Cissus Stem Gum as Potential Dispersant in Pharmaceutical Liquid Systems 2: the Emulsifying and Suspending Properties

J. Alfa*, A. Chukwu, O.K. Udeala

Department of Pharmaceutical Technology and Industrial Pharmacy University of Nigeria Nsukka, NIGERIA.

**Correspondence*

Pervenuto in Redazione
il 13 settembre 1999

ABSTRACT

The emulsifying and suspending properties of a new gum derived from the stem of *cissus rufescence family Amphelidaceae* were studied. Stability of the liquid paraffin emulsions prepared using this mucilaginous substance was compared with that containing tragacanth or acacia. The rate of globule coalescence was determined using Sherman's equation for concentrated emulsions. The suspending ability of the polymer was compared with that of *tragacanth* or *compound tragacanth*. The rate of deflocculation, K, *was* found to obey a power law equation: $\beta_t = \beta_o e^{kt}$ in zinc oxide suspensions. At concentrations above 0.75% w/v, cissus gum produced liquid paraffin emulsion with minimal separation. The rate of globule coalescence was in the order *acacia > cissus > tragacanth* and rate of creaming was *tragacanth > acacia > cissus*. At concentrations of 0.6 to 1.0 % w/v, cissus gum produced highly flocculated zinc oxide suspensions, which exhibited good redispersibility. Stability of the agglomerated, dispersed particles was similar to that produced using tagacanth mucilage.

KEY WORDS: Cissus; Emulsion; Globule coalescence; Creaming; Suspension; Flocculation

INTRODUCTION

Organic polymers used as thickeners in formulated pharmaceutical liquid systems include the natural gums, modified natural gums and synthetic polymeric substances. *Tragacanth, acacia* and *guar* gums have been used as suspending and emulsifying agents in pharmaceutical preparations [1,2]. *Mucuna* and *Xanthan* gums have been evaluated as possible dispersants in suspensions and emulsions [3,4]. Some preliminary investigations have shown that *cissus* gum could serve as binder in tablet formulations [5] and the aqueous disperse system is found to exhibit pseudoplastic flow behaviour at concentrations above 4 % w/v [6].
In this study, the emulsifying and suspending properties of *cissus* stem gum have been investigated, with a view to identifying its relevance as a local excipient for the pharmaceutical or cosmetic industries.

MATERIALS AND METHODS

Materials

Cissus gum was obtained as earlier described [6]. Acetone (Pharmachem Ltd. England); liquid paraffin (Windsor Laboratories London); acacia, zinc oxide (Merck, Darmstadt Germany); tragacanth methylparaben, compound tragacanth (Sigma Chem. Coy. U.S.A.); glycerol, nigrosin dye (BDH Chem. Ltd. England); were used as obtained from the suppliers.

Emulsifying Properties

Preliminary ExperimentPreliminary Experiment

A study was carried out so as to determine suitable oil-water ratio for liquid systems containing cissus gum as emulsifier. Liquid paraffin emulsions were prepared using oil-water ratios of 2:8, 3:7, 4:6, and 5:5 with 0.2% w/v cissus gum as emulsifier in each case. Required amount of the gum was sprinkled unto the surface of measured volume of liquid paraffin and enough volume of water added.
The mixture was blended with Silverson mixer (Gallenkamp) for 5 minutes. A 50 ml quantity of each emulsion was stored at 30°C for 24 h. Selection was based upon a general appraisal of the overall emulsion consistency and extent of separation (unemulsified) after the period of storage.

Preparation of Emulsions

Batches of liquid paraffin emulsion containing 4:6 oil-water ratios were prepared with cissus gum as emulsifier in concentration range of 0.50 to 1.25% w/v. The weighted amount of the gum was placed inside measured quantity of liquid paraffin and appropriate amount of preserved water added so as to contain 0.5% w/v methylparaben in the final product. The mixture was blended with Silverson mixer (Gallenkamp) for 10 minutes. Emulsion obtained was passed three times through a table homogenizer (Gallenkamp). Similarly, batches of liquid paraffin emulsion were prepared with 10.0% w/v acacia, and 1.0% w/v tragacanth respectively. A 50 ml quantity of each emulsion was stored at 30°C in relatively vibration free platform. Separation heights (unemulsified system) were measured on weekly intervals for four weeks.

