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A chemical approach to the quantitative determination of carboxymethyl cellulose in industrial samples

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The quantitation of carboxymethyl cellulose (CMC) by chemical analysis, with either manual or automated L-cysteine–sulphuric acid assays, was shown to be affected by the degree of substitution (DS) of the CMC; a decrease in response to the L-cysteine–sulphuric acid assay with increasing DS was observed. However, the use of a mathematical model, which corrected the CMC weight to cellulose content, combined with a prehydrolysis step for removing the carboxymethyl groups prior to either manual or post-chromatographic Biogel® P6 column automated L-cysteine–sulphuric acid assays eliminated the interference of the DS in the L-cysteine assay.

INTRODUCTION

Cellulose is the most abundant natural polysaccharide present in plant material. However, it has never been found with 100% purity in nature. Cellulose is always associated with considerable amounts of other substances, such as lignin and hemicelluloses.

Even though cellulose, which was first described 150 years ago (Payen, 1838), has been extensively investigated, there are still several aspects of the natural cellulose polymer that remain to be studied. Microcrystalline cellulose, for instance, has been applied in chemical, pharmaceutical and medical industries. However, efficient uses of cellulosic materials are still a relevant goal (Linko, 1987; Alen, 1990; Hatakeyama *et al.*, 1990).

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Once cellulose is derivatised by replacing the hydrogens of the hydroxyl groups of carbons 2, 3 and 6, it becomes less crystalline and more soluble in water in proportion to the degree of substitution (DS) and the solvating capacity of the substituent groups (Fan *et al.*, 1980). Carboxymethyl cellulose (CMC) has been used as a constituent in a variety of products and formulations for a number of industrial purposes (Nicholson & Merritt, 1985; Dahlgren, 1987). Thus, it is important for both users and producers to know the amount of CMC existing in the products and formulations. A number of methods proposed for the quantitative determination of CMC need the removal of impurities prior to assays by washing them with neutral or acidified alcohol and none of these methods is applicable to all cases.

In this work, a method for the quantitative analysis of industrial grade CMC with different DS and molecular weights (MW) has been developed.

EXPERIMENTAL

Materials

Samples of CMC with different DS, ranging from 0·63 to 2·10, and different MW, ranging from $0{\cdot}55 \times 10^6$ to $8{\cdot}71 \times 10^6$ were obtained from Courtaulds Research, Plc, Coventry, UK. Pure CMC (described as 98% pure with DS = 0·7) was also obtained from Courtaulds Research, Plc. CMC BDH (a laboratory grade CMC from BDH Laboratory reagents, DS = 0·7–0·8 and 99% pure) was used as a standard material.

General preparation of CMC samples

CMC (samples 1–16, sample 21 and BDH, 1·0 mg) was dried in a methanol dryer for 24 h, added to distilled water (1·0 ml) and stirred until completely dissolved. The samples were used either for manual or for automated assays.

Manual L-cysteine–sulphuric acid assay of CMC with different DS and MW

CMC (samples 1–9 with different DS, samples 10–16 with different MW, sample 21 and BDH, 1·0 mg) was prepared as described in the general preparation section. Aliquots (20 μl) were added to distilled water (180 μl) to give CMC solutions (20 μg per 200 μl). These solutions were added to a stoppered vials containing L-cysteine–sulphuric acid reagent (1·0 ml), mixed and incubated at 100°C for 3 min. After cooling to 25°C, absorbance was determined spectrophotometrically at 415 nm.

Automated L-cysteine–sulphuric acid assay of CMC with different DS

CMC (samples 1–9, 1·0 mg) was added to distilled water (1·0 ml) and stirred until completely dissolved. Aliquots (100 μl) were loaded into a chromatographic column (50 cm × 1·0 cm id), packed with Biogel® P6 (100–200 mesh) and connected to an automated L-cysteine–sulphuric acid assay. A Technicon peristaltic pump controlled the flow rates, the absorbance was measured at 415 nm by a Technicon colorimeter and recorded on a Technicon chart recorder T132X. The column was calibrated by preparing a solution (1·0 mg/ml) containing CMC (BDH 0·5 mg) and D-glucose (0·5 mg).

