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Research Article

EVALUATION OF SUSPENDING AND EMULSIFYING PROPERTIES OF CITRULLUS LANATUS SEEDS GUM

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ABSTRACT

The biphasic formulations were aimed with firstly to evaluate the *Citrullus lanatus* seed gum for its rheological properties secondly, compare between the flocculating behavior of *C. lanatus* and tragacanth, there suspending properties and thirdly comparison between there emulsifying activity. In this context, the gum was evaluated at different pH, temperature, RPM etc., and found that with an increase in pH and decrease in temperature viscosity increases. Then the suspensions of paracetamol were prepared and compared with different concentrations (0.5%, 1%, 1.5% and 2% w/v) of *C. lanatus* and tragacanth gum. Their sedimentation profile, pH, and rheological behavior were compared and found to show no sedimentation in 7 days study with less viscosity and comparable degree of flocculation to tracaganth. Flocculation study of *C. lanatus* seed gum reveals that flocculation ability of *C. lanatus* seed gum was more than gum tracaganth at lower concentration. For comparing the emulsifying activity, castor oil was taken as a model drug and emulsified with *C. lanatus* gum and gum acacia. *C. lanatus* gum showed good stability for the emulsion. From the study above it can be concluded that the *C. lanatus* seed gum acts as very good suspending agent than gum tracaganth and can be used in low concentration as emulsifier compared to acacia gum.

Keywords: *Citrullus lanatus*, Emulsifier, Acacia gum, Pharmaceutical excipients, Emulsion, Suspending agents, Rheology, Flocculation.

INTRODUCTION

A pharmaceutical suspension is a coarse dispersion in which insoluble solid particles are dispersed in a liquid medium, 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. A major challenge to formulation of suspensions is that of physical stability. The solid insoluble drug separates from the vehicle and settles to the bottom. It is desirable that such a formulation re-suspend easily upon shaking. Settling and aggregation may result in the formation of cakes that are difficult to re-suspend. Redispersibility of insoluble drug substance is, therefore, a critical requirement in the evaluation of suspensions. It is also a critical requirement that the drug in suspension be homogeneously mixed and remain both physically and chemically stable during the shelf life of the formulation. This is important due to the need to dispense a fairly uniform and accurate dose of the medicament [1-4].

The biological source of watermelon is *Citrullus lanatus* belonging to family Cucrbitaceae. It is cultivated in South Africa, China, Europe and India. It mainly contains proteins, vitamin-B, vitamin-C, minerals, fats, amino acid citrulline and beta carotene. Watermelon as a dietary supplement is used as diuretic. Due to its rich composition of fatty acids, watermelon seeds are used for smooth skin and also used in skin ointments and formulations. Seed oil is also used in baby formulations, creams, lotions, soaps, and eye creams [5,6].

Plant products serve as an alternative to synthetic products because of their eco-friendly nature, biodegradable and compatibility. Binders such as natural gums and mucilage's are biocompatible, cheap and easily available and have the advantage of the lack of toxicity, low cost, and availability. The objective of this study is that to evaluate the *C. lanatus* gum for is suspending and emulsifying properties.

In a study of suspending properties of *C. lanatus* seed gum was compared with tragacanth as suspending agents for paracetamol suspension at a concentration of 0.5%, 1%, 1.5% and 2% w/w. The evaluation parameters were appearance, viscosity, rheology, sedimentation volume, and flocculation studies. While for the emulsifying properties an effort was made to evaluate the efficacy of gum as an emulsifier. The emulsifying characteristics of gum was evaluated and compared with that of the emulsion prepared by using gum acacia as standard emulsifying agent.

MATERIALS AND METHODS

Paracetamol is gift sample provided by centaur Pharmaceuticals Ltd., Pune, India. All the other chemicals, solvents, reagents used were of pharmacopoeial and analytical grade. The seeds of *C. lanatus* were purchased from the local market.

