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EFFECT OF DEXTROSE IN THE INTERNAL AQUEOUS PHASE ON FORMATION AND STABILITY OF W/O/W MULTIPLE EMULSIONS

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ABSTRACT

The osmolarity of the internal aqueous phase of W/O/W multiple emulsions was varied by using different concentrations of dextrose in the internal phase. Evaluation of the stability of the emulsions was done by microscopic, viscometric and conductometric methods. Microscopic study indicated that as the dextrose concentration in the internal phase increased (0 - 2.50% W/V), the stability, in terms of coalescence of the internal droplets and rupture of the interfacial oily layer, increased from 12 hrs to 7-8 weeks. Viscometric evaluation showed the emulsions to exhibit Non-Newtonian flow and the apparent viscosities of freshly prepared emulsions

increased from 8000 to 56,000 cps as the dextrose concentration was increased; the viscosity decreased as the emulsion aged. The amount of drug released as determined by the conductometric method, correlated with the viscosity and stability of the emulsions. The reduction of globule size of the primary (W/O) phase by use of a colloid mill increased the apparent viscosity significantly and thus improved the stability of the formulations.

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INTRODUCTION

Multiple emulsions have been known for their inherent instability problems as well as potential usefulness as drug delivery vehicles (1,2,3). In an attempt to stabilize W/O/W emulsions, Omotosho and co-workers (4) utilized Span 80 and albumin as nonionic surfactants with sodium chloride in the internal aqueous phase to adjust osmolality. They reported that the osmotic pressure gradients caused swelling of the internal aqueous phase which resulted in a decrease of the oil layer thickness and consequent increase in the rate of solute release from the internal phase.

Florence et al (5) investigated long term stability of similar W/O/W emulsion droplets (liquid

foam structures formed from osmotically swollen W/O/W emulsion droplets) which had sodium chloride in the internal aqueous phase. They observed that the interfacial membrane appeared to have sufficient elasticity to respond to osmotic changes in the external aqueous phase and produced emulsions which were stable for several weeks. However, long term microscopic or viscometric evaluations were not reported. Although Kita and co-workers (6) estimated stability of W/O/W emulsions by viscometric studies, the investigation was limited to two weeks in emulsions with internal aqueous volume fractions of 20% V/V.

Coalescence of the internal water globules of the primary emulsion is known to be an instability indicator. Stability can be improved if the emulsion is prepared such that the globule size is very small. Peck et al (7) carried out a comparative study of pharmaceutical emulsification equipment to prepare W/O emulsions. They concluded that emulsions prepared with homogenizers produced samples with smaller average particle size than those prepared with agitators.

The objectives of this study were as follows:

1) To vary the osmolarity between the two aqueous phases using different concentrations of

dextrose in the internal phase using optimized type and concentration of surfactants reported earlier (8).

2) To follow the breakdown pattern of the multiple globules by photomicrography.

3) To study the viscometric changes in the emulsions and relate these to emulsion breakdown and drug release.

4) To study the release pattern of a model drug from the internal phase using a conductance method.

EXPERIMENTAL

Materials

Sorbitan sesquioleate (Span 83), extra heavy mineral oil, Ruger Chemical Co.; Polyoxyethylene sorbitan monopalmitate (Tween 40), Sigma Chemical Co.; Sodium salicylate, Fisher Scientific Co.; dextrose monohydrate, J.T. Baker Co.; deionized water. All materials were used without further purification.

Methods

Preparation of the three phase emulsions were as reported earlier (8) except that the concentration of the lipophilic surfactant (sorbitan sesquioleate) was fixed at 26% W/W and the hydrophilic surfactant (polyoxyethylene sorbitan monopalmitate) was fixed at 1% W/V in all formulations. The dextrose concentration was varied between 0-2.50% W/V relative

TABLE 1

W/O/W Formulations

W/O Primary Emulsions

Formulation	M.O(g)	% W/W Span 83	% W/V D.M.	% W/V S.A.	Aqueous Phase (ml)
A	100	26	0	1	100
B	100	26	0.250	1	100
C	100	26	0.307	1	100
D	100	26	1.250	1	100
E	100	26	2.500	1	100

M.O = Mineral Oil (Heavy)

D.M = Dextrose Monohydrate

S.A = Salicylic Acid

W/O/W Emulsion

Primary Emulsion	
Tween 40	1% W/V
Water	100 ml

to the aqueous internal phase. The formulations are shown in Table 1.

