

Emulsifying Properties of a New Polysaccharide Gum

By OSCAR E. ARAUJO

A recently developed polysaccharide gum produced by bacterial fermentation, now commercially available, was investigated as a potential emulsifying agent for pharmaceutical systems. Mineral oil emulsions having a fixed oil to water ratio were prepared using concentrations of the new gum ranging from 0.1 to 0.9 per cent. Emulsions containing 5 to 40 per cent acacia were also prepared and served as a basis for comparison. Rheological measurements, particle size studies, and organoleptic observations were performed on all the emulsions. A Stormer viscosimeter was used to obtain the flow properties, while a standard microscopic technique was employed for the particle size analysis. The results showed the plastic nature of the emulsions prepared with the new gum and the existence of a definite yield value. These emulsions showed good over-all stability over a period of 6 months, with little change in viscosity or particle size. The general appearance of the emulsions containing the new gum was creamier than that of those prepared with acacia, and the emulsions containing more than 0.2 per cent of the polysaccharide exhibited no phase separation after 6 months.

THE EVALUATION of potential suspending and emulsifying agents which would increase the stability and cosmetic elegance of heterogeneous pharmaceutical systems has been the object of much investigation.

In recent years, research by the U. S. Department of Agriculture resulted in the production of a heteropolysaccharide from the bacterial fermentation of glucose by *Xanthomonas campestris* (1). Jeanes *et al.* (2) reported several of the properties of what was at the time referred to as polysaccharide B-1459: this xantham gum has since been produced commercially.¹ These authors had shown upon analysis that the major constituents of the new polysaccharide were D-mannose, D-glucose, and D-glucuronic acid present as the potassium salt.

A study of the acute toxicity of and the skin sensitivity to the gum was carried out and no evidence of either was found (3). Government clearance for food and drug use is still pending final FDA approval.

Carnie (4) discussed some of the properties of solutions of the gum and the effects of heating, pH, concentration, and homogenization on these preparations. Kabre *et al.* (5, 6) studied the rheological characteristics of this xantham gum and evaluated it as a suspending agent for zinc oxide and sulfamethazine.

The above studies along with preliminary experimentation revealed certain unusual properties of this polysaccharide which indicated its potential as an emulsifying agent and led to the present investigation. Emulsions using acacia, a well-known naturally occurring polysaccharide, served as the basis for comparison.

EXPERIMENTAL

Preparation of Emulsions—All the emulsions were prepared using a 4:6 mineral oil to water ratio. The xantham gum was used in concentrations of 0.1 to 0.9%, in increments of 0.1%, and acacia in concentrations of 5 to 40%, in increments of 5%. The carefully measured liquids were placed in a Waring blender, the emulsifying agent added to the surface of the liquids, and the entire mixture blended for 4 min. The resulting emulsions were passed twice through a hand homogenizer to further standardize the products. The distilled water used to prepare all emulsions contained 0.2% benzoic acid as a preservative. All emulsions were allowed to equilibrate for 2 hr. before any measurements were made.

pH Measurements—A Photovolt electronic pH meter model 110 was used to measure the pH of the emulsions.

Rheological Measurements—A Stormer viscosimeter equipped with the modified cup and bob as described by Fischer (7) was employed. A weight hanger with various slotted weights provided the shearing stress. Calibration data obtained with a standard oil were used to calculate the instrumental constant, K_v , from the expression:

$$K_v = \eta \frac{r.p.m.}{W} \quad (\text{Eq. 1})$$

where W represents the shearing stress in Gm., r.p.m., the rate of shear, and η , the viscosity of the standard oil in cps.

The plastic viscosity was calculated according to the expression:

$$U = K_v \frac{W - W_f}{r.p.m.} \quad (\text{Eq. 2})$$

where W_f is the extrapolated yield value intercept in Gm., and U is the plastic viscosity in cps.

The yield value was obtained from the expression:

$$f = \frac{2\pi}{60} \times K_v \times W_f \times \frac{1}{2.303 \log R_e/R_b} \quad (\text{Eq. 3})$$

All rheological measurements were performed at $20 \pm 0.1^\circ$.

Organoleptic Observations—The emulsions were examined as to appearance, odor, and texture; any

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¹ Marketed as Kelzan by the Kelco Co., Los Angeles, Calif.

change in these characteristics over a period of 6 months was noted.