Extraction of gum from seeds [7-9]

The collected seeds were powdered into a coarse powder using multimill. 100 g of seed powder were defatted by Soxhlet extraction using petroleum ether as a solvent at temperature 60-70°C. This was repeatedly extracted using hot water till the complete gum was extracted. Then extracted solution was concentrated on a hot plate, filtered and kept aside for cooling. After cooling isopropyl alcohol was added to the solution (isopropyl to gum solution, 2:1), mixed thoroughly and left for 120 minutes at room temperature. The precipitate was sedimented, decant the solution and dry the precipitate in room temperature till it was completely dried. Obtained gum was crushed to get fine gum powder.

Characterization of gum [10,11]

Determination the pH of gum solution

0.5, 1, 1.5, 2% solution of *C. lanatus* seed gum in a distilled water was used to determine the pH with calibrated digital pH meter, model-EQ-610 (equiptronics). 100 ml solution was used to measure pH.

Determination of viscosity

Viscosity measurements were carried out using Brookfield viscometer (DV-II+Pro) in small sample adapter with spindle number 18 (Brookfield Engineering Labs. Inc.). Different concentrations of *C. lanatus* seed gum were prepared in distilled water while stirring for 2 hrs using a mechanical stirrer For test, hydrated samples were loaded into the small sample adapter and allowed to equilibrate for 10 minutes at 25±2°C for 100 rpm.

Determination of the effect of pH on viscosity

For the determination of viscosity at various pH Brookfield viscometer (DV-II+Pro) in small sample adapter with spindle number 18 (Brookfield Engineering Labs. Inc.) were used. For increase Ph 0.1 N NaOH solutions were used (0.1% gum solution).

Determination of effect temperature on viscosity

For the determination of viscosity of 0.5% w/v gum solution at various temperature Brookfield viscometer (DV-II+Pro) in jacketed small sample adapter with spindle number 18 were used for 100 rpm. For increase and decrease temperature hot and cold water were used.

Flocculation studies [12,13]

The flocculation experiments were carried out using a conventional jar test apparatus. The jar test is the most widely used method for evaluating and optimizing the flocculation processes. A volume of 300 ml of flocculent-dye solutions were agitated in a flocculator at 100 rpm for 1 minutes and then 30 rpm was quickly established for 10 minutes. After slow mixing, the beakers were carefully removed from the flocculator and allowed to settle for approximately 10 minutes. The different concentrations of flocculants were taken in the range of 5-20 mg/L. Portions of the settled solutions are then removed and tested at definite intervals. The portions of the supernatant solution taken out were analyzed spectrophotometrically. The concentrations of dye were analyzed using ultraviolet-visible (UV-Vis) spectrophotometer at wavelength 664 nm for methylene blue. The test was done at room temperature.

The % dye removal was calculated from initial concentration (C_0) and final concentrations (C_e) of test solutions.

$$\text{Dye removal }(\%) = \frac{C_0 - C_e}{C_0} \times 100$$

Preparation of suspension [13]

Compound tragacanth powder (1.0 g) and 0.75 g of paracetamol were triturated together with 20 ml of water to form a smooth paste. Sucrose (5 g) and vanillin (1 g) were added gradually with constant stirring to above solution. After that preservatives like methyl and propyl paraben were added. The mixture was transferred into a 100 ml volumetric flask, made up to volume with distilled water and then shaken vigorously for 2 minutes (thus making 1.0% w/v of the gum in the preparation). The procedure was repeated using 0.5%, 1.5% and 2.0% w/v of tragacanth powder. The above procedure was repeated with 0.5%, 1%, 1.5% and 2.0% w/v *C. lanatus* seed gum.

Preparation of emulsion [1-4]

To study the emulsifying characteristics, 2 g gum was taken in a clean and dry mortar and pestle. The emulsification of castor oil was done by wet gum method. The *C. lanatus* seed gum taken in a mortar and pestle and triturated with twice its weight of water and oil until a white primary emulsion with clicking sound was formed. From the study it was found that the primary emulsion results at the oil:water:gum ratio of 4:2:1 w/v. Now the primary emulsion was diluted to 20 ml with distilled water and kept in a clean and tightly closed container for stability study for 6 months.

