METHOD OF ANALYSIS FOR CROSCARAMELLOSE SODIUM - USP

GENERAL INFORMATION

1.0 Description:

A white free flowing powder

2.0 Solubility :

Descriptive Term	Part of Solvent Required for 1 Part of Solute
Very soluble	Less than 1
Freely soluble	From 1 to 10
soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10,000
Practically insoluble or insoluble	Greater than or equal to 10,000

Partially/slightly soluble in water: Weigh accurately about 1 g of sample and transfer in to dry stoppered 250 ml measuring cylinder. Add 100ml of water shake it thoroughly and examine.

Practically insoluble in alcohol: Weigh accurately about 0.01 g of sample and transfer in to dry stoppered 250 ml measuring cylinder. Add 100 ml of alcohol shake it thoroughly and examine. Sample should remain insoluble.

Practically insoluble in ether: Weigh accurately about 0.01 g of sample and transfer in to dry stoppered 250 ml measuring cylinder. Add 100 ml of ether shake it thoroughly and examine. Sample should remain insoluble.

3.0 Identification:

3.1 Test A

Mix 1g of with 100ml of methylene blue solution (1 in 2, 50,000), stir the mixture, and allows it tosettle. The Croscarmellose sodium absorbs the methylene blue and settles as a blue fibrous mass.

3.2 Test B

Mix 1 gm with 50 ml of water. Transfer 1ml of the Mixture to a Small test tube, and add 1ml of water and 5 drops of 1-naphthol TS. Incline the test tube and carefully add 2 ml of sulfuric acid down the side to that Forms lower Layers. Reddish-violet Color develops at the interface.

3.3 Test C

Mix 1 gm with 50 ml of water, impart an intense yellow color to a nonluminous flame.

4.0 pH:

Mix1 g with 100ml of water for 5 minutes.

5.0 Degree of Substitution:

Transfer 1.000 g to a glass-stoppered, 500 ml conical flask. Add 300 ml of a 100 g/l solution of sodium chloride then add 25.0 ml of 0.1 N sodium hydroxide. Insert the stopper, and allow to stand for 5 min,with intermittent shaking. Add 5 drops of m- Cresol purple solution and from a buret 15 ml of 0.1N hydrochloric acid from a burette. Insert the Stopper in the flask, and shake. If the solution is violet, and

0.1 N hydrochloric acid in 1 ml portions until the solution becomes yellow, shaking after each addition.Titrate with 0.1 N Sodium Hydroxide until the color turns to violet.

Calculate the net number of milliequivalents, M of Base required for the Neutralization of 1 g of Croscarmellose sodium, on the dried substance.

Calculate the degree of acid carboxymethyl substitution, A, by following formula.

$$\text{Result} = 1150 \times M / [7102 - (412 \times M) - (80 \times C)]$$

M = milliequivalents of base

C = Percentage of residue on ignition of the Croscarmellose sodium as determined in the test for residue on ignition.

Calculate the degree of sodium carboxymethyl substitution S, by the formula.

$$\text{Result} = [162 + (58 \times A)] \times C / [7102 - (80 \times C)]$$

A= Degree of acid carboxymethyl substitution, as determined above

C = Percentage of residue on ignition of the Croscarmellose sodium as determined in the test for residue on ignition.

The degree of substitution is the sum of A+S it is between 0.60 and 0.85, calculated on the dried basis.

6.0 Water soluble content:

Disperse 10 g in 800 ml of water, and stir for 1 minute every 10 min during the first 30 minutes. Allow to stand for an additional hour, or centrifuge, if necessary. Decant 200 ml of the aqueous slurry onto a rapid-filtering filter paper in a vacuum filtrate funnel, apply vacuum, and collect about 150ml of the filtrate. Pour the filtrate into a tare 250 ml beaker, weigh, and calculate the weight, in g, of the filtrate, **W3,** by difference. Concentrate on a hot plate to a small volume, but not to dryness; dry at 105°C for 4 hours; again weigh.

