Drug Development and Industrial Pharmacy, 24(3), 289-293 (1998)

COMMUNICATION

A Stable Multiple Emulsion System Bearing Isoniazid: Preparation and Characterization

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ABSTRACT

Multiple emulsions with an oily liquid membrane (w/o/w) bearing isoniazid were prepared by an improved 2 × 2 step emulsification technique. Both of the interfaces of the liquid membrane were stabilized by using microcrystalline cellulose (MCC) in external as well as internal aqueous phases. The emulsions were characterized for droplet size, percent formation of multiple emulsion, release rate, effect of Tween-80 in external phase, phase volume ratio on release, and stability during aging at various storage conditions. The droplet size was small and yield of multiple emulsion was fairly good. The increasing concentration of MCC in either internal or external phase increased the droplet size. The system holds promise in tuberculosis therapy.

INTRODUCTION

The potential applications of multiple emulsion systems in pharmaceutics as a prolonged release carrier (1) have attracted considerable attention of pharmaceutical technocrats. In spite of its potential, significant stability issues have limited the use of multiple emulsion systems at industrial and clinical levels. Attempts have been made to improve the stability of multiple emulsions by (2) (i) gelation of external or internal or both phases, (ii) formation of interfacial complex film, and (iii) hydrophilic-lipophilic balance approach. These attempts are based on the principle that a stable and reproducible system can be produced by preventing successive collisions of both aqueous and oil droplets with their neighboring droplet (3).

Isoniazid (INZ) is a very potent, selective, and effective drug in tuberculosis therapy, but serious neurological side effects and short biological half-life of drug are the limitations with INZ therapy.

The objective of this research work embodies development of a stable INZ-bearing multiple emulsion by

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modified emulsification technique (4,5). An attempt was made to further stabilize multiple emulsion by the formation of a three-dimensional network of colloidal microcrystalline cellulose (MCC) in external aqueous phase and adsorption at both the interfaces of multiple emulsions (6).

MATERIALS

Microcrystalline cellulose (Avicel RC 591, FMC Corp., Rockland, ME), Span-80, Tween-80 (Qualigens, Bombay, India), and liquid paraffin (Paras Chemicals, Pune, India) were used. Buffer ingredients were analytical reagent grade. Distilled water was used in all experiments.

METHODS

Preparation of Multiple Emulsions

Multiple emulsions were prepared according to formulae given in Table 1. They were prepared by a two-step process, each step further consisted of two substeps, mechanical stirring and ultrasonication (4,5). The internal aqueous phase was added to oil phase by stirring at 3500 rpm for 10 min. This emulsion was sonicated (Imeco Sonifiers, India) for 2 min in a round-bottom tube with a diameter of about 2 cm more than sonicator's probe, then placed in a waterbath at 70°C. The viscous primary emulsion formed was emulsified further in an external aqueous phase at 1500 rpm for 2 min, followed by sonication for 30 sec. The temperature was maintained at 70°C for the second emulsification step also. The emulsion was stirred on a magnetic table (Kumar Instruments, Bombay, India) for 10 min with gradual cooling and kept in a refrigerator at 4°C until the temperature was equilibrated.

Droplet Size, Yield, and Viscosity

The droplet size was measured photomicrographically under a Nikon HFX Labophot microscope attached to a camera and the formation percentage of w/o/w emulsions (yield) was determined by the method reported by Ohwaki et al. (7). The viscosity was determined by Brookefield Synchrolectric viscometer (Brookefield Eng. Labs, Stoughton, MA) using an appropriate spindle number.