Performance of total Globule counts of Emulsions

The stability of emulsions prepared using different concentrations of *cissus* gum was studied and thereafter, the efficiency of the gum as an emulsifier was compared with tragacanth or acacia. In order to do this, it was necessary to estimate the total number of globules/mm^3 of oil in the emulsion. A comparison was then made between the globule size in each emulsion and changes in diameter with time. The globule size was determined using an expression, which has previously been applied by Chiori and Udeala [7].

Apparatus

A hemocytometer (Hawksley and Sons BSS 748 London) with improved Neubauer ruling was used. The central area is divided into 25 large square which is further subdivided to form 16 smaller squares each with an area of 2.5×10^{-3} mm^2 and depth of 1.0×10^{-1} mm giving a volume of 2.5×10^{-4} mm^3.

Method

A globule count was performed on each emulsion 24 h after preparation and eight weeks of storage respectively. Dilution of the emulsion and subsequent globule count was as described by Chiori and Udeala [7]. A 0.1 to 0.5 ml quantity of the emulsion was taken from a depth of about 2 cm below the surface with the aid of a 1 ml syringe and transferred to 100-ml volummetric flask containing 50 ml of 80% w/v aqueous glycerin solution. The volume was made up to 100 ml with 1% w/v nigrosin solution, thoroughly mixed and the diluted emulsion was placed under the cover slip on the hemocytometer. By capillary action, it diffused slowly until the entire chamber was covered. The globules were observed with a light microscope (CK Olympus Tokyo, Japan) fitted with x 40 objective and x 10 eyepiece. The globule in 256 small squares were counted and the number N derived from each mm^3 of oil, the root mean cube diameter (d) and the rate of globule coalescence in each emulsion during the eight weeks of storage was determined.

Suspending Properties

Preliminary Experiment

In a preliminary experiment, the suspending effect of the new gum was compared with that of acacia. Suspensions of 16 % w/v concentration of zinc oxide were prepared containing 0.4% w/v of cissus or acacia gum as dispersant in the aqueous systems. A 50 ml measured volume of each suspension was transferred to 100- ml measuring cylinder and the sedimentation volume (F) described as $H_u/H_o.100$ [8] was determined after 7 days of storage. The H_u and H_o represent sedimentation height at time zero and t respectively. Re-dispersibility was evaluated by hand Shaking.

Preparation of suspensions

Batches of 16% w/v zinc oxide suspensions were prepared containing varied concentration of cissus gum between 0.2 – 1.6%. The required amount of finely powdered drug was dispersed in appropriate volume of the mucilage. Suitable volume of preserved water was added so as to contain 0.15% methylparaben in the final product. Suspensions containing 0.3% w/v tragacanth or 2% compound tragacanth respectively were similarly prepared and each batch was blended with a Silverson mixer (Gallenkamp) for 5 minutes.A 50 ml measure of each suspension was transferred into 100 ml calibrated measuring cylinders and stored at 30° in a relatively vibration free platform. The height of sediments in each sample was measured on daily basis for 1 week and thereafter monitored weekly for 7 weeks (a total of 56 days).
The performance of zinc oxide suspension formulated with 0.8% w/v composition of cissus gum was compared with those containing 0.3% w/v tragacancth and 2 % w/v compound tragacancth respectively.

Re-dispersibility

Investigators for assessment of re-dispersibility of suspensions have described various methods. The method used by Tempio and Latz [8] consisted of manually shaking the cylinders containing the suspensions whereas Farley and Lund [9] employed inversion by hand. The experimental design in this investigation involved centripetal rotation of the suspension along a vertical support until the suspension was cleared from the base. The number of revolutions required to clear the dispersion off the base was taken as the redispersibility number (rdn). This procedure was performed for all the batches of suspensions after 7 and 56 days of storage respectively.

RESULTS AND DISCUSSION

Emulsifying properties

The stability of a pharmaceutical emulsion is characterised by the absence of coalescence of the internal phase, ab-

sence of creaming and the maintenance of elegance [10]. The observation of creaming (although not a criterion of thermodynamic stability in the strict sense) and/or separation of the water and oil phases in an emulsion could serve as a practical commercial aspect of stability. Table I shows the rate of creaming of liquid paraffin emulsions formulated with different concentrations of cissus gum.
The rate of creaming decreased with concentration of the new gum.
At concentrations below 1.0% w/v of the gum, the rate of creaming was high, with the separated, unemulsified system reaching a level as high as 33% at the end of 4weeks of storage.
At higher concentrations, the emulsions were relatively stable and only 9 % of the liquid system separated, unemulsified, at the end of 4 weeks of study in the batch formulated with 1.25 % of the gum.
The rate of creaming in the batches of emulsions containing tragacanth or acacia was compared with that formulated using 1.25% cissus gum (Table II). It was observed that, the degree of creaming was in the order *tragacanth* > *acacia* > *cissus* as percentage separation after 4 weeks of storage was 49, 10 and 9 respectively.

