Research Journal of Applied Sciences, Engineering and Technology 2(1): 50-55, 2010
ISSN: 2040-7467

Submitted Date: October 08, 2009 Accepted Date: October 31, 2009 Published Date: January 05, 2010

Evaluation of the Suspending Property of *Khaya snegalensis* Gum in Co-Trimoxazole Suspensions

H.S. Mahmud, A.R. Oyi, T.S. Allagh and M.S. Gwarzo
Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

Abstract: The suspending property of *Khaya senegalensis* (Family Meliaceae) gum in Co-trimoxazole suspension was evaluated. The gum obtained from plants grown in Zaria town of northern Nigeria was processed using standard procedures and its physicochemical properties such as solubility, water sorption, pH and rheology were determined. The gum was used to formulate 4.8% w/v co-trimoxazole suspension in concentrations of 0.2-5.0% w/v. *Acacia senegal* gum was used as a standard for comparison. The sedimentation rates, sedimentation volume, ease of redispersibility and viscosity of the suspension were studied over a period of 8 weeks. Other properties evaluated were drug release profile and particle size distribution of formulated suspensions to detect crystal growth. The results show that the sedimentation rate ($4x10^{-4}$ cm/sec), sedimentation volume (50ml), degree of flocculation (1.52) and viscosity (118.8 m.pas) at 50 r.p.m are values obtained for the suspension containing 0.2%w/v khaya gum after 28 days period of storage. Particle size analysis as an exception, the values of all other parameters obtained for the suspensions containing equivalent concentrations of acacia gum were lower. The suspensions were stable, pourable and redispersible with no evidence of crystal growth. The mean particle size of the drug was 3.5 micrometer. The suspension met the pharmacopoeial requirement for drug release. On the basis of these findings, khaya gum may find application as suspending agent at 0.2%w/v concentrations.

Key words: Co-Trimoxazole, crystal growth, drug release, khaya senegalensis gum, sedimentation volume and suspension

INTRODUCTION

Pharmaceutical suspensions are liquid dosage forms that require the addition of suspending agents in order to stabilize their system. These suspending agent increase sedimentation volume, ease redispersibility, enhance pourability and prevent compact cake formation. Suspending agents are grouped into three classes. (i) Synthetic (ii) semi synthetic and (iii) the natural polysaccharides, in which class Acacia, tragacanth, karaya and Khaya gums belong.

Khaya gum is a natural polymer, obtained as exudates from *Khaya senegalensis* tree of the Family *Meliaceae*. Studies show that Khaya gum is colourless to reddish brown translucent tears and is acidic in nature (Mahmud *et al*., 2008). *Khaya senegalensis* gum has been evaluated for its tablet binding property (Mgbahurike and Igwilo, 1991) but no work has been done on *Khaya senegalensis* gum as a suspending agent in co-trimoxazole or in any liquid formulation as compared to other available natural and synthetic polymers such as acacia. The objective of this research is to investigate the suitability or otherwise of khaya gum as a suspending agent using trimethoprim and sulphamethoxazole combination as drug models.

MATERIALS AND METHODS

Khaya senegalensis gum was exuded directly from incised trunks of the plant trees in samaru, Zaria in mid November. The plant part was authenticated in the herbarium Department of Biological sciences of Ahmadu Bello University, Zaria. The gum exudates were washed and purified using the method described by Odeku and Itiola (1992).

Formulation of suspensions: Suspensions of co-trimoxazole were formulated using different concentrations of *Acacia senegal* (BDH Chemicals, Poole, England) and *Khaya senegalensis* gum powders as suspending agents. Chloroform (Sigma-Aldrich) water (double strength) and 0.1% benzoic acid were used as preservatives in the formulations.

