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Evaluation of *Abelmoschus Esculentus* Mucilage as Suspending Agent in Paracetamol Suspension

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ABSTRACT : Some excipients are currently available for the formulation of Pharmaceutical suspensions. The purpose of this study is to search for a cheap and effective natural excipient that can be used as an effective alternative for the formulation of pharmaceutical suspensions. The mucilage from the pods of *Abelmoschus esculentus* was subjected to preformulation study for evaluation of its safety and suitability for use as suspending agent. The mucilage extracted is devoid of toxicity. Suspensions of paracetamol were prepared and compared with different concentrations (1%, 2%, 3% and 4% w/v) of *Abelmoschus esculentus* mucilage, sodium CMC and tragacanth gum. Their sedimentation profile, redispersibility, degree of flocculation and rheolgical behavior were compared. The mucilage was found to be a superior suspending agent than tragacanth and is comparable to sodium CMC. Studies indicate that the mucilage of *Abelmoschus esculentus* may be used as a pharmaceutical adjuvant and as a suspending agent at 4%w/v, depending on its suspending ability and the stability of the resulting suspension.

KEYWORDS: *Abelmoschus esculentus*, suspending agents, sedimentation volume, rheology, degree of flocculation.

INTRODUCTION

A pharmaceutical suspension, like other disperse systems, is thermodynamically unstable, thus, making it necessary to include in the dosage form, a stabilizer or suspending agent which reduces the rate of settling and permits easy redispersion of any settled particulate matter both by protective colloidal action and by increasing the consistency of the suspending medium[1-3]. Suspending agents are(i) inorganic materials, (ii) synthetic compounds, or (iii) polysaccharides. Natural gums like Acacia, Tragacanth, Khaya, Karaya and *Abelmoschus esculentus* belong to the latter group[4]. Gums have been wildly used as tablet binders, emulgents and thickeners in cosmetics and suspensions as film-forming agents and transitional colloids.

Gums are widely employed in the pharmacy as thickeners, suspending agents, emulsifying agents, binders and film formers. With the increase in demand for natural gums, it has been necessary to explore the newer sources of gums to meet the industrial demands. India, due to its geographical and environmental positioning has traditionally been a good source for such products among the Asian countries[5]. There are reports about the successful use of *Ocimum gratissimum*, *Butea monospermama, Albizia zygia gum* and *Leucaena leucocephala* seed gum as suspending agent[6-9].

Gum of the *Abelmoschus esculentus* pods has been reported to have binder potential for tablet formulations[10]. The fresh fruits of *Abelmoschus esculentus* (L.) are a common component of Indian diet. In addition, the plant has been used medicinally in treatment of several disorders[11-12]. Anti-cancer, anti-microbial and hypoglycemic activities of plant are reported[13-14]. The anti-ulcer activity of fresh fruits is recently reported[15]. This is a coarse, erect, branched, more or less hairy, annual herb 0.6 to 1.5 meters in height, which is grown widely in India. The only published work so for on the potential application of this gum as a binder in tablet formulation was on sodium salicylates, a highly water soluble drug that is no longer in therapeutic usage. The present work is an attempt to extract and investigate the pharmaceutical properties of the gum to assess its suitability as a suspending agent in the pharmaceutical formulation. Suspending ability and suspension stability were used as the basis for evaluating the performance of *Abelmoschus esculentus* mucilage as a suspending agent.

MATERIALS AND METHODS

Paracetamol (Sunij Pharmaceuticals, Ahmedabad), Gum tragacanth, sodium CMC (Loba Chemie, Mumbai), was procured from the open market. All the other solvents, reagents used were of Pharmacopoeial and analytical grade. *Abelmoschus esculentus* fruits were purchased from local market. Immature pod were selected because they contain more content of mucilage compared to matured fruits.

Extraction of the Mucilage

About 2kg of fresh immature fruit of *Abelmoschus esculentus* were purchased from a local market. After removal of the seeds, the fresh immature fruits were sliced, homogenized and extracted with cold water containing 1% (w/v) sodium metabisulphate. The crude mucilage was centrifuged at 3000 rpm for 5 min and the gum was precipitated from the supernatant with acetone. The precipitated gum was washed several times with acetone; the obtained cream coloured product was dried under vacuum in a desiccator. A light brown coloured powder was obtained after complete removal of moisture. The dried gum was pulverized using end runner mill and screened through a 0.25 mm stainless steel sieve. This was stored in a well closed amber colored specimen bottle till ready for use. The yield of crude *Abelmoschus esculentus* mucilage was 10 g /kg immature fruits.

