WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.647
Volume 6, Issue 6, 302-313 Research Article ISSN 2278 – 4357

PRELIMINARY STUDIES ON THE SUSPENDING PROPERTIES OF *SIDA ACUTA* GUM IN PARACETAMOL SUSPENSION

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Article Received on
11 April 2017,
Revised on 01 May 2017,
Accepted on 23 May 2017
DOI: 10.20959/wjpps20176-9332

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ABSTRACT

The study was carried out to determine the use of *Sida acuta* gum as a suspending agent in the formulation of paracetamol suspension and to compare it with standard suspending agents. *Sida acuta* gum was isolated by isopropyl alcohol precipitation of the filtrate obtained from powdered dried *Sida acuta* leaves macerated in distilled water. Formulations of paracetamol suspensions were prepared using 0 – 1.5%w/v *Sida acuta* gum as suspending agent with or without a preservative or flocculating agent. Formulations were also prepared using different concentrations of acacia or sodium carboxymethylcellulose as standard suspending agents. The suspensions were kept on an undisturbed flat surface for two weeks. The suspensions were evaluated based on their sedimentation volume, redispersibility, pH, viscosity and microbiological load. The sedimentation volume based on the suspending agents were in the order; sodium carboxylmethylcellulose > *Sida acuta* > acacia. The pH of the suspensions increased slightly (0.07 pH units) after 14 days except for formulations PS2B, PS3B, PS5 and PS6 that showed decrease (0.59 pH units). The viscosity of the suspensions that contained *Sida acuta* gum increased markedly (from 15.8 -53.8 mPas for PS1C). Those that contained acacia increased slightly, while those that contained sodium carboxylmethylcellulose were very high (197.4 mPas) even on day 0. The order of ease of redispersion after 14 days based on the suspending agent; is sodium carboxylmethylcellulose > acacia > *Sida acuta* gum. The formulations without preservatives showed sign of microbial

growth. Paracetamol suspension was successfully formulated with *Sida acuta* gum as a suspending agent.

KEYWORDS: Suspending properties, *Sida acuta*, Paracetamol, Suspension.

INTRODUCTION

Suspensions are liquid dosage forms that are composed of solid particles dispersed throughout an aqueous or oily liquid in which the particles are insoluble or poorly soluble.

Suspensions as a dosage form has been used to handle some pharmaceutical challenges, such as people have difficulty in swallowing solid dosage forms who may require the drug to be dispersed in a liquid.[1, 2] Some drugs degrade in the presence of water and this precludes its formulation as solutions. Insoluble derivatives can be synthesized and formulated as suspension. Alternatively, a suspension of a drug in a non – aqueous vehicle may be formulated if it degrades in the presence of water.[1] The desired high surface area are produced when some drugs such as kaolin, magnesium carbonate and magnesium silicate which are required to be present in the gastrointestinal tract in a finely divided form are formulated as suspensions. Suspensions can be used to mask the taste of some drugs because the drug particles will be insoluble in the dispersion medium.[2] Drugs for parenteral administration can be made to have sustained release property by formulating them in form of suspensions. This can be done by varying the size of the dispersed particles of the active ingredient or enhanced further by using an oily instead of an aqueous dispersion medium. Suspensions can be formulated for administration through different routes such as oral, topical and parenteral.

Suspensions that are formulated well should have some desirable physical properties. There should not be fast sedimentation of the drug particles in the susupension, to allow the withdrawal of the required dose after each shaking. The sedimented drug particles should be re-suspended by moderate agitation the container. The suspended particles should be small and uniformly sized in order to give a smooth, elegant product free from a gritty texture. Increase in the viscosity of the dispersion medium, results in reduction in the rate of settling of the dispersed particles but the viscosity should not be excessively high to avoid retardation of pourability.[1]