Particle Size Analysis—The diluting medium used to prepare the slides for the microscopic analysis consisted of a 75% aqueous glycerin solution. Several other vehicles were tried, including propylene glycol as suggested by Knoechel (8); the medium selected provided the best contrast and least particle mobility.

One milliliter was withdrawn from well below the surface of the emulsion by means of a syringe equipped with an 18 gauge, 3.5 in. length needle. The sample was then introduced into a 100-ml. volumetric flask containing approximately 50 ml. of the diluting fluid, mixed thoroughly and brought to a volume of 100 ml. One drop of the diluted emulsion was then placed on a glass slide and a cover slip carefully placed over the liquid. Approximately 15 min. was permitted to elapse to allow the particles to rise and assume a fixed position on the slide.

The particle count was performed with the aid of a standard microscope equipped with a 43 \times objective, a 10 \times ocular, a movable micrometer stage, and a micrometer eyepiece. The latter contained a movable cross hair, the traverse of which was measured by a revolving drum marked into 100 divisions. By proper calibration each division was then equivalent, under the above conditions, to a fraction of a micron.

The number of particles counted for each sample varied from 400 to 1600 depending on the range of particle distribution. A preliminary scan of the entire slide provided an over-all picture of the spread of particle size, helping to establish the number to be counted. The degree of aggregation or clustering was also noted in this examination.

Using the movable micrometer stage and a table of random numbers, "coordinates" were chosen to insure a random selection of fields and to prevent duplicate measurements. The size frequency tally obtained from these counts was used to calculate the arithmetic mean diameter, d_m , the geometric mean diameter, d_g , and the volume-surface mean diameter, d_{vs} , according to the following expressions:

$$d_m = \frac{\sum n_i d_i}{\sum n_i} \quad (\text{Eq. 4})$$

$$d_g = \frac{\sum n_i \ln d_i}{\sum n_i} \quad (\text{Eq. 5})$$

$$d_{vs} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (\text{Eq. 6})$$

where n_i is the number of globules having a class diameter of d_i in microns (7, 9).

RESULTS AND DISCUSSION

Preliminary Studies—In order to evaluate effectively the emulsifying ability of the xantham gum, it was necessary not only to prepare the emulsions in exactly the same manner each time, but also to maintain a constant oil-water ratio. These precautions would eliminate all the variables affecting the quality of the emulsions except the nature and concentration of the emulsifier.

Preliminary experiments were performed to establish the range of concentrations of the new gum to be used in the study. The results showed that a

1.0% aqueous mucilage of xantham gum was a firm gel, while those mucilages containing less than 0.1% were too dilute. Therefore, the range of concentrations selected was 0.1 to 0.9%.

Emulsions of castor, cod liver, and mineral oils were prepared using equal parts of oil and water; 0.25 and 0.5% of the xantham gum served as the emulsifier. Mineral oil was selected for the investigation on the basis of the over-all appearance of the emulsions.

The oil-water ratio selected for the detailed experimentation was the result of an appraisal of the general appearance of mineral oil emulsions prepared with ratios of 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, and 8:2. Again the xantham gum was used in concentrations of 0.25 and 0.5%. Emulsions having a 4:6 oil-water ratio were chosen by the above criterion for use throughout the study.

Since emulsions containing acacia were to be used for the purpose of comparison, it was necessary to determine if the 4:6 ratio also provided satisfactory emulsions with acacia. Accordingly, a trial mineral oil emulsion with acacia was prepared and judged satisfactory.

Having established the range of concentrations of xantham gum to be used in the investigation, similar preliminary experiments were conducted with acacia, resulting in the selection of concentrations ranging from 5 to 40%.

Organoleptic Qualities—The emulsions prepared with the new gum exhibited a creamy white appearance. The lone exception involved the product containing 0.1% which appeared to be incompletely emulsified; this observation was confirmed by examining the emulsion under a microscope, which clearly showed large oil lakes throughout the sample, making a globule count impossible. No unpleasant odor developed in any of the emulsions during a 6-month shelf-life. The general texture when applied to the skin was smooth, leaving no gummy residue. This quality would seem to be important from a lotion formulation standpoint. The preparations having concentrations above 0.5% had a gel-like consistency easily broken up by shaking. Creaming was observed to a slight degree only with the 0.2% emulsion after storage of 6 months.