Emulsion stability has been evaluated on the basis of average particle size distribution and growth in globule size with time [3, 4, 7].
In this study, globule count was performed on the emulsions containing varying concentration of *cissus* gum. The number of globule (N) per mm^3 of oil dispersed in the emulsion was calculated using the expression (7):

$$N = c/256 \cdot f \cdot 100/p \cdot 10^4/2.5 \quad (1)$$

where c is total globule count, f, is dilution factor and p is percent oil content. The cube root mean diameter (d) of the globules for each batch of the emulsion was determined 24 h after preparation using the equation [11]:

$$d = 10^3 \cdot \sqrt[3]{6 \prod N} \quad (2)$$

and the results are presented in table III.

The result shows that an increase in the emulsifier concentration reduces the globule size of the emulsion formulation.
The dispersion containing 1.25% concentration of the new

Table I. Effect of concentration of cissus gum (%w/v) on the Rate of creaming of liquid paraffin emulsion

	Unemulsified system (%)			
Time (week)	0.50	0.75	1.00	1.25
0	0.00	0.00	0.00	0.00
1	7.00	5.00	2.00	1.00
2	17.00	5.00	7.00	5.00
3	37.00	29.00	15.00	7.00
4	34.00	33.00	17.00	9.00

Table II. Effect of emulsifier on the rate of creaming of liquid paraffin emulsion

	Unemulsified system (%)		
Time (week)	Cissus 1,25%	Tragacanth 1%	Acacia 10%
0	0.00	0.00	0.00
1	1.0	5.00	1.00
2	5.0	47.00	4.00
3	7.0	49.00	5.00
4	9.0	49.00	10.00

Table III. Effect of concentration of *cissus* gum on globule mean cube diameter

CONC. OF EMULGENT %W/V	DILUTION	TOTAL GLOBULE COUNT (c)	NUMBER OF GLOBULES PER MM^3 OF OIL N (X 10^6)	MEAN CUBE ROOT DIAMETER, D. (Mm)
0.75	1.200	298	2.33	9.36
1.00	1.200	321	2.51	9.13
1.25	1.200	422	3.30	8.33

gum was creamier, lighter in colour and had the smallest globule size. The globule size of the batch of emulsion containing 1.25% cissus gum and that containing 1.0% tragacanth or 10.0% acacia was determined after 24 h and 56 days of storage respectively while the rate of globule coalescence was calculated in each case using Sherman's equation for concentrated emulsions [11]:

$$InD = InD_o + Kt / 3 \quad (3)$$

where D_o and D_t represents the globule size at time zero and 56 days of storage respectively. Table 4 shows the mean cube root diameter of globules in freshly prepared / aged emulsions and the rate of globule coalescence. The batch containing tragacanth had the most coarse globule size but most stable to coalescence whereas that containing cissus or acacia had smaller sizes and exhibited high rate of globule coalescence. This result is somewhat similar to Sherman's [12] findings that small particles have inherent instability and tends to mutually coalesce with large particles.

Suspension properties

The performance of zinc oxide suspension containing cissus or acacia gum at 0.4% concentration was compared as represented in Table V. The aqueous system containing cissus gum showed evidence of flocculation, with high sedimentation volume (F) of 53.3 % after 7 days of storage. Re-dispersibility was readily achieved on slight shaking of the container. A sharp contrast was rather observed with the suspension containing acacia, where low sedimentation volume of 13.3 % was recorded, with formation of cake, which was difficult to re-disperse. The impression derived from the preliminary experiment is that, the new polymer has potential flocculating properties.
Sedimentation rate, which has been used as a parameter for evaluating stability of suspension [8] was determined and used as basis of assessment in this investigation. This was achieved by monitoring the ratio of the final height of sediment (H_u) to the initial height of sediment (H_o) within a period of time. Figure 1 shows that, the sedimentation rate of zinc oxide suspensions decreased with concentration of the gum. At concentrations lower than 0.4% w/v, the use of cissus gum resulted in unstable suspensions, with high sedimentation rate. The low sedimentation volume observed at low concentrations, with consequent formation of cake might be due to insufficient polymer coating necessary to effect flocculation of the powders. The increase in viscosity of the system with concentration of the gum [6] as noticed in our earlier study, might have contributed to greater stability of the suspensions at concentrations between 0.6 to 1.6 % w/v of cissus gum.
Tempio and Latz [8] have observed that flocculation is an