On the basis of the electrokinetic nature of solid particles, suspensions can also be classified as flocculated or deflocculated suspension.[3] Whether or not a suspension flocculated or deflocculated depends on the relative magnitudes of the electrostatic forces of repulsion and the forces of attraction between the particles.[1] The dispersed particles remain as discrete units in a deflocculated suspension and since the rate of sedimentation depends on the size of each unit, settling will be slow. The individual particles slip past each other as they sediment due to the repulsive forces between them. The slow rate of settling prevents the entrapment of liquid within the sediment which thus becomes compacted and can be very difficult to redisperse (caking or claying).[1] Aggregation of particles in flocculated suspensions leads to a much more rapid rate of sedimentation because each unit is composed of many individual particles and is therefore larger. The porosity of the aggregate dictates the settling rate, if porous, the dispersion medium can flow through as well as around each aggregate or floccule as it sediments. The structure of each aggregate is maintained after sedimentation thus enclosing a large amount of liquid phase within it. Although aggregation in the primary minimum will produce compact floccules while a secondary minimum effect will produce loose "fluffy" floccules of higher porosity; the volume of the final sediment will still be large and will easily be redispersed by moderate agitation.[1]

Thermodynamically, a pharmaceutical suspension is an unstable system therefore; it requires the addition of a suitable suspending agent for its stabilization.[4] Suspending agents are agents that help to decrease the sedimentation rate of particles in suspension. It achieves its function by increasing the viscosity of the liquid vehicle, theeby reducing settling in accordance with stoke's law. These agents are classified as natural polysaccharides, semi – synthetic or synthetic[5] A number of plant gums have been used as suspending agent in suspension formulations Reported cases include Grewia polysaccharide gum,[2] Vigna mungo bio anti- settlant agent,[3] Trigonella foenum – graecum mucilage[4] and Brachystegia eurycoma gum.[5]

Sida acuta gum is isolated from the dried powder of the leaves of Sida acuta, a shrub belonging to Malvaceae family The plant is widely distributed in the subtropical regions where it is found in bushes, in farms and around habitations.[6,7] The study by[7] suggested that Sida acuta hydrogel had physicochemical properties that indicated that it could used as pharmaceutical excipient such as binder, suspending agent, swellable hydrophilic matrix, and

for investigation in nanoformulation of some drugs in novel drug delivery alone or in combination with other biopolymers.

Therefore this study was aimed at researching into the suspending properties of *Sida acuta* gum in paracetamol formulation by evaluation of its sedimentation volume, p H, viscosity, microbial count and redispersibility.

MATERIALS AND METHODS

Materials

Isopropyl alcohol, acetone, (Guangxing Guanghua Chemical, China), absolute ethanol, chloroform (May and Baker, Dagenham, England), sodium dihydrogen orthophosphate, (BDH Chemicals Ltd Poole England), benzoic acid, paracetamol (Changsh Huagang Pharm. Co, China) was received as a gift from Orange Kalbe Limited , Lagos, acacia (T. Baker, U.S.A), Sabourand dextrose agar, nutrient agar (Titan Laboratories, India), sodium carboxymethylcellulose, were of analytical grades.

Leaves were collected from *Sida acuta* plants from bushes in the New G.R.A area of Trans – Ekulu, Enugu, Enugu state, Nigeria and processed into *Sida acuta* gum.

Isolation and Purification of Gum

The gum was isolated and purified according to the method used by.[7] The leaves from *Sida acuta* plant were dried, powdered, and passed through a sieve of aperture size 600 μm. A 200 g of the sieved dried leaves powder was mixed with 1500 ml of distilled water and allowed to macerate for 6 h. The mixture was boiled for 1 h at 100 °C to ensure complete break – up of cells to release the mucilage and kept aside for settling. After 2 h, the mixture was filtered, and to the filtrate (900 ml), equal volumes of isopropyl alcohol were added and kept in a refrigerator at 8–10°C for 6 h. To the marc left, 1000 ml of distilled water was added and kept for about 1 h to wash out the remaining mucilage. The mucilage (1200 ml) was separated from the marc using a muslin cloth and precipitated with equal volumes of isopropyl alcohol. The crude gum was soaked in two volumes excess of isopropyl alcohol. The gum-solvent slurry was allowed to stand for 30 min. The precipitate was collected by filtration using muslin cloth, washed twice with isopropyl alcohol and once with acetone.[8,9,10] Finally, it was dried in the oven at 40 °C for 8 h and stored separately in a clean, dry, and closed container.