Calculate, in g, the weight of residue by difference, **W1**

Calculate the percentage of water-soluble material in the specimen, on the dried basis,Taken by the formula:

[100 x W1 x (800+ W2)] / {W2 x W3 x [1 - 0.01 x b)]}

W1 = Weight of residue by difference (g)

W2 = Weight of the specimen taken (g)

W3 = Weight of the filtrate by difference (g)

b = Percentage loss on drying of the specimen taken

7.0 Sodium chloride:

Analysis: Transfer 5g to a 250-mL beaker. Add 50 mL of water and 5 mL of 30% hydrogen peroxide, and heat on a steam bath for 20 min, stirring occasionally to ensure hydration. Cool, and add 100 mL of water and 10 mL of nitric acid. Titrate with 0.05 N silver nitrate VS, determining the endpoint potentiometrically, using a silver-based indicator electrode and a double-junction reference electrode containing 10% potassium citrate filling solution in the outer jacket and a

standard filling solution in the inner jacket, and stirring constantly.

Calculate the percentage of sodium chloride in the specimen taken:

$$\text{Result} = (F \times V \times N)/[(100 - b) \times W]$$

F= equivalence factor for sodium chloride, 584.4

V= volume of the silver nitrate (mL)

N= normality of the silver nitrate

b = percentage of loss on drying, determined separately

W= weight of the specimen (g)

Sodium glycolate:

Standard stock solution: Transfer 100 mg of glycolic acid, previously dried in a desiccator at room temperature overnight, to a 100-mL volumetric flask. Dissolve in and dilute with water to volume, and mix.

Standard solution A: Transfer 1.0 mL of the Standard stock solution to a 100-mL volumetric flask. Add water to make 5 mL, then add 5 mL of glacial acetic acid. Dilute with acetone to volume, and mix.

Standard solution B: Transfer 2.0 mL of the Standard stock solution to a 100-mL volumetric flask. Add water to make 5 mL, then add 5 mL of glacial acetic acid. Dilute with acetone to volume, and mix.

Standard solution C: Transfer 3.0 mL of the Standard stock solution to a 100-mL volumetric flask. Add water to make 5 mL, then add 5 mL of glacial acetic acid. Dilute with acetone to volume, and mix.

Standard solution D: Transfer 4.0 mL of the Standard stock solution to a 100-mL volumetric flask. Add water to make 5 mL, then add 5 mL of glacial acetic acid. Dilute with acetone to volume, and mix.

Sample solution: Transfer 500 mg to a 100-mL beaker. Moisten thoroughly with 5 mL of glacial acetic acid, followed by 5 mL of water, and stir with a glass rod to ensure proper hydration (usually about 15 min). Slowly add 50 mL of acetone while stirring, then add 1 g of sodium chloride, and stir for several min to ensure complete precipitation of the Carboxymethylcellulose. Filter through a soft, open-textured paper, previously wetted with a small amount of acetone, and collect the filtrate in a 100-mL volumetric flask. Use an additional 30 mL of acetone to facilitate the transfer of the solids and to wash the filter cake, then dilute with acetone to volume, and mix.

Analysis Samples: Standard solution A, Standard solution B, Standard solution C, Standard solution D, and Sample solution.

Transfer 2.0 mL of the Sample solution and 2.0 mL of each Standard solution to separate 25-mL volumetric flasks, and prepare a blank flask containing 2.0 mL of a solution containing 5% each of glacial acetic acid and water in acetone. Place the uncovered flasks in a boiling water bath for 20 min to remove the acetone. Remove from the bath, and cool. Add to each flask 5.0 mL of 2,7-dihydroxynaphthalene TS, mix, add an additional 15 mL, and again mix. Cover the mouth of each flask with a small piece of aluminum foil. Place the flasks upright in a boiling water bath for 20 min, then remove from the bath, cool, dilute with sulfuric acid to volume, and mix. Determine the absorbance of each solution at 540 nm, with a suitable spectrophotometer, against the blank, and prepare a standard curve using the absorbance obtained from the Standard solutions.

Calculate the percentage of sodium glycolate in the specimen taken:

$$\text{Result} = (F \times W\,1\,)/[(100 - b) \times W2\,]$$

F= factor converting glycolic acid to sodium glycolate, 12.9

W1= weight of glycolic acid in the specimen, determined from the standard curve and theabsorbance of the Sample solution (mg)

b = percentage of loss on drying, determined separately

W2= weight of the specimen taken (g)

8.0 Settling volume:

Analysis: To 75 mL of water in a 100-mL graduated cylinder, add 1.5 g of it in 0.5-g portions,shaking vigorously after each addition. Add water to make 100 mL, shake again until all of the powder is homogeneously distributed, and allow to stand for 4 h. Note the volume of the settledmass.