Mathematical model for adjustment of the theoretical amount of cellulose present in CMC samples of known DS

An increase in DS of CMC means an increase in amount of carboxymethyl groups on cellulose, and consequently the amount of D-glucose units on a weight to weight basis will decrease. Since the analytical systems devised actually determine cellulose, a conversion factor (CF) to correct the CMC samples weight (with known DS) for the theoretical amount of cellulose was required.

The calculation of the cellulose correction factor is as follows:

$$\text{Ds 0 to 1 GE} = \frac{\text{CMC} \times \text{DS} \times \text{G}}{\text{AG-1} + \text{CM}} + \frac{\text{CMC} \times (1 - \text{DS}) \times \text{G}}{\text{AG}}$$

$$\text{Ds 1 to 2 GE} = \frac{\text{CMC} \times (\text{DS} - 1) \times \text{G}}{\text{AG-2} + 2\text{CM}} + \frac{\text{CMC} \times (2 - \text{DS}) \times \text{G}}{\text{AG} - 1 + \text{CM}}$$

$$\text{Ds 2 to 3 GE} = \frac{\text{CMC} \times (\text{DS} - 2) \times \text{G}}{\text{AG-3} + 3\text{CM}} + \frac{\text{CMC} \times (3 - \text{DS}) \times \text{G}}{\text{AG} - 2 + 2\text{CM}}$$

where GE is the weight of equivalent glucose, CE is the weight of carboxymethyl cellulose equivalent to cellulose content, CMC is the weight of carboxymethyl cellulose, DS is the degree of substitution, G is the molecular weight of glucose (= 180), AG is the molecular weight of anhydroglucose (= 162), and CM is the molecular weight of sodium carboxymethyl group ($-CH_2COONa$ = 81).

$$\text{CF} = \frac{\text{GE}}{\text{CMC}} \quad \text{and} \quad \text{CE} = \frac{\text{CMC}}{\text{CF}}$$

Effect of increasing acid concentration on acid hydrolysis of CMC samples with different DS

CMC (samples 1–9) was weighed after correcting its weight for actual cellulose content (1·0 mg—see Table 1), by the described mathematical model, and

Table 1. Weights of CMC with different degrees of substitution corrected to cellulose content

Sample no.	DS	Calculated weights of sample (mg)	Weight of cellulose in assay (mg)
1	0·63	1·137	1·0
2	0·66	1·151	1·0
3	0·69	1·166	1·0
4	0·78	1·213	1·0
5	0·92	1·293	1·0
6	0·94	1·325	1·0
7	1·31	1·457	1·0
8	1·73	1·642	1·0
9	2·10	1·825	1·0
21	0·70	1·171	1·0

added to distilled water, (0·5 ml) stirring until completely dissolved. Sulphuric acid of known concentrations (2, 4, 6, 8 and 10 M, 0·5 ml) was added to CMC solution (giving final concentrations of 1, 2, 3, 4 and 5 M) and incubated at 100°C for 3 h. After cooling to 25°C, the hydrolysate (20 μl) was added to distilled water (180 μl) to give a hydrolysate solution (20 μg per 200 μl). The hydrolysate solution was added to L-cysteine–sulphuric acid reagent (1·0 ml), mixed, and reincubated at 100°C for 3 min. After cooling to 25°C, absorbance was measured spectrophotometrically at 415 nm (see Fig. 1).

