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Evaluation of the suspending properties of *Parkia biglobosa* mucilage in a metronidazole suspension formulation

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ABSTRACT

Objectives: The aim of this work is to assess the suitability of *Parkia biglobosa* mucilage (PBM) as a suspending agent in metronidazole suspension.

Methods and Materials: Metronidazole suspension was formulated with mucilage (0.1%, 0.5%, 1%, and 2% $^{w}/_{v}$) from *P. biglobosa* seeds as the suspending agent. Similar suspensions of metronidazole that contained (0.1%, 0.5%, 1%, and 2% $^{w}/_{v}$) tragacanth or gelatin were also prepared for comparison to PBM-based formulations. The suspensions were assessed for rheological properties, sedimentation volume, degree of flocculation, ease of redispersion, flow rate (flowability), and drug-excipient compatibility using Fourier-transform infrared spectroscopy.

Results: Brownish metronidazole suspensions with a smooth and elegant appearance were obtained. After 2 weeks of storage at ambient temperature (28 ± 2°C), the ease of redispersion was in the following order: tragacanth < PBM < gelatin-containing formulations. The optimal pH of the formulation was slightly acidic and did not significantly change ($p > 0.05$) after 3 months of storage at ambient temperature (28 ± 2°C). However, the viscosity of the formulations was reduced by more than 30%, and the flow rate was significantly increased ($P < 0.05$). Suspensions formulated with 2% PBM were well-flocculated and easily redispersible. Furthermore, the sedimentation volume and sedimentation rate of suspensions formulated with 2% PBM were comparable to that of tragacanth- and gelatin-containing suspensions.

Conclusion: PBM hence has great promise as a suspending agent for pharmaceutical preparations.

Keywords: *parkia biglobosa*, suspending agent, sedimentation volume, flocculation

INTRODUCTION

A suspension is a two-phase system comprising solid particles dispersed in a liquid.[1] A suspension is often chosen as a pharmaceutical dosage form for drugs insoluble in water and aqueous fluids at the dosage required for administration and when attempts to solubilize the drug would compromise stability and safety. For oral administration, the taste of a bitter or unpleasant drug can often be masked by choosing an insoluble form of the active drug and formulating it as a suspension. The large surface area of the dispersed drug particles often facilitates dissolution and absorption due to an increase in the number of absorbing sites, which exposes more particles of the compound to absorption.[2] Pharmaceutical suspensions are thermodynamically unstable

systems that require the use of a stabilizer or suspending agent which reduces the rate of settling and permits redispersion of any settled particulate matter by increasing the consistency of suspending medium or by colloidal action.[3] Various polymers that have been used as suspending agents include natural polymers, for example, acacia, tragacanth, and xanthan; cellulose derivatives, for example, methylcellulose and carboxymethylcellulose; synthetic polymers, for example, polyvinylpyrrolidone and carbomer; and particulate colloids, for example, bentonite and veegum.[1,4] However, there is still a need for and more effective natural excipients that could be used in the formulation of pharmaceutical suspensions.

Parkia biglobosa (*Mimosoideae – Leguminosae*) commonly called the African locust bean tree has long been widely recognized as an important indigenous fruit tree in anglophone and francophone West Africa. Our previous findings show that the mucilage possesses fundamental characteristics that would make it suitable as a pharmaceutical excipient in the formulation of liquid dosage forms.[5] It has also been reported that both the extracted and modified mucilage possesses properties that make them suitable as polymers for the formulation of solid dispersions.[6] This study was therefore undertaken to investigate the suitability of *Parkia biglobosa* mucilage (PBM) as a suspending agent in metronidazole suspension in comparison to commonly used suspending agents; tragacanth and gelatin.

MATERIALS AND METHODS

Materials

Metronidazole benzoate powder was obtained from Loba chemie laboratory (Mumbai, India), PBM was obtained from a batch processed in our laboratory,[6] tragacanth (Central drug house, New Delhi), gelatin (Loba chemie laboratory reagents and fine chemicals, India), benzoic acid (Griffin and George, England), and sodium lauryl sulfate (BDH chemicals Ltd Poole England). Amber colored bottles (Available in the laboratory). All other materials and chemicals used were of analytical grade and were used as received.

