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International Journal of Pharmaceutics 124 (1995) 61–67

international journal of pharmaceutics

Preparation and characterization of cytarabine-loaded w/o/w multiple emulsions

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Received 29 November 1994; accepted 9 February 1995

Abstract

Cytarabine-loaded w/o/w multiple emulsions were prepared using nonionic surfactants of the Tween and Span types, and characterized by studying the osmotic behavior. The effect of the sonication period on the entrapment efficiency, droplet size and emulsion stability was investigated. The entrapment efficiency was up to 79% and was not affected by the loading dose. The size of droplets decreased with increase in the second sonication time. It was found that the oil layer of multiple emulsion droplets including the hydrophobic fatty acid tails of surfactants behaves as a water-permeable membrane between two aqueous phases of w/o/w multiple emulsion. The combination of Tween 20/80 and Span 20/80 as a hydrophilic and lipophilic surfactant system produced the most stable multiple emulsion. The release study showed that the multiple emulsion containing cytarabine in the internal aqueous phase was stable, exhibiting a prolonged release pattern.

Keywords: w/o/w multiple emulsion; Cytarabine; Tween; Span; Osmotic behavior; Emulsion stability; Prolonged release

1. Introduction

Multiple emulsions are emulsions in which the droplets of the dispersed phase themselves contain smaller dispersed droplets miscible with the continuous phase. They are, therefore, emulsions of emulsions and can be classified as oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) types (Whitehill, 1980; Florence and Whitehill, 1982). These multiple emulsions have shown promise in many technologies, particularly in pharmaceutics and in separation science. Their potential pharmaceutical applications include adjuvant vaccines, prolonged drug delivery systems, reservoirs in drug overdose treatments and immobilized enzymes (Brodin et al., 1978; Chiang et al., 1978; Ekman and Sjoholm, 1978; Yoshioka et al., 1982).

Cytarabine (cytosine arabinoside) is an important antimetabolite, and its effectiveness in the treatment of acute lymphocytic and granulocytic

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SSDI 0378-5173(95)00074-7

leukemias is well established (Kufe and Major, 1982; Pallavicini, 1984). For effective cancer chemotherapy, it is necessary to deliver a sufficiently high concentration of anticancer agents into the tumor site for a required period and to minimize their concentration in other tissue compartments of the body because of their adverse reactions. Several groups have studied the enhanced delivery of anticancer agents to regional lymphatics by emulsions (Davis et al., 1987; Hashida et al., 1980; Omotosho et al., 1990). Multiple emulsions can provide one drug delivery system which creates a selectively high concentration of anticancer agents in the lymphatic system for the prevention of metastasis and the treatment of malignant lymphoma. In addition, w/o/w multiple emulsions possess many advantages over w/o emulsions as well as a low viscosity due to the aqueous external phase, which makes them more convenient to handle and use, especially for injection.

The purpose of this research was to develop and to characterize cytarabine-loaded w/o/w multiple emulsion for an anticancer drug delivery system. The entrapment efficiency and droplet size were determined in order to optimize the manufacturing conditions. The osmotic behavior was studied by the determination of water transference under an osmotic pressure gradient between the aqueous phases of the multiple emulsion. Finally, the stability of the emulsion was investigated by varying the composition of the surfactants and the second sonication time.

2. Materials and methods

2.1. Chemicals

Cytarabine (98.7%) was supplied by Choong Wae Pharm. Co. (Seoul, Korea). Sorbitan monolaurate (Span 20), sorbitan monooleate (Span 80), polyoxyethylene (20) sorbitan monolaurate (Tween 20) and polyoxyethylene (20) sorbitan monooleate (Tween 80) were obtained from ICI Americas Inc. (Wilmington, DE, USA). Isoton II® (filtered 1% saline phosphate-buffered solution) was purchased from Coulter Electronics Ltd (Luton, Beds, UK). All other chemicals used were of reagent grade.

