



MUCILAGE: ISOLATION, CHARACTERISATION AND SOURCES

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ABSTRACT

Now a days, mucilage has got an importance as an pharmaceutical excipient as it is having local availability, biocompatibility, lack of toxicity, low cost, biodegradability and better patient tolerance. Mucilage is ester of sulphuric acid obtained from *Aloe vera*, *Aegle marmelos*, *Althar Officinalis*, *Cassia tora*, etc. This overview on mucilage includes isolation by cold maceration method and hot maceration technique, identification test, purity test, organoleptic test, physiochemical characterisation, micromeretic, microbial characterisation and its compatibility studies by FTIR and DSC. It is pharmaceutically important as thickening, binding, gelling, disintegrating, viscolyting and stabilizing agent.

Keywords: Mucilage, Isolation, Excipient, Food industry.

INTRODUCTION

Mucilage is most commonly used excipient in pharmaceutical preparations [1]. Mucilage based plant materials has high-molecular-weight. Mucilages are esters of sulphuric acid which is a polysaccharide compound [2]. Mucilages are complexes formed from uronic acid and sugar units. Mucilages are produced inside the cells of the plant. Mucilage is a sticky and gummy substance used as an adhesive [3].

Mucilage is comprised of protein, polar glycoprotein, exopolysaccharides, polysaccharides and uranides [4]. Mucilages are present in nearly all classes of plant like locust bean, slippery elm bark, quince seed, etc. Mucilage has various advantages as it is having local availability, biocompatible, lack of toxicity, low cost, biodegradable, better patient tolerance. The disadvantages of mucilage are its microbial contamination, batch to batch variation, changes in viscosity on storage and hydration problems [5,6].

METHODS OF ISOLATION OF MUCILAGE

a) Cold maceration method

Plant material is dried in sunlight or in oven at 105°C. Plant material is treated with petroleum ether to remove pigments and fats. Then the material is soaked in water for 5h, boiled for 30 min and allowed to stand for 30 min so that mucilage is released into water. The material is squeezed from the eight folds of muslin cloth to remove

the Marc. Equal volume of acetone is added to the filtrate to precipitate the mucilage. The mucilage is separated, dried in oven at 35°C and passed through sieve number 20 and stored in desiccator [7,8].

b) Hot maceration method

Plant material is soaked for 12 h in distilled water and crushed in blender for 15 min. The dispersion is boiled for 30 min and passed through eight folds of muslin cloth. The mucilage is precipitated from the filtrate by adding acetone.

CHARACTERISATION OF MUCILAGE

A) Identification test for mucilage

Powdered polymer is treated with ruthenium red solution and it will give pink color when observed under microscope which confirm it as a mucilage [9].

B) Test for purity

Aqueous solution of extracted mucilage is used to carry out the test for carbohydrates, monosaccharides, starch, proteins, amino acid, steroid, glycosides, saponins, alkaloids, tannins and flavonoids for the determination of purity of mucilage.

C) Organoleptic evaluation of isolated mucilage

The isolated mucilage is characterized for

organoleptic properties such as color, odor, taste and texture [10].

D) Physicochemical characterization of mucilage

I) Swelling index

Accurately weighed (1g) powdered mucilage is taken in 25 ml measuring cylinder, 25ml of fresh distilled water is added and shake the mixture thoroughly every 10 min for 1h and allowed to stand for 3h at room temperature. Swelling index can be calculated using following formula [11]:

$$\text{Swelling index} = \frac{X_t - X_o}{X_o} \times 100$$

Where X_o =initial height of the powder in graduated cylinder and X_t = height occupied by swollen gum after 24 h.

II) Water uptake capacity

Water uptake capacity can be determined by placing mucilage powder disc on agar gel. A known weight of powdered mucilage is compressed into a disc. Initial weight of the disc (W1) is recorded and disc is placed on 2 % agar gel. At regular interval of 1 h mucilage disc is removed, excess water is removed off by using filter paper. And disc is weighed until a constant weight (W2) is obtained. The Percentage water uptake can be calculated using formula [12]:

$$\text{Water uptake (\%)} = \frac{W1 - W2}{W1} \times 100$$

III) pH of mucilage

The mucilage is weighed and dissolved in water separately to get a 1% w/v solution. The pH of solution is determined by using digital pH meter [12].

