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Studies on gum of *Moringa oleifera* for its emulsifying properties

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ABSTRACT

Background: Emulsion has been a form of presenting water insoluble substances for a long period of time. Now a day, it has been a way of presenting various intravenous additives and diagnostic agents in X-ray examinations. Various substances can be used as emulsifying agent, which can be operationally defined as a stabilizer of the droplets formed of the internal phase. **Materials and Methods:** Gum from *Moringa oleifera* was evaluated for its emulsifying properties. Castor oil emulsions 30 percent (o/w), containing 2 to 4% *Moringa oleifera* gum was prepared. Emulsions containing equivalent concentration of acacia were also prepared for comparison. All the emulsions prepared were stored at room temperature and studied for stability at various time intervals for 8 weeks. The prepared emulsions were evaluated for creaming rate, globule size and rate of coalescence. 23 factorial design was chosen to investigate the effects of centrifugation, pH, temperature changes and electrolytes on the creaming rate and globule size. **Results:** The results of the investigations show that the gum of *Moringa oleifera* possesses better emulsifying properties as compared to gum acacia. **Conclusion:** Gum of *Moringa oleifera* could be used in pharmaceutical and non-pharmaceutical preparation.

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Emulsion has been a form of presenting water insoluble substances for a long period of time. Now-a-day, it has been a way of presenting various intravenous additives and diagnostic agents in X-ray examinations. Various substances can be used as an emulsifying agent, which can be operationally defined as a stabilizer of the droplets formed of the internal phase.[1,2] Stability of emulsion could then be used for determining the efficacy of an emulsifying agent. Stability can be estimated by the rate of coalescence of the dispersed globules. For this purpose, usually a long storage tests are employed and the deterioration is examined either weekly or monthly. The breakdown of an emulsion is usually slow. Attempts have been made to devise means of evaluating the stability rapidly and this includes addition of electrolytes, acids or alkalis, which results in an increase in breakdown rate of an emulsion. Sometimes elevated temperature has also been use in evaluating the emulsion stability.[3] Cockton and Wynn[4] have used centrifugal force as standard artificial breakdown stress.

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Polysaccharide gums derived from plants have been reported to be useful as suspending agents, emulsifying agents, binders, disintegrants etc., in different pharmaceutical formulations.[5-11] We have recently reported the possible utilities of *Moringa oleifera* as the gelling agent,[12] suspending agent[13] and film former.[14] The gum has got a high LD_{50} in mice indicating its safety. The present work was undertaken to find out its use as an emulsifying agent.

Materials and Methods

Castor oil, IP (Aggrawal Pharmaceutical, Delhi), acacia (SD Fines Chem. Ltd., Mumbai), benzoic acid, ferric chlorides, calcium chloride, sodium chloride (Loba Chem. Mumbai) were used for the study. All other solvents and reagents used were of analytical grade. The gum of *M. oleifera* was isolated in the laboratory as per the procedure reported earlier.[12]

Formulation of emulsion

Batches of castor oil emulsion containing 30% of castor oil in water were prepared using gum of *M. oleifera* gum at the concentration range of 2, 2.5, 3 and 4% w/v. The mucilage of the gum was

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prepared by dispersing the calculated amount of gum in water containing enough benzoic acid to give 0.1% w/v concentration of preservative in the finished product. To the prepared mucilage oil was added and mixed using a propeller mixer at an rpm of 10,000. The speed and time required for emulsification was optimized after evaluating a series of emulsions made primarily on the basis of degree of creaming and oil separation. For comparison batches of emulsions were similarly prepared with 2, 2.5, 3 and 4% w/v of acacia. All the emulsions prepared were subjected to uniform treatment during the entire study.

Determination of creaming rate

In a graduated stoppered measuring cylinder, 100 ml of each emulsion was placed and stored at room temperature. The height of the cream layer was measured weekly for 8 weeks. The creaming rate of the emulsion was determined from the relationship:

$$\text{Percent creaming} = \text{Hc/Ho} \times 100 \quad (1)$$

Where, Hc is the height of the cream layer and Ho is the height of the original emulsion.

Globule count, globule size analysis and rate of coalescence

Globule structures of the emulsions were determined by photomicroscopy at a magnification ×40. Photomicrographs were taken and all globules appearing in each micrograph were counted and sized with the aid of a photomicrograph of a calibrated stage slide, also taken at magnification ×40. Mean globule size (X) was calculated from the expression:

$$X = \sum fx / \sum f \quad (2)$$

Where '*f*' *is* the frequency of each size's. For each emulsion sample, 3 representative.

