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Quantification of Stabilizers in Food Products by a Simple Method Development and Validation

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CMC, stabilizer, incubation, validation.

Abstract
The 2,7-naphthalenediol technique was used to quantitatively evaluate the existence of sodium carboxymethylcellulose, or CMC, in ice cream by making a few modifications to the method's "clean-up" process established by H. D. Graham. In this procedure incubation was excluded and the usage of concentrated sulphuric acid is minimized comparing to the graham's method as the concentration of CMC increases viscosity also increase. This article provides in-depth analysis of CMC furthermore it discusses about the validation parameters. The λ_{max} of carboxy methyl cellulose was found to be 470nm And R^2 (0.9983). CMC used as thickening agent, emulsifying agent, and the stabilizer.

1. INTRODUCTION

Food stabilizers, often known as food additives, are compounds that are incorporated into food items to enhance their flavor, regularity, and longevity on the shelf. They aid in the preservation of food's chemical and physical qualities, avoiding unwanted alterations such as division, phase separation, or deterioration. For this reason, stabilizers such as (carboxymethylcellulose) and pectin are frequently utilized.

CARBOXY METHYL CELLULOSE

The flexible, water-soluble polymer carboxymethyl cellulose (CMC), also known as cellulose gum or sodium carboxymethyl cellulose (Na-CMC), is generated from cellulose, a naturally occurring carbohydrate that exists in plants. several its special qualities and functions, it is frequently used in a variety of sectors, including food, medicine, personal care products, as well as personal care. To create carboxymethyl cellulose, the cellulose is chemically altered by reacting with a mixture of sodium hydroxide and chloroacetic acid. This process results in the incorporation of groups of carboxymethyl groups within the cellulose framework. The characteristics and functions of CMC are governed by the degree of substitution (DS), which is the median amount of carboxymethyl groups per gluon molecule in the viscose chain. It is a white to off-white, odorless powder with different degrees of stiffness and soluble according to the grading and DS. It is extremely soluble in water and

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produces clear or faintly tinted solutions having a dense viscosity at minimal concentrations could thicken, stabilize, emulsify, suspend, and hold onto water, which makes it helpful in several purposes. It is robust throughout a wide pH range, spanning acidic to alkaline environments is frequently utilized as a practical component and nutritional supplement because of it improve texture, stability, and sensory attributes of food products It serves a purpose in a variety of foods, including milk and cheese, sauces, coverings, pastries, confections, and drinks, as a thickening, a thickener stabilizer, and structure enhancer. For reduced-fat or low-calorie food compositions, CMC can offer viscosity control, moisture retention, and fat Furthermore, it can increase the freeze-thaw stability of food goods, inhibit crystallization, and enhance texture and sensations. CMC is regarded as safe for eating by regulatory organizations, including the American Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA). It meets the average daily intake (ADI) that was set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and has been deemed as safe (GRAS) in the United States Carboxymethyl cellulose is a popular and adaptable component that offers practical qualities across a range of applications. It is a useful ingredient in numerous contexts due to its capacity to alter texture, stabilize formulas, and enhance the performance of the product.

(1) Sodium Carboxymethyl Cellulose shall be a white or slightly yellowish powder consisting of very fine particles, fine granules or fine fibers with hygroscopic nature and as described below, namely:-

Common Name	Sodium Carboxymethyl Cellulose
INS No.	466
C.A.S No.	9004-32-4
Chemical Name	Sodium salt of carboxy methyl ether of cellulose.
Empirical Formula	$[C_6H_7O_2(OH)_x(OCH_2COONa)_y]_n$

CMC could also aid in decreasing your appetite. Foods that include cellulose gum's fiber tend to make your stomach feel full longer because of this. Uses for cellulose gum are numerous. Because of its stabilizing and smoothing qualities, it may also be found in toothbrushes, medications, and even home items in addition to a few food products at 0.1 to 0.5 demonstrates its superior stabilizer in frozen desserts. The recommended amount of CMC in ice creams, according to FSSAI, is 500 mg/kg.

Adverse effects:

These consequences are more probable to manifest at greater doses or in those with existing problems with digestion. In a few cases, CMC may induce gastrointestinal discomfort, such as gas, abdominal pain, diarrhea, or stomach cramps. Allergic effects can involve a rash on the skin, itching, expanding, beehives, or breathing issues. • Certain individuals may be hypersensitive to or allergic to CMC, leading in an allergic reaction upon contact. Although exceedingly rare, serious allergic responses like anaphylaxis can happen to sensitive people. Contact directly with CMC liquids or products might temporarily irritate the eyes. Fine CMC particles could cause irritation to sensitive people's breathing passages when breathed in significant doses. Inhaling sensitivity can be reduced with proper working with, which includes employing sufficient airflow along with personal protection equipment.

