Quantitative colourimetric assessments of carboximethylcellulose in anionic and anionic-ionic food recipes

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Abstract
The paper presents a colorimetric (500 – 700 nm) quantitative method for the determination of carboxymethylcellulose (CMC) in the ionic – nonionic foods based on the acid hydrolysis of CMC and condensation with diphenylamine.

Keywords: carboximethylcellulose, anionic food recipes, nonionic food recipes, food additivation

1. Introduction
Among colloidal additivation (up to 3%) accessed in food industry in order to diminish dirt tendency to re-attach (food sanitising) or to increase the deflocculating power, carboximethylcellulose (CMC) is of great importance [1].

Chemically, it is a macromolecular compound made up of units of β-D – glucopiranose mutually grafted β – (1.4) – glycosidic. Due to the equatorial conformation of the links, the chains have a linear structure with no helices, and with a high capacity of associating through hydrogen links and of forming insoluble crystalline structures. In order to access it as a food additive, we need to improve the solubility factor through chemical processes (cellulose gums).

Solubilising cellulose can be done by etherifying the three reactive hydroxyl groups of each glucose fragment. In order to get the etherified derivative, first we solubilise the glucose in concentrated alkaline solutions, after which we etherify it heterogeneously under controlled conditions to get the desired substitution degree (Figure 1).

The most used ethers (acknowledged from a food point of view) are carboximethylcellulose (CMC), methylcellulose (MC), hydroxypropylmethyl-cellulose (HPMC) together with methyl-ethyl-cellulose (MEC) or hydroxypropyl-cellulose (HPC) (Table 1).

| Table 1. Uses of cellulose derivates in food processing [1] |
|-----------------|---|---|---|---|---|---|---|---|---|
| **Food products**       | **Cellulose derivates** | **Coloidal competences** |
|                           |   |   | A | B | C | D | E | F | G |
| Flour products           | + | + |   |   | + |   |   |   |   |
| Products from potato     | + | + | + |   | + |   |   |   |   |
| Meal, fish               | + | + | + |   | + |   |   |   | + |
| Mayonnaise, dressings    | + | + | + | + | + |   |   |   |   |
| Fruit jelly              | + | + | + | + | + |   |   |   | |

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| Fruit juice | + | + | + |
| Beer | + | + | + |
| Wine | + | + | + |
| Ice-cream | + | + | + |
| Dietetical products | + | + | + |

1. CMC natrium salt; 2. methyl cellulose; 3. HPMC; A. thickening agent; B. water-linking capacity; C. low-temperature jellifying; D. high-temperature jellifying; E. emulgator and emulsion stabiliser; F. suspension agent; G. surface-active agent; H. adsorbent; I. film-maker.

Figure 1. Ways of derivatising cellulose chemically [1]

The most important ether derivative of cellulose from a food point of view is CMC natrium salt, which differs from an assortment point of view by molecular volume and substitution degree (etherifying). CMC dissolves easily in water, forming viscous solutions whose concentrations are below 1%, and whose behaviour is pseudo-plastic when flowing (in the case of low shearing rates it behaves Newtonian-like).

Literature signals synergic effects between CMC, guar gum and locust gum, with an increase of viscosity. CMC forms gels in the presence of trivalent metallic cations (aluminium sulphate, basic aluminium acetate, ferric phosphate). By using kellation agents (citrate or malate), pH and temperature change, we can control the jellifying rate and gel texture.

The main competences of the CMC confer it diverse functionalities (linking agent, stabiliser of compounds or syneresis prevention).

As a stabilising agent, CMC plays the role of preventing protein (soy or milk) precipitation for pH values close to the isoelectric point and it controls ice crystal formation during ice-cream processing. Due to its water solubility and to the high capacity of grafting water, CMC is recommended in diet food products as a dissolvent, conferring them a pleasant texture, consistency, and melting characteristics.

It is often used in mixture with other gums (gelatine, pectin, or locust gum). The quantitative assessment method adapted and presented in this paper is based on
colourimetry within the range 490-680 nm of colour compounds obtained by condensing di-phenyl-amine with 5-hydroximethyl-furfurrol obtained through acid hydrolysis of cellulose derivatives.

Pechmann and Ilk [1885] [2, 3] studied the reaction of fructose with di-phenyl-amine in a concentrated chlorhydric environment with the formation of a blue combination. Later, the reaction was generalised in the case of pentose, hexose, di-, oligo- and poly-sugars [4-10], of uronic acids [11], of nucleic acids [12, 13], etc. For hydrolysis, they recommend chlorhydric, sulphuric, or perchloric acids in concentrations of 70%.

For the condensation phase, we can access a solution of di-phenyl-amine 0.2-10% in ethanol – sulphuric acid, ethanol – chlorhydric acid, acetic acid – chlorhydric acid, acetic acid – sulphuric acid or ethanol – acetic acid – sulphuric acid, etc., and as an organic phase, n – butanol, alcohol n – amylic, isoamylic and chloroform.

Quantitative colourimetric assessment of CMC was the main concern of many researchers [14 – 18]. In all the variants studied and suggested, they appealed to the acid hydrolysis of the product, followed by condensation with antrone [14], when they obtained a di-benzantrone derivative with absorption over the range 620-630 nm, with 2, 7 – di-hydroxi-naphthaline [15, 18], when they got a derivative of the 2, 7, 2', 7' – tetra-hydroxi – di-naphtyl – (I) – methane with absorption within the range 530-550 nm, or with phenol [16] resulting in a condensation product with maximum characteristic absorption at 490 nm.
In the present paper, we have made an option for determining quantitatively CMC in anionic and non-ionic food recipes to avoid partial hydrolysis of the cellulose derivative without going further than phase (I) (Figure 2).