Drug Release Studies

The drug release was studied using the USP Basket method. Freshly prepared w/o/w multiple emulsion (10 ml) was placed in a treated cellulose tube (Sigma Corporation, St. Louis, MO), tied at both ends, and then placed in a rotatory basket and rotated in 900 ml of phosphate buffered saline at 100 rpm maintained at 37 ± 0.1°C. Dissolution study was initially performed at pH 1.2 for 2 hr, pH 4.5 for 1 hr, pH 6.8 for 2 hr, and finally at pH 7.5 for 3 hr. Before the basket was shifted to the next pH medium, the dialysis tube was rinsed

Table 1

Composition and Nomenclature of Various Isoniazid Formulations

Formulation Code	Additives in Aqueous Phase		PV ratio (w/o/w)
	Internal	External	
In-0.5	0.5% MCC	–	1:1
ME	1.0% MCC	–	1:1
In-2.0	2.0% MCC	–	1:1
Ex-0.5	–	0.5% MCC	1:1
Ex-2.0	–	2.0% MCC	1:1
T-0.2	–	0.2% Tween-80	1:1
T-0.5	–	0.5% Tween-80	1:1
T-0.8	–	0.8% Tween-80	1:1
PV-1	–	0.5% Tween-80	1:4
PV-2	–	–	1:4

Internal aqueous phase contains 1.0% INZ.
Oily phase: liquid paraffin containing 5% Span-80.
PV: phase volume.

with 5 ml fresh buffer of respective pH value. Samples (5 ml) were withdrawn at 15-, 30-, 60-, 120-, and 180-min intervals in each dissolution medium and replaced with the same volume of respective buffer. The analysis was made spectrophotometrically at 266 nm on a Shimadzu UV-150-02 spectrophotometer.

Stability

The percentage of phase separation, change in mean droplet size, polydispersity, and percent drug leakage were considered as the parameters to evaluate stability. The stability was assessed periodically with visual inspection by the cylinder method and microscopy at 4°C and ambient conditions. The amount of drug in external phase was determined as mentioned previously.

RESULTS AND DISCUSSION

Droplet Size and Polydispersity

The increase in percentage of MCC in internal as well as external aqueous phases appreciably increased droplet size and polydispersity because of larger size of internal droplets and high shear rates required to break coarse droplets to smaller size. The polydispersity was increased because of nonuniform shear experienced by the droplets. Increase in Tween-80 concentration in external aqueous phase decreased droplet size and polydispersity due to its efficient surfactant action. The size and polydispersity of droplets with PV-1 and PV-2 formulations increased slightly with decrease in phase volume ratio because of nonuniform and low intensity of shear experienced by the droplets in the diluted external phase (Table 2).

Table 2

Physical Characteristics of Multiple Emulsion Formulations

Formulation Code	Droplet Size Mean (Pd) (μm)	Viscosity (cps)	Yield (%)
In-0.5	7.1 (23.5)	1240	71.2
ME	8.3 (30.1)	1325	78.7
In-2.0	10.8 (39.7)	1390	80.5
Ex-0.5	10.4 (29.7)	1050	72.7
Ex-2.0	13.5 (39.1)	1660	79.0
T-0.2	5.5 (24.5)	1500	76.1
T-0.5	4.8 (18.6)	1950	82.5
T-0.8	4.5 (20.0)	1980	71.3
PV-1	6.1 (29.2)	950	84.6
PV-2	13.0 (40.9)	875	88.4

Yield

The yield tended to decrease in general with the attempts to decrease droplet size because of shear-induced expulsion/breaking of internal droplets in external aqueous phase. The yield was optimum for multiple emulsions. For In-0.5 and In-2, the yield percent was low, which may be attributed to the disruption of larger droplets under drastic treatment such as ultrasonication. The increase in MCC concentration in the external phase increases the yield of multiple emulsions because of a decrease in tendency for droplet diameter to decrease. The yield of T-0.5 multiple emulsion was higher in spite of its small droplet diameter, which may be attributed to the presence of Tween-80 in a concentration which favors size reduction with spontaneous stabilization of droplets, thus avoiding expulsion/breaking of internal droplets to external phase. The decrease in phase volume ratio increases yield because of less destruction of internal droplets (Table 2).

Viscosity

Viscosity of multiple emulsions was found to increase with increase in MCC concentration in the external phase. There was little effect of increasing concentration of MCC in internal phase. Higher viscosity in the case of Tween formulations may be attributed to the small droplet size. Tween-80 might have facilitated disintegration of MCC into the primary needle-shaped particles and thereby enhanced their thickening action. Increase in external phase volume decreases viscosity (Table 2).