IV) Moisture absorption study

Mucilage powder (1g) is uniformly spread on the petridish and placed in a desiccator containing saturated solution of aluminium chloride. After 3 days, the mucilage powder is taken out and weighed. The percentage of moisture absorption is calculated as:

$$\text{Moisture absorption(\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

V) Loss on drying

Moisture content of mucilage can be determined by loss on drying method. Accurately weighed 1g sample is heated at 105°C to get a constant weight in a hot air oven and percent loss of moisture on drying is calculated using following formula [13]:

$$\text{LOD (\%)} = \frac{\text{Weight of water in sample}}{\text{Weight of dry sample}} \times 100$$

VI) Solubility

One part of dry mucilage powder is shaken with different solvents such as methanol, ethanol, hot water, cold water for the determination of solubility behavior of the mucilage.

VII) Ash values

Ash values such as total ash, acid insoluble ash and water-soluble ash can be determined according to Indian Pharmacopoeia [14].

a) Total Ash

About 3g of sample is accurately weighed and taken in a silica crucible, which is previously ignited and weighed. The powder is spread as a fine, even layer on the bottom of the crucible. The crucible is incinerated gradually by increasing temperature to make it dull red hot until free from carbon. The crucible is cooled and weighed. The procedure is repeated to get constant weight. The percentage of total ash is calculated with reference to air dried sample.

b) Acid Insoluble Ash

The ash obtained as described above is boiled with 25 ml of 2N HCl for five minutes. The insoluble ash is collected on an ash less filter paper and washed with hot water. The insoluble ash is transferred into a silica crucible, ignited and weighed. The procedure is repeated to get a constant weight. The percentage of acid insoluble ash is calculated with reference to the air-dried sample.

c) Water-soluble Ash

The ash obtained as described for the determination of total ash is boiled for 5 min with 25 ml of water. The insoluble matter is collected on ash less filter paper and washed with hot water. The insoluble ash is then transferred into silica crucible, ignited (15 min) and is weighed. The procedure is repeated to get a constant weight. The weight of insoluble matter is subtracted from the weight of the total ash. The difference of weight is considered as water-soluble ash. The percentage of water-soluble ash is calculated with reference to the air dried sample.

E) Pharmaceutical characterization of mucilage

I) Surface Characterization

Surface characterization of the dry powdered mucilage is done using scanning electron microscope (SEM) [15].

II) Flow properties

a) Particle size distribution

Polymer is dispersed in glycerin and a smear of the dispersion is made and examined under microscope. The size of particles (more than 500) is measured using a calibrated eyepiece micrometer [16].

b) Loose Bulk density

The bulk density of mucilage is measured by putting the accurately weighed powder into a graduated cylinder and the volume is calculated using following formulae [3]:

$$\text{Loose Bulk density} = \frac{\text{Weight of powder}}{\text{Volume occupied by powder}} \times 100$$

c) Tapped density

The tapped density can be determined by three tap method. Weighed quantity of powder is carefully introduced into a 10 ml graduated cylinder and is dropped on hard wood surface on tiles three times from height of 2.5 cm. It can be calculated by using formula [18]:

$$\text{Tapped density} = \frac{\text{Weight of powder}}{\text{Final volume after tapping}} \times 100$$

d) Compressibility index

It is indirectly related to the relative flow rate, cohesiveness and particle size of the powdered mucilage. It is fast, simple and popular method of predicting powder flow characteristics. It is calculated by following formula [19]:

$$\text{Compressibility index} = \frac{\text{Tapped density} - \text{Loose bulk density}}{\text{Tapped density}} \times 100$$

e) Hausner's ratio

It is an index which shows the ease of powder flow; it is determined by using the following formula:

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Loose bulk density}}$$

f) True density

True density of mucilage is determined by liquid displacement method. The weight (W1) of the clean, dry 15 mL density bottle is determined. The bottle is filled with distilled water and the top of the bottle is dried with filter paper and weighed as (W2). The procedure is repeated using carbon tetrachloride to obtain the weight of the bottle plus carbon tetrachloride (W3). Carbon tetra chloride is used as the displacement liquid. About 2 g of the mucilage powder is transferred to same clean and dried density bottle and weighed as (W4). The bottle is filled with carbon tetrachloride and the weight (W5) is measured. The density of the carbon tetrachloride is calculated using following formula:

$$\text{Density of Carbon tetrachloride } (\rho) = \frac{(W3 - W1)}{(W2 - W1)} \times 100$$

(Density of distilled water at 25°C = 0.9971 g/c)