Co-trimoxazole suspensions containing 4%w/v sulphamethoxazole (BDH Chemicals, Poole, England) and 0.8%w/v trimethoprim (Yixing City Xingyu Medicine Chemicals Co., Ltd., China) were prepared, using either acacia or khaya gum in concentrations of 0.2, 2.0 and 5.0% w/v. Mucilages of the gums were prepared by hydration using part of the vehicle. The solid components of the formulation were finely triturated with the aid of

Corresponding Author: H.S. Mahmud, Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

mortar and pestle. The suspending agent (acacia or khaya mucilage) was added to the powdered drug and triturated until homogeneous slurry was obtained. This was transferred into a 100 ml beaker and the remaining vehicle was used to rinse the mortar to make up the required volume. Samples for the different tests were prepared. The viscosity of the suspensions were taken using Brookfield viscometer DV I- Prime digital viscometer. To observe the sedimentation rate and volume, the suspension was transferred into three 100ml bottles and three 50ml-measuring cylinders. The measuring cylinders were stoppered, put on a vibration- free surface and stored at 28-30ºC.

Evaluation of suspension properties:

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 measuring the volume of the sediments in the suspension placed in the measuring cylinders, on daily basis for 7 days and thereafter weekly for 4 weeks. The sedimentation volume was recorded daily for 7 days and then weekly for 4 weeks.

The sedimentation volume (F) was calculated using the formula

$$F = V_u / Vo \quad (1)$$

Where, V_u = ultimate volume of sediment and V_o = original volume of sediment before settling occurred.

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

Degree of flocculation: It is a qualitative expression of flocculation. Degree of flocculation (β) is expressed as

$$\beta = F / F\infty \quad (2)$$

Where, F = ultimate sedimentation volume in flocculated suspension and F∞= ultimate sedimentation volume in deflocculated suspension.

Re-dispersibility of formulated suspensions: Using measuring cylinders, 50 ml quantities of the formulated suspensions were poured into bottles, stoppered and kept on a vibration free platform. The suspensions were shaken 3 times, manually by hand after 7 days to find out how much of it was re-dispersed.

Rheological assessment: Viscosities of the prepared suspensions were determined using a Brookfield DV I prime digital viscometer. Different concentrations of the prepared suspensions (0.2, 2.0 and 5.0% w/v) were put separately in a 600 ml beaker, appropriate enough to immerse the spindle groove in the fluid. Speed of rotation was varied to determine its effect on the viscosity values since drag force is known to alter with changes on the spindle size and rotational speed. Viscosity values at rotational speeds of 10, 20, 50, and 100 r.p.m were determined at room temperature and the viscometer guard leg was used. Viscosity values were recorded for different speeds of rotation. Graphs of viscosity versus speed of rotation were then plotted.

Microscopical examination: Samples of the suspensions formulated using the gums 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 sizes of the formulated suspensions were measured using microscopic method. Drops of suspensions were separately put on slides and placed on the (pre-calibrated) stage of the microscope. Using the graticles placed in the eye-pieces, particle sizes of 500 particles were measured, and from which the mean particle sizes were later computed.

Dissolution test: To determine the dissolution behaviour of the formulated suspensions, the official paddle method for dissolution testing was carried out as described by (Gohel *et al*., 2007). Dissolution rate of the suspension was determined at 37ºC in 900 ml of a pH 7.2 phosphate buffers at a paddle rotation speed of 25 rpm. Cylindrical round bottom flasks (1 litre capacity) were immersed in a multiple spindle drive at a thermostated temperature of 37ºC. The paddles were positioned at about 2.5 cm above the bottom of the flask. The suspension was then carefully introduced to the bottom of the flask with the aid of a 10 ml glass syringe. Aliquots of 10 ml dissolution medium were withdrawn at time intervals of 2, 5 and 10 min. The withdrawn volumes were replaced each time with equal volume of the dissolution medium using a 10ml glass syringe. The dissolution medium withdrawn was immediately filtered through a filter paper and the content of drug determined using spectrum lab 752s UV-visible Spectrophotometer at wavelength of 257 nm for sulphamethoxazole as recommended in the British Pharmacopoeia (2002).

