Research Article

Isolation and Evaluation of Mucilage of *Abelmoschus esculentus* as a Suspending Agent

Suja C. Jayan* and Navaneet Krishna Manoj

Department of Pharmaceutics, Crescent College of Pharmaceutical Sciences, Kannur, kerala, India.

ABSTRACT

The aim of the present work was to isolate and evaluate the mucilage of *Abelmoschus esculentus (*Okra) as a suspending agent. A pharmaceutical suspension is a coarse dispersion in which insoluble solid particles (internal phase) are dispersed in a liquid medium (external phase). Suspending agents form film around particle and decreases inter-particle attraction. The mucilage was collected from the fruits of *Abelmoschus esculentus.* Calcium Carbonate suspension was prepared by using Acacia, Tragacanth and Okra mucilage. Comparison of suspension parameters such as sedimentation volume, viscosity and flow rate, redispersibility, particle size of calcium carbonate suspension prepared by using suspending agents like acacia, tragacanth and okra mucilage was done. The mucilage obtained from the fruits of *Abelmoschus esculentus* was easy to isolate and found to remain stable for longer periods of time. From the results of the study carried out it was found that the suspension containing *Abelmoschus esculentus* mucilage as suspending agent sedimented slowly and the sediment formed was readily redispersible.

Keywords: *Abelmoschus esculentus*, suspending agent, redispersibile.

INTRODUCTION

A pharmaceutical suspension is a coarse dispersion in which insoluble solid particles (internal phase) are dispersed in a liquid medium (external phase) [1].

The internal phase consisting of insoluble solid particles having a specific range of size which is maintained uniformly throughout the suspending vehicle with aid of single or combination of suspending agent.

The external phase (suspending medium) is generally aqueous in some instance, may be an organic or oily liquid for non-oral use. Ideally, the internal phase should be dispersed uniformly within the dispersion medium and should not sediment during storage [2].

Most suspending agents perform two functions i.e. besides acting as suspending agent they also imparts viscosity to the solution. Suspending agents form film around particle and decrease inter-particle attraction.

A good suspension should have well developed thixotropy. At rest, the suspension should be sufficiently viscous to prevent sedimentation and thus aggregation or caking of the particles. When agitated the viscosity should be reduced and provide good flow characteristics so that the suspension flows smoothly from the mouth of bottle[3].

Okra *(Abelmoschus esculentus* (L) Moench) or bhendi also known as ladies finger is an important vegetable crop being native of tropical Africa. Okra *Abelmoschus esculentus* (L) *moench* is a tall annual dicotyledonous plant related to cotton and thought to be of African origin. Okra mucilage has medicinal applications; when used as a plasma replacement or blood-volume expander. The mucilage of Okra not only binds cholesterol but the bile acid carrying toxins dumped into it by the filtering liver. It also has industrial applications; when added as size to glaze paper and used in confectionary.

MATERIALS AND METHODS

Materials Used

1. Acacia
2. Tragacanth
3. Calcium carbonate
4. Okra fruit

Instruments Used

1. pH meter
2. Weighing balance
3. Ostwald viscometer
4. Compound microscope

EXPERIMENTAL WORK

Isolation of Mucilage

COLLECTION

The fruits of *Abelmoschus esculentus* were purchased from the local market and it was identified from Parassinikadavu Ayurveda Medical College.

EXTRACTION OF *A.esculentus* GUM

The pods of *A.esculentus* were washed with water and sliced with knife in to small pieces.

The slices were soaked in water for 24 hrs to extract the gum and the gum there after was filtered out of the bulk using muslin cloth .The filtrate was extracted with 96% ethanol several times to complete the extraction process. The chlorophyll was removed by rinsing the gum in hot ethanol. The gum was air dried, pulverized and packaged in polythene container for further use.[4]

PREPARATION OF SUSPENSION

5 gram of calcium carbonate was taken in a clean mortar and pestle and triturated well. Then a small quantity of water was added to make a paste like consistency and then more amount of water was added and triturated well and then it was transferred to clean 100ml measuring cylinder and made the volume up to 100ml with water. Similarly calcium carbonate suspensions containing 0.5g of different suspending agents (acacia, tragacanth, okra mucilage) were prepared.

EVALUATION OF SUSPENSION

1. Sedimentation volume

- Suspensions containing different suspending agents were prepared by taking 0.5grams of acacia, tragacanth and okra mucilage along with 5g calcium carbonate in a clean dry mortar and triturated well with the help of a pestle. Then small quantity of water was added to it to get a paste like consistency and then more quantity of water was added and triturated. This was then transferred to a measuring cylinder and the volume made up to 100ml with water.
- Then the four cylinders was stirred well height of sediment was noted immediately. Thereafter the height of sediment was noted at 5,10,20,30,40,50,60 minutes respectively.
- A plot of time in minutes on x- axis and sedimentation volume in y- axis was drawn.[16]

2. Degree of Flocculation

The degree of flocculation (β) was determined from the following equation:

$\beta = (Vu)_{floc} / (Vu)_{defloc}$, where $(Vu)_{floc}$ is the ultimate sedimentation volume in flocculated suspension and $(Vu)_{defloc}$ is the ultimate sedimentation volume in deflocculated suspension.