2.2. Preparation of w / o / w multiple emulsion

As shown in Fig. 1, w/o/w multiple emulsions were prepared by a two-step emulsification process; the first emulsification to prepare the w/o primary emulsion and the second emulsification to complete the w/o/w multiple emulsion.

To prepare the w/o emulsion, 1 ml of oily phase (Span/liquid paraffin = 3:7 w/w) was mixed with 0.4 ml of internal aqueous phase (cytarabine solution) in a round-bottomed glass tube (diameter 2.5 cm). The mixture was heated to 70° C and emulsified by vortex mixing for 10 min. This coarse w/o emulsion was then sonicated for 2 min using a probe-type ultrasonicator with 18 μm (MSE, MK2, Sussex, UK). Sonication and stirring were carried out in a temperature-controlled water bath at 70 ± 1° C.

For the preparation of the w/o/w multiple emulsion, the newly prepared w/o primary emulsion was added slowly into a 20 ml cap tube containing 4 ml of external aqueous phase (0.5%

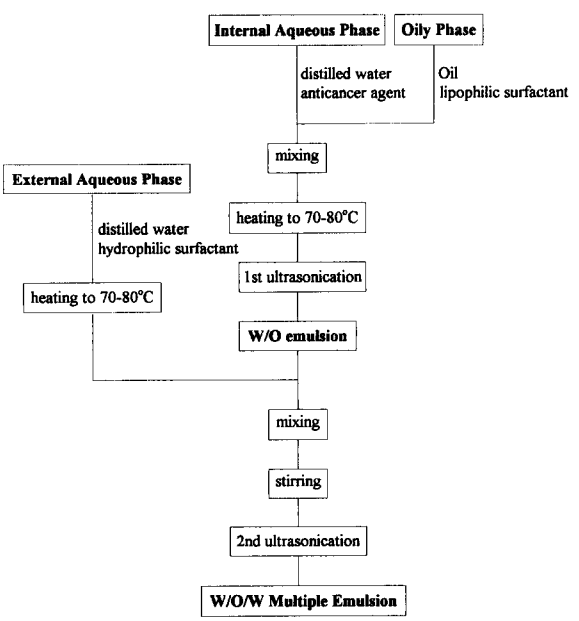

Fig. 1. Schematic diagram representing the preparation of w/o/w multiple emulsion.

Tween in distilled water) which was preheated to about 70° C. The mixture was stirred for 5 min in a water bath maintained at 70 ± 1° C using a magnetic stirrer and emulsified by ultrasonication (6 μm) for the designated time period (15, 30, 45, 60 s) at 70° C followed by cooling to about 4° C in an ice bath.

2.3. Entrapment efficiency of cytarabine

5 ml of freshly prepared w/o/w multiple emulsion was sedimented using an ultracentrifuge (Beckman 4C-90) at 180 000 *g* for 30 min at 4° C. The resulting lower solution was removed into a 1 ml syringe and diluted with 5 ml of distilled water. Each solution was filtered using a membrane filter (0.2 μm pore size) and analyzed by UV spectrophotometry (Uvikon 860, Kontron Instruments Co., Basel, Switzerland) at 267 nm. In each case, the multiple emulsion without cytarabine was treated likewise and regarded as a blank.

The amount of cytarabine entrapped is the amount of total cytarabine minus the amount of free cytarabine separated into lower part of solution by ultracentrifugation. The efficiency of cytarabine entrapment is defined as follows;

$$\text{efficiency of cytarabine entrapment (\%)} = [(\text{total cytarabine} - \text{free cytarabine})/\text{total cytarabine}] \times 100$$

2.4. Measurement of oil droplet size

For the determination of the mean diameter of dispersed oil droplets, the w/o/w multiple emulsions prepared with various second sonication time (15, 30, 45, 60 s) were dispersed in Isoton II® (Coulter Electronics Ltd, Luton, Beds, UK) as an electrolyte solution and particle sizes were measured with a Coulter counter (model TA II, Coulter Electronics Ltd) with 50 μm aperture at the designated time intervals. The instrument was calibrated with a latex solution.