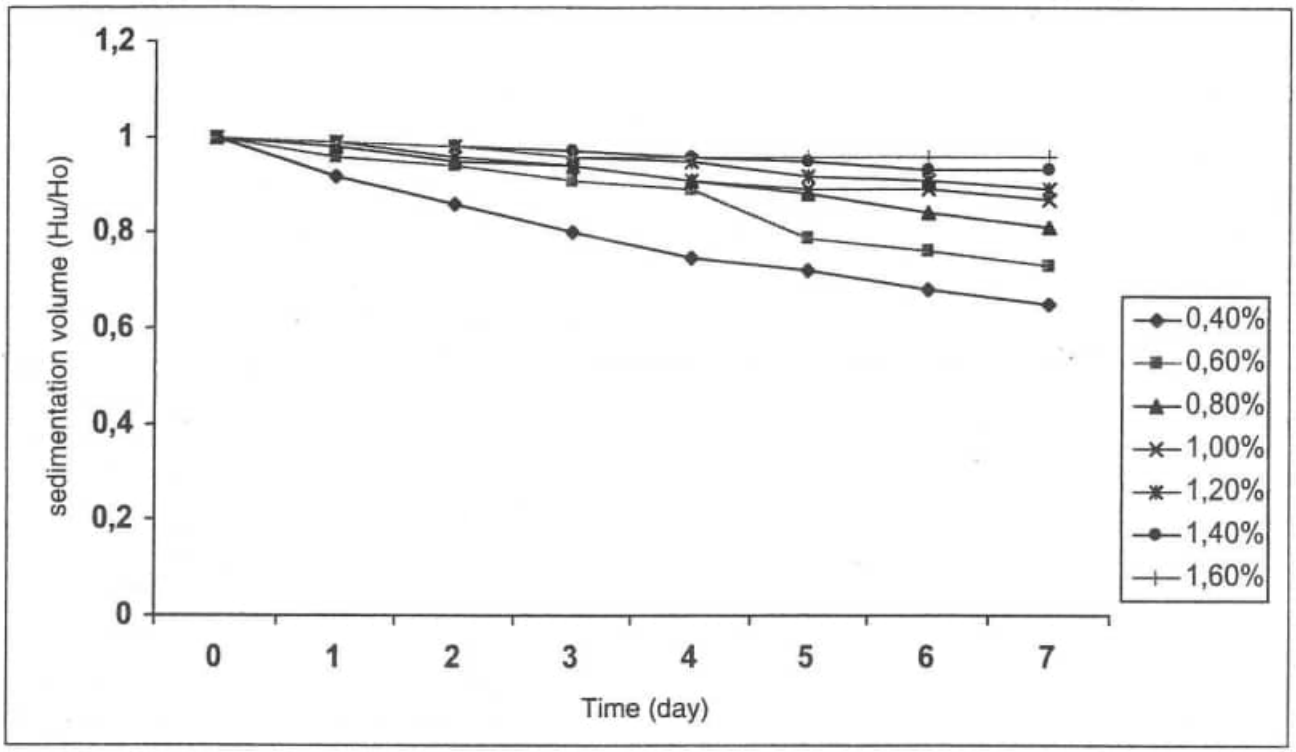
Table IV. Rate of globule coalescence in freshly prepared and aged emulsions

Emulgent (% w/v)	Dilution	Globule Count		Number of globules Per mm^3 of oil (x 10^6)		Mean Cube Root Diameter (mm)		Rate of globule coalescence K (day^{-1})
		C_o	C_t= 56 days	N_o	N_t = 56 days	d_o	d_t = 56days	
Cissus 1.25	1.200	422	300	3.30	2.34	8.33	9.30	6 x 10^3
Tragacanth 1.0	1200	59	48	0.46	0.38	16.07	17.3	3 x 10^3
Acacia 10.0	1000	921	568	35.9	22.34	3.76	4.40	8 x 10^3