9.0 Residue on ignition:

Ignite a suitable silica crucible (for example silica, platinum, porcelain or quartz) at 600º ± 50ºC for 30 minutes, allow to cool in a desiccators over silica gel or other suitable Desiccant and Weigh. Use 1.0 g of the± substance being examined in the crucible and weigh. Moisten the Substance to be examined with a small amount of sulphuric acid (usually 1ml) and heat gently at as Low a temperature as practicable until the sample is thoroughly charred.

After cooling, moisten the residue with a small amount of sulphuric acid (usually 1ml), heat gently

until white fumes are no longer evolved and ignite at 600º ± 50º C until the residue is completely incinerated. Ensure that flames are not produced at any time during the procedure. Allow the crucible to cool in desiccators over silica gel or other suitable desiccant, weigh it again and calculate the percentage of residue. The percentage of residue.

Calculation: Calculate the % of Sulphated ash in sample using the formula;

$$\text{Calculation} = \frac{[W3-W1] \times 100}{[W2-W1]}$$

W1 = Weight of crucible

W2 = Weight of crucible and test sample

W3 = Weight of crucible and residue

10.0 Loss on drying:

Weigh accurately a weighing bottle that has been previously dried at 100ºC to105º C for 30 minutes. Transfer about 1.0 g of substance being examined to the weighing bottle, replace the cover, and accurately weigh the bottle and the contents. By gentle, sidewise shaking, distribute the substance taken as evenly as practicable to a depth of not more than10 mm. Place the loaded bottle in the drying chamber, removing the stopper and leaving it also in the chamber. Dry the substance at 105ºC for 6 hours. After drying remove the bottle from the chamber, replace the cover and cool to room temperature in desiccators before weighing. Weigh the bottle and contents. **Calculation**: Calculate the % of loss on drying using the formula;

Formula: $\frac{[W2-W3] \times 100}{[W2-W1]}$

W1 = Weight of weighing bottle

W2 = Weight of sample with weighing bottle

W3 = Weight weighing bottle with sample after drying.

11.0 Microbial enumeration tests

Total aerobic microbial Count:

- Suspend 10 gm Croscarmellose sodium in Fluid Soybean – Casein Digest Medium tomake a 100 ml specimen i.e. 1:10.
- Prepare decimal dilutions until desired dilutions is obtained & for each dilutionuse fresh

sterile pipette.

- Label all sterile Petri plate with required dilution.
- Pipette 1 ml of 1:10 diluents & other dilution in to Petri plate in duplicates.
- Pour 15 to 20 ml of Soyabean Casein Digest Agar (cooled to 42-45ºc) in to the plate.
- Cover the petri dish Mix the sample with the Agar by tilting or rotating the dishes.
- And allow the contents to solidify at Room temperature.
- Invert the petri dishes and incubate at 35ºC ± 2ºC for 48 to 72 hours.

Following incubation examine the plate for growth, count the number of colonies, and express the average for the 2 plates in terms of the number of microorganisms per gramof specimen.

Yeast and Mould Count:

Suspend 10 gm Croscarmellose sodium in Fluid Soybean – Casein DigestMedium to make a 100 ml specimen i.e 1:10

Prepare decimal dilutions until desired dilutions is obtained & for eachdilution use fresh sterile pipette.

Label all sterile Petri plate with required dilution.

Pipette 1 ml of 1:10 diluents & other dilution in to Petri plate in duplicates.

Pour 15 to 20 ml of Sabouraud Dextrose Agar (cooled to 42-45ºc) in to Coverthe petri dish

Mix the sample with the Agar by tilting or rotating the dishes and allow the contents to solidify at Room temperature.

Invert the petri dishes and incubate for 25º C± 2º C 48 to 72 hours.

Following incubation examine the plate for growth, count the number of colonies, and express the average for the 2 plates in terms of the number of microorganisms per gramof specimen.