2.5. Osmotic behavior

For the determination of the osmotic behavior, the following multiple emulsions were prepared. w/o/w multiple emulsion composed of 0.5 ml of internal aqueous phase, 1 ml of oily phase (30% Span 80 in liquid paraffin) and 4 ml of external aqueous phase (0.5% Tween 80 in distilled water) was dispersed in hypertonic electrolyte solutions (0.125, 0.5, 1.0% NaCl). w/o/w multiple emulsions were prepared with the same compositions as the above multiple emulsion except the electrolyte concentrations in internal aqueous phases (distilled water, 0.5 and 1.0% NaCl) were dispersed in distilled water. The percentage changes of particle sizes under an osmotic pressure gradient were determined at the designated time intervals. The percentage change in mean diameter was then calculated as follows;

$$\text{percentage change} = [(\text{measured diameter} - \text{initial diameter})/\text{initial diameter}] \times 100$$

2.6. Emulsion stability and diffusion study

The physical stability of the w/o/w emulsion was evaluated by visual observation of the creaming process. The emulsion samples were transferred into 100 ml glass cylinders immediately after preparation. Multiple emulsions were separated into three phases; oil phase, unseparated emulsion and water phase from the top. The volume ratios of each separated phase were measured.

For the diffusion study, a benzoylated cellulose membrane (Sigma, USA) was placed between the plastic blocks. Samples were poured into one side of the membrane, and distilled water was added to the other. The set was circulated and maintained at 37 ± 1° C. Dialysates were removed, filtered and analyzed by spectrophotometrically at 267 nm.

3. Results and discussion

3.1. Entrapment efficiency

The entrapment efficiencies of cytarabine in the w/o/w multiple emulsions with various drug

Table 1
Effect of drug concentration and second sonication period on the entrapment efficiency of cytarabine

Drug concentration (μg/ml) [a]	Entrapment efficiency (%)	Sonication time (s) [b]	Entrapment efficiency (%)
20	73.6±7.7 [c]	15	57.4±2.2 [c]
40	74.1±4.7	30	78.9±3.9
80	79.3±2.2	45	75.2±2.4
160	78.9±3.9	60	67.2±3.4

[a] Formulations with first sonication time of 120 s and second sonication time of 30 s.
[b] Formulations with first sonication time of 120 s and drug concentration of 160 μg/ml.
[c] Means±S.D. of five experiments.

concentrations and second sonication periods are listed in Table 1. With increasing drug content, the entrapment efficiency increased slightly and was not affected by the drug concentration over 80 μg/ml. The emulsion with 80 μg/ml of drug content resulted in 79.3% entrapment efficiency. The w/o/w multiple emulsion with a second sonication of 30–45 s entrapped the greatest amount of cytarabine.

3.2. Size of multiple emulsion

It was found that the droplets decreased in size initially and then increased with time. The mean diameters of dispersed oil droplets in w/o/w multiple emulsions prepared with various second sonication periods are summarized in Table 2. The initial sizes of oil droplets were in the range of 1.3–1.6 μm and decreased with increasing second sonication time. The oil droplet size reached its minimum value at 4–8 days after the preparation of the multiple w/o/w emulsions. The percentage size reduction was about 12–22% and the greatest decrease in size was obtained with a 30 s second sonication. It is considered that the initial mean diameter decreased as aggregates of oil droplets dispersed completely. After the minimum size observed, the droplet size increased due to the coalescence of droplets.

3.3. Osmotic behavior

When multiple emulsion droplets are placed in various osmotic pressure gradients, the oil droplets containing the internal aqueous phase can shrink depending upon the osmolarities of the two aqueous phases. The effect of sodium chloride concentration in both the internal and external aqueous phases of the multiple emulsion on the amount of migrated water was investigated. Fig. 2 demonstrates the effect of electrolyte concentration in the external aqueous phase on the percentage change in mean diameter of w/o/w emulsion droplets. The higher electrolyte concentration in the external aqueous phase produced a larger percentage change in mean diameter and more rapid shrinkage, implying that more and faster internal water was released.