Evaluation of suspension [13-16]

Rheological study

The time required for each suspension sample to flow through 10 ml pipette was determined (in ml/seconds) and the apparent viscosity was calculated:

$$\text{Flow rate} = \eta\alpha = \frac{\text{Volume of pipette (ml)}}{\text{Flow time (seconds)}}$$

The viscosity (in poise) of the samples was determined at 25°C using the Brookfield viscometer, model DV-II+PRO (Brookfield Laboratories, Massachusetts) at 20 rpm (spindle no. 18). All determinations were made in at least triplicate and the results obtained are expressed as the mean values.

Rate of sedimentation

The rate of sedimentation 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 7 days. The sedimentation volume, F (%), was then calculated using the following equation:

$F=100\ V_u/V_o$

Where, V_u is the ultimate volume of the sediment and V_o is the original volume of the suspension.

Determination of the pH of the suspensions

The pH of each of the prepared suspension was measured using pH meter (equiptronics digital pH meter); 10 ml of each suspension was poured into four calibrated tubes, which were stored at room temperature.

Degree of flocculation

Potassium dihydrogen phosphate was added as a deflocculating agent. The degree of flocculation (β) was assessed by comparing the ultimate sedimentation volume (Fα) with control formulations in which no flocculating agent was added.

Degree of flocculation, $\beta = F/F\alpha$

Where, F is the ultimate flocculation height in the flocculated system and, Fα is the ultimate sedimentation height in deflocculated system.

Evaluation of emulsion [11]

Micromeritic measurements of emulsion stability

The particle size distribution of the emulsions was measured using a motic microscope. The emulsions were diluted with distilled water prior to analysis so that the droplet concentration was <0.02% w/v. Each sample was analyzed 3 times and the data are presented as the average.

Centrifugal method of evaluating emulsion stability

The samples of emulsion were placed in the test tube of centrifuge (Remi, C-24BL, Remi Instruments Ltd., Mumbai, India) controlled to temperature at 37°C for 10 minutes with 3500 rpm.

The emulsion stability "S" was determined from the formula:

$S = ([V_0-V]/V_0) \times 100\%$.

Where, S-emulsion stability %, V_0-volume of emulsion undergo centrifugation cm^3, V-volume of the phase given off cm^3.

Viscosity measurement of emulsion stability

The emulsions were stored at room temperature for 12 weeks and the viscosity of the emulsions were measured by using Brookfield viscometer, spindle-61 (Brookfield LVII, USA) at the interval of 0, 6 and 12 weeks.

Turbidity measurement of emulsion stability

Every emulsion was diluted 1 part to 1000, prior to the absorbance measurements. The absorbance was taken at 400 nm and 800 nm, using UV-Vis spectrophotometer (UV-1601,

Shimadzu, Japan). From the absorbance values the opacity was determined and the ratio of the absorbance at 800-400 nm, the size index (R) and stability were predicted.

RESULTS AND DISCUSSION

The extracted gum was evaluated for its physicochemical, suspension and emulsifying properties.

Determination the pH of gum solution

The pH of gum solution in distilled water was found to slightly increase with increase in concentration of gum as shown in Table 1.

Determination of viscosity

The viscosity was found to increase with increase in concentration of gum as shown in Table 2.

Table 1: pH of *C. lanatus* seed gum solution

Concentration (% w/v)	pH
0.5	6.01
1.0	6.10
1.5	6.15
2.0	6.19

C. lanatus: Citrullus lanatus

Table 2: Viscosity of *Citrullus lanatus* seed gum

S. no.	Concentration (% w/v)	Viscosity (cp)
1	0.1	9.6
2	0.2	15.0
3	0.3	21.9
4	0.4	32.4
5	0.5	39.1

C. lanatus: Citrullus lanatus

Table 3: Effect of pH on viscosity of *C. lanatus* seed gum

Ph	4.88	5.80	6.93	7.98	8.83	9.40	9.70	9.85	9.93	10.06
Viscosity (cp)	7.5	7.8	8.7	10.2	10.5	10.8	11.7	12.6	13.2	13.5

C. lanatus: Citrullus lanatus

Determination of effect of pH on viscosity

The viscosity of 0.1% w/v *C. lanatus* seed gum was found to alter with alteration in pH i.e. viscosity increased with increase in pH as shown in Table 3.