2. Experimental part

a. Materials, Reactants
- Re-constituted standard food recipes with anionic and anionic – non-ionic nutritive principles (in the Laboratory of Food Additive Technologies);
- Carboximethylcellulose (Merck);
- Diphenylamine 99% (CAS 122-39-4) (Sigma Aldrich);
- Different solvents (Sigma Aldrich).

b. Equipment
- Hydrolysis installation with efficient mechanical stirring, ascending refrigeration thermostat, dripping funnel;
- Colourimeter (Cole Parmer) (400 – 600 nm);
- FT-IR (Büchler);
- Qualitative and preparative thin-layer chromatography (Camag);
- Open-column chromatography (Quikfit).

Working method
Determining CMC natrium salt by exhaustive acid hydrolysis and condensation with diphenyl-amine

Preparation of the standard natrium salt CMC solution
In a Berzelius glass of 400 mL we introduce 180 – 200 mL distilled water. We heat up to 80°C under continuous and efficient stir when we introduce gradually 4g CMC (analytic precision). We continue heating and stirring for about 20 min at the same temperature, then the colloidal solution is put to rest for 2 hours, after which it is stirred again for 20 min without heating and is transferred quantitatively into a graded balloon of 1000 mL completing the volume up to the sign. From the solution we can get controlled dilutions within the range 10 – 100 γ/mL.

Working way. Drawing the sampling curve and determining CMC proper
We weigh with analytic precision 0.5 g of anionic and/or anionic – non-anionic food recipe in a Berzelius glass of 50 mL. We dissolve in distilled water and later on we transfer into a graded balloon of 250 mL; 3 mL of this solution is dropped together with 6 mL of diphenylamine in the testing tube. We then regulate in the thermostat coat for 30 min, at a recycling environmental temperature of 108 ± 0.02°C. We then cool...
suddenly the testing tube and then absorbance at 580 and 640 nm.

Graphically, absorbance depending on concentration results in the linearity range of the law Lambert – Beer (0 – 5 γ/mL) (Figure 3).

In parallel, we prepare a control sample using, instead of the standard CMC solution, distilled water. Transmission of the sample control should not be below 90% otherwise the diphenylamine is not proper. If necessary, one should also take into account the possible dilutions over the determination period.

The alcoholic solution of diphenylamine will be prepared at the time of the determination, and for no more than four consecutive samples (0.375 g diphenylamine p.a. are dissolved in 15 mL of glacial acetic acid and 9 mL of concentrated chlorhydric acid).

The contents in CMC of the sample to be assessed can be quantified with the formula:

\[
CMC = \frac{A \cdot 1000}{A_{1\%}^{1cm} \cdot a \cdot d} \%
\]

where:
- \( A \) - Sample absorbance;
- \( A_{1\%}^{1cm} \) - Molar extinction coefficient (g/l · cm);
- \( a \) - Analyse food recipe amount (g/l);
- \( d \) - Absorbent layer thickness (cm).

**Figure 3.** Carboximethylcellulose calibration curve: 1 – for maximum \( M_1 \); 2 – for maximum \( M_2 \)

**Figure 4.** Absorption curve of the condensation compound 5-hydroxi-methyl-furfurol with diphenylamine: \( M_1 \) – maximum absorption at 580 nm; \( M_2 \) – maximum absorption at 640 nm
3. Results and discussion

Food recipes reconstituted on a classical nutritional structure into an apricot jelly were additivated under monitoring with anionic (a) superficialactive compounds [sodium lauril sulphate (E - 487)] and anionic (b) [sodium cetyl sulphate (E – 487*)] or anionic (a) – non-ionic [polysorbates (E – 432/436)]. Saliﬁed carboximethylcellulose in the processed recipe was supplementarily controlled (Table 2).

Table 2. Results of quantitative determination of saliﬁed carboximethylcellulose in anionic, anionic – non-ionic food recipes

<table>
<thead>
<tr>
<th>No.</th>
<th>Nature of additivated food recipe</th>
<th>CMC determined (%)</th>
<th>additivated (%)</th>
<th>Final CMC (%)</th>
<th>Final theoretical CMC (%)</th>
<th>Error (%)</th>
<th>Average relative error (%)</th>
<th>Reproducibility of the method</th>
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<td>1</td>
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<td>3.17</td>
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<td>5.09</td>
<td>5.01</td>
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<td>3.10</td>
<td>+ 0.23</td>
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<td>3.40</td>
<td>-</td>
<td>3.412*</td>
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<td>4.83</td>
<td>4.89</td>
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</table>

The quantum of CMC present and additivated in the apricot jelly was assessed directly with no previous isolation of the cellulose derivative in the product. A possible error in the system was removed by comparing with non-additivated control samples.

Analysing experimental data shows that the balance of theoretical and practical (determined) global material is to be found with an acceptable error (- 1.5 ÷ +1.5); the method reproducibility is also encouraging (- 0.3 ÷ + 0.35).

4. Conclusions

The method we have described here, rapid and efﬁcient, ensures error results within the range ±1%, when recommended analytic conditions are observed.

Determination is not hindered by the presence of other anionic or non-ionic superficialactive substances accidentally or purposely added, though some of them hydrolyse in acid environments in warm conditions (sulphated fatty alcohols, soaps, etc.). Method reproducibility is ± 0.341%.

We recommend expansion of research to other nutritional systems with the...
possibility of later extrapolating and generalising.

Acknowledgment

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References