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Measurement of the yield of multiple emulsion droplets by a fluorescent tracer technique

S.S. Davis and I. Walker

Pharmacy Department, University of Nottingham, University Park, Nottingham NG7 2RD (U.K.)

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Summary

The yield of multiple droplets in emulsions made from mineral oils (liquid paraffin and squalane) and vegetable oils (sesame oil, maize oil and arachis oil) has been followed using a tracer method based on 6-carboxyfluorescein. The yield was found to be very dependent upon the nature of the oil phase for different phase volumes and processing conditions. The mineral oils gave much greater yields than the vegetable oils and of the latter group, sesame oil was superior. The yield of multiple droplets decreased with the phase volume of the original primary w/o emulsion as well as with the secondary emulsification time. The phase volume of the secondary emulsion had little effect on the yield. The results are discussed in terms of the use of multiple emulsions for drug delivery and the nature of the oil phase.

Introduction

Before an emulsion system can be used for drug delivery, the relevant pharmaceutical parameters should be adequately defined (Davis, 1976). This applies especially to the more complex systems such as multiple emulsion (water/oil/water) ($w_1/o/w_2$) which have been shown to consist of two distinct droplet populations; simple o/w and multiple $w_1/o/w_2$ droplets (Burbage, 1979; Whitehill, 1980). Thus, in order to characterize a multiple emulsion as a drug delivery system it is essential to establish the relative proportions of simple and multiple droplets and hence the yield of multiple emulsions.

Two main methods, size analysis and internal tracer technique, have been developed for evaluating the yield of multiple emulsion droplets.

Size analysis technique

The yield by number and volume percentage of simple and multiple droplets can be found by analysis of the particle size distribution of the total system (Burbage,

1979; Whitehill, 1980; Davis and Burbage, 1978). Davis and Burbage (1978) used a variety of techniques (photomicroscopy, Coulter Counter, electron microscopy) to evaluate the effect of formulation parameters upon the yield of a liquid paraffin-based multiple emulsion system ($w_1/o/w_2$). Photomicroscopy and Coulter Counter analysis indicated a bimodal distribution of droplet size (due to o/w and $w_1/o/w_2$ populations) which could be resolved into the individual populations using a graphical procedure (Lewis and Taylor, 1967). Whitehill (1980) used photomicroscopy to evaluate the ratio of multiple ($w_1/o/w_2$) to simple (o/w) droplets in multiple emulsions based upon isopropyl myristate.

Internal phase tracer technique

The use of an internal tracer can be used to establish the efficiency of entrapment of an impermeable marker molecule in the w_1 phase of the system. Matsumoto et al. (1976) used glucose to characterize systems based upon various oil phases whilsts Kita et al. (1977) used an ion selective electrode to evaluate the yield and stability of multiple emulsion systems. Other workers in the field of chemical engineering have used the rate of leakage of a marker impermeable in the oil phase to study the properties of multiple emulsions (liquid membranes) used in extraction procedures; for example, hydrogen ions (Martin and Davies, 1976; Teramoto et al., 1981; Völkel et al., 1980; Kondo et al., 1979), electrolyte (Takahashi et al., 1981; Hochauser and Cussler, 1971; Lee et al., 1978).

The two methods for the determination of yield of multiple emulsions cannot be compared directly due to inherent differences in the parameters which are measured. The impermeable marker technique measures essentially the difference in w_1 and w_2 phase concentrations. However, interchange of the w_1 and w_2 phases can occur during the second emulsification procedure (Burbage, 1979) and it is possible to have a maximum theoretical marker concentration in the external (w_2) phase (apparent zero yield) yet with many multiple drops present in the system. In contrast, the size analysis technique measures only the physical presence of multiple droplets and not the efficiency of marker (drug) entrapment. Consequently it is possible for a system to have a high yield in terms of observed multiple droplets but with relatively little marker entrapped in the internal (w_1) phase.