Determination of effect temperature on viscosity

Viscosity at decreasing and increasing temperature:

With the decrease in temperature the viscosity of *C. lanatus* seed gum was found to increase and vice versa as shown in Tables 4 and 5.

Flocculation study

Flocculation is incredibly imperative in case of pharmaceutical suspensions. The plot in Fig. 1 shows % dye removal (methylene blue) versus concentration of flocculants used. It was observed that with increase in concentration of flocculants (*C. lanatus* seed gum and gum tragcanth)% dye removal was increased up to 10% level, above that there was decrease in dye removal as concentration of flocculants increases. The maximum dye removal was found to be 81.9% at 10 mg/L concentration of *C. lanatus* seed gum. At lower concentration of flocculants in suspension caused larger amount of dye particles to aggregate and settle this is due to polymer bridging during flocculation. At higher concentrations of flocculants in dye solution particle settling was disturbed, which may be due to increase in repulsive energy between flocculants and dye solution. Whereas the exact opposite effect was seen for gum tracaganth. At lower concentration dye removal was less but after 10 mg/L concentration the dye removal was increased up to 86.7%. The results of flocculation studies reveals that flocculation ability of *C. lanatus* seed gum was more than gum tracaganth at lower concentration, whereas for tracaganth it is more in higher concentration as per Fig. 1.

Evaluation of suspension

Rheological study and rate of sedimentation

The rheological studies of the suspensions prepared with *C. lanatus* seed gum and gum tragacanth shows that the suspensions are pseudo

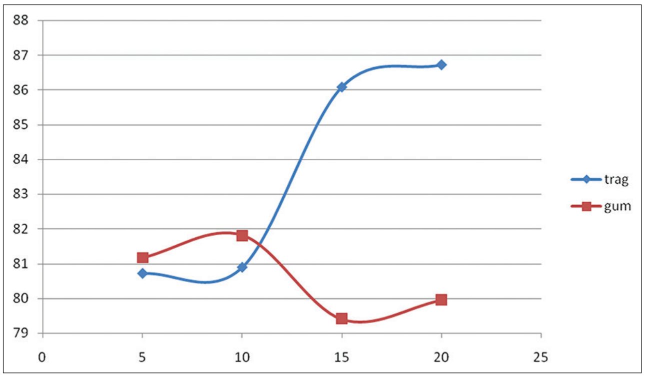

Fig. 1: Effect of concentration of flocculants on % dye removal

Table 4: Effect decreasing temperature on viscosity of *C. lanatus* seed gum

Temperature (C)	21	19	17	15	14	13	12.7	12	11.5	11	10	9.8	9.5	9.3	8
Viscosity (cp)	11.1	11.7	12.0	12.3	12.6	12.9	13.2	13.5	13.8	14.1	14.4	14.7	15.0	15.3	15.6

C. lanatus: Citrullus lanatus

Table 5: Effect increasing temperature on viscosity of *C. lanatus* seed gum

Temperature (C)	21	32.5	33.0	36.5	37.0	41.0	41.2	43.0	45.0	46.0	47.0	50.6
Viscosity(cp)	11.1	10.8	10.5	10.2	9.9	9.6	9.2	9.0	8.7	8.4	8.1	7.8

C. lanatus: Citrullus lanatus

plastic in their nature and their viscosity decreases with increase in rate of shear, which is an essential property in the formulation of suspension. Flow rate also decreases with increase in concentration of suspending agent. The results also reveal that the viscosity of suspension with *C. lanatus* seed gum was much less than gum tragacanth but showed good stability than gum tracaganth. It is quite known that the better is the suspending medium the lesser the rate of sedimentation. Suspensions are routinely evaluated for their suspending property. To evaluate the suspending properties of the *C. lanatus* seed gum, suspensions were prepared with fixed concentration of paracetamol but with varying concentration of test *C. lanatus* seed gum (0.5-2.0% w/v) as well as tragacanth. With the change in concentration of *C. lanatus* seed gum, no change was found in the sedimentation volume throughout of study. During study of 7 days rate of sedimentation was higher during first 2-3 days for and then decreases for tracaganth as shown in Tables 6 and 7.