Table V. Effect of gum on the flocculation of zinc oxide suspensions

Suspending agent (0.4% w/v)	Ho (cm) (t_o=24 h)	Hu (cm) (t =7days)	$H_u / H_o.100$	Observation
Acacia	15	2.0	13.3	formed cake
Cissus	15	8.0	53.3	formed flocs

Fig. 1. Sedimentation rate of zinc oxide suspensior



effective way of controlling the settling rate and subsequent caking of relatively coarse suspensions: The phenomenon of degree of flocculation is related to sedimentation volumes by the expression:

$$\beta = F / F_{\infty} \quad (4)$$

where F and F_{∞} represent sedimentation volume of the flocculated and deflocculated system respectively. Sedimentation rate and zeta potential have been used as indices of caking ([13]) and similarly, sedimentation volume and flow rate have been used as indices of stability and pourability in suspensions ([14]). In this evaluation, degree of flocculation and re-dispersibility were used as indices of caking and pourability as represented in Figure 2.

Caking was noticed in the region A while the second region, designated B was characterised by high degree of flocculation and ease of redispersibility. The classified third region, C had low degree of flocculation, no caking but redispersibility was not readily achieved as in the zone, B. An unsightly adherence of the particles to the wall of the container occurred here and the flow was non-regular. The implication is that uniform dosing of the formulation may not be guaranteed in the region, C.

A maximum degree of flocculation of 8.2 was obtained at 0.6% w/v of the new gum that also coincided with the lowest redispersibility number of 2. This observation is probably suggestive of polymer adsorption and bridging as postulated by Lamer and Healey ([15]).

Maximum bridging therefore occurs at some optimal concentration, in this instance, within 0.6 to 1.0 %w/v cissus gum, which consequently resulted in optimum degree of flocculation. The lower degree of flocculation and cake formation at concentrations of 0.2 to 0.4% of the gum may be attributed to insufficient polymer adsorption. The decrease in the degree of flocculation of the systems containing ≥1.2 % cissus gum may be a result of excessive polymer coating which leaves little or no space for particle-particle bridging. On the basis of these analyses, cissus gum could serve optimally as suspending agent for some pharmaceutical powders at concentrations between 0.5 to 1.0 % w/v.

Sedimentation rate of the systems as represented in figure 3 shows that the performance of zinc oxide suspension for-

Fig. 2. Effect of concentration of cissus gum on degree of flocculation and redispersibility of zinc oxide suspensions