Formulation of Paracetamol Suspensions

A 0.5 g of Sida acuta gum was weighed and transferred into a mortar. It was turned into a mucilage with about 30 ml of double strenght chloroform water. A 2.5 g of paracetamol pre – sieved with a 425 µm sieve was added to the mortar and triturated till smooth paste was formed. A 0.05 g of benzoic acid was weighed and dissolved with 10 ml of the double strenght chloroform water. The soultion transferred into the mortar with further trituration. This was transferred into a 50 ml measuring cylinder. The mortar was rinsed with more of the double strenght chloroform water and used to make up the volume to the 50 ml mark. The measuring cylinder was properly sealed with masking tape. This was labeled batch PS1A. The suspension was shaken properly and kept on a flat vibration – free table for 2 wk. The other batches were prepared according to the formula on Table 1 by increasing the concentration of the *Sida acuta* gum, omission of benzoic acid or inclusion of sodium dihydrogen phosphate. Some batches were prepared using standard suspending agents (acacia or sodium carboxymethylcellulose) in place of the *Sida acuta* gum.

Table 1: Composition of paracetamol suspensions for formulations PS 0 to PS 6.

F	PS 0	PS 1A	PS 1B	PS 1C	PS 2A	PS 2B	PS 2C	PS 3A	PS 3B	PS 3C	PS 4	PS 5	PS 6
PCM (g)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
SAG (g)	0	0.5	0.5	0.5	0	0	0	0	0	0	0.75	0	0
ACACIA (g)	0	0	0	0	0.5	0.5	0.5	0	0	0	0	0.75	0
NaCMC (g)	0	0	0	0	0	0	0	0.5	0.5	0.5	0	0	0.75
BENZOIC ACID (g)	0	0.05	0	0.05	0.05	0	0.05	0.05	0	0.05	0.05	0.05	0.05
NaH_2PO_4 (g)	0	0	0	2.5	0	0	2.5	0	0	2.5	0	0	0
DW (ml) TO	50	50	50	50	50	50	50	50	50	50	50	50	50

KEY: F = formulations, SAG = *Sida acuta* gum, PCM = paracetamol, DW = distilled water, NaCMC = sodium carboxy methyl cellulose.

Evaluation of Paracetamol Suspension

Sedimentation volume: The different batches of the paracetamol suspensions were kept on a flat vibration – free table for 2 wk. The volume of sedimentation was recorded daily for fourteen days. This was carried out in triplicate. The sedimentation volume (f), was calculated from the formula

$$F = \frac{Vu}{Vo} \quad \text{---------- 1}$$

Where Vu = ultimate volume of sediment, Vo = initial volume of sediment.

P H: The pH values of the different batches of the paracetamol suspensions were determined using a model HI 2211 pH/ORP meter (Hanna Instruments) on day 0 and on day 14. This was carried out in triplicate.

Viscosity: The viscosity of the different batches of suspensions were determined in triplicate using Brookfield Viscometer (NDJ – 5S Viscometer, England Lab science) on day 0 and day 14.

Redispersibility: A 50 ml suspension was transferred into a 100 ml bottle and kept on a vibration – free table for 14 days. This was carried out in triplicate for all the batches. On the fourteenth day, each bottle was held with the thumb at the bottom of the bottle and the index finger on top of the bottle and rotated 180^0 clockwise and anti – clockwise until the sediments redispersed. Each clockwise and anti – clockwise movement was taken as a cycle. The number of cycles it took to redisperse each suspension was recorded as its redispersion number.

Microbiological Evaluation of the different paracetamol suspension formulations: This was done by observing the change in one of the organoleptic properties (colour) and also by microbial count. The microbial count of the paracetamol suspensions was performed for the total aerobic microbial count of bacteria and fungi using the plate count method. The limit of colony forming units (cfu) for bacteria was 300 and for fungi was 100.

Plate count: A 1 ml supension was withdrawn from paracetamol suspension formulation P1A and the volume was adjusted to 10ml with sterilized water. A Serial dilution was made by transferring 1 ml of the paracetamol suspension into a test tube and making it up to 10 ml with sterilized water. Further dilutions were made to obtain 10^{-5} paracetamol suspension. These processes were repeated using formulations P1B, P2A, P2B, P3A and P3B of paracetamol suspensions.