3. Rheology

The time required for each suspension sample to flow through a 10 ml pipette was determined and the apparent viscosity was determined (n in ml/s) was calculated using the equation.[18]

Flow rate η_α= volume of pipette (ml)/ Flow time (sec)

Determination of viscosity

- Clean the Ostwald or capillary viscometer using water and acetone and dry.
- Clamp it vertically on a stand.
- Transfer a known quantity of distilled water with the help of pipette through wide limb.
- Suck the water through other limb up to a level higher than the upper mark and clamp the tube with the finger.
- Allow the water to flow down, start the stop clock when the water just passes the upper mark.
- Stop the clock when the water passes the lower mark.
- Note time required for the flow of water between the marks.
- Similarly carried out the above steps with suspension of mucilage of okra, acacia, tragacanth.
- Determine the viscosity of the suspension of mucilage, acacia, and tragacanth using the formula.

4. pH

pH of suspension was determined using pH meter.[18]

5. Redispersibility

Fixed volume (50ml) of the each suspension was kept is calibrated tubes which are then stored at room temperature for various time intervals (5,10,15,25days). At regular interval of 5 day each tube was removed and shaken vigorously to redistribute the sediment and the presence of deposit if any is recorded.[10, 11]

6. Particle size analysis

The suspensions were taken on a plane slide separately and observed under microscope and the size of about 500 particles were measured by using eye piece micrometer which was previously calibrated using stage micro meter. The particle size were divided into different size ranges and the percentage frequency and cumulative percentage frequency are calibrated .Average size was calculated using the formula.

$D_{mean} = \sum \% nd / \sum \% n$

Size distribution graph was obtained by plotting the percentage frequency and cumulative percentage frequency against average size and also percentage frequency against log average size.[12]

RESULT AND DISCUSSION

1. Isolation of mucilage and its phytochemical identification

The yield of mucilage from okra pods was 17.36% w/v. The phytochemical identification tests confirmed the absence of alkaloids, glycosides, tannins, steroids and sterols. On treatment of mucilage with ruthenium red, it showed red colour confirming the obtained as mucilage. A violet ring was formed at the junction of two liquids on reaction with Molisch's reagent indicating the presence of carbohydrates. Mucilage on treating with Ninhydrin reagent did not give purple coloration indicating the absence of proteins and amino acids. All these results of phytochemical identification tests on the isolated mucilage are summarized in Table No.1.

2. Sedimentation Volume

Sedimentation volume (F) is the ratio of the ultimate height (Hu) of the sediment as suspension settles in a cylinder under standard conditions to the initial height (Ho) of the total suspension

Sedimentation volume (F) =

$$\frac{\text{Ultimate height of sediment}}{\text{Initial height of suspension}} = \mathbf{Hu/Ho}$$

Sedimentation volume of the prepared suspensions within the time limit 5-60 minute is given in Table no.2.A plot of sedimentation volume against time is as seen in Figure No.1. While comparing the sedimentation volume values, it was found that at the initial 5 minutes, sedimentation volume was 0.99. It was higher than that of the suspension containing tragacanth and acacia and it was 0.97 and 0.98 respectively.

From the above plot it is seen that the suspension containing okra mucilage has a good suspending properties which is better than tragacanth and acacia.

2. Rheology

Flow Rate

Flow rate is the ratio of the volume of pipette (ml) to the flow time (secs)

$$\text{Flow rate } \eta_{\alpha} = \frac{\text{volume of pipette (ml)}}{\text{Flow time (secs)}}$$

Viscosity

Viscosity was determined by using Ostwald viscometer .The viscosity of suspension is as given in the Table no.3.

3. pH

The pH of the suspension was determined by using pH meter. The pH of suspensions is as given in Table no.4.

4. Redispersibility

Redispersibility of suspension was estimated by shaking the suspension. When comparing the redispersibility of the suspension it was found that suspension prepared from okra mucilage had good redispersibility characteristics than that of acacia and tragacanth.

5. Particle Size

Particle size was determined by optical microscopic method and the results are as shown in Table No.6.When comparing the particle size of calcium carbonate in the suspensions containing different suspending agents, it was found that suspension prepared from okra mucilage has calcium carbonate particles with smaller size than that of suspensions prepared from acacia and tragacanth.