Equipment

Mortar and pestle (available in the laboratory), BenchTop pH meter (Sper scientific, Arizona, United States of America), NDJ-SS Digital viscometer (SearchTech, instrument, London, United Kingdom), Rotatory shaker (M/C SR. NO. England, United Kingdom). FT-IR Cary 360 (Agilant Technologies Inc, United States).

Preparation of metronidazole suspensions

Metronidazole suspension was formulated using varying concentrations (0.1%, 0.5%, 1%, and 2% $^{w}/_{v}$) of PBM as the suspending agent. Similar suspensions of metronidazole containing same concentrations (0.1%, 0.5%, 1%, and 2% $^{w}/_{v}$) of either gelatin or tragacanth were also prepared. Twelve batches of the suspension were prepared with a detailed composition, as shown in [Table 1]. In each case, the appropriate weight of the required suspending agent for each suspension was weighed and triturated in a mortar, and then, metronidazole powder was added using the doubling-up technique (powder addition in increasing order of volume, that is, by doubling the amount of powder in the mortar at each addition.). After the inclusion of 20 mL of water to produce a smooth paste, 2 mL of sodium citrate (0.33% $^{w}/_{v}$), benzoic acid (0.13% $^{w}/_{v}$), and sodium lauryl sulfate solution (0.67% $^{w}/_{v}$) were gradually added while the mixture was constantly stirred. The slurry was poured into a pre-calibrated, labeled amber bottle, made up to volume with distilled water, and then shaken vigorously for 2 min.

Appearance and pH of the suspensions

Metronidazole suspension was re-dispersed by shaking. Then, color and homogeneity were visually examined and pH was measured using a pH meter.

Sedimentation volume

At various time intervals (5 min, 10 min, 15 min, 20 min, 30 min, 1 h, 24 h, 48 h, 72 h, 1 week, 2 weeks, and 1 month), the volume of sediments in the suspension was measured. The sedimentation volume (F) of the suspensions was calculated according to Equation 1.[7]

$$F = \frac{V_u}{V_o} \quad (1)$$

V_u = volume of sediment at time t
V_o = volume of the suspension before settling occurred

Flowability of the suspensions

Using Equation 2, the flow rate was computed and the time it took for a 10 mL sample of a freshly prepared suspension to pass through a 10 mL pipette was measured.[8] This was repeated on the samples after 1 month of storage to examine the effect of aging on the flow properties of the formulations.

$$\text{Flow rate} = \frac{\text{FV}}{\text{t}} \quad (2)$$

FV = volume of the sample in the pipette (in mL),
t = time (in seconds) required for the 10 mL suspension to totally elute out of the pipette.

Ease of redispersion assessment

Using a modified version of the procedure outlined by Ogaji and Hoag,[9] the suspensions' dispersibility was quantitatively

Table 1: Composition of formulated suspensions.

Ingredient	Batches			
	Sample 1 (0.5%)	Sample 2 (1.0%)	Sample 3 (1.5%)	Sample 4 (2.0%)
Metronidazole benzoate	2 g	2 g	2 g	2 g
Benzoic acid	0.2 g	0.2 g	0.2 g	0.2 g
Sodium citrate	0.5 g	0.5 g	0.5 g	0.5 g
Sodium lauryl sulfate	1 g	1 g	1 g	1 g
PBM/gelatin/tragacanth	0.15 g	0.75 g	1.5 g	3 g
Purified water to	150 mL	150 mL	150 mL	150 mL

PBM: *Parkia biglobosa* mucilage

assessed. The formulated suspensions were divided into two portions, each holding 50 mL of the sample in a 100 mL measuring cylinder, and shaken to achieve uniform dispersion. To allow for the formation of sediments, the first and second portions were allowed to stand for 2 and 4 weeks, respectively. After the storage time, the samples were shaken in a rotary shaker by rotating the cylinders in a 360° cycle. Re-dispersibility was measured as the number of full cycles necessary to completely re-disperse the formulation in the cylinder.

