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Isolation And Evaluation Of Borassus flabellifer Mucilage As A Natural Suspending Agent

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Abstract: Natural excipients have gained importance over synthetic excipients because they are non toxic, less expensive and freely available. The present study was undertaken to search for a cheap and effective natural excipient and to evaluate the mucilage obtained from the endosperm of *Borassus flabellifer* as a suspending agent that can be used as an effective alternative for the formulation of pharmaceutical suspensions. The suspending properties of *Borassus flabellifer* mucilage (BFM) was evaluated comparatively with tragacanth at concentrations of 1, 1.5, 2.0 and 2.5% w/v in paracetamol suspension. The physical stability of the paracetamol suspension was assessed by appearance and pourability, viscosity and rheology, sedimentation volume ratio, redispersibility, degree of flocculation and microbial load. The rheological study of the formulations indicated that as the RPM increases the viscosity decreases, confirming the shear thinning nature of the suspension. The sedimentation volume by using BFM as a suspending agent shows highest sedimentation volume than tragacanth. The BFM containing formulations were more easily redispersed than tragacanth containing formulations and exhibited a higher degree of flocculation at 2.5%w/v than tragacanth containing formulations. The drug content of all the formulations was in the range of 97.1 to 99.6%. The suspension was found to be stable during the entire period of study. The results suggested that the mucilage was found to be a superior suspending agent than tragacanth indicating that it may be a good source as pharmaceutical adjuvant. But the feasibility of isolation of BFM powder in large scale needs to be studied in future.
Keywords: suspending agent, gum tragacanth, Rheology, paracetamol, *Borassus flabellifer* mucilage.

INTRODUCTION

Pharmaceutical suspensions are solid dispersions of insoluble or sparingly-soluble drugs in an aqueous or oily vehicle. They are intended for oral administration, topical application or parenteral administration of drugs. Formulation of drugs as suspensions for oral administration is a convenient way to administer insoluble or sparingly soluble drugs to infants and the elderly that have difficulty swallowing tablets or capsules. They are also useful to mask taste, and to control the absorption rate of the drug.

A major challenge to formulation of oral suspensions is that of physical stability. The solid insoluble drug separates from the vehicle and settles to the bottom of the container. It is desirable that such a formulation re-suspend easily upon shaking. Settling and aggregation may result in the formation of cakes that are difficult to re-suspend. This is a common occurrence in deflocculated systems which do not easily settle but are difficult to re-disperse once set. Redispersibility of insoluble drug substance is therefore a critical requirement in the evaluation of suspensions. It is also a critical requirement that the drug in

suspension be homogeneously mixed and remain both physically and chemically stable during the shelf life of the formulation. This is important because of the need to dispense a fairly uniform and accurate dose of the medicament per portion of the suspension. In order to address these problems several ingredients perform different or synergistic roles in the formulation of oral suspensions. Hydrophilic colloids such as xanthan gum, acacia and the cellulose derivatives have been used as suspending agents and, like surfactants, can produce a deflocculated system when used in low concentrations. Acacia or gum Arabic is mainly used in oral and topical pharmaceutical formulations as a suspending and emulsifying agent. Sodium carboxymethylcellulose and xanthan gum are also used in pharmaceutical suspensions for their suspending effect[1-5].

Mucilages are polysaccharide macro molecules that dissolve more or less upon contact with water and form colloidal solutions. Mucilages and gums are well known since ancient times for their medicinal value. In recent years, plant gums and mucilages have evoked tremendous interest due to their diverse application in pharmacy in the formulation of both solid and liquid dosage forms as thickeners, water retention agents, emulsion stabilizers, suspending agents, binders and film formers. Apart from its use in finished medicines, newer uses have been found in the preparation of cosmetics, textiles and paint paper. Hence the demand for these substances is increasing and new sources are getting tapped. Vast application of plant mucilages and gums in various industries is because of low cost, ready availability and important properties which they confer on products. With the increase in demand for natural mucilages, it has become necessary to explore the newer sources of mucilage to meet the industrial demands[6-7].

The *Borassus flabellifer* is a tall and erect palm, with large, fan-shaped leaves which are quite unlike the pinnate leaves of other palms. *Borassus* is from a Greek word describing the leathery covering of the fruit and *flabellifer* means "fan-bearer". Synonyms of the plant include jaggery palm, Palmyra palm, toddy palm, wine palm. This species is globally distributed from Africa to Australia. Within India, it is found throughout tropical regions, especially along the peninsular coast and in West Bengal and Bihar. It is often cultivated. The Palmyra palm has long been one of the most important trees of Cambodia and India. The different parts of the plant is used for the various ailments like secondary syphilis, antiperiodic, heart burns, liver and spleen enlargement etc. Other than these pharmacological uses the juice of the plant is used in preparation of health drinks, jellies etc. The leaves are use to make baskets, hats and many other useful items. *Borassus flabellifer* contains albuminoids, fats and the fresh pulp is reportedly rich in vitamins A and C. The fresh sap is reportedly a good source of vitamin B-complex. Male inflorescence constitutes spirostane-type steroid saponins like borassosides and dioscin. It also contains 20 known steroidal glycosides and carbohydrates like sucrose. It also contains bitter compound called flabelliferrins, these are steroidal saponins[8-11]. The endosperm contains a high proportion of mucilage. The two major polysaccharides present in this endosperm are galactomannan and mannan.

The purpose of the present study was to isolate and investigate *Borassus flabellifer* mucilage as a new, effective natural suspending agent that can be used as an effective alternative for the formulation of pharmaceutical suspension.

MATERIALS AND METHODS

Paracetamol was obtained from Piramal Healthcare Ltd. Baddi, India as gift sample. *Borassus flabellifer* endosperm was procured from the local market. All the other solvents, reagents and chemicals used were of either Pharamcopoeial or analytical grade.

Methods

Isolation and purification of mucilage from *Borassus flabellifer* endosperm[12]

The endosperm of *Borassus flabellifer* fruit contains mucilage. To increase the yield of the mucilage the endosperm of *Borassus flabellifer* fruit were extracted by different solvents. The endosperm of *Borassus flabellifer* were collected, cut into small pieces and dried using tray dryer at 37°C for 24 h at room temperature, made fine powder by crushing in a mixer. The fine powder was soaked in different solvents such as water, hot-water, phosphate buffer solution (PBS) of pH 4.0, 6.8, 9.2, separately for 2-3h and heated up to 80-90°C for 30-45 min for complete release of the water soluble mucilage into the solvents. The mucilage was then extracted by using a multi layer muslin/cheese cloth bag to remove the marc and concentrated viscous solution under reduced pressure at 60-70°C. Acidified ethanol (5% HCl in 75% ethanol) was added to the concentrated viscous solution with constant stirring. The gel like precipitate was formed and separated by filtration. The precipitate was washed 2-3 times

with 75% and 95% ethanol. After complete washing of the precipitate with ethanol 95%, brownish white powder was obtained. The powder was dried in an oven at 37°C, collected, grounded, passed through a # 80 sieve and stored in a desiccator till use. The brownish white powder was considered as mucilage for pharmaceutical use Physicochemical characterization, phytochemical screening and toxicity studies of the isolated mucilage were carried out as per the reported procedure[13-16].

Drug-Excipient Compatibility study

This study has been done to check whether there is any compatibility related problems are associated with drug and excipients used for the formulation of suspension.

Fourier Transform Infrared (FTIR) Spectral analysis

FTIR spectra of pure drug and physical mixture of drug and excipients were recorded on samples prepared in potassium bromide (KBr) disks using a FTIR spectrophotometer (FTIR-8300, Shimadzu, Japan). Samples were prepared in KBr disks by means of a hydrostatic press at 6-8 tons pressure. The scanning range was 400 to 4000 cm^{-1}.