CONCLUSION

The mucilage obtained from the fruits of *Abelmoschus esculentus* was easy to isolate and found to remain stable for longer periods of time. The suspension containing *Abelmoschus esculentus* mucilage as suspending agent was readily dispersible. The suspension containing *Abelmoschus esculentus* mucilage as suspending agent showed good flow properties which is essential for the preparation of a good dosage form. The suspension prepared using this mucilage sediment slowly and the sediment formed was found to be readily redispersible. Since mucilage obtained from the fruits of *Abelmoschus esculentus* is non-toxic, less expensive and easily available, it can be concluded that the mucilage isolated from *Abelmoschus esculentus* has a very good potential as a suspending agent.

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Table 1: Phytochemical identification tests

Identification tests	Name of tests	Observations
Test for carbohydrates	Molisch's test	+
Test for proteins and amino acids	Ninhydrin test	-
Test for mucilages	Ruthenium red test	+
Test for starches	Iodine test	-
Test for alkaloids	Dragendroff's test	-
Test for glycosides	Keller-Killiani test	-
Test for tannins	Ferric chloride test	-
Test for steroids and sterols	Libermann-Burchard test	-

Table 2: Sedimentation volumes of suspensions with different suspending agents

Time(min)	Calcium carbonate +Acacia,SD	Calcium carbonate +Tragacanth,SD	Calcium carbonate +okhra mucilage,SD
0	1±1.220	1±1.432	1±1.801
5	0.981±1.255	0.97±1.135	0.994±1.39
10	0.933±1.395	0.863±1.020	0.992±1.259
20	0.892±1.410	0.791±1.572	0.981±1.225
30	0.862±1.142	0.788±1.115	0.965±1.536
40	0.857±1.035	0.781±1.156	0.933±1.419
50	0.831±1.057	0.760±1.215	0.897±1.846
60	0..809±1.127	0.753±1.174	0.882±1.490

SD=Standard deviation

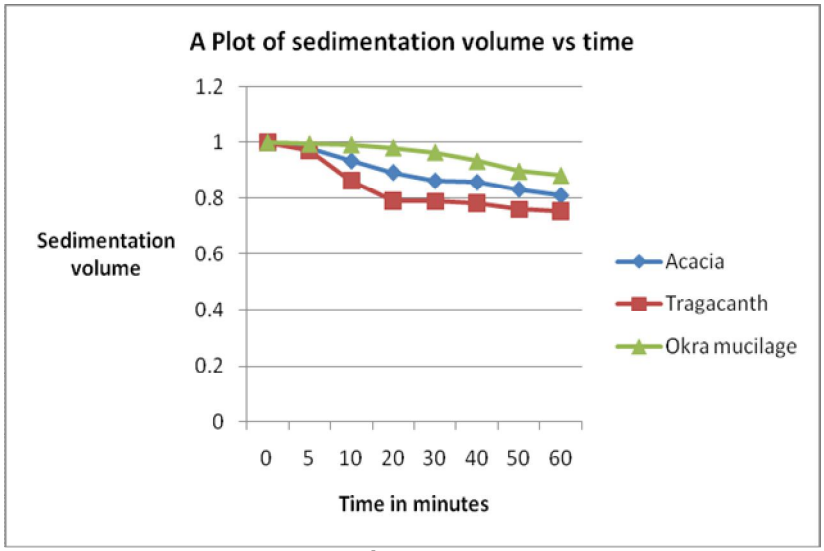

Fig. 1:

Table 2: Flow rate of suspensions with different suspending agents

Suspending agent	Flow rate (ml/sec),SD
Acacia	3.26±0.192
Tragacanth	3.45±0.047
Okra mucilage	2.22±0.124

SD=Standard deviation

Table 3: Viscosity of suspensions with different suspending agents

Suspending agent	Viscosity (centi poise),Sd
Acacia	1.06±0.249
Tragacanth	1.04±0.407
Okra gum mucilage	1.83±0.505

SD=Standard deviation

Table 4: pH of suspensions with different suspending agents

Suspending agent	pH
Acacia	8.7±0.235
Tragacanth	8.23±0.047
Okra gum	8.63±0.124

SD=Standard deviation

Table 5: Redispersibility study of suspensions with different suspending agents

Suspending agent	5th day	10th day	15th day	20th day
Acacia	No deposit	No deposit	Deposit	Deposit
Tragacanth	No deposit	No deposit	Deposit	Deposit
Okra gum	No deposit	No deposit	No deposit	Deposit

Table 6: Particle size of Calcium carbonate in suspensions with different suspending agents

Suspending agent	Average particle size(μ)
Acacia	2.23
Tragacanth	2.56
Okra gum	0.86

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