Table 6: Flow rate and viscositys of suspension

Suspending agents	Concentration (% w/v)	Flow rate (ml/seconds)	Viscosity (cp)
Tragacanth	0.5	1.2	22.5
	1.0	1.1	31.5
	1.5	1	57.0
	2.0	0.9	85.5
Citrullus lanatus Gum	0.5	1.2	12.0
	1.0	1.0	13.5
	1.5	0.85	15.5
	2.0	0.75	16.5

Table 7: Sedimentation volume (%) using different concentration of suspending agents

Time	Tragacantha				Gum			
	0.5	1.0	1.5	2.0	0.5	1.0	1.5	2.0
½ hr	100	100	100	100	100	100	100	100
6 hrs	70	72	75	79	100	100	100	100
1 day	61	64	67	70	100	100	100	100
2 day	43	45	52	61	100	100	100	100
3 day	25	30	43	52	100	100	100	100
4 day	16	28	40	46	100	100	100	100
5 day	16	28	40	46	100	100	100	100
6 day	14	24	36	40	100	100	100	100
7 day	14	26	38	44	100	100	100	100

Table 8: Determination of pH of formulation using different concentration of suspending agents

Concentration (% w/v)	pH	
	Tragacanth	Watermelon gum
0.5	5.59	6.48
1.0	5.62	6.60
1.5	5.65	6.67
2.0	5.67	6.70

Table 9: Degree of flocculation for prepared formulation

Suspending agents	Concentration (% w/v)	Degree of flocculation
Tragacanth	0.5	1.60
	1.0	2.50
	1.5	4.30
	2.0	5.20
Seed gum	0.5	1.80
	1.0	2.70
	1.5	3.40
	2.0	5.40

Determination of the pH of the suspensions

The variation in the pH of suspensions prepared with different concentrations of *C. lanatus* seed gum and gum tragacanth were recorded, formulations stored at room temperature. The pH of all the suspensions was found to be between 6.4 and 6.7 for *C. lanatus* gum whereas between 5.59 and 5.67 for tracaganth as shown in Table 8.

Degree of flocculation

An assessment of degree of flocculation (β-values) for suspensions prepared with *C. lanatus* seed gum and gum tragacanth shows slight higher values for *C. lanatus* seed gum then gum tracaganth. These observations show that *C. lanatus* seed gum is a comparable suspending agent to gum tracaganth as shown in Table 9.

Evaluation of emulsion

Micromeritic measurements of emulsion stability

The particle size distribution indicates that both the emulsions stabilized by using gum acacia and *C. lanatus* seed gum are within 0.5-5 µm ranges. The emulsion prepared by *C. lanatus* seed gum results very fine particle size distribution in comparison to gum acacia and results in stable emulsion because of fine particle size.

Centrifugal method of evaluating emulsion stability

The results of emulsion stability by centrifugal method indicate that there is no phase separation result even if with high rpm (3000) at 37°C and the percentage stability was found to be 100% in both the cases indicating the formation of stable emulsion.

Viscosity and turbidity measurement of emulsion stability

No creaming or settling of emulsion results during 6 months of storage period at room temperature, which indicate that the *C. lanatus* seed gum is very effective as emulsifying agent at low concentration in comparison to other gums as shown in Table 10.

CONCLUSION

From the study above it can be concluded it can be concluded that the *C. lanatus* seed gum acts as very good suspending agent than gum tracaganth and can be used in low concentration as emulsifier compared with acacia gum. It also shows very good rheological properties and hence can be used as sustained release ingredient.