Analytical Procedures

Micrography and conductometry methods were reported in a previous study (8).

Viscometry

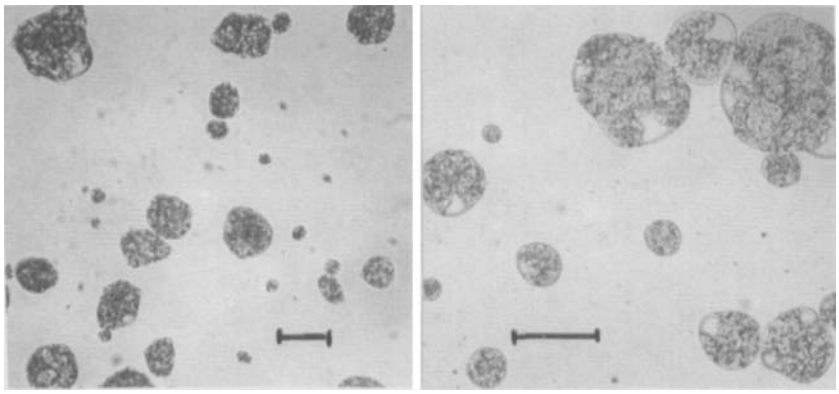
Apparent viscosity measurements were carried out during the first two hours of the emulsion life and

weekly throughout the shelf life at room temperature (25C) by using a Brookfield RVT viscometer at 2.5, 5, 10 and 20 RPM using an appropriate spindle number. Each reading was taken after equilibration of the indicator dial, (at least 1 minute).

RESULTS AND DISCUSSION

Micrographic Study of the Formation and Breakdown of W/O/W Emulsion Globules

The breakdown pattern of formulations A, B, C, D and E were monitored over a period of 30 days. Figure 1 (I,II) depicts a formulation without dextrose A and formulation B (0.250% dextrose) immediately after preparation. The emulsion globules have begun to lose the internal dispersed aqueous phase and show rupture of the interfacial layers. The total breakdown of Formulation A was observed within one day and for Formulation B was observed within 7 days. Figures 2 (I,II,III) compare the initial emulsion globules with 21 day and 30 day samples of formulation C, while Figures 2 (IV,V,VI) similarly show the effect of aging on formulation E (the most physically stable). Formulation E pattern is similar to that of formulation D which is not shown. These figures show coalescence of the dispersed aqueous phase, the



I II

Figure 1

Photomicrographs of W/O/W Emulsion A (I), Emulsion B (II) immediately after Preparation, Bar = 100 um.

weakening of the interfacial layer, the swelling of the globules due to influx of water from the external phase and subsequent rupturing of the multiple globules with formation of a simple O/W emulsion. The most physically stable formulation (E) showed globules whose structures were relatively preserved. The percent sodium salicylate released into the continuous (external) aqueous phase for each of the five formulations (A,B,C,D,E) was 73.9, 72.9, 32.9, 17.7 and 17.7%, respectively.