Differential Scanning Calorimetry (DSC) analysis

DSC analysis was performed using Shimadzu DSC-60, Shimadzu Limited Japan. A 1:1 ratio of drug and excipient was weighed into aluminum crucible. And sample was analyzed by heating at a scanning rate of 20^0C over a temperature range 40-430^0C under nitrogen environment.

Formulation of Paracetamol Suspension

Paracetamol suspension was prepared according to Formula given in Table 1. Paracetamol suspension was prepared using different concentration of BFM in the concentration 1, 1.5, 2.0 and 2.5%w/v. The solid components of the formulation were finely triturated with the aid of mortar and pestle. The required quantity of BFM powder was added to the powdered drug and little quantity of water was added and triturated until homogeneous paste was obtained. To these required quantity of preservatives like methyl and propyl paraben, then citric acid, vanillin flavor, aspartame, amaranth powder was added and further triturated to form a homogenous mixture. The mixture was transferred to a 100 ml calibrated bottle and the volume was made up using sufficient quantity of distilled water. The suspensions were stored in stoppered glass bottles. All the prepared suspensions were deflocculated. To determine the degree of flocculation, flocculated suspensions were made using magnesium aluminium silicate (0.04mol) as flocculating agent. The procedure was repeated for the preparation of suspensions containing 1, 1.5, 2.0 and 2.5%w/v of tragacanth powder.

Table 1: Composition of different batches of Paracetamol suspension

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Paracetamol (g)	1	1	1	1	1	1	1	1
BFM* (g)	1	1.5	2.0	2.5	--	--	--	--
Gum tragacanth (g)	--	--	--	--	1	1.5	2.0	2.5
Methyl paraben (mg)	150	150	150	150	150	150	150	150
Propyl paraben (mg)	100	100	100	100	100	100	100	100
Citric acid (mg)	200	200	200	200	200	200	200	200
Vanillin flavour (mg)	10	10	10	10	10	10	10	10
Aspartame (mg)	100	100	100	100	100	100	100	100
Amaranth powder(mg)	2	2	2	2	2	2	2	2
Purified water q.s. to ml	100	100	100	100	100	100	100	100

BFM* *Borassus Flabellifer* mucilage

Evaluation of paracetamol suspension

Physical test

The prepared suspension was subjected for physical test. At weekly intervals, for a period of 4 weeks, the prepared suspensions were observed for physical changes such as aggregation, caking and crystal growth formation.

Sedimentation volume and rate

The sedimentation volume of the suspensions were determined by keeping 50 ml of each suspensions in stoppered measuring cylinder and stored undisturbed at room temperature. The volume of the sediments in the suspension was noted on daily basis for 7 days and thereafter weekly for 49 days (7 weeks).

The sedimentation volume F(%) was calculated using the formula

$F = 100Vu / Vo$

Where, Vu = ultimate volume of sediment and Vo = original volume of sediment before settling occurred.

From the values of F obtained, Graphs of sedimentation volume (Vu/Vo) against time were plotted, from which sedimentation rate was calculated.

Degree of flocculation

The degree of flocculation was determined using following the equation

$\beta = F/F\alpha$,

Where F is the ultimate sedimentation volume in the flocculated suspension and Fα is the ultimate sedimentation volume in the deflocculated suspension

Redispersibility

Redispersibility can be estimated by shaking the suspension with the help of a mechanical device which simulates motion of human arm during shaking. Fixed volume (50 ml) of the each suspension was kept in calibrated tubes which were then stored at room temperature for various time intervals (5, 15, 25 and 30 days). At regular interval of 5 day, one tube was removed and shaken vigorously to redistribute the sediment and the presence of deposit if any is recorded.

Rheological assessment

The time required for each suspension sample to flow through a 10 ml pipette was determined and the apparent viscosity (ηα in ml^{-1}) was calculated using the equation:

$$\text{Flow rate} = \eta\alpha = \frac{\text{Volume of pipette (ml)}}{\text{Flow time (seconds)}}$$

The viscosity (in poise) of the samples was determined at 25^0C using Brookfield viscometer at 50 rpm by using spindle no.3. All determinations were made in at least triplicate and the results obtained are expressed as the mean values.

Microscopical examination

Samples of the suspensions formulated using the BFM and gum tragacanth as suspending agents were microscopically examined for crystal growth under a metallurgical microscope (Model: NJF-120A, Japan). A drop of each sample was put on a slide and placed on the stage of the microscope. The objects were viewed at X100 magnification from the screen attached. The photomicrographs were printed out.

Particle size analysis

The particle size distribution of paracetamol in the suspension was determined using optical microscope (Olympus LITE image). The suspensions were mixed thoroughly and a drop of the suspension was taken on a slide and spread into a thin film. A total of 200 particles were counted and their size was determined.

pH

The pH of the suspensions was determined at intervals of one week for 21 days using pH meter.

Drug content

2ml of (20mg equivalent of the drug) of the suspension was measured accurately and transferred into 100ml volumetric flask and the volume made with 0.1N HCl. From this, 5ml of the sample was transferred to 100ml volumetric flask and made up the volume with 0.1N HCl. Further from the above solution, 5ml sample was diluted to 25 ml in a volumetric flask using 0.1 N HCl. The absorbance of the resultant was measured at 243 nm on a UV-Visible spectrophotometer (Shimadzu 1601) using 0.1N HCl as a blank.

***In vitro* drug dissolution study**

The United States Pharmacopoeia (USP) rotating-paddle dissolution apparatus (Electro lab, Mumbai, India) was used to study drug release from the paracetamol suspension. Ten ml of the suspension equivalent to 100 mg of paracetamol was measured and suspended in 900 ml of 0.1 N HCl, was stirred at 50 rpm and the temperature was maintained at 37°±0.5°C. 2ml of aliquots were withdrawn at the time intervals of 2 minutes and replaced with the fresh dissolution medium. The aliquots were then filtered the drug content in the

filtrate was determined by using UV-Visible spectrophotometer (Shimadzu1601) at 243 nm against 0.1N HCl as a blank.

Microbiological evaluation

The microbial loads of suspension formulations containing 2.5%w/v of suspending agents were determined according to the BP 2010 method for assessing microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use. This was done on day 0 of formulation and on day 14 following storage under ambient conditions.

Stability study

The prepared formulations were stored in accelerated storage condition of 40 ± 2^0C and 75±5% RH for a period of 90 days and observed for changes in physical appearance, drug content and *in vitro* release profile.

RESULT AND DISCUSSION

In the present study, paracetamol suspensions were prepared by using natural suspending agent such as isolated BFM and its efficiency was compared with standard suspending agent like gum tragacanth.

The mucilage was extracted using solvents such as distilled/demineralised water, hot water, PBS pH 4.0, pH 6.8 and pH 9.2 and the yield of the dry water soluble mucilage was varied depends upon the solvents used. Percent yield of the dry water soluble mucilage was 45%, 60%, 22%, 30% and 35% in distilled/demineralised water, hot water, PBS pH 4.0, PBS pH 6.8, and PBS pH 9.2 respectively. The solvents like distilled/demineralised water, hot water and phosphate buffer pH 9.2 could be used for extraction for better yield

Preformulation Studies of Drug and Excipients

The Preformulation study of drug was carried out by conducting various parameters viz. solubility, melting point and pH determination, and spectral analysis.