The size of droplets can be increased by employing the hypertonic internal aqueous solution. For the w/o/w emulsion made from the w/o primary emulsions containing 0.5 and 1.0%

Table 2
Mean diameter of dispersed oil droplets in multiple w/o/w emulsion with various second sonication periods

Sonication time (s) [a]	Initial diameter (μm)	Minimum diameter (μm)	Decrease (%)	Time to reach minimum size (day)
15	1.60 ± 1.16 [b]	1.28 ± 0.66 [b]	20.0	4
30	1.55 ± 1.05	1.21 ± 0.58	22.2	5
45	1.40 ± 0.81	1.22 ± 0.49	13.2	6
60	1.35 ± 0.65	1.12 ± 0.49	11.7	8

w/o/w emulsions composed of 0.5 ml of internal aqueous phase, 1 ml of oily phase (Span 80/liquid paraffin = 3:7) and 4 ml of external aqueous phase (0.5% Tween 80 in distilled water).
[a] Formulations with first sonication time of 120 s.
[b] Means ± S.D. of five experiments.

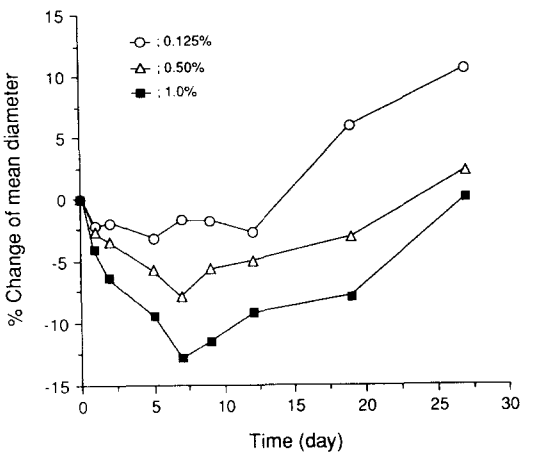

Fig. 2. Effect of external electrolyte concentration on the percentage change in mean diameter of dispersed oil droplets in w/o/w multiple emulsion.

sodium chloride solutions as internal aqueous phases, the size of droplets increased initially due to the migration of water from the external to the internal phase as shown in Fig. 3. At the plateau the amount of water migrated from the external to the internal phase may be same as that from the internal to external phase. Finally, the size increased due to coalescence. These results lead to the conclusion that the oil layer of multiple emulsion droplets acts as a water-permeable

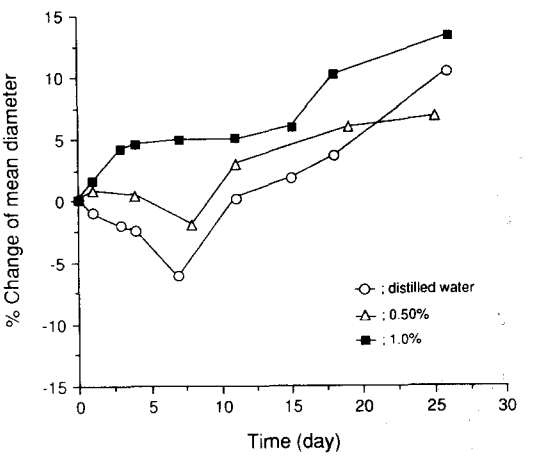

Fig. 3. Effect of internal electrolyte concentration on the percentage change in mean diameter of dispersed oil droplets in w/o/w multiple emulsion.

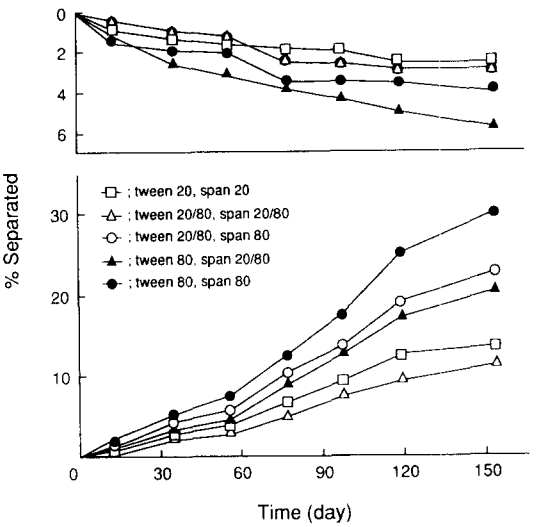

Fig. 4. Effect of surfactant composition on the phase separation of w/o/w multiple emulsion with the first sonication period of 120 s and the second sonication period of 30 s at room temperature. Upper, oil phase separated; lower, water phase separated.