For bacteria, nutrient agar was prepared at about 45 ^{0}C and poured into eighteen Petri dishes of 10 cm diameter respectively and they were allowed to solidify. A 0.1 ml of the 10^{-5} formulation P1A paracetamol suspension was transferred into three of the Petri dishes respectively. This was repeated using the other paracetamol suspension formulations (P1B, P2A, P2B, P3A and P3B) respectively. They were spread on the surface of the solidified medium in a Petri dish using a glass spreader. The paracetamol suspensions were allowed to

drain into the agar. The Petri dishes were inverted and incubated at 35 °C for 1 day. The number of colonies formed was counted and the results calculated using the average count for the respective three plates, up to a maximum of 300.

For fungi, Sabouraud glucose agar was prepared at 45 ^{0}C and poured into eighteen Petri dishes of 10 cm in diameter respectively and they were allowed to solidify. A 0.1 ml of the 10^{-5} paracetamol suspension formulation P1A was transferred into three of the Petri dishes respectively. This was repeated using10^{-5} paracetamol suspensions formulations P1B, P2A, P2B, P3A and P3B respectively. They were spread on the surface of the solidified medium in a petri dish using a glass spreader. The paracetamol suspensions were allowed to drain into the agar. The Petri dishes were inverted and incubated at 28 °C for 3 days.

The number of colonies formed was counted and the results calculated using the dish with not more than 100 colonies.

RESULTS AND DISCUSSION

Sedimentation volume of the paracetamol suspensions

The sedimentation volume of the various formulations of paracetamol suspension is as shown on Figure 1. Formulations of paracetamol suspensions that contained sodium carboxylmethyl- cellulose as suspending agent (PS3A, PS3B, PS3C and PS6) have the highest sedimentation volume among the three suspending agents from day 1 to 14. The disperse phase settled very slowly. Among the NaCMC - contained formulations, PS6 (1.5% w/v of NaCMC) had the highest sedimentation volume until day 9 when it fell below formulation PS3C (1.0% w/v of NaCMC) which contained a flocculating agent.

Formulations PS1A, PS1B, PS1C and PS4 that contained *Sida acuta* as suspending agent had sedimentation volumes that were less than those that contained NaCMC but higher than those that contained acacia as the suspending agent. Formulation PS4 (1.5 % *Sida acuta*) had higher sedimentation volume than the others formulated with 1 % *Sida acuta* gum. This may be due to increase in viscosity as a result of increase in the concentration of the suspending agent.

Formulations PS2A, PS2B, PS2C and PS5 that contained acacia as the suspending agent had the least sedimentation volume and these were almost similar to PS0 that contained no

suspending agent. Their disperse phase settled very fast. This may be due to the low viscosity of the suspensions which allowed free movement of the suspended particles.

The order of sedimentation volume based on the suspending agents were; NaCMC > *Sida acuta* > acacia.