Loss on drying

The value of loss on drying was found to be 0.23% which was found to comply with the specification of Pharmacopoeia (NMT 0.5 %) of its weight.

Solubility

The solubility of the pure drug was determined with various solvents. It revealed that it is insoluble in water but soluble in, ethanol, acetone and methanol.

Melting point determination

Melting point of Paracetamol was determined by capillary method. The melting point of Paracetamol was found to be in the range 171-175°C, which complied with BP standards, indicating purity of the drug sample.

Drug-Excipients Compatibility Study

Fourier transform infrared (FTIR) analysis

FTIR spectra were recorded to assess the compatibility of the drugs and excipients. FTIR spectra of drug (s), physical mixture of drug with different excipients, were recorded and examined. FTIR spectra of paracetamol, showed characteristic O-H, N-H, C=O (amide) stretching bands at 3326.98 cm^{-1}, 3413.77 cm^{-1}, 1654.81 cm^{-1}, respectively. Whereas, amide II band, C-N-H group and para-disubstituted aromatic rings at 1560.30 cm^{-1}, 1259.43 cm^{-1} and 837.05 cm^{-1}, respectively were also observed. The observed FTIR spectrum of drug was matched with reference spectra.

All characteristic peaks of drug(s) were observed in the FTIR spectra of physical mixture of drug and different excipients. The results showed no chemical interaction and changes took place in FTIR spectra of the drug and various excipients alone or in combination exhibiting compatibility of the drugs with all excipients. The FTIR spectra of pure drug and physical mixture of drug and different excipients are shown in figure 1 and 2 respectively.

Differential Scanning Calorimetry (DSC)

DSC provides information about the physical properties of the drugs and demonstrates a possible interaction between drug and other excipients used in tablet formulation. Thermal analysis using DSC was carried out on drug (s), physical mixture of drug (s) with different excipients using Shimadzu DSC-60 Thermal Analyzer.

The thermogram of differential scanning calorimetry showed sharp endothermic peaks of paracetamol at 175.97°C, corresponding to the melting range of paracetamol (174-176°C) in the crystalline form. DSC studies were carried out to confirm that no interaction took place between drug and other excipients used in the formulation. According to the thermograms, paracetamol showed sharp endothermic peaks at 175.97°C which corresponds to the melting point of drug in the crystalline form. In the DSC curve of physical mixture drug and different excipients showed the

characteristic peaks of drug. The result showed that drugs were compatible with excipients. It could also be conferred that tablet preparation processes did not change the nature of drugs in tablet. The DSC thermograms of pure drug and physical mixture of different excipients are shown in figure 3 and 4 respectively.

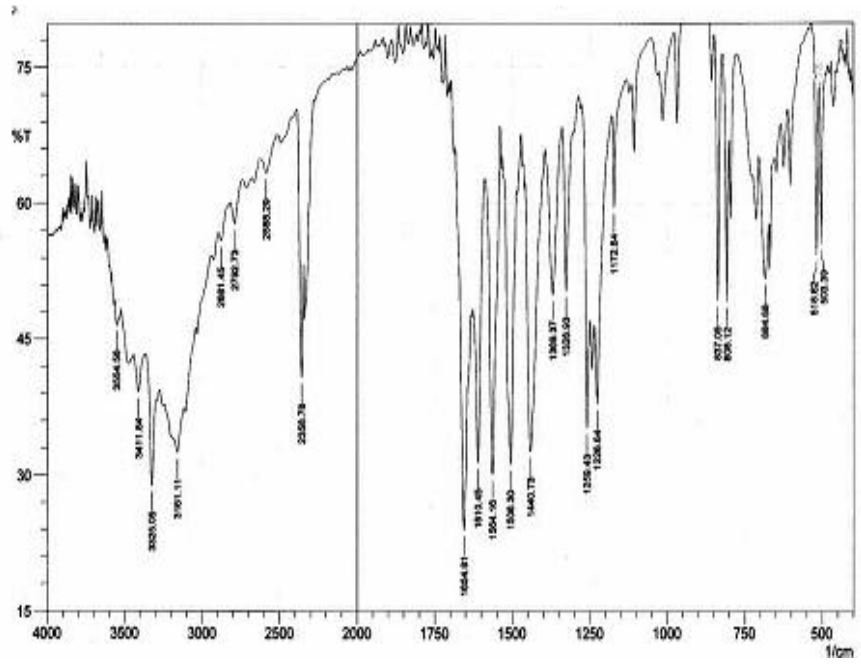


Figure 01: FTIR spectrum of paracetamol

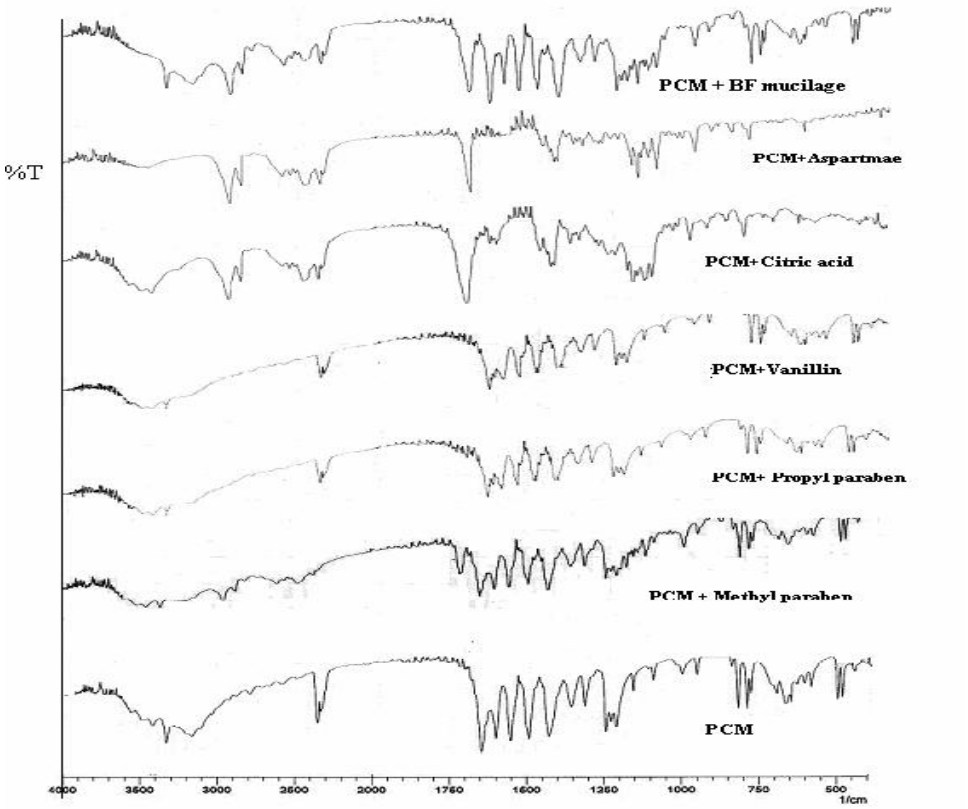


Figure 02: FTIR Spectra of physical mixture of paracetamol and different excipients