Escherichia coli :

Sample Preparation and Pre-incubation:

- Suspend 10 gm Croscarmellose sodium in Fluid Soybean – Casein Digest Mediumto make a 100 ml specimen.
- Incubate at 37ºC ± 2ºC for 18 to 24 hours.

Selection and Subculture:

- Shake the container, transfer 0.1 ml of Soybean – Casein Digest Medium to 10 mlof Macconkey Broth and incubate at 35ºC ± 2ºC for 24 to 48 hours.
- Sub culture on a plate of Macconkey Agar at 35ºC ± 2ºC for 18 to 72 hours.

Interpretation: Possible presence of E.coli is indicated by the Brick red colonies surrounded by zone of precipitated bile. This is confirm by identification test.

Identification test:

- By means of an inoculating loop, to the surface of Levine Eosin – Methylene Blue Agar Medium,plated on petri dishes.
- If numerous colony colonies are to be transferred, divide the surface of each plate in to quadrants, each of which may be seeded from a separate colony.
- Cover and invert the plates, and incubate.
- If none of the colonies exhibits both a characteristic metallic sheen under reflectedlight and a blue black appearance under transmitted light, the specimen meets therequirements of the test for the absence of E.coli.

PREPARATION OF REAGENTS / SOLUTIONS USED IN ANALYSIS

1N Acetic acid: Add 58 ml glacial acetic acid to sufficient purified water to make 1000 ml after Coolingto room temperature

2-7 Dihydroxynaphthalene TS: Dissolve 100 mg of 2-7 Dihydroxynaphthalene TS in 1000ml of sulphuric acid, Allow the solution to stand until the yellow color disappear. If the solution is very dark discard it & prepare new solution from a different supply of a sulphuric acid. This solution is stable forapproximately 1 month if store in a dark bottle.

Methylene blue TS: Dissolve 125mg of methylene blue in 100ml of alcohol, & dilute with alcohol to 250ml.

1-Naphthol TS: Dissolve 1g of 1naphthol in 25ml of methanol .Prepare this solution Fresh.

Potassium pyroantimonate solution: Dissolve 2 g of potassium pyroantimonate in 85 ml of hot water cool rapidly, and add 10 ml of solution of Potassium hydroxide (3 in 20). Allow to stand for 24hrs, filter& add sufficient water produce 100ml.

15% W/V potassium carbonate: Dissolve 15g of potassium carbonate in 100ml of water.

0.1N hydrochloric acid: Dilute 8.5 ml of hydrochloric acid to 1000ml with water.

0.1N sodium hydroxide: Dissolve 4g of sodium hydroxide to 1000ml with water.

M-cresol purple TS: Dissolve 0.1g of met cresol purple in 13ml of 0.01N sodium hydroxide, dilute with water to 100ml & Mix.

0.05 M Silver nitrate: Dissolve 8.5 g. Silver Nitrate in to 1000 with Distilled water.

1N Acetic acid: Add 58 ml glacial acetic acid to sufficient purified water to make 1000 ml after Cooling to room temperature.

6N Ammonium hydroxide: Dilute 400 ml of ammonia water, with water to make 1000 ml.

6N Hydrochloric acid: slowly with stirring 510 ml of Hydrochloric acid to 450 ml of purified Water anddilute to 1000 ml with purified water.

Soyabean Casein Digest Medium: Suspend 3.0 grams in 100 ml purified / distilled water.Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 Ibs.

Soyabean Casein Digest Agar: Suspend 4.0 grams in 100 ml purified / distilled water. Heatto boiling to dissolve the medium completely. Sterilize by autoclaving at 15 Ibs.

Sabouraud Dextrose Agar: Suspend 6.5 grams in 100 ml purified / distilled water. Heat toboiling to dissolve the medium completely. Sterilize by autoclaving at 15 Ibs.

Macconkey Agar: Suspend 5.153 grams in 100 ml purified / distilled water. Heat to boiling todissolve the medium completely. Sterilize by autoclaving at 15 Ibs.

Levine Eosin – Methylene Blue Agar: Suspend 3.745 grams in 100 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 Ibs.

For Sigachi Industries Ltd.,

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AGM - QA

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