Phytochemical Examination

Preliminary tests were performed to confirm the nature of mucilage obtained. The chemical tests that were conducted are: Ruthenium red test, Molisch test, test for reducing sugars and Ninhydrin test[16].

valuation of Toxicity

Toxicity studies were carried out according to the method of Knudsen and Curtis[17]. The animals used in the toxicity studies were sanctioned by the Institute animal Ethics Committee. The male albino rats of Wistar strain weighing 160-200 gm were divided into different groups comprising of six animals each. The control group received normal saline 20ml/kg i.p. The other groups received 500, 1000, 2000, 3000 and 4000 mg/kg of gum suspension in normal saline orally. The animals were observed continuously for the behavioral changes for the first 4 hours and then observed for mortality if any for 48 hours. Since no mortality, no toxic manifestations were observed and behavioural pattern was unaffected. In chronic toxicity studies, 12 animals were used, divided in to two groups, 6 as control and 16 as test animals. In the test group a dose of 250 mg/kg was administered daily for a period of 30 d. body weights were recorded for both the groups at an interval of 10d. And at the end, hematological parameters were studied in both the groups.

Preparation of Paracetamol Suspension

Compound tragacanth powder (1.0 g) and 10 g of paracetamol were triturated together with 20 ml of Raspberry syrup to form a smooth paste. Benzoic acid solution (2 ml) and 1ml of amaranth solution were added gradually with constant stirring and then mixed with 50 ml of chloroform water double strength. The mixture was transferred into a 100 ml amber bottle, made up to volume with distilled water and then shaken vigorously for 2 min (thus making 1.0 %w/v of the gum in the preparation). The procedure was repeated using 2.0, 3.0 and 4.0%w/v of tragacanth powder. The above procedure was repeated with sodium CMC and *Abelmoschus esculentus* mucilage. All the suspensions were deflocculated. To determine the degree of flocculation, flocculated suspensions were made using potassium dihydrogenphosphate (0.004 mol) as flocculating agent.

EVALUATION OF SUSPENSION

Rate of separation

The rate of separation of the suspensions were determined by keeping 50 ml portion of each suspensions in stoppered measuring cylinder and stored undisturbed at room temperature. The separation of clear liquid was noted at intervals of 5 d up to 45 d.

The sedimentation volume, F (%), was then calculated using the following equation[18]

F = 100Vu/Vo

Where Vu is the ultimate volume of the sediment and Vo is the original volume of the suspension.

Degree of Flocculation

The degree of flocculation was determined[19] following the equation $\beta = F/F\alpha$, where F is the ultimate sedimentation volume in the flocculated suspension and $F\alpha$ is the ultimate sedimentation volume in the deflocculated suspension.

Redispersion

Fixed volume of each suspension (50 ml) was kept in calibrated tubes which were stored at room temperature for various time intervals (5d, 10…45 d). At regular interval of 5 d, one tube was removed and shaken vigorously to redistribute the sediment and the presence of deposit if any was recorded.

Rheological Study

The rheological behavior of the suspensions prepared with gum tragacanth, sodium CMC and Abelmoschus esculentus mucilage were studied using Brookfield synchroelectric viscometer, spindle number 1 of low viscosity type with gear speed ranging 0.3 to 0.6 rpm. The dial readings for both up-curve and down curve were recorded and the experiment was repeated for three times. Using these observations, the rate of shear was calculated. The results were recorded and the rheogram were obtained by plotting rate of shear, G/sec vs shearing stress F, Dyne/cm^2.

Particle Size Analysis

After shaking, 10 ml of each sample was separately transferred into 200 ml cylinder. Distilled water (150 ml) was then added, mixed, and 10 ml aliquot was removed at a distance of 10 cm below the surface of the mixture and at 1, 5, 10, 15, 20, 25 and 30 min. This was transferred into an evaporating dish and evaporated to dryness in an oven at 105 oC and the residue weighed. The particle diameter (d in cm) was then calculated using the Stokes equation14:

$$d = \frac{18hh}{(\rho s - \rho_0)\, gt}$$

Where h is the distance of fall of the particle (cm), t is the time (s), h is the viscosity of the dispersion medium (poise), $\rho s - \rho_0$ is the density gradient between the dispersed particles and the liquid (g cm^{-3}) and g is the gravitational constant (cms^{-2}).

