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ANALYSIS OF MICROCRYSTALLINE CELLULOSE AND THEIR PRODUCTS

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ABSTRACT

Microcrystalline cellulose (MCC) has been widely used as an excipient for direct compression due to its good flow ability, compressibility, and compatibility. The analysis of microcrystalline cellulose and their products and Water testing is also performed. Take 90ml SCDM (soyabean casein digest medium) for enrichment of finished product. Add 10gm of sample in SCDM broth, now inoculate 1ml of sample in petri plate and pour soya bean casein agar on the surface of Petri plate then kept it for 15minutes to settle down and after settling incubate it at 37 degree centigrade for 18-48 hours for TAMC (total aerobic microbial count) and 18–72hours for TYMC (total yeast and mould count). Now transfer the 1ml sample in macckonky broth and also 0.1ml in RVSE broth and also streak on the cetrimide agar plate and Mannitol salt agar plate and incubate it for 18 to 24hours and note the result either bacteria are present or absent, it shows the product is good.

KEYWORDS: microcrystalline cellulose powder, cellulose powder, microcrystalline cellulose and carboxy methyl cellulose sodium, silicified microcrystalline cellulose, spirullina powder, S.aureous, E.coli, Salmonella, Pseudomonas.

INTRODUCTION

Cellulose is the most abundant polymer in nature formed by glucose units linked through a β-1, 4 glycosidic linkages. The linear chains of this polymer are bound together forming microfibrils which structure the cell wall in most plants. Microcrystalline cellulose (MCC) is produced by acid hydrolysis of wood pulp. During this process, the amorphous regions of the microfibrils are eliminated leaving the most crystalline parts intact. The resulting product is

washed and spray dried to get a powder of the desirable size, density, and moisture content. MCC is widely used as a pharmaceutical aid for direct compression, wet and dry granulation. It is also employed for the production of solid dosage forms due to its good compressibility, compatibility, and loading capacity of drugs. Further, it renders tablets of good hardness without the need of using high compression forcesand these compacts usually shows a low friability. Microcrystalline cellulose (C6H10O5)n is refined wood pulp. It is a white, free-flowing powder. Chemically, it is an inert substance, is not degraded during digestion and has no appreciable absorption. In large quantities it provides dietary bulk and may lead to a laxative effect. Increasing application of microcrystalline cellulose in various industries is a major factor propelling growth of the global microcrystalline cellulose market. Microcrystalline Cellulose (MCC) is partially depolymerized specialty cellulose prepared by treating α-cellulose. MCC is widely used in pharmaceutical, food & beverage, cosmetic and other industrial applications, owing to its broad spectrum of properties. MCC is used a suspension stabilizer and an excipient, owing to its chemical inertness and non-toxic nature. Microcrystalline cellulose (MCC) is a free-flowing crystalline powder (a non-fibrous microparticle). It is insoluble in water, dilute acids and most organic solvents, but slightly soluble in the alkali solution of 20%. It has a wide range of uses in the pharmaceutical excipients and can be directly used for tabletting of dry powder. It is widely used as pharmaceutical excipients, flow aids, fillers, disintegrating agents, antisticking agents, adsorbents, and capsule diluents. www.entrepreneurindia.co Microcrystalline cellulose is widely used in pharmaceuticals, primarily as a binder/diluent in oral tablet and capsule formulations where it is used in both wet-granulation and direct-compression processes. In addition, wide ranges of chemical, technical, and economic merits are attached with its utilization. It has found its applications in the cosmetics & personal care industry, as well as in the food sector. When it comes to food production, it acts as an anti-caking agent, a fat substitute, an emulsifier, an extender, as well as a bulking agent and as far as the beverage sector is concerned, it acts as a gelling agent, stabilizer, anti-caking agents and suspending agents. It is widely employed as a fat substitute, thickener, binder in cosmetics industry. Besides all that, it has applications in the pharmaceutical industry as it displays chemical inertness and deficiency of taste and aroma. As an excipient, it is utilized in almost every type of oral dosage entailing pellets, tablets, capsules, sachets, and others. The MCC market is projected to grow from USD 885.1 million in 2018 to USD 1,241.4 million by 2023, at a CAGR of 7.0% between 2018 and 2023. Microcrystalline cellulose is partially depolymerized cellulose in pure form, which is synthesized from -cellulose precursor. There are several