membrane between the two aqueous phases of the multiple emulsion under the osmotic pressure gradient.

3.4. Stability and diffusion study

Since w/o/w multiple emulsions are not thermodynamically stable but kinetically stable systems, they were separated spontaneously into three phases; oil, unseparated emulsion and water phases from the top. For the multiple emulsions with various surfactant compositions, the volume of separated oil and water phases was measured separately for up to 150 days and plotted in Fig. 4. Generally, the percentage of water separated was about 5-fold greater than that of oil separated, implying that the lower separation is more related to the physical stability of w/o/w multiple emulsion than the upper separation. The multiple emulsion prepared with Tween 80 (HLB 15.0) and Span 80 (HLB 4.3), as hydrophilic and lipophilic surfactant, respectively, was most unstable, and the multiple emulsion with Tween 20 (HLB 16.7) and Span 20 (HLB 8.6) was relatively stable. With the Tween 20/80 mixture (1:1, HLB

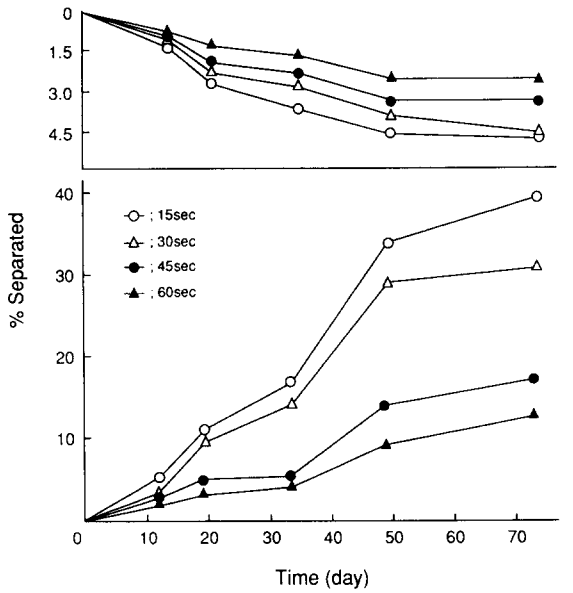

Fig. 5. Effect of second sonication time on the phase separation of w/o/w multiple emulsion with the first sonication period of 120 s at 36 ± 1° C. Upper, oil phase separated; lower, water phase separated.

15.8) and Span 20/80 mixture (1:1, HLB 6.5) the multiple emulsion was most stable. Fig. 5 shows the percentages of phase separation with respect to the second sonication period. With a longer sonication time, phase separation was slower.

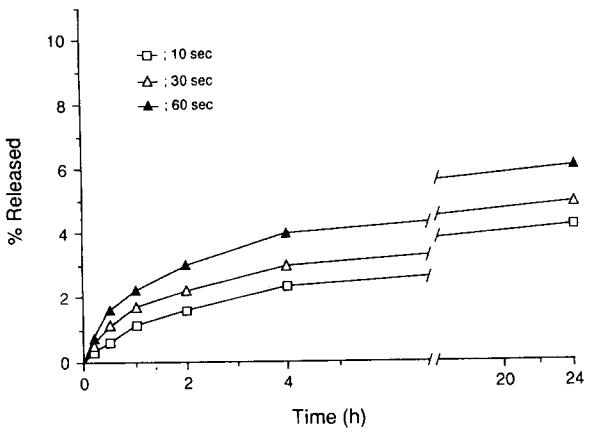

Fig. 6. Effect of second sonication time on the diffusion of cytarabine from w/o/w multiple emulsion with the first sonication period of 120 s.