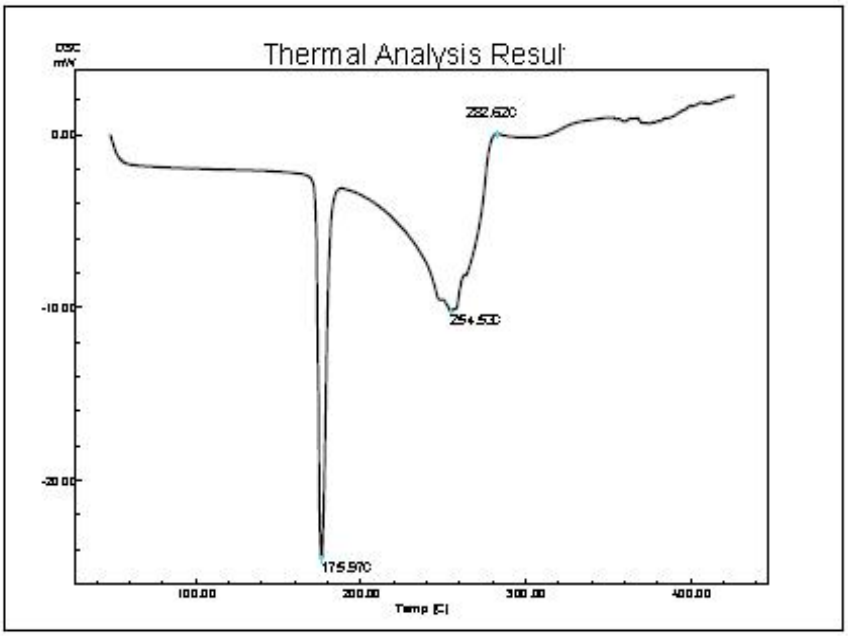


Figure 03: DSC thermogram of paracetamol

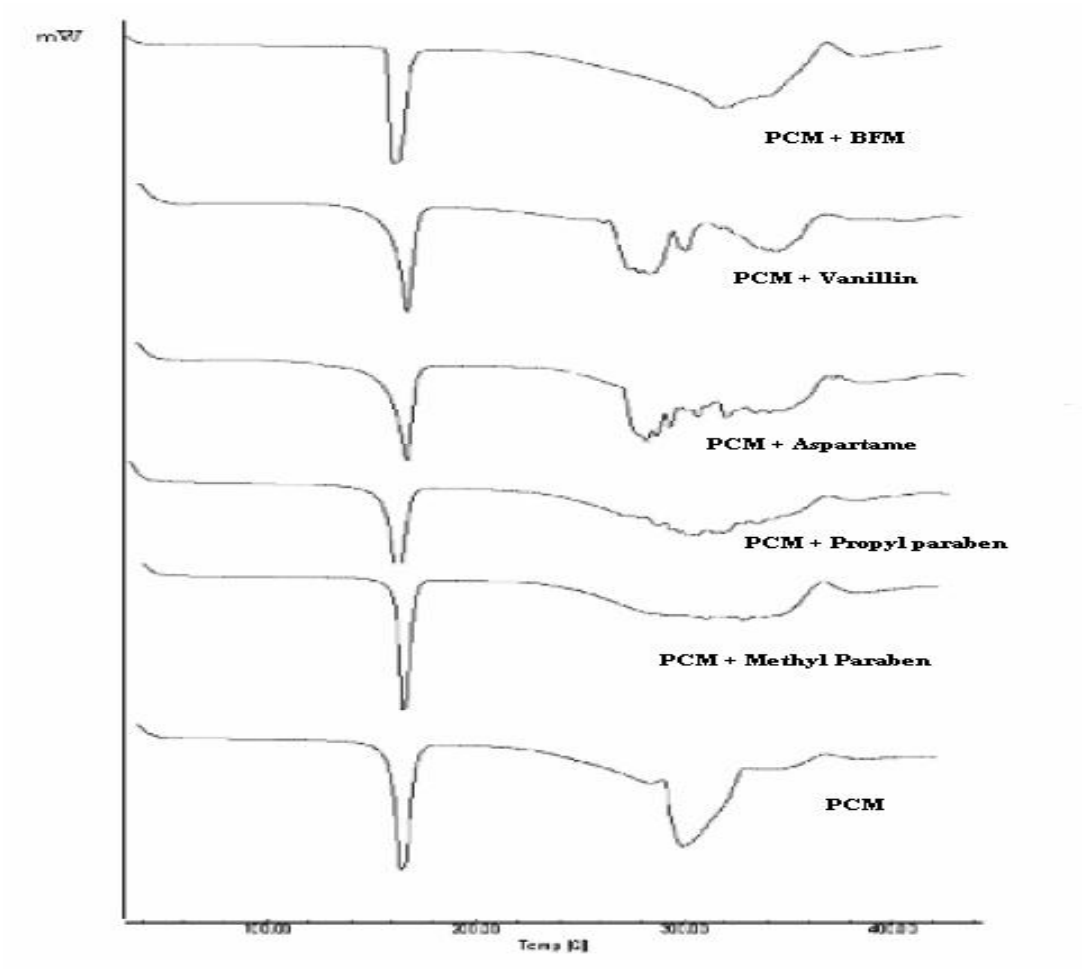


Figure 04: DSC thermogram of physical mixture of paracetamol and excipients

Evaluation of paracetamol suspension

Sedimentation volume

To evaluate the suspending properties of the isolated BFM, paracetamol suspensions were prepared with varying concentration (1.0, 1.5, 2.0 and 2.5% w/v) of the test mucilage, as well as the commonly used suspending agent, like tragacanth. The sedimentation volume (F) of these formulated suspensions prepared with the BFM was compared with the suspension prepared using commonly used suspending agents like tragacanth. The sedimentation volume (F) profile of these suspensions prepared with gum tragacanth and BFM are presented in figure 5 and 6 and in table 2 respectively. The sedimentation volume of the suspensions prepared with BFM is comparable with the suspensions prepared using gum tragacanth. The dispersed particles sediment at a faster rate in suspensions containing 1.0% and 1.5% w/v of suspending agent and the initial sedimentation during first 20 days are much faster than afterwards. The suspensions prepared with 2.0% suspending agent shown a constant decline in sedimentation volume up to 20 days whereas, the decline was minimized after 25 days. However the suspensions prepared with 2.5% suspending agent the change in sedimentation volume was minimum throughout the 45 days of study. BFM was a better suspending agent than gum tragacanth at all concentrations. The ability to suspend particles varies according to the ability of the mucilage to impart viscosity. The sedimentation volume has been used as a measure of flocculation and highly flocculated systems sediment to give large sedimentation volumes. BFM shows its ability to suspend the insoluble drug particles. The suspended dispersed solute in a continuous medium will settle is a matter of time. The characteristics of both the dispersed and the continuous phases are important in the rate and extent of this phenomenon. Dense particles (due to gravity) and, conversely, low viscosity (low resistance to movement of solute) of continuous medium will aid fast settling while less dense and more viscous medium will favor longer suspension of the solute in the medium without segregating.

It is desirable in pharmaceutical suspensions that the solutes are suspended long enough to ensure withdrawal of uniform doses and of such viscosity that the suspension is pourable. It seems that the observed sedimentation volumes of the pediatric suspensions are due more to the characteristics of the dispersion medium than of the active pharmaceutical ingredient.