Table 10: Viscosity and turbidity of emulsion

Parameter particulars	Emulsion with gum acacia (day in weeks)			Emulsion with watermelon gum (day in weeks)		
	Immediate	After 12 weeks	After 24 weeks	Immediate	After 12 week	After 24 week
Viscosity in cp	42.3	52.5	61.2	29.1	31.5	40.2
Turbidity of 1 in 1000 dilution						
At 400 nm	0.199	0.222	0.250	0.066	0.105	0.160
At 800 nm	0.220	0.251	0.285	0.056	0.073	0.106
Size index	0.91	1.11	1.87	1.17	2.09	2.86

REFERENCES

1. Troy DB, Beringer P. Remington: The Science and Practice of Pharmacy. 21st ed., Vol. I. Philadelphia: Lippincott Williams Wilkins, a Wolters Klewer Company; 2005. p. 759-66, 767-73.
2. Lachman L, Liberman HA. The Theory and Practice of Industrial Pharmacy. Special Indian edition. New Delhi: CBS Publisher and Distributors; 2009. p. 479-94, 502-31.
3. Aulton ME. Aultin's Pharmaceutics: The Science of Dosage form Design. 3rd ed. New York: Lippincott Williams Wilkins, a Wolters Klewer Company; 1998. p. 383-405.
4. Allen LV, Popovich NG, Ansel HC. Ansel's Pharmaceutical Dosage Form and Drug Delivery System. 8th ed. UK: Churchill Livingston Elsevier Science; 1988. p. 386-404, 404-15.
5. Candolle A. Water-melon. Origin of Cultivated Plants. Cambridge: Cambridge University Press; 1882. p. 262.
6. Wada M. Über citrulline, eine neue aminosäure im presssaft der watermelon, citrullus vulgaris schrad. Biochem Zeit ;224:420.
7. Farahnaky A, Darabzadeh N, Majzoobi M, Mesbahi GH, Rezvani E, Schleining G. Production and rheological properties of locust bean gum from Iranian carob seeds. Inside Food Symposium; April, 2013. p. 1-5, 9-12.
8. Arul Kumaran KS, Palanisamy S, Rajasekaran A, Hari A. Evaluation of *Csassi roxburghii* seeds as binder in tablet formulation of selected drugs. Int J Pharm Sci Nanotechnol 2010;2(4):726-32.
9. Razavi SM, Mortazavi SA, Matia-Merino L, Parvar SH, Motamedzadegan A, Khanipour E. Optimisation study of gum extraction from Basil seeds (*Ocimum basilicum* L.). Int J Food Sci Technol 2009;44:1755-62.
10. Singh AK, Selvam PR, Sivakumar T. Isolation, characterisation and formulation properties of a new plant gum obtained from *Mangifera indica*. Int J Pharm Biomed Res 2010;1(2):35-41.
11. Kumar R, Patil SR, Patil MB, Paschapur MS, Mahalaxmi R. Isolation and evaluation of the emulsifying properties of tamarind seed polysaccharide on castor oil emulsion, scholars research library. Der Pharm Lettre 2010;2(1):518-27.
12. Mishra A, Bajpai M. The flocculation performance of *Tamarindus mucilage* in relation to removal of vat and direct dyes. Bioresour Technol 2006;97(8):1055-9.
13. Sudam N, Manish B, Ritesh M, Sachin P, Ratnaparkhi MP, Shilpa C. Evaluation of various natural suspending agents for its suspending behaviour using paracetamol as model drug for suspension. Asian J Pharm Clin Res 2012;5(4):183-6.
14. Mahmud HS, Oyi AR, Allagh T. Evaluation of the suspending properties of *Khaya Senegalensis* gum in paracetamol suspensions. Niger J Pharm Sci 2009;8(1):128-34.
15. Femi-Oyewo MN, Adedokunf MO, Olusoga TO. Evaluation of the suspending properties of *Albizia zygia* gum on sulphadimidine suspension. Trop J Pharm Res 2004;3(1):279-84.
16. Arul Kumaran KS, Vimalson DC, Palanisamy S, Jagadeesan M. Evaluation of suspending properties of *Cassia Roxburghii* mucilage on sulphamethoxazole suspension. Int J Pharm Bio Sci 2010;1(2).