The in vitro and in vivo drug release characteristics of a $w_1/o/w_2$ emulsion will depend both on the number and distribution of multiple droplets and the drug entrapment capacity, thus when assessing the yield of a multiple system, both methods should not be considered in isolation. In the present investigation a fluorescent tracer method for assessing the yield of multiple emulsion droplets is described together with its application in assessing the effect of the nature of the oil phase. 6-Carboxyfluorescein, used as the tracer, has been used widely in studies on the integrity and release characteristics of liposomes in vitro and in vivo (Weinstein et al., 1977; Gregoriadis and Davis, 1979; Kirby et al., 1980) and to investigate the effect of liposomal entrapment upon lymphatic uptake and distribution in the rat (Parker et al., 1981). Due to an increased polarity the half-time of leakage of 6-carboxyfluorescein from liposomes is greater (weeks compared to minutes) than that of sodium fluorescein (Weinstein et al., 1977). Our preliminary experiments

indicated that in solution at pH 7.2 6-carboxyfluorescein would not diffuse through thin oil lamellae produced using a variety of oil phases. Therefore it was considered a suitable marker molecule to evaluate the yield of various $w_1/o/w_2$ multiple emulsion systems.

Experimental

Materials

All materials were of the highest available quality reagent grade and were used without further purification. 6-Carboxyfluorescein (6-CF) was obtained from Eastman Kodak, Rochester, NY, U.S.A. Tween 80, Span 80 were obtained from Honeywill Atlas, Carshalton, Surrey.

The oil phases were obtained as follows: light liquid paraffin from Fisons Scientific Apparatus, Loughborough, squalane from BDH Chemicals, Poole, maize oil and arachis of BP quality from Evans Medical, Greenford and sesame oil (USP quality) from Alembic Products, Sale, Manchester. Each was used without further purification.

Production of emulsions

The internal phase consisted of 1 mg/ml 6-CF in pH 7.2 phosphate buffer while the oil phase comprised 10% Span 80 w/w, 2% Tween 80 w/w (as emulsifiers) and oil to 100% w/w. The external phase was 2% Tween 80 w/w in pH 7.2 phosphate buffer.

The internal phase was shaken with the appropriate oil phase in a glass vial for a few seconds and then mixed on a VM20 vortex mixer (Chiltern Scientific Enterprises, Leighton Buzzard) at maximum speed for 5 min to produce the primary w_1/o emulsion. The primary system was then mixed on VM20 mixer at maximum speed for an appropriate time with enough external phase to produce a final $w_1/o/w_2$ emulsion. 20 ml batches of multiple emulsion were prepared.

Assay procedure

100 μl of the multiple emulsion system were mixed with 4.9 ml pH 7.2 phosphate buffer and after allowing the system to cream it was filtered through a 1.2 μm pore size 13 mm Millipore filter unit (Millipore, Bedford, MA U.S.A.) to remove the primary w_1/o droplets. Initial investigations showed that this procedure did not cause disruption of the w_1/o droplets. The diluted external phase was then assayed for 6-CF content using a Perkin Elmer 1000 fluorescence spectrophotometer (Perkin Elmer, Beaconsfield, Bucks) ($\lambda_{exit} = 473$ nm, $\lambda_{emiss} = 520$ nm). The results are expressed as a percentage of the theoretical maximum 6-CF that could be entrapped. Each experiment was performed 3 times. In general the results were highly reproducible and in the figures the mean and standard error of the mean is shown, the latter when it exceeds the size of the symbol.

Results and Discussion

Evaluation of the effect of formulation parameters

The effect of 3 formulation parameters: (i) primary phase volume ratio ϕ w_1/o; (ii) secondary emulsification time t_2; and (iii) secondary phase volume ratio ϕ $w_1/o/w_2$, upon the efficiency of entrapment of 6-CF has been investigated using 5 oils of different sources and chemical characteristics—(a) a mineral oil (light liquid paraffin BP); (b) a chemically defined hydrocarbon oil (squalane); and (c) 3 vegetable oils (arachis oil, sesame oil, maize oil) all of pharmacopoeial quality.

The experimental design provided for one formulation parameter to be varied, while the others were chosen to give as high a yield of multiple emulsion droplets as possible.

The effect of primary phase volume ratio (ϕ w_1/o) upon yield

The yield of 5 different oil-based multiple emulsion systems was markedly dependent upon both ϕ w_1/o and the nature of the oil phase (Fig. 1), the individual curves being somewhat sigmoidal in shape. The phase volumes that give rise to 50% release are given in Table 1 for the different oils. These results are in contrast to those reported by Matsumoto et al. (1976) who found that the yield of a liquid paraffin-based multiple emulsion system was relatively insensitive to changes in ϕ w_1/o.