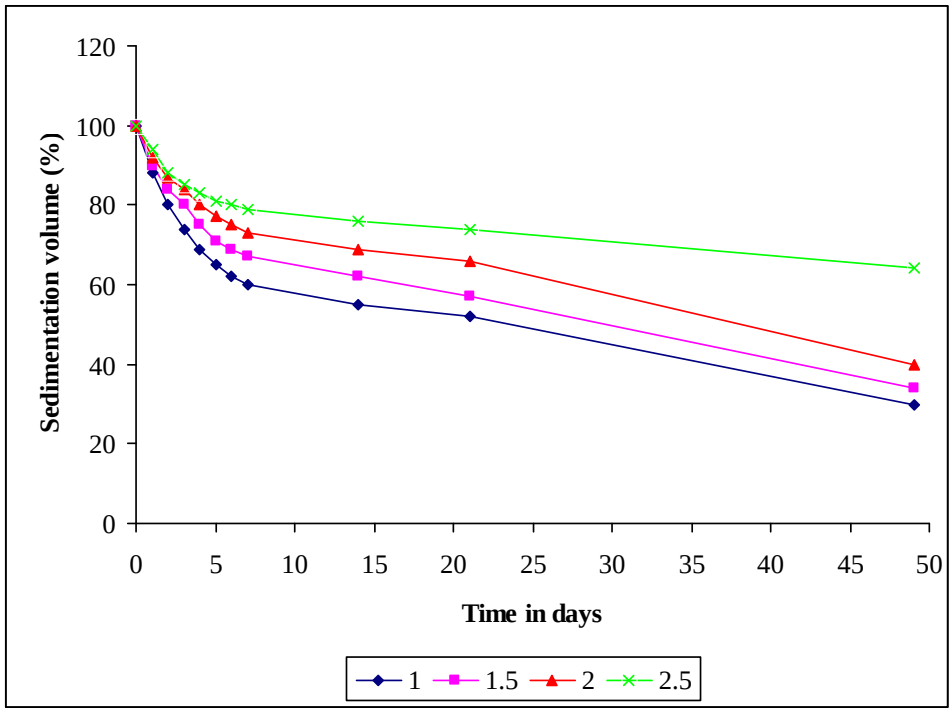


Figure5: Sedimentation profile of paracetamol suspension with different concentrations of gum tragacanth

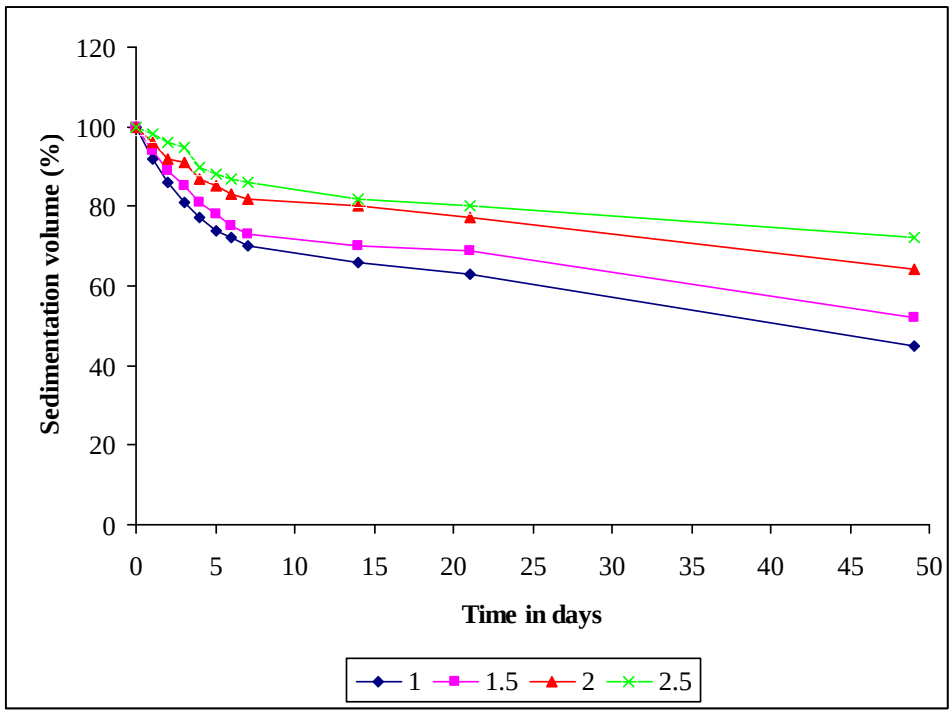


Figure6: Sedimentation profile of paracetamol suspension with different concentrations of BFM.

Table 2: Results of sedimentation volume of different batches of paracetamol suspensions

Time	Tragacanth (%)				BFM (%)			
	1.0	1.5	2.0	2.5	1.0	1.5	2.0	2.5
0 day	100	100	100	100	100	100	100	100
1 day	88	90	92	94	92	94	96	98
2 day	80	84	87	88	86	89	92	96
3 day	74	80	84	85	81	85	91	95
4 day	69	75	80	83	77	81	87	90
5 day	65	71	77	81	74	78	85	88
6 day	62	69	75	80	72	75	83	87
7 day	60	67	73	79	70	73	82	86
14 day	55	62	69	76	66	70	80	82
21 day	52	57	66	74	63	69	77	80
49 day	30	34	40	64	45	52	64	72

Degree of flocculation

The sedimentation volume (F) gives only a qualitative account of flocculation since it lacks a meaningful reference point. The degree of flocculation is more fundamental parameter than F since it relates the volume of flocculated sediment to that in a deflocculated system. The degree of flocculation (β) is more useful parameter, which is the ratio of ultimate sedimentation volume in the flocculated and deflocculated system. The degrees of flocculation (β) were determined for all the formulated suspensions using different concentrations of BFM and the commonly used suspending agent like gum tragacanth. The values of the degrees of flocculation for all formulated suspensions are presented in table 3. The comparative values of the degree of flocculation (β) indicated that the BFM showed higher values when compared to tragacanth. These observations showed that the BFM was a better suspending agent than tragacanth.

Redispersibility

Since the suspension produces sediment on storage it must be readily dispersible so as to ensure the uniformity of the dose. If sediment remains even

after shaking vigorously for specified time, the system is described as caked. While redispersion is one of the recognized quality attributes of pharmaceutical suspensions, its evaluation has been qualitative and subjective. The present effort at quantitative determination was to make the evaluation more objective when comparing a new suspending agent with an established one. Easily redispersed sediments in a suspension allow withdrawal of uniform doses. Patients or their representatives with the same effort will require less time to achieve complete redispersion of paracetamol-containing BFM. The results of redispersibility of suspensions prepared with different suspending agents are shown in table 4. The suspensions with 1.0% w/v tragacanth as suspending agents have caked after 30 days. However, the suspensions with the BFM found to be easily redispersible, irrespective of their concentrations. Suspension prepared using 2.5% BFM and gum tragacanth showed a redispersibility cycle of 6 as compared to 9 of gum tragacanth. The redispersing ability of the suspending agents were in the order of BFM > tragacanth.

Rheology

The viscosity of suspensions is a factor of great importance for stability and pourability of suspensions. Suspensions are the least stable dosage form due to sedimentation and cake formation. As the viscosity of the suspension increases, the terminal settling velocity decreases thus the dispersed phase settles at a slower rate and remains dispersed for a longer time yielding higher stability to the formulated suspension. Viscosity affects the ease with which a suspension is withdrawn for administration. The less viscous suspension tends to pour more easily than the more viscous ones, and the rheological study can help us gain insight into the structure of the system. The majority of the viscosity measurements is made at the quality control level and consists of a single data point using one spindle at one speed, and this is a good bench mark for decision making in a production setting. However, many fluids exhibit a characteristic change in viscosity with a change in applied force that cannot be captured with a single viscosity measurement. The rheological behavior of the suspensions prepared with BFM and gum tragacanth reveal that the suspensions are pseudoplastic in their behavior this implies that with minimum agitation the suspension will be easily redispersed and a stable dose can be withdrawn and their viscosity decreases with increase in shear rate, which is an essential property in the formulation of suspension. Rheology study shows that the suspensions prepared using BFM at 2% had better viscosity compared to the suspensions prepared using gum tragacanth, of all concentrations. The viscosity of the suspensions were in the order of BFM >gum tragacanth. The flow rate was inversely proportional to the viscosity of the suspension and were in order of gum tragacanth >BFM. The results of flow rate and viscosity of paracetamol suspensions were shown in table 5.