Degree of flocculation

The degree of flocculation (β) was calculated from Equation 3.[7]

$$\beta = \frac{F}{F_{\infty}} \quad (3)$$

F = sedimentation volume in flocculated suspension (suspension with no deflocculant)
F_{∞} = sedimentation volume in deflocculated suspension (suspension in which 0.54% $^{w}/_{v}$ potassium dihydrogen phosphate was added as a deflocculant).

Preliminary assessment of the suspensions revealed that suspension containing 2.0% $^{w}/_{v}$ PBM exhibited the most optimal properties (moderate flocculation and the highest sedimentation volume and flow rate), hence, was selected as the optimal formulation. A second version of the optimal formulation containing Syrup BP (British Pharmacopoeia) was formulated, as shown in [Table 2].

The optimized formulation with and without Syrup BP was further examined when freshly prepared and after 3 months' storage for appearance, pH, and flow rate as previously described.

Viscosity

The viscosity of the suspensions was examined when freshly prepared and after 3 months' storage using a digital viscometer with spindle number 1 at a shear rate of 30 rpm.

Table 2: Formula for metronidazole suspension containing 2.0% $^{w}/_{v}$ of PBM with syrup BP.

Ingredient	Quantity
Metronidazole benzoate	2 g
Benzoic acid	0.2 g
Sodium citrate	0.5 g
Sodium lauryl sulfate	1 g
PBM	0.25 g
Syrup BP	10 mL
Purified water to	50 mL

PBM: *Parkia biglobosa* mucilage, BP: British Pharmacopoeia

Drug-excipient compatibility

The spectra of the freshly prepared formulations and after 3 months of storage at room temperature (28 ± 2°C) were generated using Fourier-transform infrared spectroscopy (FTIR). The spectra were examined for peaks shifting to either higher or lower frequencies or disappearance of some bands. The spectra were obtained over the range 650–4,000 cm^{-1}.

Statistical analysis

Software from IBM SPSS Inc., Chicago, Illinois, USA, was used to conduct the statistical analysis. Using one-way analysis of variance and the Bonferroni *post hoc* test, mean comparisons between the means of several groups were performed. At $P < 0.05$, the results were considered significant.

RESULTS

The suspension formulated with PBM was dark brown, had a homogenous appearance, and was foamy with white, tiny sediments at the bottom of the bottle. The sedimentation volume of all the formulations is shown in [Table 3]. Among the PBM-based suspensions, the suspension containing 2.0% $^{w}/_{v}$ of PBM exhibited the highest sedimentation volume after 1 h of dispersion. All the PBM-based suspensions gave small negative slope, as can be seen in the graph of

Table 3: Sedimentation volumes of formulated suspensions.

Time	Tragacanth (%w/v)				Gelatin (%w/v)				PBM (%w/v)			
	0.5	1	1.5	2	0.5	1	1.5	2	0.5	1	1.5	2
Minutes												
0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5	0.47	0.48	0.49	0.49	0.49	0.50	0.50	0.50	0.94	0.90	0.97	0.97
10	0.46	0.46	0.48	0.48	0.46	0.48	0.47	0.48	0.90	0.75	0.90	0.95
15	0.45	0.45	0.47	0.47	0.42	0.44	0.44	0.47	0.75	0.70	0.86	0.90
20	0.44	0.43	0.46	0.46	0.40	0.42	0.42	0.44	0.70	0.60	0.80	0.84
30	0.42	0.43	0.46	0.45	0.35	0.37	0.37	0.38	0.65	0.55	0.70	0.75
Hours												
1	0.20	0.28	0.30	0.35	0.15	0.17	0.17	0.18	0.50	0.40	0.55	0.70
24	0.10	0.28	0.17	0.25	0.01	0.02	0.03	0.04	0.008	0.004	0.10	0.11
48	0.10	0.13	0.17	0.23	0.01	0.02	0.03	0.04	0.008	0.004	0.10	0.11
72	0.10	0.12	0.17	0.22	0.01	0.02	0.03	0.04	0.008	0.004	0.10	0.11
Weeks												
1	0.10	0.12	0.17	0.22	0.01	0.02	0.03	0.04	0.008	0.004	0.10	0.11
2	0.10	0.12	0.17	0.22	0.01	0.02	0.03	0.04	0.008	0.004	0.10	0.11
4	0.12	0.12	0.17	0.20	0.01	0.02	0.03	0.04	0.008	0.004	0.10	0.11

PBM: *Parkia biglobosa* mucilage

Table 4: Flow rate and redispersibility of formulated suspensions.