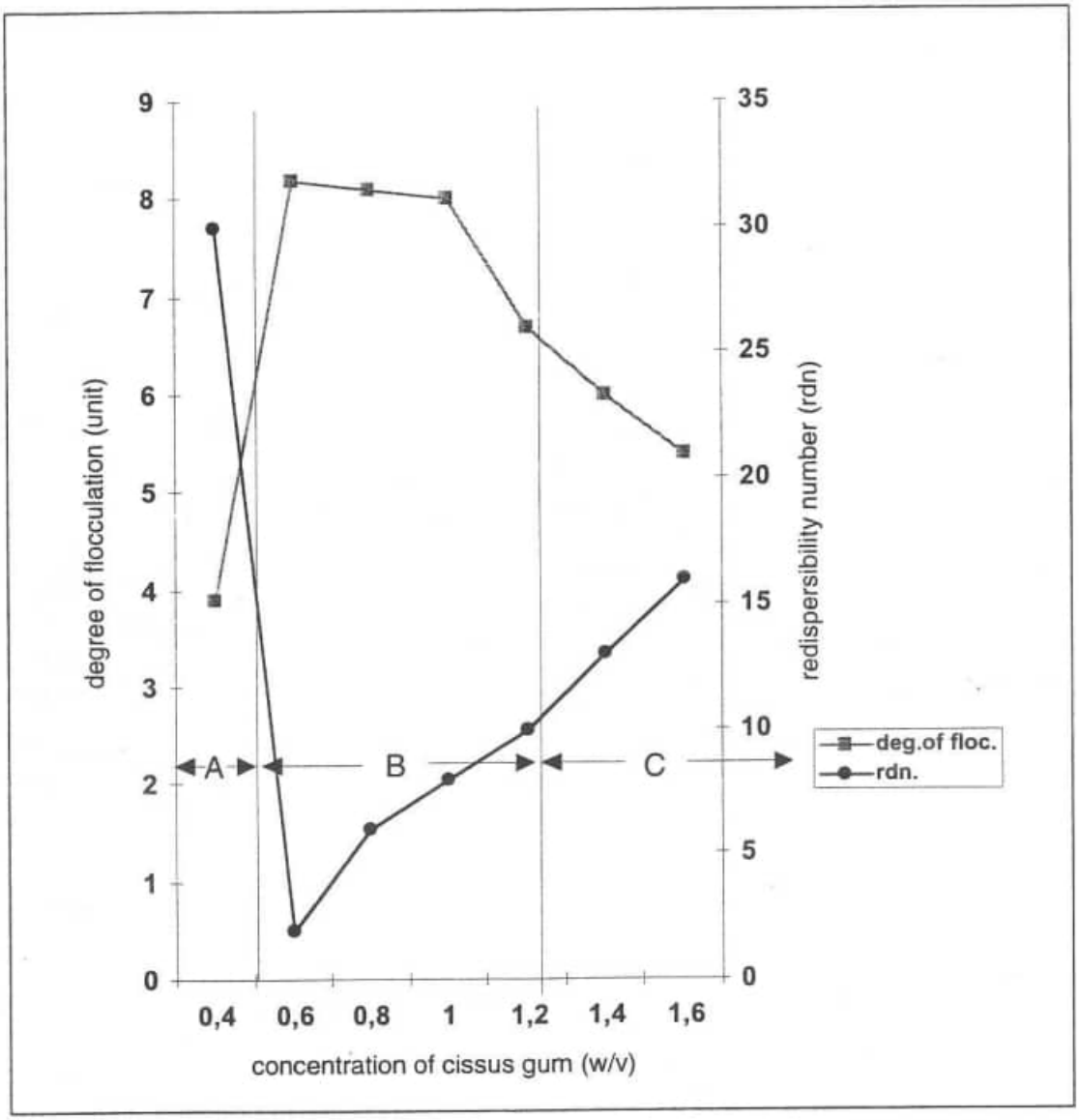
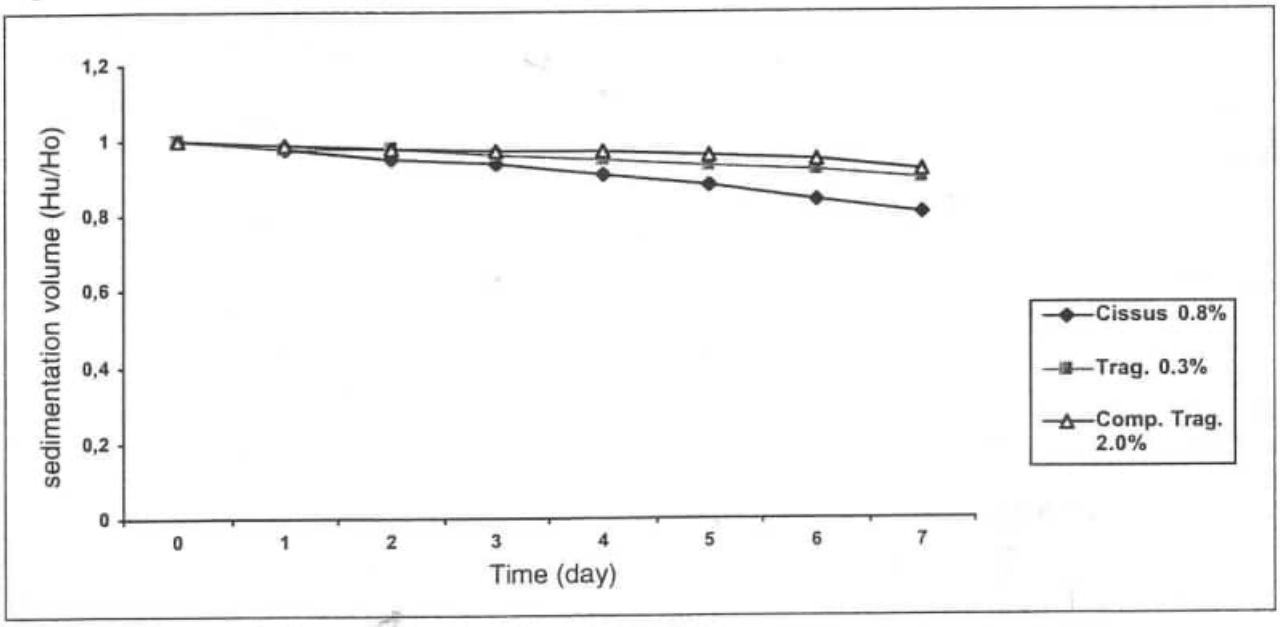


Fig. 3. Sedimentation rates of zinc oxide suspensions



mulated with 0.8 % w/v cissus is close to that prepared using 0.3 % tragacanth or 2.0 % compound tragacanth. There was no cake formation and all re-dispersed with ease after one week of storage.