In Fig. 6, the percentage of cytarabine diffusion was plotted with respect to the sonication period. The amount of drug diffused out was relatively small, indicating that the oil layer of the multiple emulsion acted as a stable diffusion barrier, thus the drug was released mainly by permeation through this membrane. Slightly faster diffusion was observed with a longer period of second sonication, since the multiple emulsion sonicated for a long period might include the smaller droplet size and the larger total surface area. However, the differences in the percentage release at 22 h were less than 2%. Thus, the drug incorporated into the internal aqueous phase of the w/o/w emulsion was found to be relatively stable, which induced the prolonged release of cytarabine from the multiple emulsion.

4. Conclusion

In conclusion, a cytarabine-loaded w/o/w emulsion has been prepared and found to have an entrapment efficiency of up to 79%. The multiple emulsion possessing more internal aqueous phase showed better entrapment efficiency. With a longer second sonication time, smaller droplets dispersed in the multiple emulsion were obtained. The oil layer of the multiple emulsion droplets acted as a water-permeable membrane under the osmotic pressure gradient, which can allow drug release from the internal aqueous to external aqueous phase. The multiple emulsion prepared with the Tween 20/80 and Span 20/80 mixtures was the most stable. The stability of the w/o/w multiple emulsion was proportional to the length of the second sonication period. The release study showed that the multiple emulsion containing cytarabine in the internal aqueous phase was stable, exhibiting a slow release pattern.

Acknowledgements

This research work was supported in part by a research grant from the Research Center for New Drug Development in Seoul National University

and Korea Science and Engineering Foundation (KOSEF 94-1400-03-01-3).

References

Brodin, A.F., Kavaliunas, D.R. and Frank, S.G., Prolonged drug release from multiple emulsions. *Acta Pharm. Suec.*, 15 (1978) 1–2.

Chiang, C.W., Fuller, G.C., Frankenfeld, J.W. and Rhodes, C.T., Potential use of liquid membranes for drug overdose treatment: in vitro studies. *J. Pharm. Sci.*, 67 (1978) 63–66.

Davis, S.S., Illum, L. and Walker, I.M., The in vivo evaluation of emulsion formulations administered intramuscularly. *Int. J. Pharm.*, 38 (1987) 133–137.

Ekman, B. and Sjoholm, I., Improved stability of proteins immobilized in microparticles prepared by a modified emulsion polymerization technique. *J. Pharm. Sci.*, 67 (1978) 693–696.

Florence, A.T. and Whitehill, D., The formulation and stability of multiple emulsions. *Int. J. Pharm.*, 11 (1982) 277–308.

Hashida, M., Heui Lias, M., Muranishi, S. and Sezaki, H., Dosage form characteristics of microsphere-in-oil emulsion: II. Examination of some factors affecting lymphotropicity. *Chem. Pharm. Bull.*, 28 (1980) 1659–1666.

Kufe, D.W. and Major, P.P., Studies on the mechanism of action of cytosine arabinoside. *Med. Pediatr. Oncol.*, (Suppl.) 1 (1982) 49.

Omotosho, J.A., Florence, A.T. and Whateley, T.L., Absorption and lymphatic uptake of 5-fluorouracil in the rat following oral administration of w/o/w multiple emulsions. *Int. J. Pharm.*, 61 (1990) 51–56.

Pallavicini, M.G., Cytosine arabinoside: molecular pharmacokinetic and cytokinetic considerations. *Pharmacol. Ther.*, 25 (1984) 207–211.

Whitehill, D., Multiple emulsions and their further uses. *Chem. Druggist*, 26 (1980) 130–135.

Yoshioka, T., Ileuchi, K., Hashida, M., Muranishi, S. and Sezaki, H., Prolonged release of bleomycin from parenteral gelatin sphere-in-oil-in-water multiple emulsion. *Chem. Pharm. Bull.*, 30 (1982) 1408–1415.