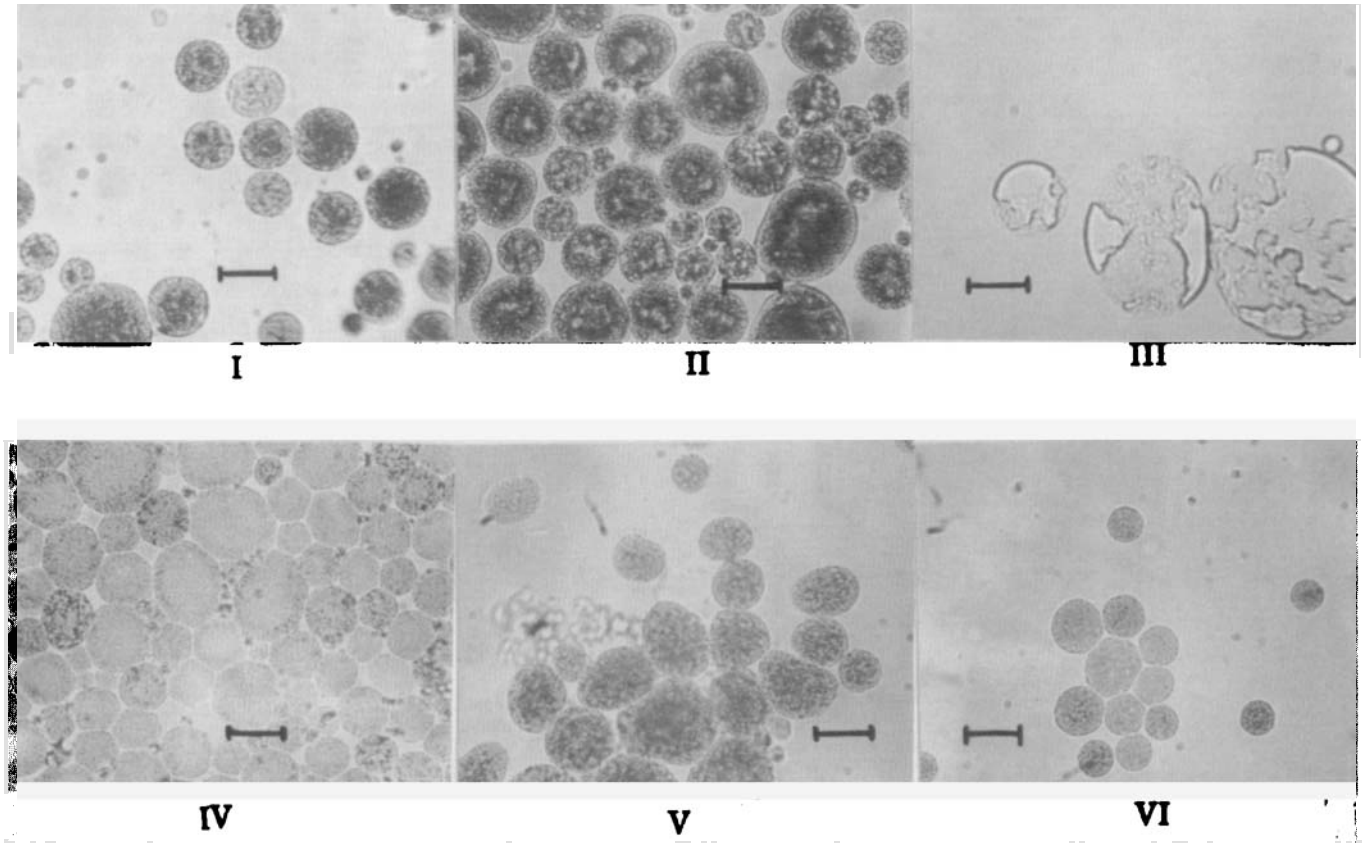


Figure 2
Photomicrographs of W/O/W Emulsion C immediately after Preparation (I), 21 days (II), 30 days (III) after Preparation and Emulsion E immediately after preparation (IV), 21 days (V) and 30 days (VI) after preparation.
Bar (I,II,IV,V) = 20 um, Bar (III,VI) = 40 um

