

**ANALYTIC METHOD FOR DETERMINING DEGREE OF SUBSTITUTION IN THE PRODUCT
(A.S.T.M. METHOD)**DOCUMENT CODE
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1 of 3**1.0 Objective.**

Establish the steps for determining the degree of substitution (D.S.) in the product, according to the A.S.T.M. "A" method.

2.0 Scope.

This method applies to the product in process and for finished goods resulting from the CMC and/or PAC fabrication process.

3.0 Reference document.

A.S.T.M., D-1439-03 (American Society for testing and materials)

4.0 Responsibilities.

The Laboratory Head is responsible for verifying the application of this document.

The analysis technicians are responsible for exercising the method as described in this document and inform the Head of Quality Assurance, head of Production and Head of research and development of any discrepancies that might arise.

5.0 Terminology.

Degree of substitution (D.S.):

Represents the number of the carboxymethyl groups which are in the molecular unit of the anhydroglucose units.

6.0 Procedure.**6.1 Sample preparation.**

An approximately 300 gram sample is taken from the product to evaluate, it is homogenized inside a bag, the required amount for the test is taken afterwards.

6.2 Equipment, materials and reagent preparation.

- Magnetic stirrer
- Hotplate
- Oven at 105°C ±3°C
- Analytical balance
- Gooch "C" 30 ml. filter
- Desiccator (with blue silica gel)
- 250 ml. beaker
- 250 ml. Erlenmeyer flask
- 25 ml. burette
- 80°GL ethyl alcohol
- Anhydrous methanol

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- Chloric acid 0.3N ±0.02
 - Sodium hydroxide 0.3 ±0.02
 - Concentrated nitric acid
 - Diphenylamine reagent
(0.5 g of diphenylamine in 120 ml. of sulfuric acid)
 - Wash bottle with distilled water
 - 25 ml volumetric pipette
 - bulb-type safety pipette filler
 - Phenolphthalein
- 6.3. Weigh 4 g. of the sample in a 250 ml. beaker and add 75 ml. of 95% ethyl alcohol. Agitate the specimen for 5 min. Then add 5 ml. of nitric acid and place on hotplate and bring to a boil, being careful not to burn it. Take the specimen from the hotplate and continue stirring for 10 minutes.
- 6.4. Decant the liquid and pass the precipitate through the filter, using vacuum, placing 150 ml. of 80% ethyl alcohol (60°C). Wash the beaker and precipitate with the 80% ethyl alcohol at 60°C to remove all the acid from the specimen.
- 6.5. Wash as many times as necessary with 80% ethyl alcohol at 60°C, to obtain from the test negative with diphenylamine, which is absence of nitrite (in total washing five times is enough in almost all cases). In each wash the specimen should be stirred for 10 minutes.
- 6.6. The diphenylamine test is done with a portion of the precipitate, it is taken with the spatula, adding a drop of diphenylamine the result is positive when it turns blue (nitrite presence) and negative when color is undisturbed (nitrite absence).
- 6.7. Wash the precipitate with a small quantity of anhydrous methanol and apply vacuum until all the alcohol is removed, dry the filter at 105°C for 3 hours, cool in a desiccator for half an hour.
- 6.8. Weigh 1 to 1.5g of acid of the dry carboxymethylcellulose in a 250 ml. flask add 100 ml. of water and 25 ml of hydroxide 0.3N with agitation, heat to boil and maintain it 15 to 20 minutes.
- 6.9. Value it with the chloric acid solution (HCl) 0.3N (the solution to value should be hot), add a few drops of phenolphthalein indicator, the final point of the valuation is when a color change is observed from Mexican pink (dark pink) to colorless.
- 6.10. Calculus.
To calculate the degree of substitution, the following math is done:

$$A = \frac{BC - DE}{F} \quad \text{Degree of Substitution} = \frac{(0.162) \times A}{1 - (0.058 \times A)}$$

Where:

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- A = milli-equivalents of consumed acid per gram of specimen.
B = Millimeters of added Sodium hydroxide.
C = Normal sodium hydroxide.
D = Millimeters of consumed chloric acid.
E = Normal chloric acid.
F = Specimen grams used.
162 = Molecular weight of the anhydrous glucose unit.
58 = Net increment in the anhydrous glucose unit for every substituted carboxymethyl group.