Photomicrographs were used in the size analysis. The count was conducted 24 h after the preparation of emulsion and then after 2 weeks interval for 8 weeks. These emulsions were stored at room temperature 28 ± 2°C. The average rate of increase in globule size was taken as a measure of the globule coalescence rate and hence a measure of instability of the emulsions.

Effect of centrifugation

The emulsion was placed in calibrated centrifuge tubes and subjected to centrifugation at a speed of 2000 rpm. The creaming height after 5 and 20 min of centrifugation was noted and percentage creaming globule count and mean globule size (X) were calculated.

Effect of pH

The pH of three sets of emulsion were adjusted to 4, 7 and 9 respectively using 1N HCl and 1N NaOH solution, as desired. The creaming heights after 1, 6, 24, 48 and 72 h were noted and mean globule size (X) were calculated.

Effect of temperature

The prepared emulsion taken in calibrated test tubes securely stoppered were placed at temperatures, viz. 4°C, room temperature and 50°C, respectively. The creaming heights after 12, 24, 36 and 48 h were noted and percentage creaming was calculated.

Effect of electrolytes

The effect of ionic strength on the stability of the emulsion was determined at various ionic strengths (0.1 M) using NaCl, $CaCl_2$, $FeCl_3$. The ionic strength (μ) of each electrolyte was calculated from the relationship.[2,15]

$$\mu = \frac{1}{2} \sum C_i Z_i \quad (3)$$

Where, C is the concentration in moles/liter and Z_i is the valence of ion i. The mean globule sizes (X) were calculated.

Data analysis

Factorial design was chosen to evaluate the factors that significantly influence emulsion stability. The factorial design consists of 9 experiments, with two level factorials. The three factors are X1, percentage of emulgent, X2, pH and X3, temperature and are represented as −1 and +1, analogous to low and high values.

The following equation described the response:

$$Y_1 = a_1 + a_1X_1 + a_2X_2 + a_2X_3 + a_{12}X_1X_2 + a_{23}X_2X_3 + a_{13}X_1X_3 + a_{123}X_1X_2X_3 \quad (4)$$

Where, 'Y' is response, 'X' factors and 'a' coefficient of each term. The responses studied are creaming rate and globule size after 48 h for the emulsions prepared with gum of *M. oleifera*.

Results and Discussion

Creaming rate

The isolated gum of *M. oleifera* was found to be hydrophilic in nature and thus can be used for preparation of o/w type of emulsion. Preliminary experiments performed for effective evaluation of the emulsifying properties of *M. oleifera* gave an idea of the concentrations of gum to be used in the study. Emulsions were prepared with *M. oleifera* gum and acacia 2, 2.5, 3 and 4 per cent w/v. The emulsion prepared with 2% gum acacia shows least stability. Of the four concentrations of *M. oleifera* used, the emulsion made with 4% w/v showed the least creaming of about 18.4%, whereas the emulsion made with 2% acacia gum showed maximum creaming of 51.3% after 8 weeks of storage [Figure 1]. The overall results show that the emulsion prepared with *M. oleifera* gum were more stable when compared with emulsion prepared with gum acacia. The differences in the creaming rate may be attributed to their difference in emulsifying properties. Lower the emulsifying property, larger the oil globules and thereby greater the rate of creaming. In addition to this, the creaming in emulsion is also dependent on the differences in densities between the dispersed phase and dispersion medium and the viscosity of dispersion medium and the force of gravity.[16] Creaming together with other form of instability is used in assessing the stability of emulsion. At the same time, creaming may not be considered a serious instability when it gets redispersed uniformly upon shaking. In such case, the oil globules are surrounded by a protective sheath of emulsifying agent.[2,16] All the emulsions creamed after 8 weeks of storage and got reconstituted readily upon shaking.

Globule count, globule size analysis and rate of coalescence

The number of globules decreased with prolonged storage time and depends on the concentration of the emulsifier used. The globule size increased progressively with storage time [Tables 1 and 2]. The changes in the globule size and globule count could be attributed to the fact that an emulsion is thermodynamically unstable and is prone to flocculation and coalescence when stored.