Suspending agent	Concentration (%w/v)	Flow rate (mL/sec)		Redispersibility (number of full cycles)	
		Day 1	4 Weeks	2 Weeks	4 Weeks
PBM	0.5	3.86	1.64	16	22
	1.0	4.77	0.88	10	15
	1.5	3.97	0.74	7	12
	2.0	4.89	0.43	3	6
Gelatin	0.5	0.52	0.42	11	14
	1.0	0.54	0.19	7	12
	1.5	0.66	0.05	4	9
	2.0	0.70	0.01	2	4
Tragacanth	0.5	1.63	1.40	20	24
	1.0	1.09	0.41	16	14
	1.5	0.28	0.08	8	12
	2.0	0.16	0.05	4	6

PBM: *Parkia biglobosa* mucilage

sedimentation volume against time presented in [Figure 1]. As shown in [Table 4], gelatin-based formulations were most easily re-dispersible compared to other formulations. On the other hand, formulations prepared using PBM gave the highest flow rate. In general, the degree of flocculation of the suspensions was in the following order: tragacanth > PBM > gelatin-containing formulations, as shown in [Figure 2]. The pH, viscosity, and flow rate of the optimized formulation (with and without Syrup BP) when freshly prepared and after 3 months of storage at ambient temperature are presented in [Table 5]. While the pH of the optimized formulations was not significantly ($P = 1$) altered after 3 months of storage, the viscosity decreased, and the flow rate increased significantly ($P < 0.05$). [Figure 3] displays the FTIR spectra of metronidazole active ingredient and the optimized formulation (with and without Syrup BP) shortly after they were prepared and after 3 months of storage at room temperature (28 ± 2°C). Metronidazole produced characteristic peaks at 1265 cm^{-1} (C-O stretch), 2879 cm^{-1} (aliphatic C-H stretch), 1624 cm^{-1} (C-C stretch), and 1357 cm^{-1} (C-N vibration).

DISCUSSION

A pharmaceutical suspension should have an elegant appearance.[10] Brownish metronidazole suspensions with a homogenous and smooth appearance were formulated using PBM as the suspending agent. In terms of elegance,

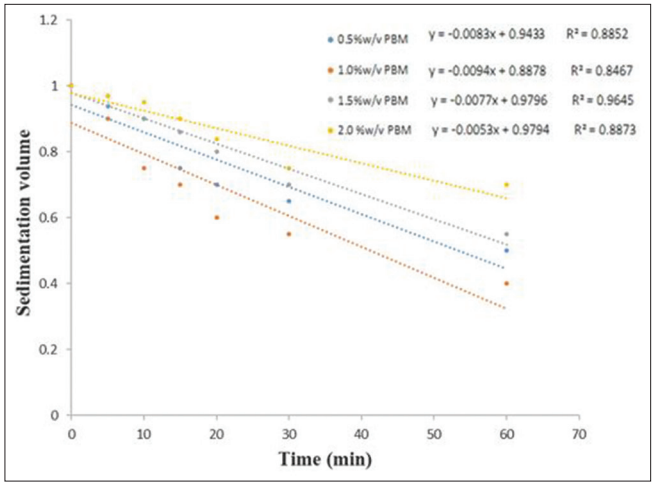

Figure 1: Sedimentation profiles of different *Parkia biglobosa* mucilage-based suspensions.

