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A Comparison of Some Methods for the Determination of the Degree of Substitution of Carboxymethyl Starch

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The degree of substitution (DS) markedly affects the properties of sodium carboxymethyl starch (Na-CMS). In this work Na-CMS samples with different DS starting from both potato and corn starch were synthesized in an ethanol/water mixture and their DS was determined using three methods: direct titration of the acid form of the carboxymethyl starch (H-CMS), Cu salt precipitation and back titration. It was found that direct titration gave smaller DS values, while the Cu salt precipitation method gave higher DS values than the back titration method, which was found to be the most accurate. The values of the DS obtained by these methods were similar for lower DS while for higher values the discrepancy was more pronounced. Under the same experimental conditions the CMS obtained from corn starch had a higher DS than the one obtained from potato starch and on average, the discrepancies between the DS values obtained by the employed analytical methods were smaller for the CMS samples synthesized from corn starch.

Keywords: Sodium carboxymethyl starch; Direct titration; Cu salt precipitation; Back titration

1 Introduction

Sodium carboxymethyl starch (Na-CMS) is a water soluble starch derivative finding wide application. The degree of substitution (DS), i.e. the average number of sodium carboxymethyl groups bound per anhydroglucose unit, markedly affects the properties of this compound. Convenient methods for the determination of the DS have been developed, most of them being very similar to those already developed for sodium carboxymethyl cellulose.

One of the oldest methods used for the determination of the DS of carboxymethyl cellulose is the direct titration of the carboxyl groups [1] and it was applied to carboxymethyl starch much later [2, 3]. In this method the acid form of carboxymethyl starch (H-CMS) is dispersed in water and the carboxyl groups are titrated with an aqueous sodium hydroxide solution of known molarity.

The DS of CMS can also be determined by the back titration method. This method was first applied for determination of the DS of carboxymethyl cellulose in 1947 [1]. Later, it was also used for the determination of the DS of carboxymethyl starch in a number of studies [4–7]. In this method, Na-CMS is first treated with an acid (HCl or H₂SO₄) to obtain the H-CMS. After that, alkali solution is added to an aqueous dispersion of a weighed amount of

H-CMS converting it into the soluble sodium salt. The excess alkali is then back titrated, permitting the calculation of the amount of carboxymethyl groups. This method was recommended as a standard procedure for the determination of DS of carboxymethyl starch by the Work Group 3 of the International Standards Organization Technical Committee 93 (ISO/TC 93/WG3) [8].

The colorimetric J acid (6-amino-1-naphthol-3-sulphonic acid) method [9] for the determination of formaldehyde and formaldehyde releasing compounds was found to be useful for the determination of the DS of CMS [4, 10]. The method involves dissolution of the Na-CMS sample in sodium hydroxide solution, addition of a J acid solution, further acidification with sulphuric acid and heating under reflux to produce glycolic acid. A colorimetric method was used for the determination of glycolic acid in an aliquot of the resulting solution.

A salt precipitation method, developed for Na carboxymethyl cellulose by *Conner* and *Eyler* [11] in 1950, was improved and adjusted for Na-CMS by *H. Kessel* [12] in 1985. In his method of analysis, the –CH₂COONa groups of the carboxymethyl starch are determined by precipitation of their copper salt (Cu-CMS) and back titration of the excess copper in the filtrate with ethylenediamine tetraacetate using Murexide as the indicator. This method is based on the experimentally proved fact that the copper salt of carboxymethyl cellulose contains an amount of copper, that is very close to the theoretical one, i.e. one mole per two carboxyl groups [11].

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In the last five years, the DS of carboxymethyl starch has been determined using $^1\text{H-NMR}$ [13, 14], HPLC [14–16] and capillary electrophoresis [14]. For the application of these methods, the CMS has to be partially or completely depolymerized. As a result, the methods give not only the DS values but also the molar fractions of differently functionalized repeating units.

The purpose of this work was to synthesize CMS samples with different degrees of substitution starting from potato and corn starch, to determine the value of the DS of the synthesized samples using three different methods: the back titration method, the Cu salt precipitation method and the direct titration of free carboxyl groups and to compare the results obtained by the last two methods with those obtained by the back titration method, which has been recommended as the standard procedure for the determination of the DS. These methods were chosen because they demand neither depolymerization of the CMS nor expensive equipment. The direct titration of free carboxyl groups and Cu salt precipitation method have not been used so widely although they appear to be simple and accurate methods.

2 Materials and Methods

2.1 Chemicals

The following types of starch were used for carboxymethylation: corn starch (25% amylose, “Jabuka” Starch Industry, Pancevo, Serbia) and potato starch (20% amylose, CHP Carbohydrate Pirna GmbH & Co. KG, Pirna, Germany).