Determination of the pH of the suspensions

The pH of each of the prepared suspension was measured using pH meter (Systronics Digital pH meter. Sr.No.272,μ pH system 361), at weekly intervals for 4w. for ease of redispersibility , 10 ml of each suspension was poured into four calibrated tubes, which were stored at room temperature for 1,2,3 and 4 w. at the end of each storage period, each tube was shaken at constant moderate rate of 30 shake/min. the time (s) taken to redisperse the sedimented suspension was recorded.

The method essentially consisted of holding the sample tube straight in upright position between two fingers with thumb at the bottom and the middle finger at the top followed by the almost uniform rotation through 180^0 and brought back through the same path. The pair of successive upward and downward movement each of approximately equal force, constituted one complete shake. The number of shakes required for complete elimination of sediment from the bottom of the tube was recorded. At this juncture the sample was observed for homogeneity of the suspension and the total time(s) recorded to redisperse the sedimented suspension. This was based on the empirical understanding that not more than that force should be required and the same that is routinely applied by the consumer in the event of "shake well before use" maximum care was taken to exert approximately the same amount of force every time and the same time interval.

RESULTS AND DISCUSSION

The average yield of dried mucilage obtained from *Abelmoschus esculentus* fruit was 10g/kg immature fruits. The mucilage obtained was subjected to physicochemical characteristics the results of which are summarized in table 1.

Phytochemical tests carried out on *Abelmoschus esculentus* mucilage confirmed the absence of alkaloids, glycosides and tannins. On treatment of mucilage with ruthenium red, it showed red colour confirming the obtained product 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 could not reduce Fehling's solution, so the sugars present were non reducing sugars. It reduced Fehling's solution after hydrolysis for 1hr with concentrated sulfuric acid under reflux. Mucilage on treating with ninhydrin reagent does not give purple colouration indicating the absence of amino acids. The results of phytochemical screening of mucilage are summarized in table 2.

To determine the safety level of the extracted *Abelmoschus esculentus* mucilage, acute toxicity and chronic toxicity studies were carried out. In both toxicity study of the gum revealed no behavioral changes for first four hours and no mortality, no toxic syndromes were observed even at the dose level 4000mg/kg body weight after 24 hours, indicating the safety of the gum. To assess the suitability of gum for the oral delivery we have recorded the body weight profile for the animals during the chronic toxicities at regular intervals of 10 d. it was found that the body weight of both test and control and rate of increase were also comparable. Hence it is concluded that chronic administration of the gum might not influence either the food intake or growth. Hematological parameters that were determined at the end of 30 d of continuous administration were also found to be comparable to that of control rat. The effect of *Abelmoschus esculentus* mucilage on hematological parameters is summarized in table3.

The effective concentration of the suspending agent in the conventional dosage from normally does not exceed 2% of the formulation, which is approximately 5-10 mg/kg dose. Hence this excipient is not likely to exert any toxic effect on the body.

It is quite understand that the better is the suspending medium the lesser the rate of sedimentation. Suspensions are routinely evaluated for their rate of separation which indicates its suspending property. To evaluate the suspending properties of the gum, suspensions were prepared with fixed concentration of paracetamol but with varying concentration of test mucilage (1.0 to 4.0%w/v) as well as the traditional suspending agents such as tragacanth and sodium CMC. The sedimentation volume profile of the suspensions prepared with sodium CMC, *Abelmoschus esculentus* mucilage and gum tragacanth are shown in figure 1, 2 and 3 respectively. Here *Abelmoschus esculentus* mucilage shows its superiority over tragacanth. *Abelmoschus esculentus* mucilage showed a comparable result to that of sodium CMC.

The dispersed particles sediment at a faster rate in suspensions containing 1% and 2% w/v of suspending agent and the initial sedimentation during first 20 days are much faster than afterwards. The suspensions prepared with 3% 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 4% suspending agent the change in sedimentation volume was minimum throughout the 45 days of study.