RESULTS AND DISCUSSION

In pharmaceutical suspensions, polymers play a vital role as flocculating agents with an advantage over ionic flocculating agents for their reduced sensitivity to added electrolytes; hence accommodating a wide range of excipients.

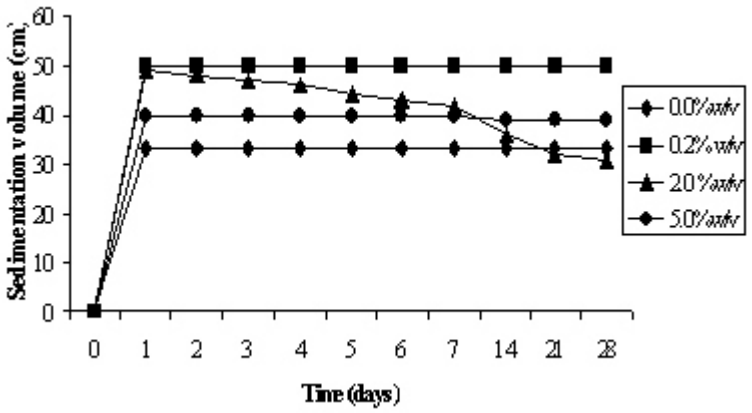

Fig 1: Sedimentation volume versus time of co-trimoxazole suspensions using different concentrations of Khaya gum

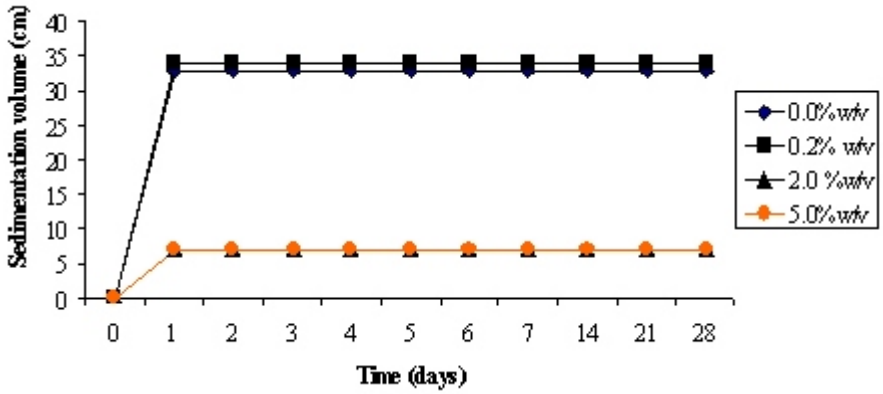

Fig 2: Sedimentation volume versus time of co-trimoxazole suspension using different concentrations of Acacia gum

The affinity of a polymer for the particle surface as well as the charge, size and orientation of the polymer molecules in a continuous phase determines its effectiveness as a stabilizing agent in any suspension. Most pharmaceutically useful polymers contain polar functional groups that are separated by a hydrocarbon backbone. This structure provides the polymer molecule with many active centres that permit interaction with a particle surface. At very low concentration of polymer, a large number of sites on the surface of the dispersed solids are available for adsorption of the polymer. The simultaneous adsorption of the polymer molecule on to the surfaces of different particles creates a bridge. At low polymer concentration the number of particle-particle bridges is relatively low. At an intermediate concentration, sufficient binding sites are still available on the particles, permitting more bridges to form. It is this intermediate concentration that results to optimum flocculation and sedimentation volume. At a high concentration of polymer, there is complete coverage of the particles by the polymer and insufficient binding sites remain on the particles to form interparticulate bridges. This consequently leads to deflocculation due to formation of adsorbed layers of polymer on different particles hence preventing close attraction (Gennero *et al.,* 2000). These perhaps offer an explanation for the inconsistency in sedimentation volumes of co-trimoxazole suspensions suspended with different concentrations of different suspending agents.