All the other chemicals used in this investigation (NaOH, $\text{ClCH}_2\text{COONa}$, $\text{CH}_3\text{CH}_2\text{OH}$, CuSO_4 , FeCl_3 , EDTA, CaCl_2 , NaCl, HCl, Murexide) were obtained either by Fluka (Buchs, Switzerland) or Merck (Darmstadt, Germany) and were used as received.

2.2 Synthesis of carboxymethyl starch

The synthesis of CMS was carried out in two steps as described earlier [17]. In the first step, alkalization was performed by mixing 40 g starch, 120 cm^3 ethanol and 28 cm^3 aqueous 11.5 M NaOH solution at 25°C. The mixture was stirred for 20 min, sodium monochloroacetate (Na-MCA) was added and the reaction mixture was heated to 58°C and stirred at that temperature for 100 min. The desired values of the DS were achieved by adjusting the amount of added Na-MCA (Tab. 1).

The synthesized Na-CMS samples were purified by dissolving in water, neutralizing the solution with 0.1 M HCl solution and precipitating with ethanol. The precipitated Na-CMS was filtered, dried under vacuum at 50°C and then ground. The obtained Na-CMS was dispersed in acetone by stirring with a magnetic stirrer, and then converted to the acid form (H-CMS) by adding an aqueous solution of 30 cm^3 6 M HCl per 10 g of the sample, with continued stirring for 30 min. The dispersion was filtered. In order to remove the excess acid, the precipitate was washed with a methanol-water solution (80 mass% of methanol) until the conductivity of the filtrate was about 25 $\mu\text{S}/\text{cm}$. Then the precipitate was again dispersed in acetone, filtered, dried under vacuum at 50°C, and ground. The obtained H-CMS was used for the DS determination.

Tab. 1. The mass of Na-MCA used for the synthesis of CMS, the DS values of CMS obtained by the three different methods and the relative difference of the DS values obtained by the direct titration, δ' , and by the Cu salt precipitation method, δ'' , in relation to the results of the back titration method.

Starch origin	Sample	Mass of Na-MCA [g]	DS			δ' [%]	δ'' [%]
			Direct titration	Back titration	Salt precipitation		
Potato	CMS1	15	0.35 ± 0.02	0.40 ± 0.01	0.36 ± 0.05	–13	–11
	CMS2	28	0.70 ± 0.10	0.81 ± 0.01	1.1 ± 0.20	–14	31
	CMS3	43	0.76 ± 0.08	0.90 ± 0.01	1.2 ± 0.30	–15	36
Corn	CMS4	15	0.40 ± 0.04	0.50 ± 0.01	0.51 ± 0.05	20	2.4
	CMS5	28	0.66 ± 0.05	0.70 ± 0.01	0.84 ± 0.1	5.7	20
	CMS6	43	0.95 ± 0.11	1.1 ± 0.01	1.3 ± 0.15	14	22

2.3 Determination of water content

The water content of each CMS sample was determined using a Moisture Analyzer, Sartorius MA 40 (Sartorius, Edgewood, NJ, USA).

2.4 Determination of the degree of substitution

2.4.1 Direct titration

An accurately weighed sample of H-CMS (about 2 g) was dispersed in 100 cm³ of 2% NaCl solution by stirring for 15 min and then titrated with 1 M NaOH at room temperature. A titroprocessor 636 Metrohm automatic titrator (Herisau, Switzerland) was used for the titrations. The titration was performed to pH = 12 and the end point was calculated by the instrument as the inflection point of the obtained pH vs. V_{NaOH} curve. The procedure was repeated three times for each sample and the average value of the equivalent volume was taken for the DS calculation. The DS was calculated from the following equation:

$$DS = \frac{162 \times n_{\text{COOH}}}{m_{\text{ds}} - 58 \times n_{\text{COOH}}} \quad (1)$$

where 162 g/mol is the molar mass of an anhydroglucose unit (AGU); n_{COOH} (in mol) is the amount of COOH calculated from the obtained value of the equivalent volume, V_e , of known molarity NaOH (1 M); 58 g/mol is the net increase in the mass of an AGU for each carboxymethyl group substituted, and m_{ds} (in g) is the mass of dry sample calculated from known sample mass m_s (in g) and the water content, $w_{\text{water}}\%$:

$$m_{\text{ds}} = \left(1 - \frac{w_{\text{water}}}{100}\right) \times m_s \quad (2)$$