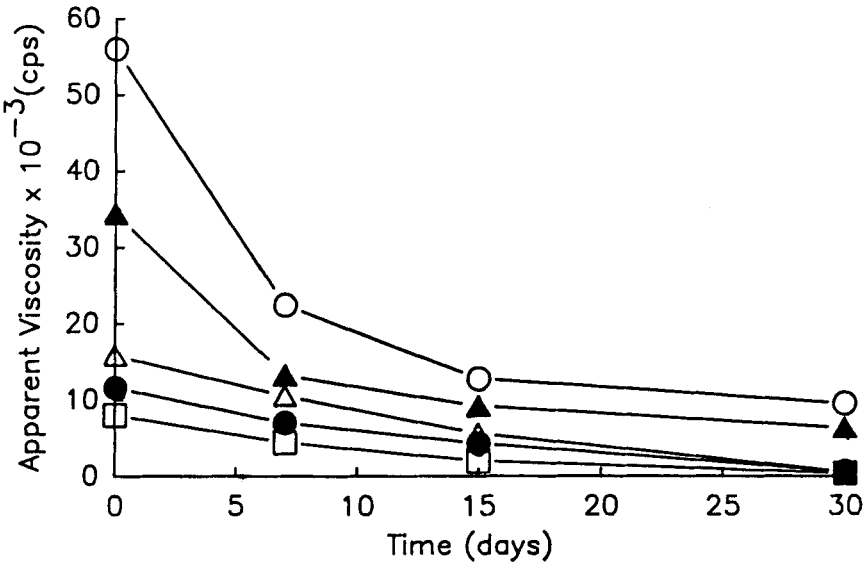


Figure 3

Effect of Aging on the Apparent Viscosities (at 2.5 rpm) in W/O/W Emulsions A,B,C,D,and E)
□= Formulation A, ●= Formulation B,
△= Formulation C, ▲= Formulation D,
○= Formulation E

Effect of Aging on the Apparent Viscosity of the Emulsions

The effect of aging on apparent viscosities of the emulsions A, B, C, D and E are shown in Figure 3. As depicted, the apparent viscosities decreased as the emulsion aged.

The amount of dextrose in the internal phase, an index of osmolarity, affected the dispersion state of the dispersed water droplets. The variation in the

apparent viscosities of the emulsions was due to the degree of swelling of the vesicular structure in the O/W internal phase. This swelling resulted in a higher O/W phase volume ratio which is known to cause an increase in apparent viscosity (9). Formulation E, which had the maximum concentration of dextrose, had the highest viscosity due to the migration of water from the external phase to the internal phase resulting in the swelling of the oil globules. This is in contrast to formulation A which had no dextrose in the internal phase and had the lowest viscosity all through the aging period. The changes in viscosity with time for formulations B, C, and D lie within those of Formulations A and E.

The apparent viscosities of the five formulations A, B, C, D and E at 2.5 rpm, 2 hours after preparation were 8, 11.8, 15.8, 34.4 and 56 x 10^3 cps, respectively. The analysis of viscometric changes as done by Kita et al (6) to follow stability cannot be applied to this study because Matsumoto's emulsions had a combined W/O volume fraction not more than 0.1 and the flow curves (9) followed a Newtonian pattern. The combined O/W volume fraction of the formulations reported here were at least 0.67 and the emulsions had pseudoplastic flow properties. The viscosity curves

reflect the phase volume ratio of the combined inner phases to the external aqueous phase, i.e. W-O/W, therefore, the volume fraction of the dispersed aqueous phase affects the flow property of the emulsions because it determines the volume of the W/O internal phase after mixing, depending on the extent of breakdown.

Effect of Primary Emulsion Particle Size

The relationship between the particle size of the primary emulsion and the apparent viscosity of the W/O/W emulsion during the first 2 hours of emulsion life is shown in Figure 4. Formulation E was used to investigate this parameter. The apparent viscosity of the W/O/W emulsions in which the primary emulsion was prepared with a colloid mill and contained very small globules (2 um) was significantly higher than emulsions prepared without a colloid mill, (average globule size 9 um) and whose apparent viscosity remained constant after an initial fall. In the latter case, the large internal globules of the primary emulsion coalesced rapidly causing rapid breakdown of the oily phase.

Also represented in Figure 4 is the apparent viscosity of W/O/W emulsion without dextrose in the internal phase, but whose primary emulsion was