The emulsions prepared with acacia did not have a creamy appearance and possessed an off-white coloration. A gummy film characteristic of acacia preparations remained on the skin upon drying. The acacia emulsions having concentrations of 25% and less showed creaming after a period of 6 months, the extent of which increased with decreasing concentration.

pH Considerations—The pH of each emulsion was taken initially and after 6 months. The pH of the emulsions with the xantham gum ranged from 3.5 to 4.0 while that of those prepared with acacia varied from 4.2 to 4.4. The measurements indicate essentially no change in the pH of the emulsions prepared with either emulsifier.

Rheological Observations—The emulsions prepared with the new gum showed certain unusual rheological characteristics. Figure 1 clearly indicates the plastic nature of the emulsions and the existence of a definite yield value. As the shearing stress is increased, a value is reached for each of the emulsions above which a constant plastic viscosity is given by the linear portion of

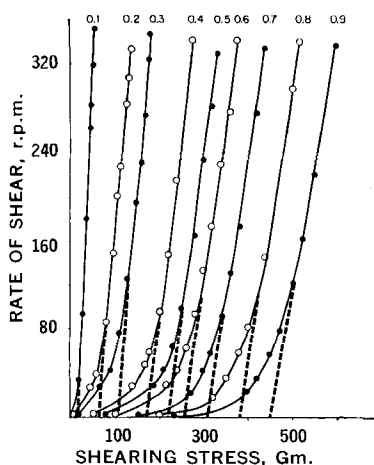


Fig. 1—Rheograms of emulsions containing various per cent concentrations of xantham gum.

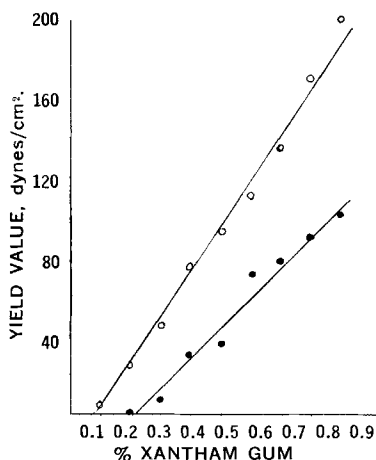


Fig. 2—Yield values of emulsions containing the xantham gum. Key: ●, minimum yield value; ○, Bingham yield value.

the rheogram. Extrapolation of this linear segment to the shearing stress axis gives the Bingham yield value, in Gm.

A minimum yield value was established for each of the xantham gum emulsions by determining the minimum number of Gm. which produced rotation of the bob on the Stormer viscosimeter. Each emulsion, except the 0.1% semiemulsified product, displayed a minimum yield value, thereby precluding any pseudoplasticity.

The minimum and extrapolated yield values for the various concentrations of the xantham gum are shown in Fig. 2. There appears to be a linear relationship between concentration and both yield values over the range studied. In addition, the Bingham yield value for the most concentrated emulsion is less than 200 dynes/cm.², well within the range of the stress of a hand-shaken bottle.

Figure 3 indicates that the plastic viscosities increase with increasing concentrations of the gum, as did the yield values. The increase, however,

appears to decrease exponentially so that the overall range of plastic viscosity was only 25 to 86 cps. Consequently, the 0.9% xantham gum emulsion which has the highest plastic viscosity still has sufficient mobility upon agitation to be easily poured from a bottle.

Rheologically, the ideal emulsifying agent should possess the same qualities as those of the ideal suspending agent: high viscosity at the negligible shear of shelf storage and low viscosity upon agitation, causing the preparation to be free-flowing and easily spread on the skin (10).

The new polysaccharide appears to lack one desirable feature of an emulsifying agent, that of thixotropy. Since the upcurve and downcurve of the rheograms were found to be coincidental, there was no time-dependence in the rheological reconstitution of the emulsions upon removal of the shearing stress. This property held true for all concentrations of gum but only affects pourability in those emulsions of concentrations above 0.6%, where agitation must be maintained to remove the contents from the bottle.

The rheograms for the acacia emulsions are shown

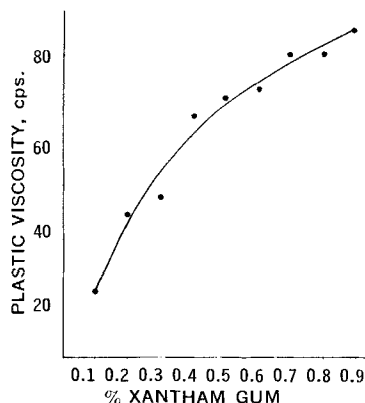


Fig. 3—Plastic viscosities of emulsions containing xantham gum.

