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COMMUNICATION

Effects of Drug Concentration in Inner Aqueous Phase and Additives in Oleaginous Phase on Release and Bioavailability of Isoniazid from Multiple Emulsion

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ABSTRACT

The effects of drug concentration in internal aqueous phase of stabilized w/o/w emulsion and additives in oleaginous phase on release characteristics of isoniazid were investigated. The release was significantly effected by both of the formulation variables. The release was enhanced initially with increasing concentration of drug in internal aqueous phase followed by a steady release at high concentration of isoniazid. The release declined substantially in the presence of aluminum tristearate, cetostearyl alcohol, and cholesterol, and it increased with egg lecithin and oleic acid in oily phase. The bioavailability was increased with a multiple-emulsion formulation.

INTRODUCTION

The potential of multiple-emulsion systems as prolonged and sustained-release carriers is richly substantiated by various reports (1). One of its drawbacks, the instability, has also been improved successfully by various methods used by a number of researchers (2). In our previous paper (3), we developed a stable multiple-emulsion system encapsulating isoniazid (INZ) in its internal aqueous compartment. As an extension of the previous report, the present study was undertaken to evaluate release characteristics of a stable INZ formulation as a function of two variables (*a*) drug concentration in inner aqueous compartment, and (*b*) effect of oleophilic additives in oily phase. This was done with a view to design a formulation for oral administration with an

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acceptable volume, in addition to the optimized release properties needed to attain prolonged plasma levels.

MATERIALS

Colloidal microcrystalline cellulose (MCC) (Avicel, FMC Corp.), Span-80, Tween-80 (Qualigens, Bombay, India), liquid paraffin (Paras Chemicals, Pune, India), egg lecithin (PE) (Sigma Chemical Co., St. Louis, MO), cetostearyl alcohol (CSA), cholesterol (CH), aluminum tristearate (ATS), and oleic acid (OA) (Loba Chemie, Bombay, India) were used. Distilled water was used in all experiments. The ingredients of the buffer were analytical reagent grade.

METHODS

Preparation of w/o/w Emulsion

A w/o/w emulsion bearing INZ in internal aqueous phase was prepared by 2 × 2 step emulsification technique as reported previously (3). The internal phase was drug solution containing 1% MCC, the oily phase was liquid paraffin containing 5% Span-80, and additives and external phase consisted of 1% MCC and 0.5% Tween-80. Formulations with 1, 2, 4, 6, and 8% drug concentration in internal aqueous phase (code: INZ-ME, INZ-2, INZ-4, INZ-6, and INZ-8) and ATS, CSA, CH, PE, and OA in oily phase (code: INZ-ATS, INZ-CSA, INZ-CH, INZ-PE, and INZ-OA) were prepared.

Physical Characteristics

The droplet size, polydispersity, viscosity, and yield of the multiple emulsion were determined as reported previously (3).

In Vitro Release Characteristics

The in vitro release characteristics were assessed by the methods reported previously (3). The release data were fitted to modified Higuchi (Magdassi and Garti) (4) and Higuchi (5) models.

Bioavailability Study

A urinary excretion study was undertaken to assess the bioavailability of selected preparations. The formulations INZ-CSA, INZ-ME [formulation T-0.5 from a previous study (3)], and INZ capsule containing free drug in a dose equivalent to 100 mg were administered orally to six healthy male volunteers (previously identified as fast acetylators) aged between 24 and 28 years (mean 25.6 years) and weighing between 52 and 65 kg (mean 56 kg). The volunteers gave their written consent after the object and procedure of the trial had been fully explained to them. No abnormalities were found after clinical examination. A total crossover study was conducted. A preparation was administered to each subject with 100 ml of water following an overnight fast. The volunteers continued fasting for 4 hr after the dose was given, however, water (100 ml/hr) was allowed to maintain the normal volume of urine. The urine samples were collected at 0, 1, 2, 3, 4, 6, 8, 12 hr post administration and stored at 4°C until analyzed. Following a washout period of 7 days, the volunteers recieved another preparation and the series of samples was taken following the same schedule as described. The urinary concentration of total INZ was estimated by the reported method (6).