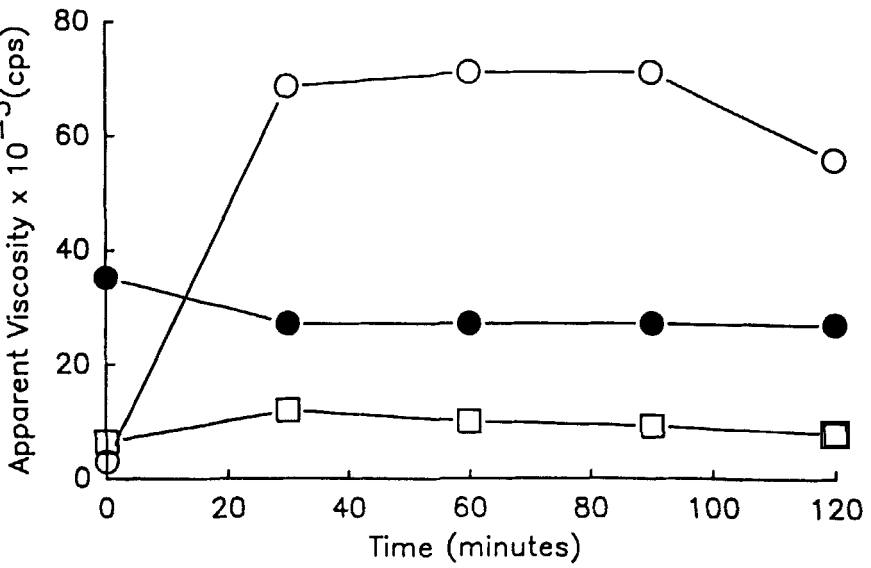


Figure 4

Effect of Primary Emulsion Particle Size on Changes in Apparent Viscosity of W/O/W Emulsion E during the first 2 hours of Emulsion Life.
○ = Primary Emulsion of W/O/W Emulsion emulsified with colloid mill.
● = Primary Emulsion of W/O/W Emulsion emulsified without colloid mill.
□ = Primary Emulsion of W/O/W Emulsion (without dextrose) emulsified with colloid mill.

prepared with a colloid mill. The apparent viscosity was very low, and decreased with time due to further loss of the internal phase and less resistance to flow.

Effect of Dextrose Concentration on Drug Release

Figure 5 depicts the release patterns of the five formulations containing 0, 0.250, 0.307, 1.250,

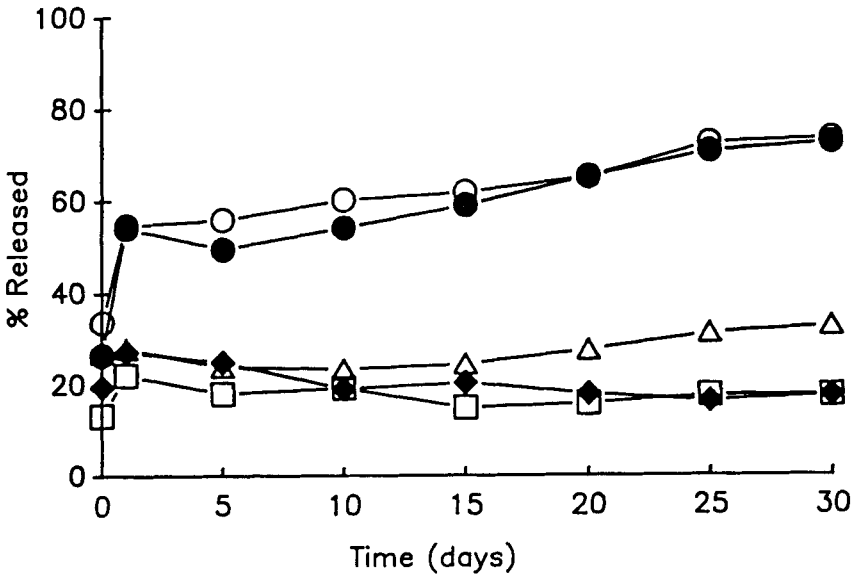


Figure 5

Effect of Dextrose Concentration on the Release Patterns of Sodium Salicylate in Aging W/O/W Emulsions.
○ = Formulation A, 0% Dextrose
● = Formulation B, 0.25% Dextrose
△ = Formulation C, 0.307% Dextrose
◆ = Formulation D, 1.25% Dextrose
□ = Formulation E, 2.50% Dextrose

and 2.50% W/V dextrose concentrations over a period of 30 days. As shown in the Figure, salicylate released into the external phase of the W/O/W emulsion was least (17.7%) in Formulation E within the period of study.