Table 3: Results of degree of flocculation (β) values of paracetamol suspension

Suspending agent	Concentration(% w/v)	Degree of flocculation(β)
BFM	1.0	2.11 ± 0.08
	1.5	2.7 ± 0.04
	2.0	4.76 ± 0.49
	2.5	5.33 ± 0.39
Tragacanth	1.0	2.13 ± 0.18
	1.5	3.95 ± 0.26
	2.0	4.00 ± 0.17
	2.5	5.17 ± 0.12

[1]Data was recorded after 49 d keeping at room temperature;
[2]All values are expressed as mean of five observations ± SD

Table 4: Results of redispersibility of different batches of paracetamol suspension

Suspending agents	Concentration	Rate of redispersibility (cycles)			
		5 days	15 days	25 days	30 days
Tragacanth	1.0	8	12	15	caked
	1.5	8	9	13	15
	2.0	5	6	9	10
	2.5	4	5	8	9
BFM	1.0	6	7	9	10
	1.5	5	6	8	9
	2.0	4	5	6	7
	2.5	3	4	5	6

Table 5: Results of flow rate and viscosity of paracetamol suspension

Suspending agent	Concentration (%w/v)	Flow rate (ml s^{-1})	Viscosity (poise)
Tragacanth	1.0	1.16	0.85
	1.5	0.94	0.95
	2.0	0.80	1.10
	2.5	0.66	1.25
BFM	1.0	0.97	1.00
	1.5	0.84	1.10
	2.0	0.73	1.20
	2.5	0.61	1.45

pH and drug content

The pH of the paracetamol suspensions was determined at intervals of one week for 21 days of storage at room temperature using pH meter. The pH of the suspensions made with BFM and gum tragacanth ranged from 7.10 to 8.20 and 7.55 to 8.62 respectively, indicating the basic nature of the suspensions. The variation in the pH of the suspension prepared with BFM was higher as compared with that recorded in the suspensions prepared with tragacanth. The change in pH may be due to hydrolysis or microbial decomposition. The microbial decomposition of the suspension made with BFM seems to be more feasible given their neutral character. The drug content of all the formulations was in the range of 97.1 to 99.6%. The results of pH and drug content of paracetamol suspensions were shown in table 6.

Particle size analysis

For physical stability of suspensions, it is relevant to consider particle size. Particles of small diameter tend to settle slowly while large particles will settle fast. Small particles may form conglomerates and if not flocculated will tend to cake. Suspensions that contain high quantities of solids tend to be more viscous and thixotropic as a result of inter particle interactions. From the particle size distribution data it indicated that the suspension is a coarse dispersion with particle size predominantly lying between 6.03 to 7.58μ**.** The mean particle sizes are in the range for coarse dispersion. The results of particle size analysis of paracetamol suspension containing 2.5% concentration of BFM and gum tragacanth is shown table 7 and 8 respectively.

Crystal growth analysis

Crystal grow in any suspension is an indication of instability as a result of dissolution of smaller particles leading to reduced solubility of the total drug in the suspension and growth of the larger particles. Dosing such suspensions orally will be expected to reduce absorption because of a reduction in dissolution rate. This is of vital importance in storage of suspended products. Large conglomerates, appearing as fluffs due of flocculation in suspensions prepared with tragacanth gum on day 14 were observed while gum particles were seen hydrating on day 1 of formulation. This same occurrence was observed with BFM but no floccules were seen, both suspensions had no crystal growth. Therefore reduction drug absorption is not expected. The Photomicrographs of paracetamol suspensions formulated with 2.5% w/v concentrations of BFM and gum tragacanth on day 1 and day 14 of formulation are presented in figure 7.

Microbiological evaluation

The ability of BFM powder to increase solution viscosity accounts for their use as suspending agents in oral pharmaceutical suspensions. However, natural mucilage can show a gradual reduction in the viscosity of their dispersions or solutions with age due to bacterial or mould growth. In the initial batches of paracetamol suspension containing BFM and gum tragacanth the preservatives were not included in the formulation.The microbial load and/or growth in suspension formulations containing 2.5%w/v of suspending agent was evaluated on day 0 and on day 14 of storage and the results are presented in table 9. Microbial growth evident by colony forming units or cloudiness and darkening of the nutrient agar is indicative of positive result while a clear and transparent nutrient agar is indicative of a negative result. The results show that all the suspension formulations contain some degree of microbial contamination from day 0 of formulation. After 14 days the probable number of bacteria per ml of formulation increased for each suspension formulation indicating microbial growth and multiplication. This result provides an explanation for reduction in viscosity of the suspension formulations after storage for 14 days. Consequently in the formulation of oral pharmaceutical suspensions containing BFM will need to be preserved similar to the requirements of the other agents tested. Hence it was decided to incorporate preservatives in the formulation of paracetamol suspensions containing BFM and gum tragacanth as they found to support the microbial growth on long term storage.

***In vitro* drug dissolution studies**

The release rate of paracetamol was instantaneous for suspensions using the two test suspending agents. It has been observed that 100% drug was released within 25 minutes in case of the suspension containing BFM at 2.5%w/v. The release profile with suspensions of tragacanth gum was gradual. All suspensions containing the two test suspending agents released more than 70% of their drug content which meets Indian Pharmacopoeia specifications. The Percentage drug release profile of paracetamol suspensions formulated with 2.5% w/v of BFM and gum tragacanth are shown in figure 8 and table 10 and 11 respectively.

Table 6: Results of pH and drug content of paracetamol suspension

Suspending agents	Concentration	pH after storage for				Drug content (%)
		0^{th} day	7^{th} day	14^{th} day	21^{th} day	
Tragacanth	1.0	7.96	7.83	7.70	7.55	97.1
	1.5	8.37	8.22	8.09	7.94	98.7
	2.0	8.7	8.93	8.41	8.28	99.2
	2.5	9.2	8.92	8.79	8.62	99.6
BFM	1.0	7.46	7.28	7.20	7.10	98.4
	1.5	7.68	7.58	7.45	7.37	99.3
	2.0	8.18	8.02	7.95	7.88	98.2
	2.5	8.55	8.48	8.36	8.20	99.16

Table 7: Results of particle size analysis of paracetamol suspension (F4)

Sl.No.	Size range		Mid value (d)	No. of particles (n)	% no. of particles	nxd
	No. of EPM division	«m				
1.	1-1	3.6-7.2	5.4	173	86.5	934.2
2.	2-3	7.2-10.8	9	21	10.5	189
3.	3-4	10.8-14.4	12.6	4	2	50.4
4.	4-5	14.4-18	16.2	2	1	32.4

*EPM: Eye piece micrometer $\sum n=200$ $\sum nd= 1206$

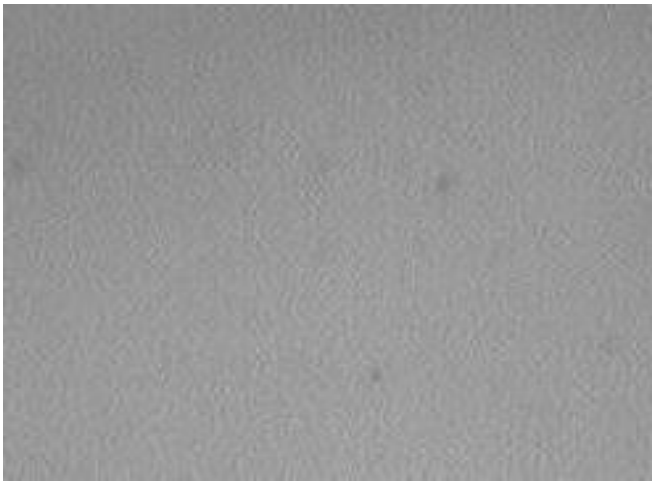
Average diameter (d_{av}) = $\sum nd/\sum n$ = 1206/200 = **6.03«**