According Martin *etal.*[18] the sedimentation volume provides only a qualitative account of flocculation. The degree of flocculation (β) is more useful parameter, which is the ratio of ultimate sedimentation volume in the flocculated and deflocculated system. A comparison of β values (Table 4) of suspensions prepared with *Abelmoschus esculentus* mucilage, gum tragacanth and sodium CMC shows a slight higher values at the 3 and 4% w/v level for *Abelmoschus esculentus* mucilage and tragacanth. These observations show that *Abelmoschus esculentus mucilage* is a better suspending agent than Sodium CMC.

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. The suspension with 1 and 2%w/v tragacanth have shown to be caked after 25 and 35 days respectively. Indicating its effectiveness as suspending agent at this concentration. However the suspensions with sodium CMC and *Abelmoschus esculentus* mucilage were found to be redispersible irrespective of their concentration.

The rheological behavior of the suspensions prepared with *Abelmoschus esculentus* mucilage, gum tragacanth and sodium CMC reveal that the suspensions are pseudoplastic in their behavior and their viscosity decreases with increase in shear rate, which is an essential property in the formulation of suspension.

The change in the pH of suspensions prepared with different percentages of *Abelmoschus esculentus mucilage*, sodium CMC and *gum* tragacanth were recorded after 24 and then weekly up to 4 w of storage at room temperature. The pH of the suspensions made with *Abelmoschus esculentus mucilage* and gum tragacanth ranged from 4.50 to 5.25 and 4.10 to 4.45 respectively at concentration levels under consideration (4%w/v), thus indicating the acidic nature of the suspensions. The variation in the pH of the suspension prepared with *Abelmoschus esculentus mucilage* were 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 *Abelmoschus esculentus* mucilage seems to be more feasible given their neutral character.

The prepared suspensions were also assessed based on the viscosity, flow rate and particle size analysis. The results showed that viscosity and particle size were found to be directly proportional to the concentration of the suspending agents. The reverse was the case for the flow rate. All the formulations were observed to obey the Stoke's law when subjected to particle size analysis. The results are given in table 5.

CONCLUSION

The extracted mucilage of *Abelmoschus esculentus* is non toxic, has the potential as a suspending agent even at lower concentration (4%w/v) and can be used as a pharmaceutical adjuvant. In view of these properties, mucilage of *Abelmoschus esculentus* can be employed as stabilizer and thickener of choice when high viscosity is desired especially in cosmetic, pharmaceutical and food industries. The binding properties of the gum in tablets are being studied.

FUTURE PERSPECTIVES

The present investigation is a primary platform to indicate the suitability of *Abelmoschus esculentus* mucilage as a suspending agent. The work can further be extended for evaluation of its suitability as disintegrating agent, gelling agent, emulsifying agent and other similar pharmaceutical applications considering the easy and ample availability of the plant. The work can go a long way to evaluate herbal pharmaceutical excipients.

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Table 1: Physicochemical characterization of mucilage of *Abelmoschus esculentus*

Parameters	Observation
Solubility	Slightly soluble in water, practically insoluble in alcohol, chloroform and acetone. Forms thick gel in water.
pH (1% w/v solution)	5.5
Loss on drying	2.5%
Ash value	5.68%
Water soluble ash	4.3%
Acid insoluble ash	1.0%
Sulphated ash	4.03%

Parameters	Observation
Test for foreign matter	Less than 0.1%
Test for arsenic	Less than 1ppm
Swelling ratio	
In water	8.0
In 0.1 N HCl	5.0
In phosphate Buffer 7.4	6.0
True density	1.8g/dl
Bulk density	0.48 g/cc
Tapped density	0.56 g/cc
Compressibity index	16.33%
Hausner ratio	0.15
Description	Powder: light brown coloured granular powder
Angle of repose	23.25

Table 2: Phytochemical screening of mucilage of *Abelmoschus esculentus*

	Tests	Observation
1.	Test for Carbohydrates(Molisch's test)	+
2.	Test for Tannins(Ferric chloride test)	-
3.	Test for proteins (Ninhydrin test)	-
4.	Test for alkaloids (Wagner's test)	-
5.	Test for glycosides(Keller – Killaini test)	-
6.	Test for mucilage (Ruthenium red test)	+
7.	Test for flavonoids (Shinoda test)	-
8.	Test for reducing sugar (Felhing's test)	-
9.	Mounted in 95% alcohol	Transparent angular masses under microscope
10.	Mounting in the iodine	No blue colored particles (starch absent)
11.	Test with cupric –tartaric solution	Red precipitate is produced
12.	Warming with 5M sodium hydroxide	A brown color is produced
13.	Test for chlorides(silver nitrate test)	-ve
14.	Test for sulphates (barium chloride test)	-ve