Effect of time on acid hydrolysis of CMC samples with different DS

CMC (samples 1–9) was weighed after correcting its weight for the actual cellulose content (1·0 mg—see Table 1), by the mathematical model, and added to distilled water, (0·5 ml) stirring to complete dissolution. Sulphuric acid (10 M, 0·5 ml) was added to CMC solution (giving final concentration of 5 M) and incubated at 100°C for 0, 2, 5, 10, 15, 30, 60, 120 and 180 min. Hydrolysis time was staggered in order to analyse all the samples together. After cooling to 25°C, the hydrolysate (20 μl) was added to distilled water (180 μl) to give a hydrolysate solution (20 μg per 200 μl). The hydrolysate solution was added to L-cysteine–sulphuric acid reagent (1·0 ml), mixed, and reincubated at 100°C for 3 min. After cooling to 25°C, absorbance was determined spectrophotometrically at 415 nm (see Table 2).

RESULTS AND DISCUSSION

CMC can be hydrolysed by cellulase enzyme complexes. The simpler, smaller molecular units obtained by such enzymic hydrolyses can be quantitated by different methods. Cellulose and its derivatives can also be converted into smaller units by acids, which serve as catalysts for the breakdown of 1,4-β-D-glucosidic linkages of the polysaccharide.

Table 2. Time taken for testing hydrolysis of carboxymethyl cellulose with different degrees of substitution

Sample no.	DS	Time of hydrolysis (h)
1	0·63	0·5
2	0·66	0·5
3	0·69	0·5
4	0·78	0·5
5	0·92	0·5
6	0·94	1
7	1·31	1
8	1·73	2
9	2·10	1

When cellulose is completely hydrolysed into its constituent sugars, the main product obtained is D-glucose. A complete hydrolysis of CMC gives D-glucose as a product but mono-, di-, and/or tri-carboxymethyl glucose are also obtained. The quantitative analysis of CMC with different DS by the manual L-cysteine-sulphuric acid assay showed a decrease in response to L-cysteine with increasing DS (see Fig. 2). This result could be explained by the fact that on the one hand 3 min of incubation reaction was not sufficient to obtain the same rate of hydrolysis of the CMC with higher DS and on the other hand 3 min was not long enough to remove the carboxymethyl groups from the D-glucose units, (mainly the di- and tri-carboxymethylated D-glucose units) making them less reactive to the L-cysteine. Differences in the molecular size of CMC with the same DS = 0·7 did not affect the quantitative analysis by either manual or automated L-cysteine–sulphuric acid assays (see Table 3). In this case, after 3 min of reaction, a uniform production of D-glucose units occurred even with a large difference in molecular size.

A statistical analysis using Student's *t* test was made

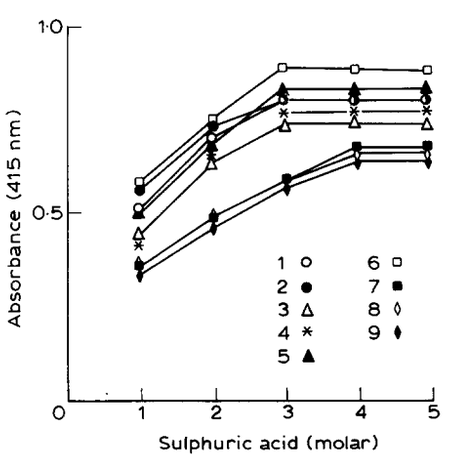

Fig. 1. Effect of sulphuric acid concentration on the acid hydrolysis of CMC samples, with DS ranging from 0·63 to 2·10 measured by the L-cysteine assay.

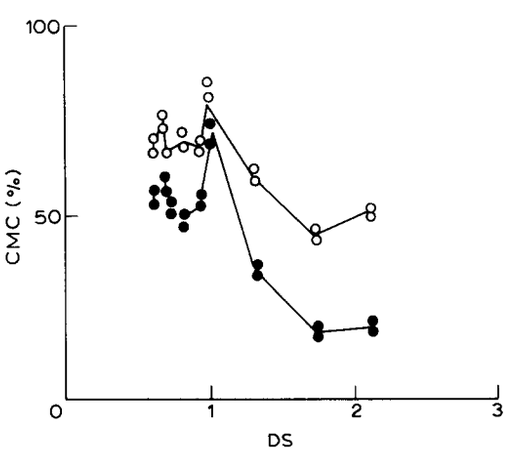

Fig. 2. Effect of DS on CMC hydrolysis (●) without and (○) with correction of sample weight to cellulose content prior to manual L-cysteine assay.