This is in contrast to formulation A (without dextrose), in which about 50% of the drug was released

within the first 24 hours of emulsion life, and over 70%, within the time of study. Formulation B contained the least amount of dextrose (0.275 g) in the internal phase and it released almost the same quantity of its drug content as formulation A. Formulation C was, surprisingly, much slower in the release of its drug content despite the low concentration of the dextrose, this concentration may be critical. Thirty-three percent of the drug was released within the 30 days study period. Formulation D, with 1.25% dextrose, released a lesser amount of drug than formulations A, B, and C (17.7%), but the same as formulation E.

The percentages of drug released in the five formulations, namely, 73.8%, 72.9%, 32.9%, 17.7% and 17.7% correlated with the physical shelf stability observed microscopically in which substantial breakdown was observed after 12 hours, 36 hours, 30 days, 42 days, and 56 days, respectively.

Calculated osmotic pressures in the internal phases of the five formulations, A, B, C, D, and E are 1.6, 1.97, 2.05, 3.5 and 5.3 atmospheres, respectively, while that of the external phase containing only 1% W/V Tween 40 is near 0. Osmotic pressure caused by Tween 40 was negligible because of

the extremely high molecular weight of its micelles (10). From these osmotic pressure values, the migration of water should be toward the internal phase (except in Formulation A), assuming there is no escape of the salicylate ion into the external phase at the onset of the experiment. This flux of water into the internal phase should cause swelling of the multiple globules in the order of E D C B A. The swelling, however, apparently had little effect on the rupture of the interfacial film because the formulations with the highest osmotic pressure were the most stable. Therefore, increased stability was not due to the difference in osmotic pressure *per se* but to the increased viscosity of the emulsions, E being the most viscous and A the least. The increasing viscosity observed with increasing amount of dextrose in the internal phase was due to the increased volume of internal phase (from the influx of water).

One hundred percent release was not observed within the test period even in formulations where, microscopically, there appeared to be a total loss of the internal phase, namely A and B. No doubt a small proportion of the W/O phase remained intact.

Partition Studies

The results of the distribution of the drug between the internal aqueous phase and the oily phase

TABLE 2

Partition Coefficient Studies

Mean Absorbance	Conc mg/ml	Partition Coefficient k(Co/Cw)	Comments
0.440	10.22		Aqueous Solution
0.420	9.76	0.046	Equilibration in the presence of surfactant and dextrose.
0.429	9.97	0.025	Equilibration without surfactant and dextrose.
0.422	9.80	0.042	Equilibration without surfactant but with dextrose.

are shown in Table 2. The observed partition or distribution coefficient (k-O/W-) was 0.05 in the presence of surfactant in the oily phase, and dextrose in the aqueous phase. In the absence of surfactant but with dextrose in the aqueous phase, k-O/W- was 0.04, while in the absence of both surfactant and dextrose, it was 0.025.

According to pH theory, salicylic acid, a weakly acidic drug with pKa 2.98 and pH 5-6, is 99% ionized. However, from the partition coefficient study, 5% of undissociated drug was distributed in the oil phase.

The net percent drug recovered in the external phase should therefore be at least 90% in a total breakdown of the emulsion. From the results shown in Figure 8, this was not realized in the experiments utilizing dextrose formulations. It is unlikely that the hydrophobic surfactant (Span 83) formed a complex with the salicylic acid (which could have been responsible for the incomplete release) because, at the lowest concentration, when there was total breakdown of the emulsion, the amount of drug released into the external phase was the same (90%) as when maximum concentration of the Span 83 (31%) was used. See previous report (8).

CONCLUSIONS

W/O/W formulations with 0 or 0.31% dextrose concentration in the internal aqueous phase were least stable as shown by the coalescence of the internal phase, rupture of the interfacial layers and higher drug release. Increasing the dextrose concentration beyond 0.31% up to 2.5% led to increase in relative stability, an effect caused by an increase in apparent viscosities of the emulsions. The apparent viscosities decreased with time in all the emulsions.

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FOOTNOTES

1 Brookfield Synchro-Lectric RVT Viscometer, Brookfield Engineering Laboratories, Inc., Stoughton, Massachusetts, USA, 1961.

2 Colloid Mill, Speco, Inc., 58 Rantoul St. Beverly, Massachusetts, 01915.

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