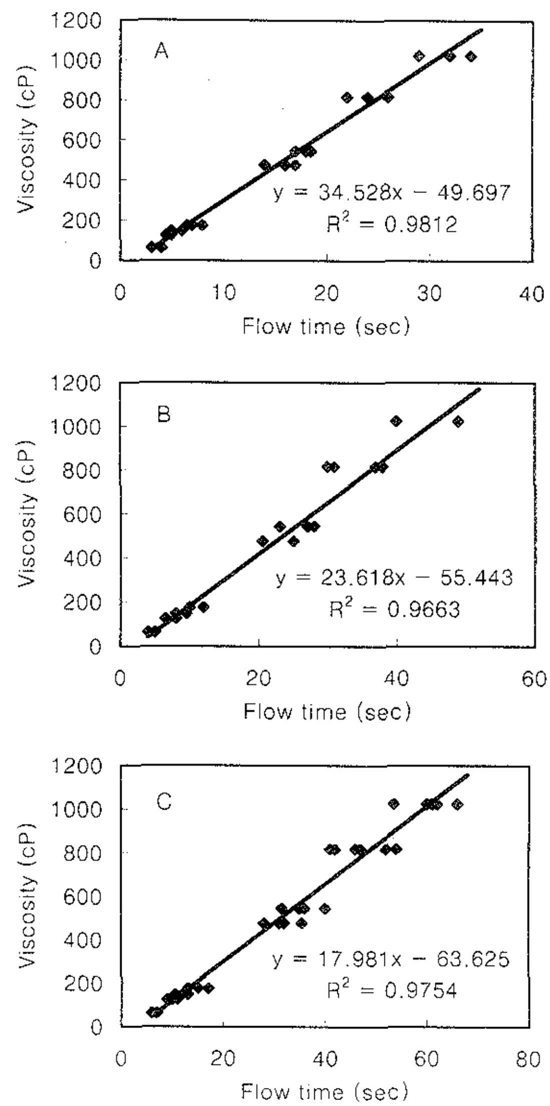

Fig. 1. Relationships between viscosity and flow time of 1% chitosan solution. Viscosity was measured with a Brookfield viscometer and flow time was measured with A: 4 sec, B: 5 sec, and C: 6 sec group of pipettes.

sion analysis revealed high linear relationships (R^2=0.9812, 0.9663, and 0.9754) between viscosity and flow time measured with 4 (A), 5 (B), or 6 sec (C) group pipettes, respectively. This indicates that the viscosity of chitosan can be accurately estimated by measurement of flow time using pipettes. Table 2 shows viscosity values actually measured with a viscometer and calculated from regression equations.

To confirm whether the linear regression equations established with the above nine chitosans can be actually applied to other chitosan samples, the viscosities of two additional different chitosans (K1 and K2) were measured with a viscometer and compared with those calculated from the linear regression equations (Table 3). Results indicated that the viscosity of chitosan samples could be readily estimated from flow time.

CONCLUSIONS

This investigation has revealed that the viscosity of chitosan can be rapidly and accurately established by measuring flow time using pipettes if a standard viscometer instrument is not available. However, since each pipette may have dissimilar inherent flow times, measurements using several pipettes are recommended to reduce possible variations in the analysis. This study again demonstrates the great variability in viscosity among commercial chitosan products and the need for regular monitoring of product quality using the simplified and accurate method presented in this paper. This method is an effective monitoring tool for analyses of production efficiency, especially any slight changes in the processing that can significantly affect the chitosan viscosity.

Table 2. Viscosities measured with a viscometer and calculated from regression equations

Chitosan sample	Viscosity (cP) measured[1)]	Flow time (sec)[2)] of 9 ml of 1% acetic acid		
		4	5	6
		Viscosity (cP) calculated[3)]		
C1	62	64	56	48
C2	125	116	122	116
C3	543	565	559	575
C4	1,025	1,045	1,031	1,024
L1	817	779	764	771
L2	817	779	788	789
L3	475	492	500	506
L4	147	147	157	150
L5	174	199	211	206

[1)]Viscosity was measured with 1% chitosan solution in 1% acetic acid using a Brookfield viscometer.
[2)]Measured with 10 ml pipettes (Superior Class B Mohr pipettes calibrated "To Deliver/Blow out," W. Germany).
[3)]Viscosity was calculated from regression equations in Fig. 1.

Table 3. Comparison of viscosities measured with a viscometer and calculated from regression equations

Chitosan sample	Viscosity (cP) measured[1)]	Flow time (sec)[2)] of 9 ml of 1% acetic acid		
		4	5	6
		Viscosity (cP) calculated[3)]		
K1	84	99	86	73
K2	397	423	405	386

[1)]Viscosity was measured with 1% chitosan solution in 1% acetic acid using a Brookfield viscometer.
[2)]Measured with 10 ml pipettes (Superior Class B Mohr pipettes calibrated "To Deliver/Blow out," W. Germany).
[3)]Viscosity was calculated from regression equations in Fig. 1.

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