Table 3. Analysis of CMC with different molecular weights and DS = 0·7 by either manual or automated L-cysteine–sulphuric acid assay

Sample no.	MW × 10^6 (by GPC)	Glucose produced (%)	
		Manual assay	Automated assay
10	0·55	93·1 ± 0·7	92·3 ± 0·5
11	1·11	84·7 ± 0·5	84·0 ± 0·7
12	1·43	86·2 ± 0·5	85·7 ± 0·7
13	1·74	86·2 ± 0·5	87·6 ± 0·5
14	2·51	93·5 ± 0·7	95·2 ± 0·5
15	3·16	86·1 ± 1·0	88·1 ± 0·3
16	8·71	88·9 ± 1·0	90·5 ± 0·7

for each CMC analysed by manual assay with no significance ($P > 0·1$) and for each CMC analysed by automated assay with no significance ($P > 0·1$). Also, a statistical treatment was realised for all CMC analysed by manual assay and all CMC analysed by automated assay. A comparison between them has shown no significance with $P > 0·1$.

Since the L-cysteine–sulphuric acid method was calibrated with D-glucose and since the product of hydrolysis of CMC contains carboxymethylated D-glucose, which seems to give different responses to the L-cysteine assay (see Fig. 2), a mathematical model was developed in order to correct the weight of CMC for cellulose content. It was expected that correcting the weight of CMC with different DS, with the use of the mathematical model to obtain the same cellulose content (see Table 2), the interference of the DS would be eliminated. Improved results were obtained, but total elimination of the DS effect was not (see Fig. 2).

Removal of the carboxymethyl groups by acid hydrolysis prior to L-cysteine–sulphuric acid assay was thought to be a method of eliminating the interference of DS in the L-cysteine assay. Several acids have been proposed for hydrolysis of cellulose, e.g. hydrochloric acid (Makooi & Goldstein, 1985; Shambe & Kennedy, 1984, 1985), phosphoric acid (Stone, 1969), etc. Since the L-cysteine assay occurs in sulphuric acid, this acid was chosen for the prehydrolysis step. Different concentrations of sulphuric acid were used to hydrolyse CMC samples with different DS. The concentration of sulphuric acid of 10·0 M (which in contact with the CMC samples, gives a final concentration of 5·0 M) was found to be the concentration in which the curves (extent of hydrolysis versus acid concentration) reach their plateaux for the whole range of DS (see Fig. 1).

The extent of acid hydrolysis of CMC was also analysed as a function of time. Table 2 shows that the time for complete hydrolysis increases with increasing DS, and that 2 h was the longest time needed for the hydrolysis of CMC sample 8. However, it can also be seen in Table 2 that even for the optimum concentration of acid (5·0 M) and for a longer time than the 2 h considered best, the absorbance decreases with increasing DS. This unexpected behaviour could be explained by the fact that the CMC samples did not have their weights corrected to cellulose content by the mathematical model. Hence, if the same amount of CMC is weighed, the higher the DS of the CMC, the lower the cellulose content. The acid hydrolysis of CMC samples (weights corrected to cellulose content) with sulphuric acid (final concentration 5·0 M) for 2 h was introduced prior to the manual L-cysteine–sulphuric acid assay. This process of using a combination of the mathematical model and the acid prehydrolysis, eliminated the interference of the DS to the manual L-cystein method (see Fig. 3).