2.4.2 Back titration

About 0.5 g (accuracy of weighing ± 0.1 mg) of the H-CMS sample was dissolved in 20 cm³ of 0.2 M NaOH and 50 cm³ of bidistilled water was added. The solution was transferred to a 100 cm³ volumetric flask, which was then filled up to the mark with bidistilled water. 25 cm³ of the solution was transferred to an Erlenmeyer flask and diluted by addition of 50–100 cm³ of bidistilled water. The excess of NaOH was back-titrated with standard 0.05 M HCl using phenolphthalein as the indicator. The titration was repeated three times and the average value of the HCl volume was used for the calculations. A blank was also titrated. The DS was calculated using Equation (1), the amount of COOH being equal to:

$$n_{\text{COOH}} = (V_b - V) \cdot c_{\text{HCl}} \cdot 4 \quad (3)$$

where: V_b (in cm³) is the volume of HCl used for the titration of the blank; V (in cm³) is volume of HCl used for titration of the sample; c_{HCl} (in mol/dm³) is the HCl concentration and 4 is the ratio of the total solution volume (100 cm³) and the volume taken for titration (25 cm³).

2.4.3 Cu salt precipitation

For Cu salt precipitation Na-CMS had to be used, which was obtained by neutralizing the H-CMS with NaOH solution. The amount of NaOH necessary for the neutralization was calculated from the results obtained by back titration. After neutralization, the Na-CMS was precipitated by addition of ethanol, the precipitate was dried in an oven at 60°C and was then ground. A weighed sample of Na-CMS was dissolved in water and precipitated by adding a CuSO₄ solution in excess. After the precipitation, the excess of CuSO₄ was determined by titration with EDTA (ethylenediaminetetraacetic acid) in the presence of Murexide as an indicator. The blank was also titrated. From the difference of the volume of EDTA used for the titration of the Na-CMS, V_s , and that used for the blank, V_b , the amount of bound Cu²⁺ was determined. The procedure was repeated three times for each sample and the average value of the EDTA volume difference was used for the calculation of the amount of –CH₂COONa groups, $n_{\text{–CH}_2\text{COONa}}$, in the following way:

$$n_{\text{–CH}_2\text{COONa}} = (V_s - V_b) \cdot c \cdot 2 \cdot 2.5 \quad (4)$$

where c is the concentration of the EDTA solution (0.5×10^{-4} mol/L); 2 is the ratio of the number of moles of –CH₂COONa and number of moles of EDTA used for the titration of Cu in the solution and 2.5 is the ratio of the solution volume (250 cm³) and the volume taken for titration (100 cm³).

The DS is calculated from an equation similar to Equation (1):

$$DS = \frac{162 \times n_{\text{–CH}_2\text{COONa}}}{m_{\text{ds}} - 80 \times n_{\text{–CH}_2\text{COONa}}} \quad (5)$$

where 80 g/mol is the net increase in the mass of the AGU for each –CH₂COONa group substituted.

3 Results and Discussion

The values of the DS of all six synthesized samples (three from corn and three from potato starch) were determined using the three described methods. Direct titration gave the titration curves, $\text{pH} = f(V_{\text{NaOH}})$, shown in Fig. 1 for potato starch and in Fig. 2 for corn starch. As can be seen the obtained curves had two inflection points. This was probably the consequence of the CMS samples being

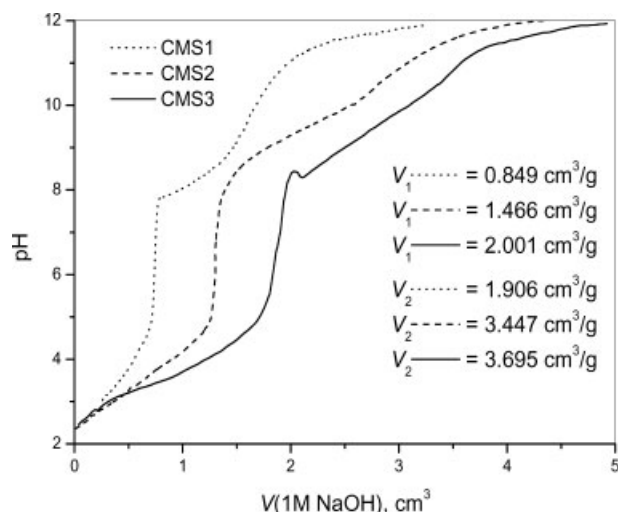


Fig. 1. Direct titration curves for CMS samples obtained from potato starch.