2. METHODOLOGY

Active pharmaceutical ingredient:

Carboxy methyl cellulose was procured as gift sample by Aparna Medicare limited, hyderbad.

Reagents and chemicals

Papain buffer mixture, Sodium chloride, Calcium chloride, Cetyl pyridinium chloride, Distilled water, Sodium thiosulphate, Sulphuric acid Phenol,2,7 - naphthalenol

Preparation of reagents:

Papin Buffer Mixture:8gm of papain pure drug in 100ml of 0.01M sodium acetate-acetic acid buffer(pH-5.5),3.5g of EDTA, 1.5g of cysteine hydrochloride monohydrate in 250 ml water and adjust the PH to 6 by using 1N sodium hydroxide.

1n Sodium Hydroxide :4g of NaOH in 100ml of distilled water.

1M Cacl2: 36.75g if calcium chloride in 100ml of distilled water.

0.01%CPC-0.01M Nacl: take the 0.0584 grams of Nacl and put in 500ml volumetric flask and make up the volume with distilled water in that solution add 1mg of cetylpyridinium chloride powder.

30%H2SO4 Solution: Take hot H2SO4 In 100ml beaker and add 70ml od distilled water.

Phenol- H2SO4 test: take 30 ml H2SO4 in beaker and 2ml of phenol solution

2,7- napthalendiol reagent preparation:

weigh 50mg of 2,7 napthalendiol drug and dissolve in 100ml of concentrated H2SO4 appears yellow color.

Diluents: distilled water,2,7-napthalendiol reagent

λ_{max} determination

preparation of standard solution

Weigh 10 mg of pure carboxymethyl cellulose in 10 ml volumetric flask and add 4 ml of 2, 7-napthalendiol reagent and make up with distilled water and perform the calibration curve

Preparation of working standard:

2,7-napthelendiol and distilled water was diluted from its standard stock solution using water, and its maximum concentration was determined by scanning using an ultraviolet-visible spectrophotometer across the spectral range of 400-800nm against blank 2,7-napthalendiol reagent. The

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absorption curve shows characteristic λ_{max} at 470nm for carboxymethyl cellulose

Calibration curve

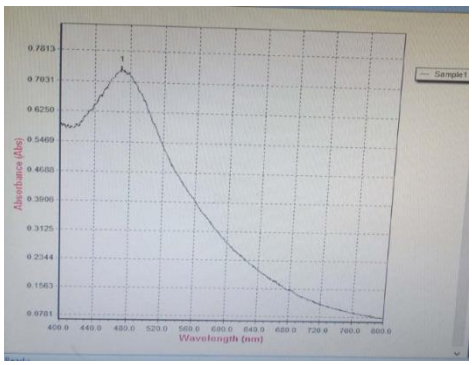
2,7-napthalendiol as a solvent for the preparation of calibration curve at λ_{max} 470 using ELICO UV-Visible spectrophotometer.

Standard stock solution

By precisely weighing, a standard stock sample is prepared. 10mg of carboxy methyl cellulose in 10ml volumetric flask and dissolve in 4ml of 2,7-napthalendiol and 6ml of distilled water. Then 500µg/ml -1200µg/ml were prepared with 2,7-napthalendiol reagent through serial dilutions and the absorbance were taken at λ_{max} 470nm against the blank. The data obtain from concentration v/s absorbance gives standard calibration curve reported.

Extraction procedure

Weigh 10g of sample add 6g Nacl next add 25ml of papain buffer add2ml of Cacl2, 10 min and filter the mixture. To filtrate add 20ml of distilled water add 2g CPC and perform the filtration. Wash the residue with25ml of 0.1%CPC-0.01M Nacl add 100ml of distilled water, filter it from the filtration take 10ml of solution add 10ml H2SO4(phenol-H2SO4 Test Negative) Allow it to cool add 50ml of H2SO4.