Sedimentation pattern of flocculated suspensions have been shown to consist of an initial phase and a final phase as reported by Cerstensen and Su ([16]). The initial phase involves a sharp drop in sedimentation volume followed by gradual settling of particles in the second phase. The first phase was found to follow a first order decay pattern represented mathematically by the equation:

$$a = x_o e^{-kt} \quad (5)$$

were a is height of sediment at time, t and x_o is height of the sediment at time zero.

The behaviour of the suspended particles in this phase might be related to an initial deaggregatation of the flocs followed by a gradual settling of the discrete particles in the second phase, which may eventually form a compact mass (cake). The initial phase that is reported to follow a first order decay pattern could be related by the expression:

$$\beta_t = \beta_o e^{-kt} \quad (6)$$

where b_t is the degree of flocculation at time t, β_o is the degree of flocculation at time zero and *k* is the rate constant. This relationship, when expressed in logarithmic form, results in the following expression:

$$\text{Log}b_t = \text{Log}\beta_o - \frac{Kt}{2.303} \quad (4)$$

Taking the time t_o as 7 and t to be 14, 21, 28, 35, 42, 49, or 56 days respectively, the corresponding degrees of flocculation b_o or b_t were calculated using equation 4 and the results are presented in Table VI.

The rate of deflocculation, *k* of each liquid system was determined as slope of the Graph of $\log b_t$ versus t and the result is represented in Table VII.

Cissus gum exhibited the highest initial degree of flocculation as shown in Table VI, that is, 8.1 for cissus compared to 3.3 and 3.7 for compound trgacanth and tragacanth respectively. There was no cake formation in all the batches after 7 days of storage and the suspensions redispersed with ease. However, after 56 days of storage, suspensions containing compound tragacanth and cissus gum were more easily redispersible than that prepared with tragacanth.

The rate of deflocculation, k, was equivalent in formulations containing cissus or tragacanth while that containing compound tragacanth was most stable to the deflocculation process. The low degree of flocculation and difficulty in redispersibility noticed in zinc oxide suspension formulated with tragacanth after 56 days of storage might be associated with cake formation on storage due to lower coating of the zinc oxide particles. Although the rate of deflocculation in suspension containing cissus was similar to that formulated with tragacanth, there was no cake formation in the former. The presence of starch and acacia in compound tragacanth ([17]) might have contributed to the high stability of the flocs in the liquid system containing compound tragacanth as flocculant. Interestingly, after 8 weeks of storage, the degree of flocculation of suspension formulated with cissus gum was found to be relatively close to the initial values of those prepared using tragacanth or compound tragacanth as in table VI. The flocculating effect of cissus gum may thus be considered as being superior to those of the other two agents used in this study.

Table VI. Degree of flocculation of zinc oxide suspensions after 7 and 56 days of storage

Suspending agent	Degree of Flocculation	
	7 days = t_o	56 days = t
Cissus 0.8% w/v	8.1	2.5
Tragacanth 0.3% w/v	3.1	1.1
Compound tragacanth 2% w/v	3.3	3.1

Table VII. Rate of deflocculation in zinc oxide suspensions

Suspending agent	Rate of deflocculation (day^{-1})
Cissus 0.8% w/v	0.02
Tragacanth 0.3% w/v	0.02
Compound tragacanth 2% w/v	0.001

CONCLUSSION

Cissus gum exhibited good emulsifying properties at concentrations of between 1 to 1.5 % w/v. The consistency of the emulsion formed with the new polysaccharide gum is similar to that prepared with acacia. The flocculating effect of the highly viscous material was found to be superior to that of tragacanth or compound tragacanth and the flocs redispersed with ease after a period of storage. The overall impression is that the polymeric gum derived from the edible cissus plant could strongly find application as dispersant in some pharmaceutical and cosmetic disperse systems.

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