The data in Table 1 and Fig. 1 can be used to predict the effect of the formulation parameter upon yield when designing a multiple emulsion system, e.g. for a sustained release preparation, namely that the drug in the W_2 external phase will be released much faster than drug in the internal w_1 phase and that by varying the ratio of (drug in w_1)/(drug in w_2) (e.g. by varying ϕ w_1/o) the drug release properties of the multiple emulsion system can be varied.

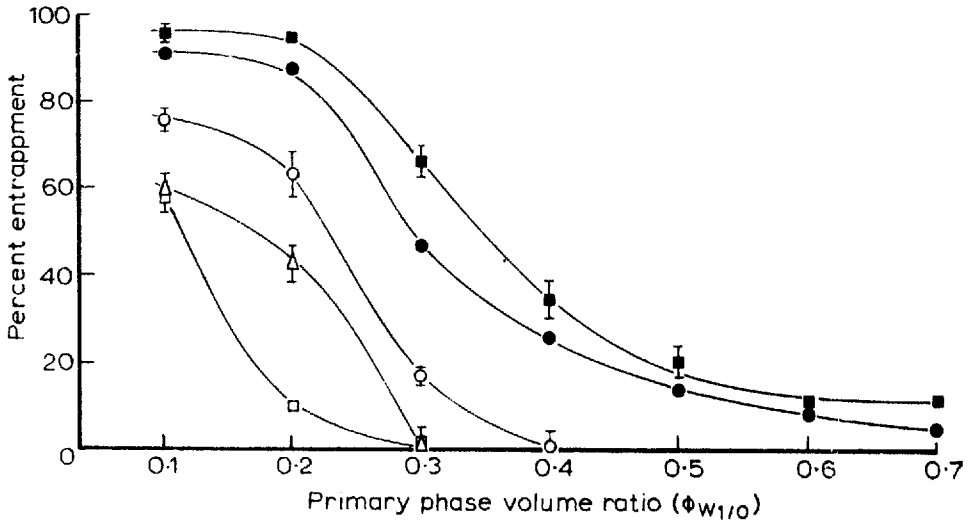

Fig. 1. The effect of primary phase volume ratio upon yield of multiple emulsion. ■, liquid paraffin; ●, squalane; △, arachis oil; ○, sesame oil; □, maize oil. ϕ $w_1/o/w_2$, t_1 and t_2 were kept constant at 0.5, 5 min and 0.25 min, respectively.

TABLE 1

THE EFFECT OF PRIMARY PHASE VOLUME RATIO (ϕ w_1/o) UPON THE EFFICIENCY OF ENTRAPPMENT OF 6-CARBOXYFLUORESCEIN BY MULTIPLE EMULSIONS BASED ON DIFFERENT OIL PHASES

ϕ w_1/o	% encapsulation of 6-carboxyfluorescein		
	Mean	Standard deviation	Standard error of the mean
Light liquid paraffin – 10% Span 80 – 2% Tween 80 (w/w)			
0.1	96.55	0.26	0.15
0.2	94.81	0.673	0.39
0.3	66.03	8.88	3.97
0.4	33.0	8.36	4.18
0.5	19.97	7.14	4.12
0.6	10.31	1.59	0.92
0.7	10.70	2.43	1.40
ϕ w_1/o for 50% entrappment 0.35			
Squalane – 10% Span 80 – 2% Tween 80 (w/w)			
0.1	91.54	1.67	0.96
0.2	87.89	0.30	0.17
0.3	46.17	7.86	3.93
0.4	26.55	20.12	10.06
0.5	14.53	4.42	2.55
0.6	9.37	3.30	1.91
0.7	4.33	3.44	1.98
ϕ w_1/o for 50% entrappment 0.29			
Maize oil – 10% Span 80 – 2% Tween 80 (w/w)			
0.1	59.17	8.93	5.16
0.2	9.62	2.08	1.20
0.3	– 1.11	4.49	2.59
ϕ w_1/o for 50% entrappment 0.11			
Sesame oil – 10% Span 80 – 2% Tween 80 (w/w)			
0.1	75.79	6.91	3.46
0.2	61.30	19.88	7.51
0.3	16.46	2.10	1.21
0.4	0.75	4.24	2.45
ϕ w_1/o for 50% entrappment 0.22			
Arachis oil – 10% Span 80 – 2% Tween 80 (w/w)			
0.1	58.38	3.10	1.79
0.2	42.27	6.73	3.88
0.3	0.52	5.71	2.85
ϕ w_1/o for 50% entrappment 0.15			