An increase in emulsifier concentration reduces the globule size of an emulsion formation and a decrease in globule size should enhance emulsion stability. However, small particles have inherent instability and tend to mutually coalesce or coalesce with larger particles. Thus, the rate of coalescence has been used as one of the parameters to evaluate the stability of emulsion.[17] From the data obtained [Tables 1 and 2], it can be seen that as the gum concentration increases the rate of coalescence decreases. At the equivalent concentrations of gum used, *M. oleifera* gum showed much lower rate of creaming compared with acacia indicating better stability.

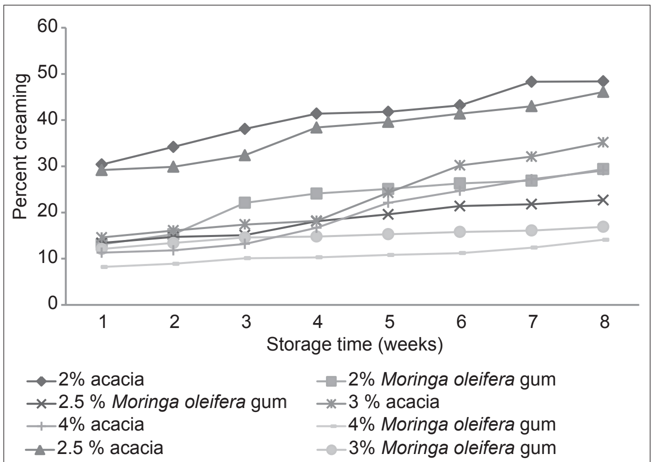

Figure 1: Creaming behavior of emulsions

Effect of centrifugation

Upon centrifugation, the increase in globule size was least in case of emulsions prepared with 4% *M. oleifera* gum, whereas emulsions prepared with 2% gum acacia was maximum after 20 min of centrifugation [Table 3].

Effect of pH

The effect of pH was observed with emulsion prepared with 2% *M. oleifera* and acacia gum. Higher numbers of globules were found in the case of emulsions prepared with both the emulgents at pH 7[Table 4].

Effect of temperature

Emulsions prepared with *M. oleifera* and *acacia* gum showed higher per cent creaming at 50°C than stored at room temperature or at 4°C. This is because at higher temperatures the number of collisions increases between the dispersed globules, which results in enhanced the creaming. On the other hand, an increase in the kinetic energy of droplets facilitates coalescence, which results in a higher percentage of creaming.[1] Among all the emulsion the emulsion prepared with gum of *M. oleifera* showed least percent of creaming at all the temperatures in comparison to emulsion prepared with gum acacia [Figure 2].

Table 1: Globule size analysis of emulsions prepared with various concentrations of *Moringa oleifera* gum

Gum percent w/v	Storage period in weeks									
	0		2		4		6		8	
	Size (μm)	Number	Size (μm)	Number	Size (μm)	Number	Size (μm)	Number	Size (μm)	Number
2.0	3.73	439	3.98	367	4.01	354	4.18	305	4.52	264
2.5	3.66	462	3.74	445	3.78	392	4.09	341	4.18	311
3.0	3.26	631	3.31	612	3.42	578	3.51	537	3.68	479
4.0	2.21	1116	2.24	1105	2.29	1089	2.31	1074	2.33	1056

Table 2: Globule size analysis of emulsions prepared with various concentrations of *acacia* gum

Gum percent w/v	Storage period in weeks									
	0		2		4		6		8	
	Size (μm)	Number	Size (μm)	Number	Size (μm)	Number	Size (μm)	Number	Size (μm)	Number
2.0	4.62	581	5.04	451	5.51	348	6.25	231	6.75	187
2.5	4.34	644	4.86	538	5.12	488	5.76	328	6.09	247
3.0	4.07	822	4.32	758	4.74	673	4.95	609	5.22	548
4.0	3.95	934	4.03	873	4.21	763	4.41	648	4.71	558

Table 3: Globule size analysis of emulsions prepared with various concentrations of *Moringa oleifera* and *acacia* gum following centrifugation at 2000 rev/min

Time (min)	*Moringaa oleifera* gum				*Acacia* gum			
	5		20		5		20	
	Size (μm)	Number	Size (μm)	Number	Size (μm)	Number	Size (μm)	Number
2.0	3.96	366	4.76	208	5.41	367	5.61	171
2.5	3.72	438	4.07	337	4.67	564	5.98	283
3.0	3.41	608	3.51	489	4.22	688	5.32	378
4.0	3.23	688	3.38	564	4.06	865	4.81	524