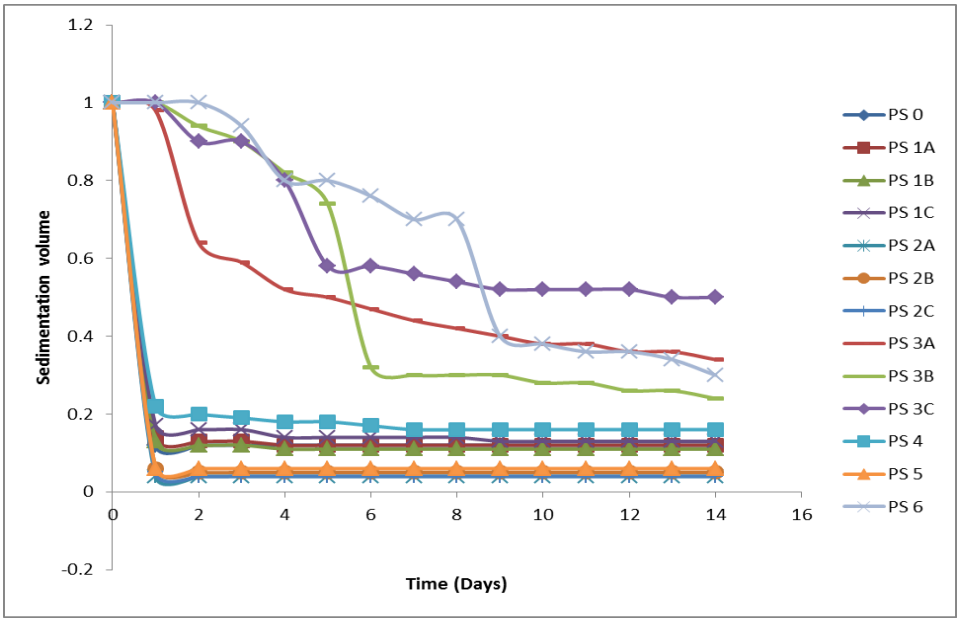

Fig. 1: Sedimentation volume for paracetamol suspensions formulations PS0 to PS6.

Key: PS0 = Suspending agent (0%), benzoic acid (0%) and NaH_2PO_4 (0%), PS1A = SAG (1%), benzoic acid (1%) and NaH_2PO_4, (5%), PS1B = SAG (1%), benzoic acid (0%) and NaH_2PO_4, (5%), PS1C = SAG (1%), benzoic acid (1%) and NaH_2PO_4, (0%), PS2A = Acacia (1%), benzoic acid (1%) and NaH_2PO_4, (5%), PS2B = Acacia (1%), benzoic acid (0%) and NaH_2PO_4, (5%), PS2C = Acacia (1%), benzoic acid (1%) and NaH_2PO_4, (0%), PS3A = NaCMC (1%), benzoic acid (1%) and NaH_2PO_4, (5%), PS3B = NaCMC (1%), benzoic acid (0%) and NaH_2PO_4, (5%), PS3C = Acacia (1%), benzoic acid (1%) and NaH_2PO_4, (0%), PS4 = SAG (1.5%), benzoic acid (1%) and NaH_2PO_4, (0%), PS5 = Acacia (1.5%), benzoic acid (1%) and NaH_2PO_4, (0%), PS6 = NaCMC (1.5%), benzoic acid (1%) and NaH_2PO_4, (0%).

pH of Paracetamol suspensions

The pH of the suspensions increased slightly (decreased acidity) between day 0 and day 14 as shown on Table 2 except for formulations PS2B, PS3B, PS5 and PS6 that recorded decrease in pH (increased acidity).

Viscosity of the paracetamol suspensions

As shown on Table 2, the viscosity of the paracetamol suspensions that contained *Sida acuta* gum as the suspending agent (formulations PS1A, PS1B, PS1C and PS4) showed a significant increase during storage from day 0 to day 14. This may be due to complete hydration and subsequent swelling of the gum on storage.

The viscosity of the paracetamol suspensions that contained acacia as the suspending agent (PS2A, PS2B, PS2C and PS5) showed slight (insignificant) increase in viscosity on storage between day 0 and 14.

The viscosity of the paracetamol suspensions that contained sodium carboxylmethylcellulose as the suspending agent (formulations PS3A, PS3B, PS3C and PS6) was very high even on day 0 (197.4 mPas) except for formulation PS 3B (33.8 mPas). They showed a significant increase during storage from day 0 to day 14. This may be due to complete hydration and subsequent swelling of the gum on storage.

The higher the viscosity of the suspension, the lower the rate at which the particles or the dispersed phase settle down. However, increase in viscosity also decreases pourability. One of the qualities of a good suspension is that of pourability from the container. Therefore a balance should be struck between increase in viscosity and good pourability.