The effect of secondary emulsification time (t_2) upon the yield

ϕ w_1/o, ϕ $w_1/o/w_2$ and t_1 were kept constant at 0.1, 0.5 and 5 min, respectively. The results (Fig. 2) indicate that the yield decreases in a zero-order fashion as a function of increasing t_2. This is in agreement with previous reports that have noted an apparently zero-order rupture of multiple emulsion droplets as a function of

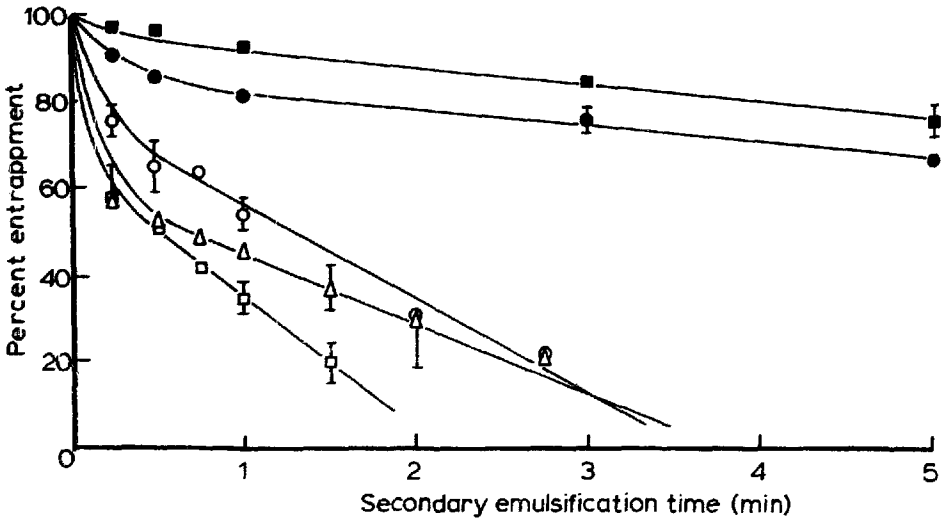

Fig. 2. The effect of secondary emulsification upon yield of multiple emulsions. As for Fig. 1.

extraction (and hence agitation) time for liquid membrane extraction systems (Takahashi et al., 1981; Kondo et al., 1979; Frankenfeld et al., 1981; Völkel et al., 1980; Li and Shrier, 1972; Yang and Rhodes, 1980; Chilamkurti and Rhodes, 1980; Cahn et al., 1981).

In a patent, Collings (1968) implied that during secondary emulsification drug can leak out of the w_1 internal phase and that this was greatest with systems of low particle size (large surface area), hence suggesting that an increase in secondary emulsification time not only reduced the particle size of the system but also increased the amount of drug in the external w_2 phase (thus decreasing the yield).