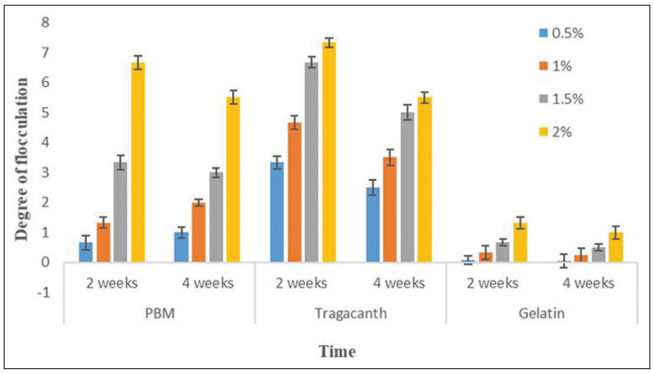

Figure 2: Degree of flocculation of formulated suspensions.

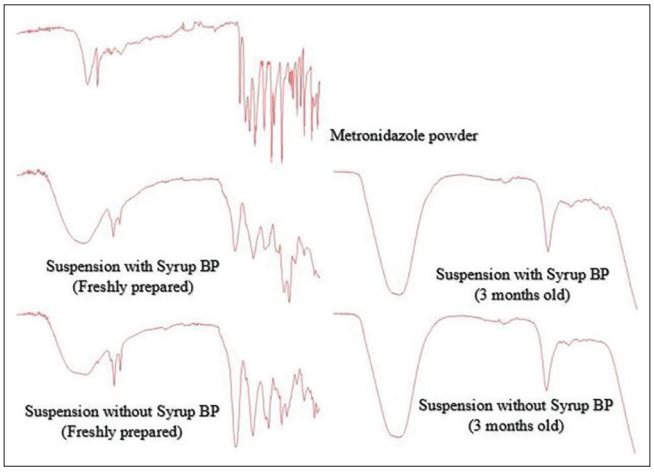

Figure 3: Fourier-transform infrared spectroscopy of pure metronidazole and the optimized formulation (with and without Syrup BP) when freshly prepared and after 3 months of storage at ambient temperature.

suspensions made with PBM compared quite favorably to the formulations made with gelatin and tragacanth.

Sedimentation is the term used to describe the gravitational settling of particles or floccules in a liquid dosage form.[11] Sedimentation volume is the ratio of the final or ultimate volume of sediment to the original volume of sediment before settling. The sedimentation volume only provides a qualitative description of flocculation. Sedimentation volume typically ranges from 1 to <1, with 1 denoting an ideal suspension (no clear supernatant) and 0 denoting complete sedimentation (totally unstable suspension). Sedimentation volume should ideally be 1 or very close to 1.[11] The slope of the graph of sedimentation volume against time is often used to assess stability. A zero slope, for instance, denotes zero sedimentation; such a system will stay suspended for a long time, and resuspension will only require a small amount of shaking. However, a suspension with a substantial negative slope may present challenges since it may sediment too quickly before a consistent dose can be withdrawn.[1] Up until the 1st h of redispersion, PBM-based suspensions had the largest sedimentation volume. They all likewise showed small negative slopes, which suggest a moderate rate of sedimentation. However, the formulation containing 2.0% $^{w}/_{v}$ PBM was the best since it yielded the highest sedimentation volume and the least negative slope, indicating a formulation with controlled flocculation and satisfactory sedimentation characteristics. Ease of redispersion assessment is another test often linked to sedimentation volume measurement. It is the number of agitations necessary to fully resuspend a system after storage.[1] The ease of redispersion was generally of the order of tragacanth<PBM<gelatin-containing formulations. The suspension containing 2.0% $^{w}/_{v}$ PBM was the most easily dispersible as compared to all other PBM-based formulations. Easily redispersed sediments in a suspension allow for withdrawal of uniform doses. Compared to the product that contains tragacanth, users of this product will require less effort to achieve complete redispersion. Small particles in flocculated suspensions form large clusters called flocs, which frequently sediment quite quickly to generate loose sediments that are easy to redisperse. In contrast, a deflocculated system retains dispersed particles as discrete units with a slow sedimentation rate that inhibits liquid entrapment, resulting in compact sediments that are frequently highly challenging to redisperse.[10] PBM-based formulations had lower flocculation than tragacanth-containing formulations but higher flocculation than gelatin-based formulations. In particular, the suspension containing 2.0% $^{w}/_{v}$ PBM demonstrated a satisfactory level of flocculation and produced a product that was easily dispersible and had a moderate and acceptable volume and rate of sedimentation. Due to their capacity to develop a gel-like network within a system, adsorb to the surfaces of the dispersed particles, and so maintain them in a flocculated condition, a good number of polymeric materials have

Table 5: Some physicochemical characteristics of the optimized formulation (with and without Syrup BP) when freshly prepared and after 3 months of storage at ambient temperature (28±2°C).