ways to synthesize microcrystalline cellulose such as reactive extrusion, acid hydrolysis, steam explosion and enzyme mediated synthesis. Microcrystalline cellulose has large scale applications in the pharmaceutical, food & beverage, and personal care industries. In the food & beverage industry, microcrystalline cellulose is added to the processed food to create a creamy and smooth mouth feel. This is mostly used to prepare low-fat dairy products such as frozen yogurt, whipped cream, and ice cream. In the pharmaceutical industry, it is used as fillers, disintegrating agents, anti-sticking agents, adsorbents, and capsule diluents.

METHOD AND MATERIALS

Sample preparation and method for finished product

Sample preparation: - Take 90ml SCDM (soyabean casein digest medium) for enrichment of finished product. Add 10gm of sample in SCDM broth, now inoculate 1ml of sample in petri plate and pour soya bean casein agar on the surface of petri plate then kept it for 15minutes to settle down and after settling incubate it at 37 degree centigrade for 18-48 hours for TAMC (total aerobic microbial count) and 18–72hours for TYMC (total yeast and mould count). Now transfer the 1ml sample in macckonky broth and also 0.1ml in RVSE broth and also streak on the cetrimide agar plate and Mannitol salt agar plate and incubate it for 18 to 24hours and note the result either bacteria are present or absent.

Water sampling locations

Take various sample of water from different places such as feed water, potable water, barine water, purified water.

Sample preparation and method for water

Take 100ml SCDM (soyabean casein digest medium) for enrichment of various kinds of water. Add 10ml of sample in 10ml SCDM broth, now inoculate 1ml of sample in petri plate and pour soya bean casein agar on the surface of Petri plate then kept it for 15minutes to settle down and after settling incubate it at 37^0 C for 18-48 hours for TAMC (total aerobic microbial count) and 18–72hours for TYMC (total yeast and mould count). Now transfer the 1ml sample in macckonky broth and also 0.1ml in RVSE broth and also streak on the cetrimide agar plate and Mannitol salt agar plate and incubate it for 18 to 24hours and note the result either bacteria are present or absent.

Sample preparation and method for spirullina powder

Take 90ml SCDM (soyabean casein digest medium) for enrichment of spirullina powder. Add 10gm of sample in SCDM broth, now inoculate 1ml of sample in Petri plate and pour soya bean casein agar on the surface of petri plate then kept it for 15minutes to settle down and after settling incubate it at 37 degree centigrade for 18-48 hours for TAMC (total aerobic microbial count) and 18–72hours for TYMC (total yeast and mould count). Now transfer the 1ml sample in macckonky broth and also 0.1ml in RVSE broth and also streak on the cetrimide agar plate and Mannitol salt agar plate and incubate it for 18 to 24hours and note the result either bacteria are present or absent.

Gram Staining for the presence of bacteria

Place your slide on a slide holder or a rack. Flood (cover completely) the entire slide with crystal violet. Let the crystal violet stand for about 60 seconds. When the time has elapsed, wash your slide for 5 seconds with water. The specimen should appear blue-violet when observed with the naked eye. Now, flood your slide with the iodine solution. Let it stand about a minute as well. When time has expired, rinse the slide with water for 5 seconds and immediately procede to step three. At this point, the specimen should still be blue-violet. This step involves addition of the decolorizer, ethanol. Step 3 is somewhat subjective because using too much decolorizer could result in a false Gram (-) result. Likewise, not using enough decolorizer may yield a false Gram (+) results. To be safe, add the ethanol dropwise until the blue-violet color is no longer emitted from your specimen. As in the previous steps, rinse with the water for 5 seconds.The final steps involves applying the counterstain, safranin. Flood the slide with the dye as you did in steps 1 and 2. Let this stand for about a minute to allow the bacteria to incorporate the saffranin. Gram positive cells will incorporate little or no counterstain and will remain blue-violet in appearance. Gram negative bacteria, however, take on a pink color and are easily distinguishable from the Gram positives. Again, rinse with water for 5 seconds to remove any excess of dye.