In order to facilitate rapid and accurate analyses, quantitative methods can be automated, particularly when chromatographic column separations are employed. The post-column detection of the total sugar content of CMC samples was estimated by the automated L-cysteine–sulphuric acid assay (White & Kennedy, 1986). It can be observed from the analyses of CMC (samples 1–9) that, as expected, a decrease in response to the automated L-cysteine assay occurred with increasing DS (see Fig. 4). This could be explained on the same basis of the manual L-cysteine assay, in which 3 min reaction times were insufficient for a uniform rate of hydrolysis of either the 1,4-β-D-glycosidic linkages of the CMC molecule or the ether linkages of the carboxymethyl groups. Thereby, the higher the DS is, the less the removal of the substituent groups is obtained and consequently, the more difficult the reaction of the carboxymethylated fragments with the L-cysteine assay. Gel permeation chromatography permits the analysis of intact CMC. Thereby, it was possible to follow the conversion of high-molecular-weight CMC samples into their individual oligo-, tri-, di-, and monosaccharide components by the concentrated sulphuric acid present in the L-cysteine assay. The chromatographic column and consequently the post-column automated L-cysteine–sulphuric acid method were calibrated with D-glucose. We know from the manual L-cysteine assay that

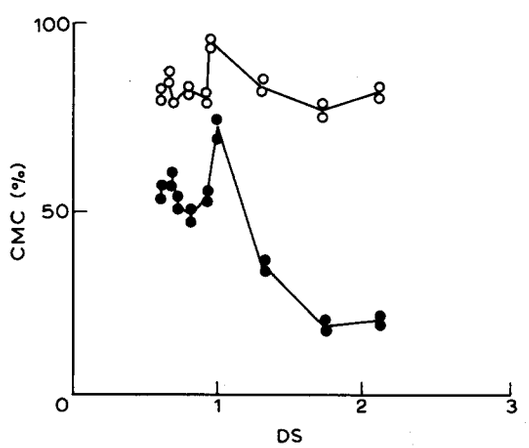

Fig. 3. Effect of DS on CMC hydrolysis (●) without and (○) with correction of sample weight to cellulose content plus 2 h of prehydrolysis prior to manual L-cysteine assay.

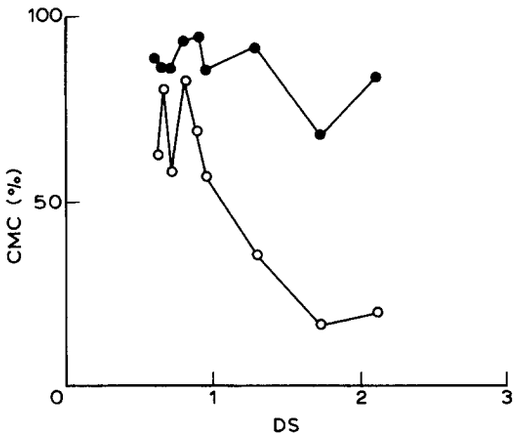

Fig. 4. Effect of DS on CMC hydrolysis (○) without and (●) with correction of sample weight to cellulose content plus 1 h of prehydrolysis prior to automated L-cysteine assay.

the fragments obtained by the acid hydrolysis of CMC contain carboxymethylated D-glucose, which seems to give different responses to the L-cysteine assay (see Fig. 2). Thereby, the mathematical model used in the manual L-cysteine assay for correcting the weight of CMC to cellulose content was also used here, combined with a modified automated L-cysteine–sulphuric acid system in which a 1 h prehydrolysis step was introduced. It can be seen from Fig. 4 that the prehydrolysis reaction time of 1 h is sufficient to eliminate the interference of DS to the L-cysteine assay. The slightly lower response observed for CMC sample 8 in Fig. 4 (automated assay), compared to the corresponding sample in Fig. 3 (manual assay) could be due to the fact that CMC sample 8 was the only sample which needed 2 h of prehydrolysis in the manual assay (Table 2). This lower response of CMC sample 8 to the L-cysteine assay was also found using only 1 h of manual prehydrolysis (see Fig. 5). Table 3 shows that the differences in molecular sizes of CMC, (all