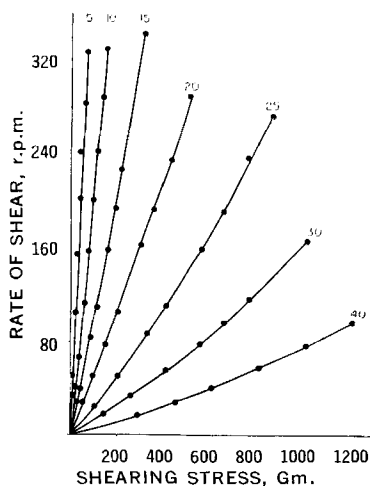


Fig. 4—Rheograms of emulsions containing various per cent concentrations of acacia.

TABLE I—PARTICLE SIZE OF EMULSION GLOBULES

% Xantham Gum	Diam., μ			% Acacia	Diam., μ		
	d_m	d_g	d_{vs}		d_m	d_g	d_{vs}
0.2	1.38	1.13	11.1	5	7.53	5.33	19.7
0.3	1.46	1.18	12.1	10	6.47	5.37	11.6
0.4	1.81	1.21	25.9	15	5.81	4.40	13.7
0.5	2.00	1.27	28.9	20	2.39	2.10	3.60
0.6	2.08	1.24	33.3	25	2.05	1.84	2.84
0.7	2.26	1.30	31.5	30	1.83	1.72	2.26
0.8	2.52	1.38	27.2	40	<1.00
0.9	3.19	1.55	26.4				

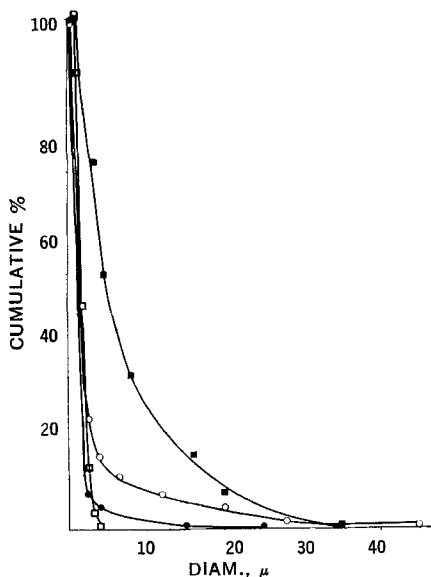


Fig. 5—Particle size distribution of emulsions containing extremes of concentration of acacia and xantham gum. Key: ■, 5% acacia; □, 30% acacia; ●, 0.2% xantham gum; ○, 0.9% xantham gum.

in Fig. 4. The quasi-Newtonian characteristics of the dilute emulsions and the pseudoplastic nature of the more concentrated ones are evident. The rheological pattern is quite similar to that of the aqueous mucilages of the gum (11).

Regardless of the emulsifying agent used or its concentration, the products showed no significant change in rheological properties after a 6-month shelf-life, indicating good consistency stability.

Particle Size Studies—Table I gives the mean globule diameters for all the emulsions prepared in this study. The ranges of the arithmetic and geometric mean diameters of the emulsions prepared with the xantham gum from low to high concentration are narrower than those for the acacia emulsions. However, the emulsion containing 40% acacia contained only particles of 1 μ and smaller; no larger particles were observed even when the entire slide was scanned.

The volume-surface mean diameters are much larger for the emulsions prepared with the new polysaccharide, indicating the presence of a number of particles in the 25 to 45 μ range. However, in Fig. 5, which shows the cumulative frequency distribution curves for the extremes of concentration of both emulsifiers, it can be seen that the

xantham gum emulsions have a considerably larger percentage of 1- μ diameter globules than do the acacia emulsions.

Figure 6 shows the log normal plots for the emulsions prepared with acacia. Whereas these emulsions appeared to follow a log normal distribution, those prepared with the new gum did not. This may be explained by the fact that in most cases more than 90% of the globules in the xantham gum emulsions had a diameter of less than 1 μ .