Co-trimoxazole suspensions formulated with different concentrations of *K. senegalensis* gum as shown in Fig. 1 had the highest sedimentation volume occurring with the 0.2%w/v suspension. It was the most stable, flocculated and aesthetically pleasing with no sediment or supernatant formed. The suspension remained flocculated throughout the period of storage. The suspension containing 5.0%w/v gum remained stable and achieved some level of flocculation while that formulated with 2.0% deflocculated as the storage period increased. On the other hand, co-trimoxazole suspensions formulated with acacia gum (Fig. 2) yielded deflocculated suspensions at 2.0 and 5.0% while the suspending agent

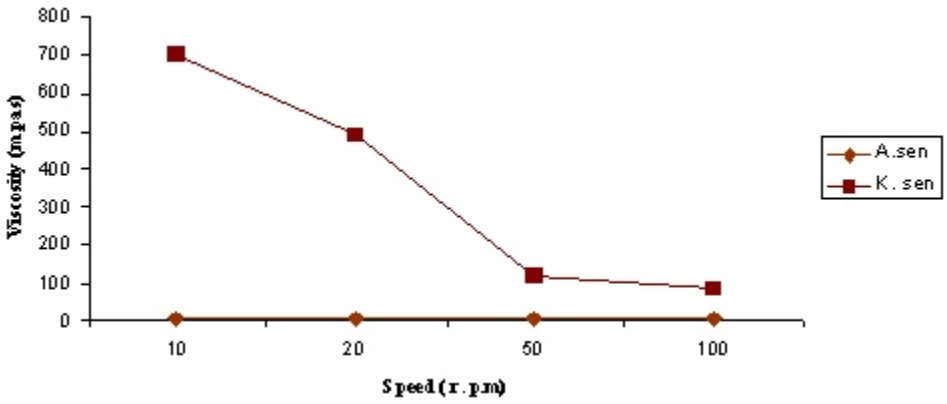

Fig 3: Effect of speed of rotation on the viscosity of co-trimoxazole suspension formulated with 0.2% w/v concentrations of test gums

did not have much impact on the suspension at 0.2% had sedimentation volume of 34 cm as compared to the control which had 33 cm.

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. The decreased viscosity values observed with increasing speed of rotation (Fig. 3) could be attributed to the nature of the mixture which is said to be pseudo plastic (Anonymous, 2007). This implies that with minimum agitation the suspension will be easily re dispersed and a stable dose can be withdrawn.

Crystal growth analysis: Crystal growth formation 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 (Carter, 2005). Dosing such suspensions orally will be expected to reduce absorption because of a reduction in dissolution rate (Gennero *et al*., 2000). This is of vital importance in storage of suspended products. Large conglomerates, appearing as fluffs due of flocculation in suspensions prepared with *K. senegalensis* gum on day 14 (Fig. 4, Plate Id) were observed while gum particles were seen hydrating on day 1(Fig. 4, Plate Ic) of formulation. This same occurrence was observed with *A. senegal* but no floccules were seen (Fig. 4, Plate Ia, b), both suspensions had no crystal growth. Therefore reduction drug absorption is not expected.

The amount of drug in the systemic circulation (bioavailability) of a suspension is determined by the extent of absorption of the drug through the GIT which can be affected by wettability, viscosity and effect of suspending agent on the suspension. Suspensions containing xanthan and tragacanth gum as suspending agents was highly viscous and showed inhibitory effect on dissolution (Gohel *et al*., 2007).

As shown in Fig 5. The release rate of co-trimoxazole was instantaneous for suspensions using the two test gums. The release profile with suspensions of acacia gum was rapid while the drug release with khaya gum was gradual. All suspensions containing the two test gums released more than 70% of their drug content which meets British Pharmacopoeia (2002) specifications.