Table 8: Results of particle size analysis of paracetamol suspension (F8)

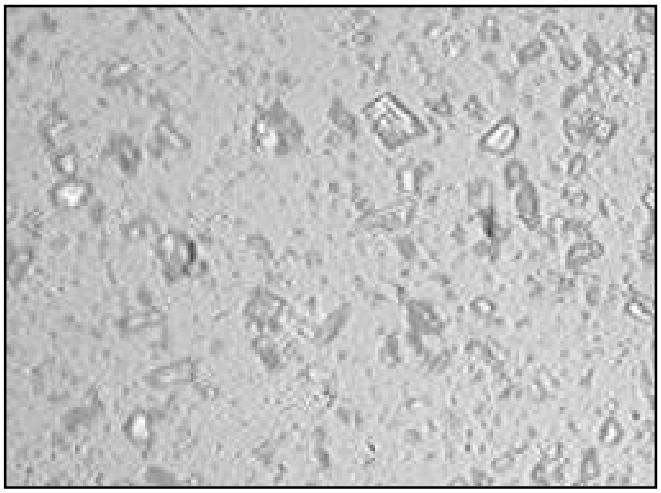
Sl.No.	Size range		Mid value (d)	No. of particles (n)	% no. of particles	nxd
	No. of EPM division	«m				
1.	1-1	3.6-7.2	5.4	98	49	529.2
2.	2-3	7.2-10.8	9	92	46	828
3.	3-4	10.8-14.4	12.6	4	2	62.4
4.	4-5	14.4-18	16.2	6	2	97.2
				∑n=200	∑nd= 1516	

Average diameter (d_{av}) = ∑nd/∑n = 1516/200 = **7.58«**

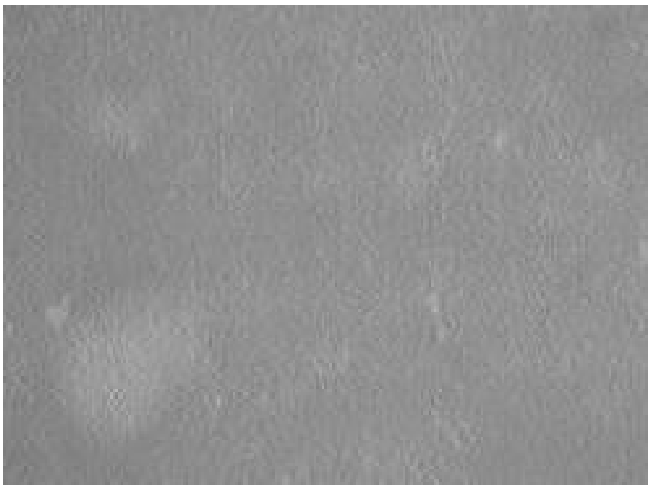
F8 on day 1



F8 on day 14



F4 on day 1



F4 on day 14

Figure7: Photomicrographs of paracetamol suspensions formulated with 2.5% concentration of BFM and gum tragacanth

Table 9: Microbial evaluation of paracetamol suspension

Formulation	**Results for each quantity of suspension formulation at 0th day**			**Probable number of bacteria per ml of formulation**
	0.1 ml	0.01 ml	0.001 ml	
BFM	+	-	-	$<10^2$ and >10
Gum tragacanth	+	+	-	$<10^3$ and $>10^2$
Formulation	**Results for each quantity of suspension formulation at 14th day**			**Probable number of bacteria per ml of formulation**
	0.1 ml	0.01 ml	0.001 ml	
BFM	+	+	+	$>10^3$
Gum tragacanth	+	+	+	$>10^3$

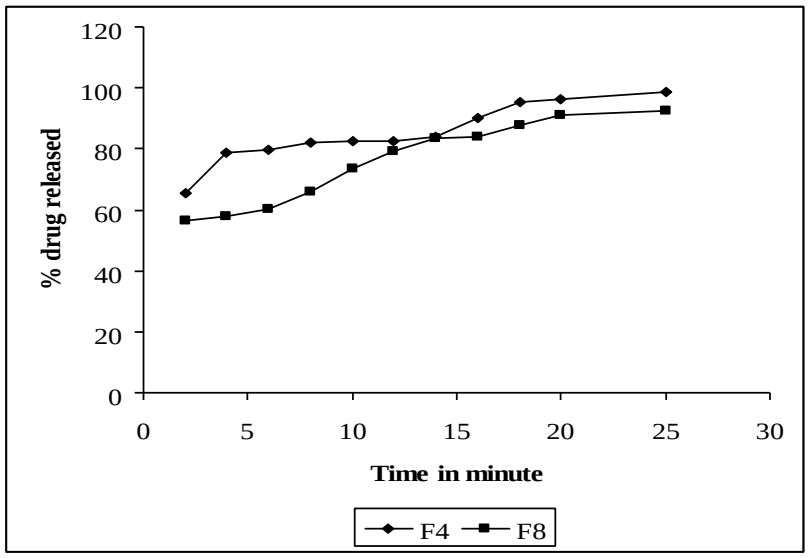


Figure 8: *In vitro* dissolution profile of paracetamol suspensions formulated with 2.5% w/v of BFM and gum tragacanth

Table 10: Dissolution data for paracetamol suspension with 2.5% of BFM (F4)

Time (min)	**Absorbance**	**Concentration (mcg/ml) 'C'**	**Amount of drug released Cx900xDF/1000**	**% drug released**
2	0.3791	7.25	65.28	65.28
4	0.4588	8.73	78.6	78.6
6	0.4628	8.87	79.8	79.8
8	0.4752	9.11	82.1	82.1
10	0.4777	9.16	82.4	82.4
12	0.4795	9.19	82.7	82.7
14	0.4864	9.32	83.9	83.9
16	0.5221	10.01	90.1	90.1
18	0.5508	10.59	95.1	95.1
20	0.5588	10.72	96.5	96.5
25	0.5708	10.96	98.6	98.6

Table 11: Dissolution data for paracetamol suspension with 2.5% w/v of tragacanth (F4)

Time (min)	Absorbance	Concentration (mcg/ml) 'C'	Amount of drug released Cx900xDF/1000	% drug released
2	0.3312	6.26	56.35	56.35
4	0.3353	6.40	57.65	57.65
6	0.3502	6.69	60.25	60.25
8	0.3821	7.31	65.80	65.80
10	0.4259	8.15	73.43	73.43
12	0.4586	8.79	79.11	79.11
14	0.4828	9.25	83.33	83.33
16	0.4850	9.30	83.72	83.72
18	0.5078	9.74	87.68	87.68
20	0.5275	10.12	91.11	91.11
25	0.5347	10.26	92.37	92.37

Stability study

To check the stability of the prepared formulations at elevated conditions, they were stored in accelerated storage condition of 40 $\pm 2^0$ C and 75 ±5% RH for a period of 90 days and observed for changes in physical appearance, pH, viscosity, drug content and *in vitro* release. From the data on the viscosity, pH, physical appearance and drug content of the suspension which was evaluated during the stability studies indicated that there are no considerable changes in these parameters. The suspensions were stable in accelerated storage conditions and the drug release does not vary to larger extent during the period of study. The results of stability studies of paracetamol suspension are shown in table 12 and figure 9.