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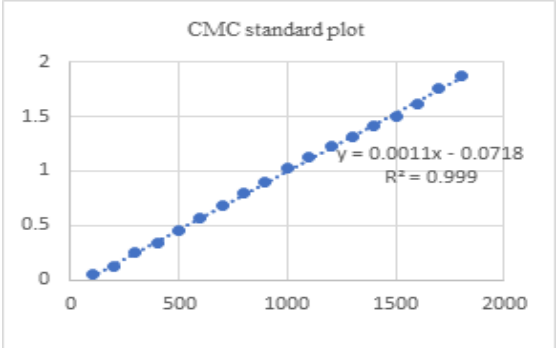
CMC Standard Plot Curve

3.RESULTS AND DISCUSSION:



Linearity

Carboxymethylcellulose Standard Preparation

Pipette out from 1000ppm stock solution and transfer to separate10ml volumetric flasks and make up to the mark with diluent to yield 500,550,600,650,700,750,800,850,900,950,1100,1150,1200 ppm solution respectively.

Concentration	absorbance
500ppm	0.7623
550ppm	0.9524
600ppm	1.0935
650ppm	1.3798
700ppm	1.4164
750ppm	1.4367
800ppm	1.5261
850ppm	1.7352
900ppm	1.7673
950ppm	1.8752
1000ppm	1.8862
1050ppm	2.1342
1100ppm	2.1873
1150ppm	2.2941
1200	2.3563

Precision

6 replicates of 500ppm Standard solutions of Carboxy methyl cellulose absorbance's was noted at wavelength 470nm % RSD was calculated and obtained results are within the limits as per ICH Guidelines.

Absorbance	concentration
500	0.7623
500	0.7962
500	0.7945
500	0.7653
500	0.7894
500	0.7991
MEAN	0.784466667
SLOPE	0.0728

Detection Limit:

According to ICH rules, the limit of detection was estimated using the formula 3.3 x / S, where stands for the response's standard deviation (Y-intercept), and S for the calibration curve's slope. The findings are shown below.

DL= 3.3 x SD/slope.

= 3.3x 0.598444957/0.232

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= 8.5123636125µg/ml

Quantification Limit (QL):

According to ICH recommendations, the limit of quantification was established using the formula 10 x / S, where stands for the response's standard deviation (Y-intercept), and S for the calibration curve's slope. The findings were tabulated.

QL= 10 x SD/slope.

= 10x 0.598444957/0.232

concentration	Abs at 469nm	Abs at 470nm	Abs at 471nm
500	0.6364	0.7828	0.5943
500	0.6435	0.7736	0.5789
500	0.6343	0.7846	0.5645
500	0.6374	0.7865	0.5897
500	0.6745	0.7769	0.5934
500	0.6347	0.7865	0.5978
Mean	0.93466667	1.98181667	1.86433333

QL = 25.79504125µg/ml

Robustness

It is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. It was determined by measuring the absorbance of sample (500µg/ml) at different wavelength 469nm, 470nm and 471nm. The Percentage Relative Standard Deviation (%RSD) was calculated and results are within the limits as per ICH Guidelines

Ruggedness

It refers to the degree of consistency of test findings acquired by analyzing the same material under various conditions, such as different laboratories or analysts. It was determined by measuring the absorbance of Standard(500ppm) at 470nm by different analysts. The Percentage Relative Standard Deviation (%RSD) was calculated and results obtained are within the limits as per ICH guidelines

concentration	Analyst-1	Analyst-2
500	0.7678	0.6364
500	0.7632	0.6343
500	0.7945	0.6343
500	0.7765	0.6372
500	0.7675	0.6463
500	0.7956	0.6346
Mean	0.777516667	0.643466667
%RSD	1.87262093	0.98418276854

Quantification Of CMC in Extracted Food Products

Take 2ml from sample extraction in 10 ml, volumetric flask to that add 4ml 0f 2,7 napthalendiol and add 6ml of distilled water check the absorbances

Sample name	absorbances	% found as per FSSAI
marketed butter milk	1.1268	600mg/kg
delight chocolate ice cream	1.8020	930mg/kg
Mango ice cream	0.9552	550mg/kg
Vanila milkshake	0.9622	565mg/kg
Crystal foam ice cream	1.2991	620mg/kg

3. CONCLUSION:

In the current study, we created and verified a UV-visible spectroscopic method. ICH guidelines were followed in the approval of the procedures. For each UV Spectroscopic approach, the linearity ranged from 500 to 1200 g/ml, indicating an R2 of 0.9983. The linearity, accuracy, robustness, and ruggedness of the UV Spectroscopic technique were all accepted**.**

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