Gram staining

A smear of the strain was prepared on a clean glass slide & the smear was allowed to air dry & then heat fixed. The heat fixed smear was flooded with crystal violet & after one minute, it was washed with water & flooded with mordant Gram's iodine. The smear was decolorized with 95% ethyl alcohol, washed with water & then counter – stained with safranin for 45sec.

After washing with water, the smear was dried with tissue paper & examined under oil immersion (100X).

b) Motility

The motility is an important part in identification of bacteria. Motility of the given isolates, was observed by using 'Hanging drop Method' as per standard protocol. On the basis of motility the organism were observed for their motility as no-motile, sluggishly motile, actively motile or highly motile. The bacterial isolates were further followed for biochemical characteristics.

3) Biochemical characteristics

1) **Sugar fermentation test**: - The bacterial isolates were tested for their ability to utilize a variety of sugars such as glucose, lactose, sucrose and mannitol as a source of carbon and energy to be tested production of gas. The sugar to be tested was added in peptone water medium to the concentration of 1% and autoclaved. The isolated was inoculated and incubated in the medium at 37^0c for 24 hours.

2) Indole test:-This test is performed to determine the ability of an organism to split indole from tryptophan molecule. Certain organism are capable of hydrolyzing and deaminating the amino acid tryptophan that mediate the production of indole .Tryptone water a mediam for detection of indole production which is rich in tryptophan was inoculated with the bacterial isolate and incubated at $37^{0.}$c for 24 hour .At the end few drops of kovac's reagent was added and tube was observed for formation of red coloured ring. The presence of red coloured ring gives positive test while absence of red coloration gives negative test.

2)Methyl red test:- This test provide important key point for the identification of bacteria that produce strong acid from glucose as well can maintain low pH after prolonged incubation by buffering system. Two drops of methyl red reagent was added after 24hrs of incubation at 37^0c. The change of colour of medium to bright red colour after the addition of methyl red indicator. Show positive test while no change in colour shows negative test.

3) Vogus proskauer test:-This test is used to detect the production of acetyl methyl carbinol. The tube containing glucose phosphate broth was inoculated with bacterial isolate and incubated at $37^{0.}$c for 24 hour. After incubation about 0.6 ml of alpha naphthol solution followed by 0.2 ml of 40% KOH solution was added to 2.5 ml of incubated broth. The tubes

were shaken well and kept for 10-15 min. The positive test is indicated by development of red colour after the addition of barrit's reagent. The red colour is due to production of acetyl methyl corbinol. The formation of red colour gives positive test while no change in colour gives negative test.

4) Citrate utilization test:-This test is used to determine the ability of an organism to use sodium citrate as a sole source of carbon for metabolism & growth. Koser's citrate broth which is devoid of protein and carbohydrate. Except citrate was inoculated with organism and incubated at 37^0c for 24 hour. The broth used for the test was koser's citrate broth which contains bromothymol blue indicator which is yellow at acidic pH green at neutral pH and blue at alkaline pH. The presence of blue colour in the broth indicate positive test while absence of blue colour indicate negative test.

5) Urease test:-This test is used to determine the production of enzyme urease by micro-organism. For this test urea agar base is used. It contains urea which gets hydrolyzed by the growing bacteria. Hydrolysis of urea results in the formation of ammonia, which is identified by the change in colour from yellow to pink because of the indicator incorporated in the medium it hat is phenol red. The urea agar base slant was inoculated with the bacterial isolated and was incubated at 37^0c for 24 hour. After 24 hour the formation of pink colour indicated positive test while no change in colour indicates negative test.

6) Triple sugar iron agar: - Triple sugar iron agar is used for the differentiate of enteric pathogens by ability to determine carbohydrate fermentation and hydrogen sulphide production. Organism that ferment glucose produce a variety of acid turning the colour of the medium from red to yellow, more amount of acids are liberated in butt(fermentation) than in the slant (respiration). Growing bacteria also from alkaline products from the oxidative decarboxylation of peptone and these alkaline products neutralize the large amount of acid present in the butt. Thus the appearance of an alkaline (red) slant and an acid (yellow), but after incubation indicates that the organism is a glucose fermenter butt is unable to ferment lactose and for sucrose. Bacteria that ferment lactose or sucrose (or both) in addition to glucose produce large amount of acid enables no reversion of pH in that region and thus bacteria exhibit an acid slant and acid butt. Gas production (co_2) is detected by the presence of cracks or bubbles in the medium, when the accumulated gas escapes. This sulphate is reduce to hydrogen sulphide by several species of bacteria and H_2S combine with ferric ions of ferric salt to produce the insoluble black precipitate of ferrous sulphide. Reduction of

thiosulphate proceeds only in an acid environment and blackening usually occurs in the butt of the tube.