The true density of powder is calculated from following formula:

$$\text{Density of sample} = \frac{(W4 - W1)}{[(W3 - W1)/\rho] - [(W5 - W4)/\rho]} \times 100$$

g) Surface tension

The surface tension of the mucilage can be determined by drop count method, using a stalagmometer. The stalagmometer is filled with purified water above the upper mark. The flow rate is adjusted to 10-15 drops/min with the help of screw pinch cork. Then, number of drops of water is counted between the marks of the stalagmometer (n_1). The water is removed and the stalagmometer is filled with the mucilage dispersion (1% w/v) and number of drops is counted (n_2). The surface tension of the mucilage is determined using formula given below:

$$\text{Surface Tension } (\gamma_1) = \frac{n_1 \rho_2 \gamma_2}{n_2 \rho_1}$$

Where,

n_1 =number of drops of water,

n_2 =number of drops of sample,

ρ_1 =density of water (0.9956 g/mL),

ρ_2 =density of sample

γ_1 =surface tension of water (71.1 dynes/cm)

h) Angle of repose

Flow properties of powdered mucilage can be determined by the angle of repose technique. Angle of repose is determined by the fixed funnel method. A funnel with the end of the stem cut perpendicular to its axis of symmetry is fixed at a given height (h) above the graph paper placed on a flat horizontal surface. The gum powder is carefully poured through the funnel until the apex of the conical pile just touched the tip of the funnel. The radius (r) of the base of the pile is determined and the tangent angle of repose (θ) is calculated by following equation:

$$\tan \theta = h/r$$

F) Microbial characterization

1g of the substance being examined is suspended in peptone water to produce 150 ml in a sterile conical flask. Later the conical flask is incubated at 37°C for 60 minutes and allowed the material to settle down to get the clear solution. Sterile nutrient agar and Sabouraud dextrose agar media plates are prepared as per standard procedures. 0.1ml of the clear supernatant from the incubated tube is pipetted on the sterile nutrient agar and Sabouraud dextrose agar media plates. Sample on the surface of the medium are spreaded using sterile autoclaved spreader uniformly maintaining aseptic conditions, in laminar air flow cabinet. Petri dishes are kept in invert position and incubated for 24 hours to 48 hours at 37°C \pm 1°C for bacteria and at 28°C \pm 1°C for fungi, respectively. Plates are examined for microbial growth; the number of colonies are counted and expressed in terms of colony forming units per gram of the substance (CFU/gm) [20]:

G) Fourier Transform Infrared Spectroscopy (FTIR)

FTIR mucilage can be determined by Fourier Transform Infrared Spectroscopy method using KBr disc method. The samples are gently triturated with KBr and compacted into disc using IR press at 10 tons. The sample is placed into sample holder and scanned from 4000-400 cm^{-1} at a resolution of 4 cm^{-1} .

H) Differential Scanning Calorimetry (DSC)

The DSC thermogram of mucilage is recorded using a differential scanning calorimeter. The instrument is calibrated using Indium (156°C), Tin (232°C) and Zinc (419°C) as internal standards. Each sample (2.0-10.0 mg) is accurately weighed into a 40 μL aluminium pan and sealed. The probes are heated from 25 to 400°C at a rate of 10K/min under nitrogen atmosphere [21].

APPLICATION OF MUCILAGE**a) Mucilage in pharmaceutical industry**

Plant mucilages are pharmaceutically important polysaccharide with wide range of applications such as

- ▲ Thickening agent
- ▲ Gelling agent
- ▲ Binding agent
- ▲ Disintegrating agent
- ▲ Suspending agent
- ▲ Film former
- ▲ Viscolyting agent
- ▲ Coating agent

- ▲ Emulsifying agent
- ▲ Stabilizing agent
- ▲ Gelling agents

b) Mucilage in food industry

Plant mucilages are used in food industry as stabilizing agent for

- ▲ Ice creams
- ▲ Meat products
- ▲ Instant pudding, dairy, confectionery, beverages, backed product and sauces.

Table 1. Sources of Mucilage

Biological source	Family	Plant part
<i>Cydonia vulgaris</i>	Rosaecae	Seed
<i>Remusatia vivipara</i>	Araceae	Bark
<i>Delonix regia</i>	Fabaceae	Seed
<i>Cassia sophera</i>	Caesalpinaceae	Seed
<i>Aegle marmelos</i>	Leguminosae	Fruit
<i>Eulophia herbacea</i>	Orchdaecae	Tubers
<i>Cassia tora</i>	Leguminosae	Seed
<i>Cassia auriculata</i>	Caesalpinaceae	Seed
<i>Althar officinalis</i>	Malvaceae	Flower, Fruit, Leaves
<i>Abelmoschus moschatus</i>	Malvaceae	Roots, Leaves
<i>Abelmoschus esculenta</i>	Malvaceae	Fruit
<i>Aloe vera</i>	Liliaecae	Leaves
<i>Mussaenda frondosa</i>	Rubeaceae	Leaves
<i>Prosopis juliflora</i>	Mimosaceae	Seeds

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