Interestingly, the emulsions with xantham gum increase in particle diameter and in the number of very large globules as the concentration is increased; the acacia emulsions, on the other hand, show a decrease in globule size with an increase in concentration. The explanation is probably involved in the rheological nature of the two emulsifying agents. The xantham gum may produce a strong film around the globules and as the concentration is increased, the well protected particles resist breakdown by the blender and homogenizer. At lower concentrations of the new gum, where the film may be more vulnerable, the larger particles may be broken down, producing a lower mean diameter. It was further noted that samples of the xantham gum emulsions, when viewed microscopically, show clusters of globules which remained in this aggregated state without coalescing. Some globule coalescence was observed with the acacia emulsions, as two globules in close proximity would eventually merge, sometimes within 24 hr. There did not appear to be as many clusters of particles in the

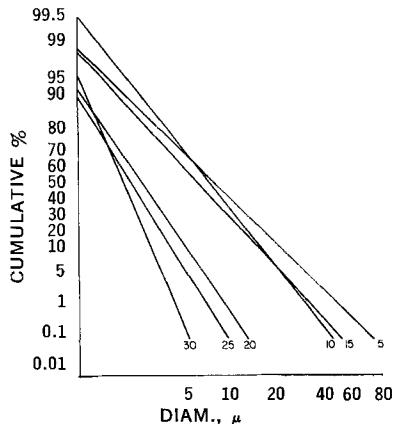


Fig. 6—Log probability plot of particle size distribution of emulsions containing various per cent concentrations of acacia.

acacia emulsions. Regardless of the emulsifier used, re-examinations of all emulsions showed no significant change in globule pattern or over-all mean diameters after 6 months on the shelf. These results were corroborated by the previously mentioned rheological stability of the emulsions after 6 months. If a general increase in particle size due to coalescence had occurred, the over-all viscosity of the emulsions would have decreased (12).

The above findings further contribute to the belief that thickness and strength of the emulsifier film adsorbed at the globule surface play a more important role in the stability of emulsions than does the initial particle size distribution.

SUMMARY

Mineral oil emulsions with a 4:6 oil to water ratio were prepared using various concentrations of a new polysaccharide.

The emulsions prepared with the new xantham gum demonstrated desirable rheological characteristics from a stability standpoint. The plastic nature of the xantham gum emulsions, as well as the corresponding yield values, were examined in detail. Their particle size distribution was broader in general than the emulsions prepared with acacia. Globule coalescence of the emulsions containing the xantham gum appeared to be minimal.

The emulsions containing more than 0.2% of the new gum did not exhibit creaming after 6 month's

storage. The xantham gum did not leave a tacky film on the skin after drying. There appeared to be no evidence of bacterial decomposition in any of the emulsions, as evidenced by no significant change in pH or viscosity.

A much lower concentration of xantham gum than of acacia was required to produce stable emulsions. As a result of this investigation, it was felt that the new polysaccharide showed good potential as an emulsifier. For the mineral oil emulsions prepared in this study, the optimum concentration of the xantham gum appeared to be 0.4 to 0.5%.

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Effect of Probenecid on Riboflavin Absorption and Excretion in Man

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An oral dose of probenecid given 1 hr. prior to oral administration of riboflavin or riboflavin-5'-phosphate (FMN) to normal humans produced a marked decrease in the excretion rate of the vitamin during the early excretion phase but had no significant effect during the later (post-absorptive) phase. Oral administration of probenecid prior to intramuscular injection of FMN increased the apparent "half-life" of the initial rapid phase of riboflavin excretion. The urinary recovery of riboflavin following oral or intramuscular administration of the vitamin was usually somewhat decreased by probenecid. These results are consistent with our earlier suggestion that riboflavin is excreted in part by renal tubular secretion. It appears that probenecid also inhibits the specialized transport process responsible for the intestinal absorption of riboflavin in man. The complex kinetics of riboflavin distribution and elimination in man are discussed and additional evidence is presented in support of enterohepatic cycling of the vitamin.

THE AUTHORS have shown recently that riboflavin and riboflavin-5'-phosphate (FMN) are absorbed from the gastrointestinal tract of

man by a site-specific and saturable specialized transport process rather than by passive diffusion (1, 2). An analysis of published data has led also to the conclusion that the renal excretion of riboflavin occurs not only by glomerular filtration but apparently also by renal tubular secretion (3).

The purpose of the study to be described here was to determine the effect of probenecid on the excretion of riboflavin. Probenecid inhibits a

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