Test	Formulation	Freshly prepared	3 months	*P*-value
pH	Suspension without syrup	5.6±0.00	5.8±0.17	1.00
	Suspension with syrup	5.5±0.20	5.5±0.20	1.00
Viscosity (mPa s)	Suspension without syrup	3.4±0.17	2.3±0.35	0.02*
	Suspension with syrup	3.8±0.10	1.5±0.20	0.00*
Flow rate (mL/sec)	Suspension without syrup	4.77±0.18	0.62±0.01	0.00*
	Suspension with syrup	4.86±0.50	0.66±0.02	0.00*

*Indicates a significant mean difference at a 5% level (i.e. $P<0.05$)

been utilized to control flocculation. Pourability is one of the characteristics of a good suspension.[9] A suspension should flow and pour freely if it needs to be moved via pipes, a bottle's aperture, or a syringe needle during processing, production, and packaging. All formulations exhibited a speedy flow, passing through a 10 mL pipette in <10 s.

The formulation containing 2.0% $^{w}/_{v}$ PBM was selected as the best formulation because it was a well-flocculated suspension with a high sedimentation volume and flow rate. Syrup BP is a concentrated (66.7% $^{w}/_{v}$) solution of sucrose, which is capable of retarding microbial growth (by virtue of its osmotic effect) and imparting a sweetening effect (due to its high concentration of sucrose).[12] After 3 months of storage, the pH of the optimized formulation remained weakly acidic and showed no significant change ($P > 0.05$). There was however a significant decrease in the viscosity and an increase in the flow rate. A formulation with rapidly declining viscosity could point to structural instability in the system.[9] Such a formulation will pour faster than the patient is accustomed to and may be unappealing to the patient even if no harmful changes have taken place.

The obtained metronidazole FTIR spectra are consistent with those reported by Yahaya *et al.*,[13] The FTIR spectra of the freshly prepared optimized formulations showed all the metronidazole characteristic absorption bands, suggesting the absence of chemical interactions between the drug and the other formulation excipients. However, after 3 months of storage, changes in the FTIR spectra of the formulations were observed, ranging from the wavelength of the C-N stretching peak shifting to a higher frequency (i.e., from 1535.7 cm^{-1} to 1636.3 cm^{-1}), the disappearance of the aliphatic C-H stretch absorption peaks in the formulation with syrup BP, and the disappearance of C-O stretch and C-H stretch absorption peaks in the formulation without syrup BP. Changes in spectral shape can indicate changes in a sample's overall composition as a result of chemical interaction or degradation.[14] Metronidazole has been reported to undergo hydrolytic degradation in an aqueous solution.[1] Water plays a dominant role in drug degradation, and in many cases, it is implicated passively as a solvent vector between two reacting species in a solution.[15] As a result, further studies are necessary to investigate ways of protecting the formulation against hydrolytic degradation, such as using complexing agents or employing techniques to reduce the system's dielectric constant.

CONCLUSION

Metronidazole suspension was successfully made using PBM. When used at a concentration of 2.0% $^{w}/_{v}$, a well-flocculated suspension that was easily redispersible, with a moderate and acceptable sedimentation volume, and rate comparable to tragacanth and gelatin-based suspensions were produced. The locally sourced PBM offers the benefit of using less-processed, affordable, non-toxic, and nutritionally acceptable raw materials that can be used to produce a stable and suitable suspension. Hence, PBM is a promising substitute for other commonly used suspending agents. However, further studies are needed to investigate ways to protect the formulation from instability due to the changes observed in the FTIR spectra of the formulations stored for 3 months.

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Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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None.

Conflicts of interest

There are no conflicts of interest.

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