Redispersibility of the paracetamol suspensions

The redispersibility number for the various formulations were as shown on Table 2 Formulation PS0 that was prepared without any suspending agent had the least redispersibility number (2.5 ± 0.71) on day 14. Formulations PS1A, PS1B, PS1C and PS4 that contained *Sida acuta* as the suspending agent have redispersibility numbers that ranged from 22.5 ± 17.68 to 38.5 ± 13.44. From 7 ± 4.24 to 16.5 ± 9.19 were the range of redispersibility numbers for formulations PS2A, PS2B, PS2C and PS6 that contained acacia as the suspending agent. Formulations prepared with sodium carboxylmethylcellulose as the suspending agent, PS3A, PS3B, PS3C and PS6 have redispersibility numbers that ranged from 4 ± 1.41 to 5.5 ± 2.12. One of the qualities of a good suspension is that upon sedimentation, a minimal agitation should be needed to redisperse the sediments. The lower the redispersibility number, the greater the ease of redispersion of the sediments back into the suspension. For all the formulations, the order of ease of redispersion based on the

suspending agent is; zero suspending agent > sodium carboxylmethylcellulose > acacia > *Sida acuta* gum.

Table 2: Evaluation of formulations PS0 to PS6 of paracetamol suspensions.

Formulation	Viscosity (mPas)		pH		Redispersibility
	Day 0	Day 14	Day 0	Day 14	Day 14
PS0	2	2.2	2.17 ± 0.01	2.21 ± 0.07	2.5 ± 0.71
PS1A	13.6	50.8	3.21 ± 0.25	3.24 ± 0.20	36 ± 1.41
PS1B	4.8	30.6	5.95 ± 0.13	6.02 ± 0.22	22.5 ± 17.68
PS1C	15.8	53.8	3.12 ± 0.00	3.32 ± 0.28	38.5 ± 13.44
PS2A	2.8	2.2	2.80 ± 0.29	2.82 ± 0.25	9 ± 7.07
PS2B	2.2	2.4	3.22 ± 0.86	2.63 ± 0.02	16.5 ± 9.19
PS2C	2.4	2.4	2.81 ± 0.06	2.85 ± 0.01	7 ± 4.24
PS3A	197.4	197.4	3.74 ± 0.11	3.75 ± 0.1	4.5 ± 0.71
PS3B	33.8	197.4	5.34 ± 0.46	4.89 ± 0.18	5 ± 2.83
PS3C	197.4	197.4	3.49 ± 0.05	3.51 ± 0.01	4 ± 1.41
PS4	20.2	67.0	3.81 ± 0.16	4.38 ± 0.65	32.5 ± 10.61
PS5	2.2	3.4	2.82 ± 0.08	2.77 ± 0.15	11 ± 9.90
PS6	197.4	197.4	3.92 ± 0.01	3.86 ± 0.00	5.5 ± 2.12

Microbiological evaluation of the paracetamol suspensions

As shown on Table 3, formulations PS1A, PS2A and PS3A that contained 0.1 % benzoic acid (preservative) did not contain any bacteria or fungi. They showed no sign of microbial growth.

Formulations PS1B, PS2B and PS3B did not contain any preservative. Formulation PS1B which contained *Sida acuta* gum as the suspending agent showed sign of microbial growth (changing of colour from light brown to dark brown and evolution of gases) from day 4. The formulation was contaminated by both bacteria and fungi. Formulation PS2B which contained acacia as the suspending agent showed sign of microbial growth (slight discolouration) from day 5. As shown on Table 3, it was contaminated by both bacteria and fungi. Formulation PS3B that contained sodium carboxylmethylcellulose as suspending agent manifested sign of microbial contamination from day 10 (slight colour change). However, unlike the other two formulations, PS3B was contaminated by only slight quantity of bacteria. This finding was in tandem with already existing knowledge that natural polymers are susceptible to microbial attacks, and this can be prevented by the addition of preservatives.

Table 3: Microbial count for Formulations PS1A, PS1B, PS2A, PS2B, PS3A and PS3B.

Formulation	No. of Bacteria (cfu/ml)	No. of Fungi (cfu/ml)
PS1A	0.00	0.00
PS1B	4.00×10^5	1.26×10^7
PS2A	0.00	0.00
PS2B	5.60×10^6	6.80×10^6
PS3A	0.00	0.00
PS3B	1.00×10^5	0.00

CONCLUSION

Paracetamol suspension was successfully formulated using *Sida acuta* gum as the suspending agent. The suspension formulated was comparable to that produced using standard suspending agents. Increase in concentration of suspending agent increases the viscosity of suspension which ultimately reduces sedimentation and contributes to the stability of suspension.

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