RESULTS AND DISCUSSION

A stable multiple-emulsion system based on the small droplet size and use of MCC gel in internal and external phase to control the coalescence of internal droplets or creaming of multiple emulsion was prepared. As reported in our previous study, the internal phase contained 1% drug, which was arbitrarily chosen. Although the release was prolonged, the use of this multiple emulsion would require an unacceptably large volume of emulsion in a single dose. Therefore, an attempt was made to vary the concentration of drug in internal aqueous phase. The transport of hydrophilic drug INZ through an oily liquid membrane is the rate-controlling step. Hence, the effect of additives in the oily phase on release of drug in order to control INZ release was studied.

The droplet size, polydispersity, and viscosity remained almost the same and the yield was slightly increased with an increase in drug concentration in the internal aqueous phase. Similar results were observed with INZ-CSA and INZ-CH. For INZ-ATS, the size, polydispersity, viscosity, and yield were increased because of gelling of the oil phase. For INZ-PE and INZ-OA, size, polydispersity, viscosity, and yield were slightly decreased, probably because of cosurfactant action of these lipids.

An increase in drug concentration in internal aqueous phase was found to increase the release of INZ from 1 to 8%. Following this, the enhancement factor was very

Table 1

Drug Release from Multiple Emulsions at Various Time Intervals

pH	Sampling Time (hr)	Cumulative Percent Drug Release									
		INZ-2	INZ-4	INZ-6	INZ-8	INZ-OA	INZ-PE	INZ-CSA	INZ-CH	INZ-ATS	INZ-ME
	0.25	10.5	10.2	11.2	11.8	12.1	2.5	8.7	9.6	0.9	9.9
	0.50	16.3	16.9	17.1	17.5	13.2	14.1	10.3	12.5	1.6	15.8
1.2	1.00	21.4	22.1	22.5	23.2	20.7	22.6	11.5	13.3	2.1	21.3
	2.00	28.6	28.7	29.8	30.0	28.1	29.5	14.4	15.2	3.3	26.7
	2.25	29.2	30.6	31.5	32.3	29.8	32.4	16.6	17.5	3.5	27.0
4.5	2.50	31.5	33.2	33.9	34.6	32.9	36.5	17.9	18.7	4.3	27.5
	3.00	34.0	35.0	35.3	36.2	36.8	37.6	20.8	21.1	5.5	28.7
	3.25	34.1	35.2	35.8	36.7	37.2	37.9	21.3	21.7	5.8	30.4
	3.50	34.6	35.4	36.0	37.1	37.7	38.3	21.9	22.5	6.5	32.6
6.8	4.00	34.9	35.4	36.1	37.4	38.1	38.9	22.3	23.5	7.1	34.5
	5.00	35.5	36.0	37.6	39.9	39.3	39.5	23.6	24.7	9.4	36.0
	5.25	35.9	36.6	37.9	40.1	40.2	41.3	23.9	25.1	10.2	36.2
	5.50	36.2	37.3	38.5	40.8	41.1	42.6	24.4	25.9	10.3	36.5
	6.00	36.8	39.5	42.8	44.3	41.5	44.1	25.5	26.9	10.3	37.2
7.5	7.00	38.5	40.8	44.2	46.5	46.7	48.5	27.2	27.8	10.8	37.8
	8.00	40.3	42.1	45.5	48.2	47.1	49.7	28.4	29.2	11.6	38.1

Average of three determinations

low and was nonsignificant (Student's t-test, $p = 0.05$), indicating that on increasing drug concentration toward the saturation solubility value, the rate of release also moved toward attainment of equilibrium.

To study the effect of additives in the oil phase, formulation INZ-4 was selected for study. The release was found to decrease substantially with addition of ATS, CH, and CSA in the order INZ-ATS < INZ-CH < INZ-CSA. ATS interacts with INZ and therefore decreases its release. The effect of fatty alcohols or cholesterol could be explained by the formation of the liquid crystalline complex at the interface as a result of molecular association between Span-80, Tween-80, and CSA or CH molecules (Table 1).