Release Rate

The increase in concentration of MCC in internal phase was found to decrease release rate, probably due to increased size and polydispersity of internal droplets. The decrease in release rate with increase in MCC concentrations in external phase occurs due to diffusion control from viscous MCC network. High reproducibility of results with Tween emulsions can be attributed to the homogeneity of the multiple emulsion. The formulations were quite viscous and the larger surface area for partitioning of INZ because of small droplet size gave faster drug release. Decrease in phase–volume ratio was found to decrease release rate compared to formulation of similar composition, perhaps because of the time required for the drug molecules to diffuse from the droplet surface to the dialysis membrane through a viscous network (Table 3). The release profile indicates

Table 3

Drug Release from Multiple Emulsions at Various Time Intervals[a]

pH	Sampling Time (hr)	Cumulative Percent Drug Release									
		In-0.5	ME	In-2	Ex-0.5	Ex-2	T-0.2	T-0.5	T-0.8	PV-1	PV-2
	0.25	6.2	4.6	3.0	6.5	2.8	8.9	9.9	10.8	6.5	3.7
	0.50	9.8	8.5	5.5	10.7	5.4	13.0	15.8	14.5	10.7	5.4
1.2	1.00	14.6	11.8	9.0	14.5	8.8	19.6	21.3	17.5	13.5	8.5
	2.00	18.2	15.2	11.4	19.4	10.5	24.5	26.7	21.0	16.2	10.8
	2.25	20.0	16.4	12.9	20.7	10.9	25.0	27.0	21.2	17.7	12.6
4.5	2.50	21.3	17.5	13.5	22.5	12.2	25.3	27.5	21.7	18.5	13.7
	3.00	23.2	19.6	14.6	24.5	13.6	26.6	28.7	22.0	19.0	14.5
	3.25	24.0	21.8	15.7	25.3	14.2	28.2	30.4	22.6	19.6	15.1
	3.50	24.9	22.5	17.5	26.1	14.5	28.9	32.6	23.2	20.5	16.3
6.8	4.00	25.7	24.3	19.8	25.8	15.5	30.8	34.5	25.8	21.8	17.1
	5.00	27.1	26.1	21.2	28.5	16.0	32.5	36.0	29.4	23.3	19.0
	5.25	28.5	26.9	22.0	29.2	17.4	33.0	36.2	29.6	24.0	19.7
	5.50	29.3	27.0	23.1	30.6	17.9	33.4	36.5	30.0	24.2	20.3
	6.00	27.8	28.2	28.9	31.0	19.8	34.2	37.2	30.5	24.5	21.5
7.5	7.00	31.4	29.4	25.0	33.5	21.5	35.1	37.8	31.7	26.0	22.0
	8.00	34.5	30.0	26.8	35.7	24.3	36.0	38.1	32.2	28.5	22.6

[a]Average of three determinations.

only a slight reduction in rate of drug release as the pH increases. The release profile satisfactorily fitted the Higuchi pattern ($Q \propto \sqrt{t}$) because of increased viscosity of the external phase which acts as a matrix system. It can be inferred that the release rate depends on mixed factors such as droplet size, presence of MCC in external or internal phase, and the viscosity of multiple emulsion.

Stability

There was a slight increase in mean droplet size and polydispersity, and negligible phase separation of multiple emulsion upon storage over a period of 90 days. The drug leakage was less than 10%. This may be due to high viscosity and greater thickness of the diffusion layer. The temperature-dependent decrease in viscosity and coalescence of emulsion droplets led to slightly higher phase separation and drug leakage at room temperature than for those kept at 4°C.

CONCLUSION

The investigations presented lead us to conclude that the multiple emulsion prepared by 2 × 2 step emulsification technique containing MCC in internal and external aqueous phases was highly uniform, reproducible, and stable. The release was prolonged so that it may be useful in maintaining therapeutic plasma levels for long periods. The studies are underway to characterize it further, both in vitro and in vivo.

ACKNOWLEDGMENTS

Financial assistance by Council for Scientific and Industrial Research (C.S.I.R.), New Delhi, Government of India, to Mr. A. J. Khopade is gratefully acknowledged.

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