Table 4: Globule size analysis of emulsions prepared with 2% of *Moringa oleifera* and *acacia* gum at different pH levels on storage for 72 h

pH	*Moringa oleifera* gum		*Acacia* gum	
	Size (μm)	Number	Size (μm)	Number
4.0	3.87	418	4.91	481
7.0	3.72	451	4.76	557
9.0	3.92	371	5.07	451

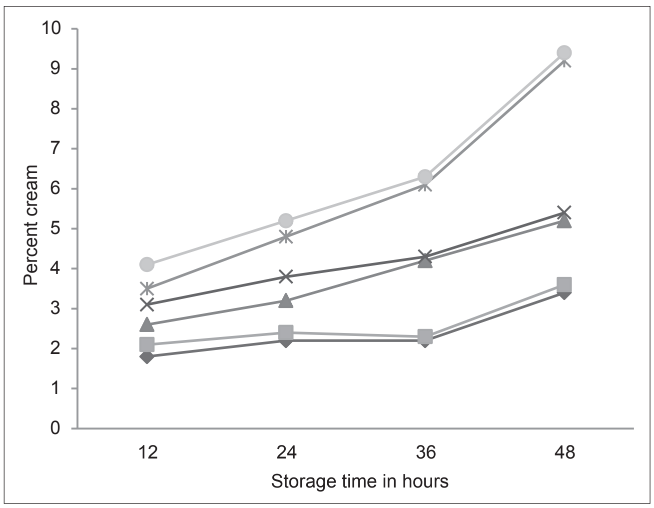

Figure 2: Effect of temperature on creaming behavior of emulsions prepared with 2% of *Moringa oleifera* (4°C-♦-, RT-▲-, 500 C-✳-) and *acacia* gum (4°C-▪-, RT-■-, 500 C-●-)

Effect of electrolytes

When different electrolytes are added to the emulsion there was an increase in globule number in both *M. oleifera* and acacia gum. With the decrease in globule number, increase in globule size was observed which was much higher in emulsions prepared with acacia than gum *M. oleifera* [Table 5].

Data analysis

Following equations were generated for creaming rate Equation 5 and globule size Equation 6.

$$Y_1 = 12.73 - 2.27X_1 - 0.20X_2 + 0.28X_3 - 0.24X_1X_2 - 0.04X_2X_3 - 0.01X_1X_3 - 0.13X_1X_2X_3 \quad (5)$$

Table 5: Effect of ionic charge on globule size of emulsions prepared with 2% of *Moringa oleifera* and *acacia* gum after 48 h

Electrolyte (0.1 M)	*Moringa oleifera* gum		*Acacia* gum	
	Size (μm)	Number	Size (μm)	Number
NaCl	3.86	471	4.92	489
$CaCl_2$	5.37	374	5.41	377
$FeCl_3$	4.21	315	6.16	254

Table 6: Experimental domains and coding of variables

Variables	Levels	
	−1	+1
Emulgent % (X_1)	2	4
pH (X_2)	4	9
Temperature °C (X_3)	4	50

Table 7: Matrix and response

Run	X_1	X_2	X_3	Creaming rate	Globule size
1	−1	−1	−1	14.4	3.98
2	−1	−1	1	14.82	4.01
3	−1	1	−1	14.29	3.92
4	−1	1	1	15.07	4.06
5	1	−1	−1	10.1	2.31
6	1	−1	1	11.01	2.41
7	1	1	−1	9.57	2.55
8	1	1	1	9.73	2.76
Check	1	1	1	9.76	2.675

$$Y_2 = 3.2 - 0.73X_1 + 0.08X_2 + 0.04X_3 + 0.08X_1X_2 + 0.01X_2X_3 + 0.005X_1X_3 - 0.01X_1X_2X_3 \quad (6)$$

The predicted response and the experimental response at the highest level of independent factor (1,1,1) were found to be 9.76 and 9.73 for creaming rate, similarly 2.67 and 2.76 for globule size. The coding, matrix and response was as per [Tables 6 and 7].

Conclusion

From the above studies it may be concluded that gum from *M. oleifera* possesses better emulsifying properties compared with gum acacia. The emulsion formulated from *M. oleifera* is less sensitive to centrifugation, pH, temperature changes and electrolytes. Hence the gum of *M. oleifera* has got immense potential to be used as an adjuvant in pharmaceutical and non-pharmaceutical preparation. The experimental designs were found to be valid and optimized.

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