The addition of OA or PE was found to increase the release substantially. This may be attributed to the transport of drug solution-loaded inverse micelles or swollen micelles of both hydrophilic and lipophilic surfactants across the liquid membrane (Table 1).

For drug release from formulations containing different amounts of drug in internal phase, the Higuchi model appeared to provide best fit. The Higuchi model also fitted satisfactorily to INZ-ATS, INZ-CSA, and INZ-CH, and in the presence of PE and SA (i.e., INZ-PE and INZ-OA), best fit was found to be the Magdassi and Garti model. The release pattern was, in fact, found to be very complex, probably as a result of mixed mechanisms involving reverse and mixed micellar transport and diffusion through outer MCC gel matrix.

The mean urinary excretion rate of total INZ, time to peak, peak excretion rate, and area under excretion rate–time curve are shown in Tables 2 and 3. Following the administration of INZ alone, the maximum value of mean urinary excretion rate of total INZ appeared in a 1- to 2-hr period, whereas it was between 2 and 3 hr for

Table 2

Mean Excretion Rates of INZ After Administration of 100 mg Test Formulations

Time[a] (hr)	Mean Excretion Rates ± SD		
	INZ	INZ-ME	INZ-CSA
0.5	8.69 ± 1.88	7.64 ± 3.86	9.54 ± 1.68
1.5	19.92 ± 7.05	14.68 ± 5.80	13.68 ± 2.69
2.5	12.59 ± 4.33	17.52 ± 4.46	15.72 ± 2.14
3.5	8.46 ± 0.63	9.74 ± 1.88	11.87 ± 1.68
5.0	4.26 ± 3.60	5.26 ± 3.60	8.64 ± 1.55
7.0	1.13 ± 0.98	3.89 ± 1.19	7.13 ± 1.71
10.0	0.74 ± 0.77	2.93 ± 1.61	5.28 ± 1.22

[a]Midpoint of time interval.
SD: standard deviation.

Table 3

Pharmacokinetic Parameters Based on Excretion Profile Following Oral Administration of INZ Test Formulations

Formulation Code	Maximum Time[a] (hr)	Maximum Excretion Rate (mg/hr)	$AUC_{0-24\ hr}$[b] (mg)
INZ	1.5	19.92 ± 7.05	60.99 ± 23.48
INZ-ME	1.5	17.52 ± 4.46	74.43 ± 27.19
INZ-CSA	2.5	15.72 ± 2.14	92.25 ± 17.12

[a]Midpoint of time interval.
[b]$AUC_{0-24\ hr}$: area under curve, 0–24 hr.

INZ-CSA and INZ. The mean excretion rate at this maximum was found to be 1.24 and 1.39 times as great as for INZ alone.

The bioavailability of INZ from INZ-CSA and INZ-ME was 1.20 and 1.51 times greater than for plain INZ capsules, respectively. A very small difference between INZ-ME and INZ capsule formulation may be due to the breakdown of multiple emulsion droplets and better absorption of drug from the gastrointestinal tract. Better in vivo stability of the INZ-CSA formulation because of solidification of oily phase perhaps gave better control of drug release.

CONCLUSION

The prolonged release of INZ may be achieved by maneuvering the oily phase of multiple emulsions and drug concentration in internal aqueous phase, thereby optimizing the volume of formulation required per dose and increasing the bioavailability of the formulation.

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REFERENCES

1. S. Nakhare and S. P. Vyas, Drug Dev. Ind. Pharm., 21, 869 (1995).
2. K. P. Oza and S. G. Frank, J. Disp. Sci. Technol., 10, 163 (1989).
3. A. J. Khopade and N. K. Jain, Drug Dev. Ind. Pharm., 24(3), 289–293 (1998).
4. S. Magdassi and N. Garti, J. Controlled Release, 3, 273 (1986).
5. W. I. Higuchi, J. Pharm. Sci., 51, 802 (1962).
6. H. G. Boxembam and S. Reighelman, J. Pharm. Sci., 63 1991 (1974).