Table3:Hematological values of male rats receiving mucilage of *Abelmoschus esculentus* for one month

Parameters	Mucilage treated	Control
RBC($\times 10^6$cells/mm^3)	9.42±0.43	9.36±0.50
WBC ($\times$103 cells/mm^3)	4.68±1.08	4.65±0.98
Hemoglobin (g/dl)	16.32±0.88	16.29±0.55
Platelet ($\times 10^3$ cells/mm^3)	959±95	956±120
Neutrophil (%)	18.39±4.58	18.26±5.62
Eosinophil (%)	1.60±0.58	1.57±0.58
Lymphocyte (%)	65.33±9.11	64.90±6.21
Monocyte (%)	10.70±4.67	10.65±4.59
Basophil (%)	4.44±2.13	4.16±2
Hematocrit (%)	51.13±2.77	50.64±2.28
MCV (µm3/red cell)	58.77±1.60	57.93±2.21
MCH (pg/red cell)	19.67±0.63	19.23±0.51
MCHC (g/dl RBC)	32.72±0.46	32.57±0.37

Table 4: Degree of flocculation (β) of various suspending agents[1].

Suspending agent	Concentration(% w/v)	Degree of flocculation(β)[2]
Sodium CMC	1	2.13 $\pm$ 0.18
	2	3.23 $\pm$ 0.29
	3	3.95 $\pm$ 0.36
	4	4.00 $\pm$ 0.19
mucilage of *Abelmoschus esculentus*	1	1.90 $\pm$ 0.09
	2	2.9 $\pm$ 0.08
	3	4.56 $\pm$ 0.39
	4	5.23 $\pm$ 0.19
Tragacanth	1	2.03 $\pm$ 0.14
	2	2.35 $\pm$ 0.04
	3	4.41 $\pm$ 0.35
	4	5.15 $\pm$ 0.15

[1]Data was recorded after 45 d keeping at room temperature; [2]All values are expressed as mean of three observations $\pm$ SD

Table 5: Effects of the type and concentration of suspending agents on the flow rate and viscosity of paracetamol suspensions.

Suspending agent	Concentration (%w/v)	Flow rate (ml s^{-1})	Viscosity (poise)
Abelmoschus esculentus mucilage	1	0.65	2.25
	2	0.74	2.78
	3	0.80	3.23
	4	Too viscous; indeterminable	Too viscous; indeterminable
Tragacanth	1	1.25	1.10
	2	1.01	1.35
	3	0.85	1.45
	4	0.76	1.65
Sodium CMC	1	1.67	0.85
	2	1.43	0.98
	3	1.25	1.12
	4	0.91	1.34

Fig 1. Sedimentation profile of suspensions made with different concentrations of sodium CMC

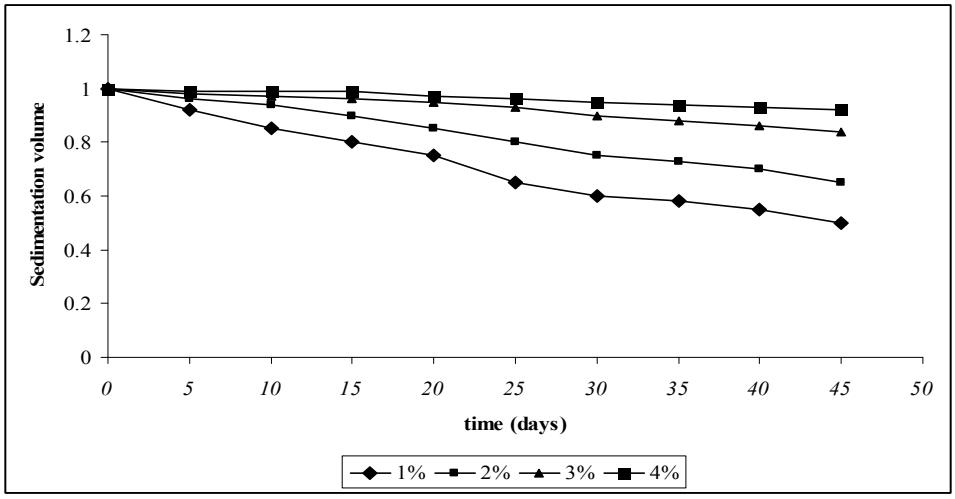


Fig 2. Sedimentation profile of suspensions made with different concentrations of mucilage of *Abelmoschus esculentus*