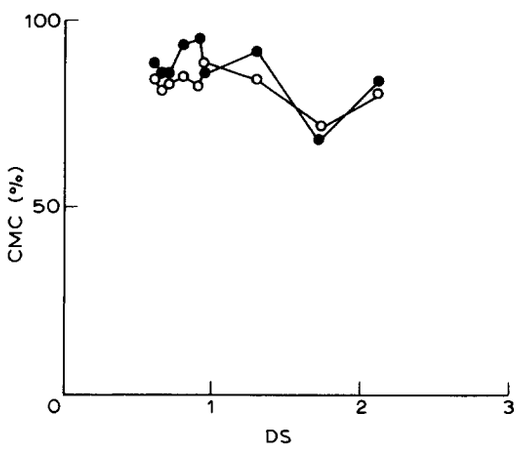

Fig. 5. A comparison between (○) manual and (●) automated systems with correction of sample weight to cellulose content plus 1 h prehydrolysis prior to L-cysteine assay.

samples with DS = 0·7) did not affect their quantitative analyses by the automated L-cysteine–sulphuric acid assay.

Figures 3, 4 and 5 have shown CMC contents of 78–98% as the best results from this test method. The samples are commercialised by the industries as high purity samples (98–99%). So, the results suggest that some industrial CMC products are with lower purities than those stated by the manufactures.

CONCLUSIONS

The quantification of CMC by chemical analysis with either manual or automated post-chromatographic column L-cysteine–sulphuric acid assay was affected by the DS, a decrease in response to the L-cysteine–sulphuric acid assay with increasing DS was observed. However, the use of a mathematical model for the correction of the CMC weight to cellulose content combined with a prehydrolysis step for removing the carboxymethyl groups prior to either manual or post-chromatographic column automated L-cysteine–sulphuric acid assay eliminated the interferences of the DS to the colorimetric L-cysteine assay. The molecular size of the CMC samples did not affect the response to both manual and automated systems.

REFERENCES

Alen, R. (1990). In *Cellulose Sources and Exploitation*, eds J.F. Kennedy, G.O. Phillips & P.A. Williams. Ellis Horwood Ltd, Chichester, UK, pp. 453–64.

Dahlgren, L. (1987). In *Wood and Cellulosics*, eds J.F. Kennedy, G.O. Phillips & B.A. Williams. Ellis Horwood Ltd, Chichester, UK, pp. 427–39.

Fan, L.T., Lee, Y.-H. & Beardmore, D.H. (1980). *Adv. Biochem. Engng*, **14**, 101–17.

Hatakeyama, H., Hirose, S., Yano, S., Nakamura, K. & Hatakeyama, T. (1990). In *Cellulose Sources and Exploitation*, eds J.F. Kennedy, G.O. Phillips & P.A. Williams. Ellis Horwood Ltd, Chichester, UK, pp. 465–71.

Linko, M. (1987). In *Wood and Cellulosics*, eds J.F. Kennedy, G.O. Phillips & P.A. Williams. Ellis Horwood Ltd, Chichester, UK, pp. 231–42.

Makooi, F.B. & Goldstein, I.S. (1985). In *Cellulose and Its Derivatives*, eds J.F. Kennedy, G.O. Phillips & P.A. Williams. Ellis Horwood Ltd, Chichester, UK, pp. 135–41.

Nicholson, M.D. & Merritt, F.M. (1985). In *Cellulose Chemistry and Its Applications*, eds T.P. Nevell & S.H. Zeronian. Ellis Horwood Ltd, Chichester, UK, pp. 363–83.

Payen, A. (1838). *CR Acad. Sci.*, **7**, 1052.

Shambe, T. & Kennedy, J.F. (1984). *Enzyme Microb. Technol.*, **6**, 169–74.

Shambe, T. & Kennedy, J.F. (1985). *Enzyme Microb. Technol.*, **7**, 115–20.

Stone, J.E. (1969). *Adv. Chem. Ser.*, **95**, 219–41.

White, C.A. & Kennedy, J.F. (1986). In *Carbohydrate Analysis — A Practical Approach*, eds M.F. Chaplin & J.F. Kennedy. IRL Press, Oxford, UK, pp. 37–54.