Multiple emulsions can be advocated as potential drug delivery systems with

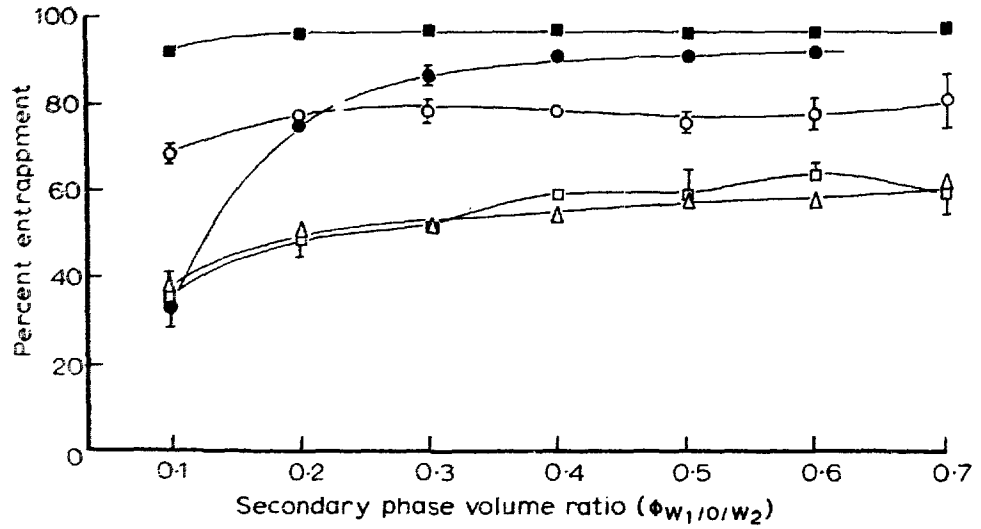

Fig. 3. The effect of secondary phase volume ratio upon yield of multiple emulsion. As for Fig. 1.

'instant' release of drug occurring from the w_2 phase, followed by slow release of drug from the w_1 phase. The linear relation between entrapment of 6-CF and secondary emulsification time could be used to predict the value of t_2 needed to produce a given drug concentration in the w_1 and w_2 phases. Naturally, consideration must be given to the effect of t_2 upon the particle size distribution of such systems since a low t_2 value might produce an unacceptably large particle size. It is

TABLE 2

THE EFFECT OF SECONDARY EMULSIFICATION TIME UPON THE EFFICIENCY OF ENTRAPMENT OF 6-CARBOXYFLUORESCEIN BY MULTIPLE EMULSIONS BASED UPON DIFFERENT OIL PHASES

Secondary emulsification time (min)	% Encapsulation of 6-carboxyfluorescein		
	Mean	Standard deviation	Standard error of the mean
Light liquid paraffin – 10% Span 20 – 2% Tween 80 (w/w)			
0.25	96.55	0.26	0.15
0.5	95.95	0.22	0.13
1.0	92.30	0.29	0.17
3.0	84.37	3.21	1.61
5.0	74.42	6.30	3.15
Squalane – 10% Span 80 – 2% Tween 80 (w/w)			
0.25	91.54	1.67	0.96
0.5	86.1	2.55	1.47
1.0	82.18	1.84	1.07
3.0	74.35	8.44	4.22
5.0	67.05	3.39	1.96
Sesame oil – 10% Span 80 – 2% Tween 80 (w/w)			
0.25	75.78	6.91	3.46
0.50	65.12	12.45	7.19
0.75	63.91	2.31	1.34
1.0	54.12	7.56	3.78
2.0	30.47	2.18	1.26
2.75	21.06	3.44	1.98
Arachis oil – 10% Span 80 – 2% Tween 80 (w/w)			
0.25	58.38	3.10	1.79
0.5	52.59	1.49	0.86
0.75	48.44	1.80	1.04
1.0	45.54	2.74	1.58
1.5	37.89	10.59	6.12
2.0	30.57	22.39	10.00
Maize oil – 10% Span 80 – 2% Tween 80 (w/w)			
0.25	59.17	8.93	5.16
0.5	50.74	1.50	0.86
0.75	41.82	1.56	0.90
1.0	34.69	5.91	3.41
1.5	20.50	10.15	5.08

TABLE 3

THE EFFECT OF SECONDARY PHASE VOLUME RATIO (ϕ $w_1/o/w_2$) UPON THE EFFICIENCY OF ENTRAPPMENT OF 6-CARBOXYFLUORESCEIN BY MULTIPLE EMULSIONS BASED UPON DIFFERENT OIL PHASES