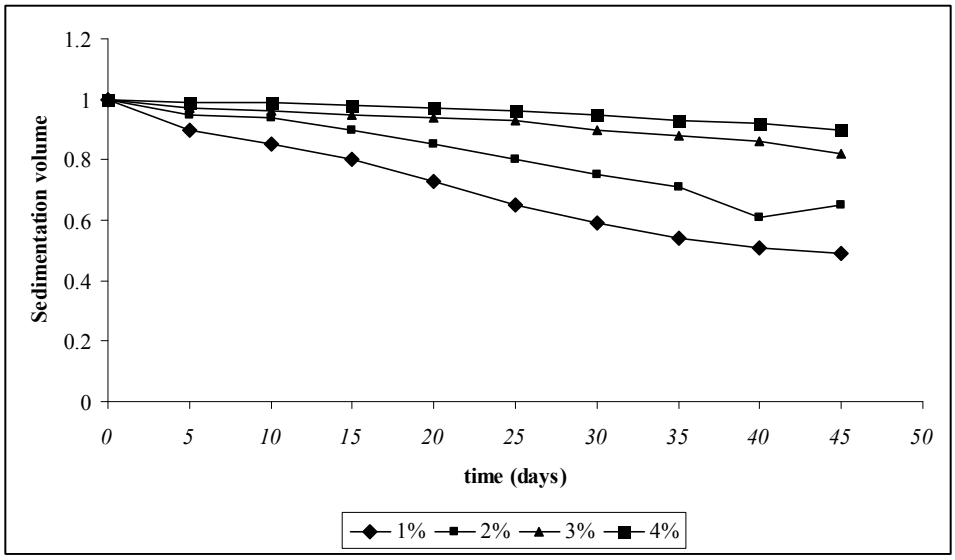
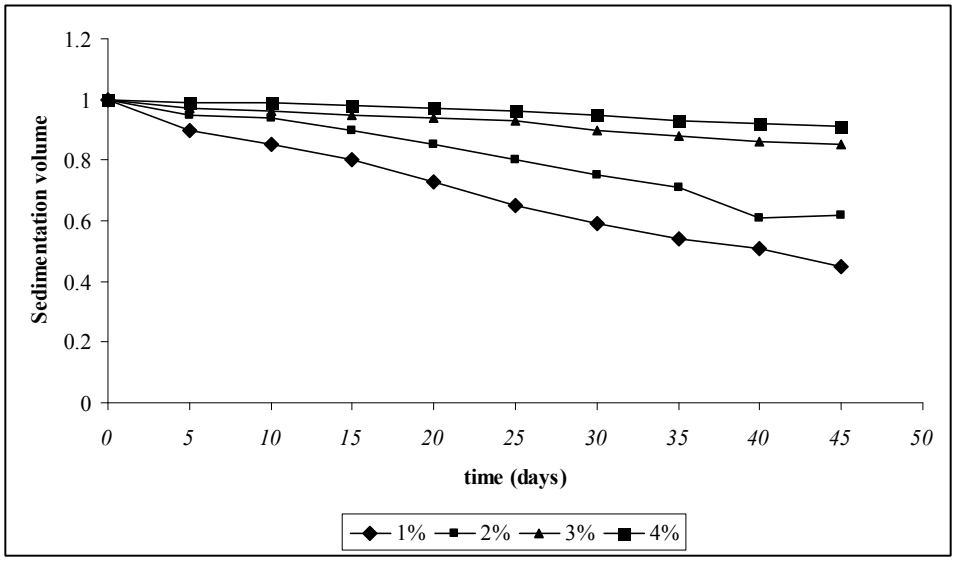


Fig 3. Sedimentation profile of suspensions made with different concentrations of tragacanth.



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