Table12: Results of stability studies on optimized batches of paracetamol suspension (F4 and F8) at 40±2^{0}C/75±5%RH

Formulation	Temperature/ humidity	days	color	odor	taste	pH	Viscosity	Drug content
F4	40 $\pm 2^0$C and 75±5% RH	0^{th} day	NC*	NC	NC	8.55	1.45	99.16
		30^{th} day	NC	NC	NC	8.48	1.42	99.18
		60^{th} day	NC	NC	NC	8.21	1.36	99.0
		90^{th} day	NC	NC	NC	8.15	1.30	98.7
F8	40 $\pm 2^0$C and 75±5% RH	0^{th} day	NC	NC	NC	9.20	1.25	99.6
		30^{th} day	NC	NC	NC	9.08	1.22	99.01
		60^{th} day	NC	NC	NC	9.01	1.15	98.96
		90^{th} day	NC	NC	NC	8.9	1.10	98.85

NC* = No change

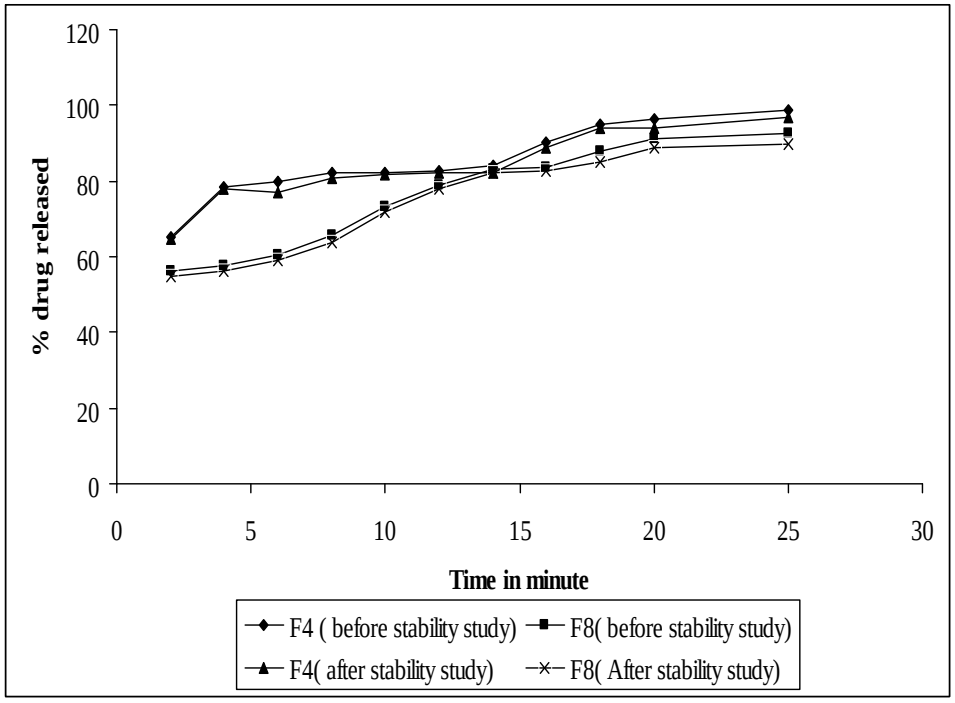


Figure 9: Comparison of drug release profile of optimized batches of paracetamol suspension after stability study

CONCLUSION

The extracted mucilage from *Borassus flabellifer* endosperm is non toxic and edible. The present study was carried out to check the suspending property of *Borassus flabellifer* mucilage. paracetamol was selected as model drug and BFM was used as suspending agent in different concentration of 1%, 1.5%, 2.0 and 2.5% w/v. The present study indicates that *Borassus flabellifer* mucilage appeared to exhibit the best suspendability for paracetamol suspensions, compared with gum tragacanth. The results showed that sedimentation volume, viscosity and particle size were directly proportional to the concentration of the suspending agents. The reverse case was observed with the flow rate. The present study reveals that BFM at 2.5%w/v (F4) concentration has excellent suspending properties in paracetamol suspension formulations, compared to the traditionally used gum tragacanth as it produced a stable, redispersible, flocculated suspension with all the features of an ideal suspension. Hence by considering, all above evaluation parameters it can be concluded that BFM used in the concentration of 2.5% w/v was proving as good suspending agent. Due its high viscosity it could be employed as stabilizer and thickener of choice in pharmaceutical suspension preparation and also in cosmetic, pharmaceutical and food industries. BFM may provide a suitable alternative to gum tragacanth as suspending agent in pharmaceutical oral suspensions, providing a more readily available and affordable option in the countries where it is found growing abundantly, wild or cultivated. Thus, it can be concluded that the extracted mucilage from *Borassus flabellifer* endosperm has the potential of a suspending agent and it can be used as a pharmaceutical adjuvant.

REFERENCES

1. Bummer P.M., Remington: The Science and Practice of Pharmacy, Philadelphia College of Pharmacy and Science, 2005, 21st ed., 280.
2. Martin A., Swarbrick J., Cammarata A., Physical Pharmacy, Lea and Febiger, Philadelphia and London, 1993, 4th ed., 477-511.
3. Banker S.G., Rhodes C.T., Modern Pharmaceutics, Marcel Dekker, Inc., New York, 1998, 3rd ed., 311.
4. Billany MR. Suspensions and emulsions. In: Aulton, ME, editor. Pharmaceutics: the design and manufacture of medicines. 3rd ed.

Philadelphia: Churchill Livingstone; 2007, 383-405.
5. Siahi MR, Barzegar-Jalali M, Monajjemzadeh F, Ghaffari F, Azarmi S. Design and evaluation of 1- and 3-Layer matrices of verapamil hydrochloride for sustaining its release. AAPS PharmSciTech 2005; 6 (4): E626-32.
6. Trease G.E. and Evans W.C., Pharmacognosy, Saunders, 2008, 15th ed., 206.
7. Shirwaikar A.A., Prabu L.S., Mahalaxmi R., Rajendran K., Studies of disintegrant properties of seed mucilage of *Ocimum gratissimum*, Ind. J. Pharm. Sci, 2007, 753-758.
8. Pullaiah T., Flora of Andhra Pradesh (India), Vol-III, Scientific Publishers, Jodhpur, 1997,1015.
9. Nadkarni KM., Indian Materia Medica, Vol-1, Popular Prakashan, Bombay, 2002,209,210.
10. Morton JF., Notes on Distribution, Propagation and Products of Borassus Palms (Arecaceae) Economic Botany,1988, 42(3), 420-41.
11. Masayuki Y. Fengming Xu., New Spirostane-Type Steroid Saponins with antidiabetogenic Activity from *Borassus flabellifer*, Chem Pharm Bull, 2007,55(2),308-16.
12. Kapoor LD., Hand Book of Ayurvedic Medicinal Plants, CRC Press, Newyork**,** 2005, 82.
13. Kokate C.K., Practical Pharmacognosy, 4th edition, Vallabh Publication, Delhi, 2006, 123.
14. Indian Pharmacopoeia, the Indian Pharmacopoeia Commission, Ghaziabad. 2007.
15. Martin Alfred., Physical Pharmacy, 4th ed, Maryland, USA: Lippincott Williams & Wilkins, 1991, 423.
16. Knudsen L.F., Curtiss J.M. The use of the angular formulation in biological assays, J Am Stat Soc, 1947, 42:282-96.

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