As depicted in Table 1, formulated suspensions of co-trimoxazole that flocculated were re dispersed with ease whereas the deflocculated suspensions required vigorous agitation or did not re disperse at all due to compact cake formation. 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 interparticle interactions (Gennero *et al*., 2002). Co-trimoxazole suspensions had smaller particles on day 14 of formulation while larger particles dominated the formulation on day 1. The mean particle sizes are in the range for coarse dispersions. The

Table 1: Other evaluated parameters of the formulated Co- trimoxazole suspensions

Parameters	Gums	
	A. senegal	*K. senegalensis*
Degree of flocculation	1.03	1.52
Mean particle size (µm) of Suspension containing 0.2%w/v gum	5.54	3.50
Re dispersibility		
0.2% w/v	+ + +	+ + +
2.0% w/v	- - -	+ +
5.0% w/v	- - -	+ + +

Key = + + = re-dispersible with vigorous agitation and stable enough for adequate dose withdrawal, + + + = easily re-dispersed with minimum agitation and stable enough for adequate dose withdrawal, - - - = Not re-dispersible, formed hard cake.

0.2%w/v *A.senegal*

Plate Ia

Plate Ib

0.2% *K.senegalensis*

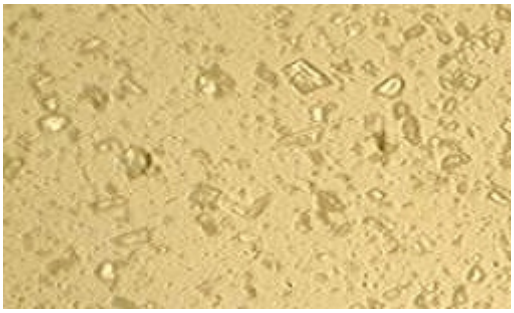

Plate Ic

PlateId

Fig 4: Plates of co-trimoxazole suspensions formulated with 0.2% w/v concentrations of *A.senegal* and *K.senegalensis* gums on day 1 (Ia, Ic) and day 14 (Ib, Id) of formulation

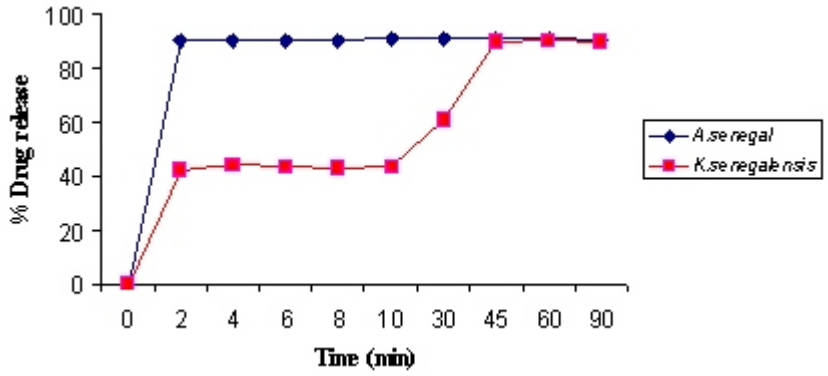

Fig 5: Percentage drug release profile of Co-trimoxazole suspensions formulated with 2.0% w/v *A.senegal and K.senegalensis* gums

degree of flocculation was higher with co-trimoxazole suspension containing khaya gum. This implies that the suspension is more stable.

CONCLUSION

In conclusion, it is necessary to have suspensions in flocculated form for long term stability. The concentration of gum to be used as wetting and suspending agent must be determined to achieve maximum flocculation. *Khaya senegalensis* gum at 0.2%w/v was able to suspend co-trimoxazole. The suspensions remained flocculated, aesthetic in appearance and stable through out the 4 weeks period of storage. Therefore, the locally sourced gum has potential to be used at 0.2% w/v concentrations as suspending agents. The gradual release rate associated with khaya gum suspension may provide potentials for its use in sustained release suspensions.

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