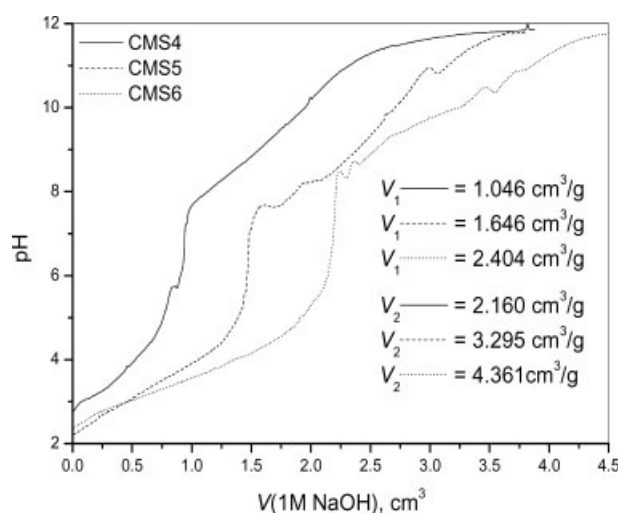


Fig. 2. Direct titration curves for CMS samples obtained from corn starch.

only dispersed in water, which presumably led to the formation of aggregates consisting of a few granules. At the surface of the aggregates there were free carboxyl groups, the number of which could be calculated from the first inflection point. It might be assumed that the $-\text{COOH}$ groups at the surface of the granules inside the aggregates formed strong hydrogen bonds and so were not available for titration at the beginning of the titration. However, when the pH was equal to 8, the solubility of the CMS increased inducing disruption of the hydrogen bonds between the $-\text{COOH}$ groups inside the aggregates. These free carboxyl groups increased the acidity of the dispersion thus slowing down the rate of the increase of the total pH. The second inflection point appeared. The

total amount of $-\text{COOH}$ groups was calculated from the second inflection point according to Equation (1) and the obtained results are presented in Tab. 1.

The values of the DS obtained by the other two methods are also presented in Tab. 1. It can be seen that values of the DS obtained by direct titration are always the lowest ones and the values obtained by the Cu salt precipitation method are the highest ones, except for CMS1. The differences between the DS values are smaller for lower values of the DS. Similar results were obtained by Roushdi [4] who determined the DS of CMS using back titration, as well as the colorimetric J acid and uranyl nitrate precipitation method. His results also showed that the salt precipitation method gave the highest values of the DS and that the difference between the values of the DS obtained by different methods was the smallest for lower DS.

Since the main goal of this work was to compare the results obtained by the direct titration and the Cu salt precipitation method with the results obtained by the back titration method, the relative difference, δ , of the results obtained by these two methods was calculated taking the results obtained by back titration method to be the most accurate. The obtained values are also shown in Tab. 1.

The obtained values for δ show that no unequivocal conclusion about the validity of the direct titration and the Cu salt precipitation method could be drawn. It is obvious that both methods have some disadvantages which result in random errors.

The main disadvantage of the direct titration lies in the fact that the acid form of CMS was only dispersed in water and the $-\text{COOH}$ groups were not all equally available for titration. This resulted in titration curves with two inflection points (Fig. 1 and Fig. 2). The volume of the second inflection point needed for the DS calculation was very difficult to determine precisely, because pH values did not change abruptly.

The main disadvantage of the Cu salt precipitation method was that there was no sharp change in color of Murexide at the end-point of the titration and human error was very possible. It might also be assumed that during the precipitation of the Cu-CMS salt a certain amount of Cu ions were also co-precipitated leading to higher values of the DS and/or that a certain amount of CuSO_4 was spent for oxidation of the end groups of CMS.

The influence of the starch origin on the obtained results was twofold. First, the differences between the values of the DS obtained by the three methods were, on average, smaller for the CMS samples obtained from corn starch

than those obtained from potato starch. Second, under the same conditions of carboxymethylation, the DS values of the Na-CMS obtained from corn starch were slightly higher than those of the Na-CMS obtained from potato starch. These differences between corn and potato starch might be explained by the known fact that corn starch granules are smaller than potato starch granules, which means that the surface area of the corn starch granules is larger enabling the presence of more OH groups at the surface. This leads to higher DS values since the synthesis was performed under heterogeneous conditions.

4 Conclusions

Three methods were used for the determination of the DS of carboxymethyl starch synthesized starting from corn and potato starch. In two of the methods (back titration and Cu salt precipitation) an aqueous solution of sodium carboxymethyl starch was used for the DS determination and in the third method (direct titration of –COOH groups) an aqueous dispersion of the acid form of carboxymethyl starch was employed. The values of the DS obtained by these methods disagreed, especially at the higher values of the DS. Analyzing the obtained results it was concluded that the back titration method was the most accurate one confirming the work of the ISO/TC 93/WG3.

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