Secondary phase volume ratio (ϕ $w_1/o/w_2$)	% encapsulation of 6-carboxyfluorescein		
	Mean	Standard deviation	Standard error of the mean
Light liquid paraffin – 10% Span 80 – 2% Tween 80 (w/w)			
0.1	92.67	0.49	0.28
0.2	97.05	0.30	0.17
0.3	96.99	0.63	0.36
0.4	97.28	0.78	0.45
0.5	96.55	0.26	0.15
0.6	96.82	2.08	1.04
0.7	97.92	0.32	0.19
Squalane – 10% Span 80 – 2% Tween 80 (w/w)			
0.1	all 34.57	9.86	4.63
0.2	75.97	2.04	1.18
0.3	87.15	4.43	2.56
0.4	91.35	1.04	0.6
0.5	91.54	1.67	0.96
0.6	92.46	3.11	1.79
Sesame oil – 10% Span 80 – 2% Tween 80 (w/w)			
0.1	68.70	5.16	2.58
0.2	77.92	1.54	0.89
0.3	78.22	6.74	3.89
0.4	78.61	2.36	1.36
0.5	75.78	6.91	3.46
0.6	77.10	9.86	4.93
0.7	81.31	12.40	7.16
Arachis oil – 10% Span 80 – 2% Tween 80 (w/w)			
0.1	37.97	5.80	3.35
0.2	50.95	2.59	1.49
0.3	51.95	2.55	1.47
0.4	54.65	3.75	1.68
0.5	58.38	3.10	1.79
0.6	57.82	2.96	1.65
0.7	61.13	3.41	1.71
Maize oil – 10% Span 80 – 2% Tween 80 (w/w)			
0.1	35.52	3.92	2.26
0.2	49.65	6.80	3.93
0.3	51.61	2.74	1.52
0.4	39.22	2.47	1.43
0.5	59.17	8.93	5.16
0.6	63.56	4.53	2.62
0.7	59.19	4.80	4.90

interesting to note that, as in the effect of ϕ w_1/o, the nature of the oil phase is extremely important in determining how a formulation parameter can affect the yield of a multiple emulsion system. The effect of the oil upon yield when altering t_2 is in the order:

light liquid paraffin > squalane > sesame oil > arachis oil > maize oil

The effect of secondary phase volume ratio (ϕ $w_1/o/w_2$) upon yield (ϕ w_1/o, t_1 and t_2 were kept constant at 0.1, 5 min and 0.25 min, respectively)

Fig. 3 shows that although the yield is markedly dependant upon the nature of the oil phase (light liquid paraffin > squalane > sesame oil > maize/arachis oil), it is relatively independent of ϕ $w_1/o/w_2$. In contrast, Matsumoto et al. (1976) found that the yield of liquid paraffin-based multiple emulsion system increased from 55 to 95% as ϕ $w_1/o/w_2$ was increased from 0.1 to 0.5. The squalane systems deviate in their behaviour from that of the other oils at lower values of ϕ $w_1/o/w_2$. These results are more in agreement with the proposals of Matsumoto et al. (1976).

The marked dependence of the yield of multiple emulsion upon ϕ w_1/o and t_2 indicate that it is the nature of the stability of the w_1/o droplets which largely determines the yield of a multiple system. Benoy (1974) found that it was impossible to prepare multiple ($w_1/o/w_2$) emulsion systems from unstable w_1/o primary emulsions. Several workers (Boyd et al., 1972; Kirikou and Sherman, 1979; Kavalunias and Frank, 1978; Boyd et al., 1976) have noted that the stability of emulsions stabilized by mixed emulsifiers increases with the degree of interaction of the emulsifier at the oil–water interface, and it is probable that the nature of the oil phase affects the degree of interaction of the emulsifiers at the oil–water interface, thus affecting the stability of the primary w_1/o emulsion and hence the yield of multiple droplets. Interfacial tension and surface rheology studies on the effect of oil phase upon the nature of the Span 80/Tween 80 mixed film at the oil–water interface will give valuable information about the mechanisms of instability of primary emulsion and the subsequent yield of the multiple emulsion systems examined (Matsumoto and Sherman, 1981). The present study suggests that the degree of interaction of the emulsifiers with mineral oils consisting of mixed hydrocarbons or a pure hydrocarbon is much greater than the interaction with vegetable oils containing various concentrations of triglyceride. It is realized that although the oils were of high purity they could still contain small quantities of surface-active material (e.g. monoglyceride) that might compete at the o/w interface during the primary emulsion stage. There would appear to be no correlation between the physical properties of the various oils and the subsequent yield of multiple emulsion droplets.

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