RESULT AND DISCUSSIONS

Result of Finished product, Water and Spirullina powder

The bacteria were not obtained from microcrystalline powder, water and spirullina powder sample. The morpholological characters are still widely used for characterizing genera. Gram positive bacteria were observed in microscopic study of gram staining slide with purple colour, rod shape and filamentous structure. Isolate were tested by biochemical tests for the identification of bacteria.

Biochemical characteristics of isolated gram positive rod shape bacteria

Result for E.coli

1) Sugars test

Test	Observation	Result
Glucose	Acid & Gas Production	Negative
Lactose	Acid Production	Negative
Mannitol	Acid Production	Negative
Sucrose	Acid & Gas Production	Negative

From the above observation it was found that the no acid & gas production in glucose, lactose, sucrose, mannitol.

IMViC Test

Test	Observation	Result
Indole	Cherry red colour ring	Negative
MR	Red colour	Negative
VP	No Red colour	Negative
Citrate	Blue colour	Negative
Urease	Pink colour	Negative
H_2S Production(TSI Slant)	No H_2S Production	Negative

From the above observation it was found that all tests are negative. Tha E.coli is not present our microcrystalline product, water and spirullina powder.

Result for Pseudomonas

1) Sugars test

Test	Observation	Result
Glucose	Acid & Gas Production	Negative
Lactose	Acid Production	Negative
Mannitol	Acid Production	Negative
Sucrose	Acid & Gas Production	Negative

From the above observation it was found that the no acid & gas production in glucose, lactose, sucrose, mannitol.

IMViC Test

Test	Observation	Result
Indole	Cherry red colour ring	Negative
MR	Red colour	Negative
VP	No Red colour	Negative
Citrate	Blue colour	Negative
Oxidase	Purple colour	Negative
H_2S Production(TSI Slant)	No H_2S Production	Negative

From the above observation it was found that all tests are negative. The Pseudomonas is not present our microcrystalline product, water and spirullina powder.

Result for Salmonella

1) Sugars test

Test	Observation	Result
Glucose	Acid & Gas Production	Negative
Lactose	Acid Production	Negative
Mannitol	Acid Production	Negative
Sucrose	Acid & Gas Production	Negative

From the above observation it was found that the no acid & gas production in glucose, lactose, sucrose, mannitol.

IMViC Test

Test	Observation	Result
Indole	Cherry red colour ring	Negative
MR	Red colour	Negative
VP	No Red colour	Negative
Citrate	Blue colour	Negative
Urease	Pink colour	Negative
H_2S Production(TSI Slant)	No H_2S Production	Negative

From the above observation it was found that all tests are negative. The Salmonella is not present our microcrystalline product, water and spirullina powder.

Result for S.aureus

1) Sugars test

Test	Observation	Result
Glucose	Acid & Gas Production	Negative
Lactose	Acid Production	Negative
Mannitol	Acid Production	Negative
Sucrose	Acid & Gas Production	Negative

From the above observation it was found that the no acid & gas production in glucose, lactose, sucrose, mannitol.

IMViC Test

Test	Observation	Result
Indole	Cherry red colour ring	Negative
MR	Red colour	Negative
VP	No Red colour	Negative
Citrate	Blue colour	Negative
Coagulase	Pink colour	Negative
H_2S Production(TSI Slant)	No H_2S Production	Negative

From the above observation it was found that all tests are negative. The S.aureus is not present our microcrystalline product, water and spirullina powder.

CONCLUSION

Cellulose, because of their industrial importance, is the best known products of pharma industry The primary objective was to identify the microcrystalline product that it's good to use. The taxonomical studies which confirmed the typical biochemical behaviours resembling of E.coli, S.aureus, pseudomonas, Salmonella. These tests helped to design good production of microcrystalline poweder and spirullina powder. The water is using for the preparation of both powder is too good.

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