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Multiple emulsions containing rifampicin

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A stable multiple emulsion containing rifampicin in the internal aqueous phase was prepared by the incorporation of additives in both aqueous and oily phases. The formulation and process variables were optimized for primary and secondary emulsification. Drug release studies were performed on selected multiple emulsions to observe the effect of dilution. The release data were fitted to the Higuchi equation for slab and spherical geometry and effective diffusion coefficients were calculated. Stability studies for three months revealed good stability of the multiple emulsions with respect to creaming, phase separation, viscosity change, drug leakage, change in droplet size upon storage and exposure to osmotic and shear stress. The *in vivo* studies performed with stable multiple emulsions administered orally in human volunteers showed prolonged plasma drug levels. The multiple emulsions were found suitable for an improved tuberculosis therapy.

1. Introduction

Tuberculosis is a major public health problem in tropical as well as European countries. With the increasing number of HIV infections and AIDS, the HIV-associated tuberculosis has become a much greater public health problem in developing countries because of its basic high prevalence even without HIV infections and the fact that *Mycobacterium tuberculosis* is a common opportunistic pathogen in HIV-infected patients. Drug treatment of tuberculosis always involves multiple drug regimens since the organism easily becomes resistant to single drugs [1, 2].

Rifampicin is indicated as first line drug in the treatment of tuberculosis by the World Health Organisation (WHO) and the International Union Against Tuberculosis and Lung Disease (IUAT) [2]. The relatively large doses required, side-effects and a long-term treatment schedule are the major limitations in its use [3]. Attempts have been made to alleviate these limitations using multiple emulsion systems. The use of multiple emulsions as potential candidates for prolonged and targeted drug delivery vehicles is richly substantiated by various reports [4–6]. Their use, especially in the delivery of antituberculosis drugs have recently been realized and the results are appreciating. Different multiple emulsion formulations were developed containing rifampicin for administration through nasal [7], i.m. [8] and i.v. [9] routes. We have already described multiple emulsions bearing isoniazid for oral administration [10, 11]. The rationale for their oral administration is mainly because of following reasons: high encapsulation, protection of bioactive present in the internal phase from pH and enzymatic environment of the G.I. tract, prolonged drug release and *in vivo* therapeutic response and finally, improved G.I. absorption of poorly absorbed drugs due to high lymphotropicity [12].

2. Investigations, results and discussion

Multiple emulsions are characterized by low thermodynamic stability due to their high interfacial free energy. Our primary aim was to determine optimum parameters for primary emulsification by investigating the adsorption phenomena on the water-oil interface concurrently with reduction of coalescence of aqueous drops. The secondary emulsification parameters were optimized by investigating size, yield and viscosity for obtaining a stable multiple emulsion with small droplet size ($<10\ \mu m$), highest possible yield, patient acceptable viscosity and volume for required administration, and appropriate drug release to show prolonged pharmacodynamic effects. The optimum parameters were selected on the basis of balance among the evaluating parameters. Liquid paraffin was selected as it gives a high yield compared to other oils [13]. The hydrophobic surfactant, Span® 80, was selected based on earlier report that a very steep concentration-interfacial tension relationship at the paraffin-water interface is obtained with a sharp break, which is a prerequisite for stable emulsion formation [14]. The hydrophilic emulsifier, Tween® 80, gave a stable emulsion when Spans are used as lipophilic emulsifiers [15]. Cetostearyl alcohol (CSA) is used for the control of drug release and to improve stability of the liquid membrane by gelling the oil phase due to liquid crystalline phase formation [16]. Colloidal microcrystalline cellulose (MCC) was used in external and internal aqueous phases as it is reported to stabilize the o/w interface by formation of a three dimensional network in aqueous phase as well as by orientation at the interface to form a mechanical barrier [17]. Span 80 further facilitates wetting of MCC by oil. The results obtained from the experimental investigation, envisaged logically, as described above are as follows:

2.1. Preparation studies

Results of optimization studies for primary emulsification are shown in Table 1. An increase in MCC concentration in the internal phase increased adsorption at the o/w interface with an additional advantage of formation of a gel like network. At 2% concentration particles were traced in the oily phase. The viscosity and droplet size increased with increasing MCC concentration and the flowability was reduced. The optimum concentration was found to be 1%. Increase in Span 80 concentration improved interfacial adsorption. It reduced the droplet size of the internal phase, as a result, viscosity was increased and flowability was reduced. Although 10% Span 80 gave a very good primary emulsion, the MCC tends to incorporate into the oil phase due to droplet size reduction. Therefore, 5% Span 80 was considered as optimum concentration. An increase in sonication time above optimum do not necessarily reduce droplet size but the chances of incorporation of MCC in the oily phase were increased. The viscosity and flowability also remained almost the same. A decrease in phase volume ratio do not change adsorption at the interface. When droplet size was increased, the viscosity was reduced and the flowability was fairly good. At 1:2 phase volume ratio emulsion was very viscous and was not flowable. At 1:7 and 1:9 ratio oil formed a distinct layer upon storage.

Table 1: Optimization of formulation and process variables of primary emulsification for the preparation of multiple emulsions bearing rifampicin

Formulation and process variables	MCC (I) (% w/v)	Span 80 (O) (% w/v)	Sonication time (min)	PV ratio	Observation		
					Adsorption at interface	Droplet size reduction	Flowability/viscosity
	0.5				+	++	+
MCC (I)	1.0	5.0	2	1:5	++	++	++
(% w/v)	2.0				++[a]	+++	++
		1.0			++	+++	++
Span 80 (O)	1.0	5.0	2	1:5	++	++	++
(% w/v)		10.0			++[a]	+	+++
			1		++	+++	+
Sonication	1.0	5.0	2	1:5	++	++	++
time (min)			3		++[a]	++[a]	++
			4		++[a]	++[a]	++
				1:2	++[a]	+	++++
				1:3	++[a]	+	+++
PV ratio	1.0	5.0	2	1:5	++	++	++
				1:7	++	+++	+
				1:9	++	+++	+

MCC (I): Colloidal microcrystalline cellulose in internal aqueous phase
Span 80 (O): Span 80 in oily phase
PV: Phase volume
[a] Incorporation of MCC crystals in oily layer

Adsorption at interface
+ Good, multilayered with no gel network
++ Excellent, multilayered with gel network in aqueous compartment

Droplet size reduction
+ Fairly good
++ Good
+++ Excellent

Flowability/viscosity
+ Flowable/fairly viscous
++ Flowable/Viscous
+++ Fairly flowable/Very viscous
++++ Not flowable/Very viscous

The results of optimization studies for secondary emulsification are shown in Table 2. The increase in MCC concentration in the external aqueous phase characteristically increased droplet size and polydispersity as well. The high energy was required for the desired size reduction. The yield was increased as the size reduction tendency decreased. The viscosity was also increased due to the gel-network structure of MCC. The percent of drug released in 8 h decreased due to the increase in networking in the external phase at high MCC concentration which act as

Table 2: Optimization of formulation and process variables of secondary emulsification for the preparation of multiple emulsions bearing rifampicin

Formulation and process variables	MCC (E) (% w/v)	Tween 80 (E) (% w/v)	Sonication time (s)	PV ratio	Observation			
					Droplet size μm (pd)	Yield (%)	Viscosity (cps)	Release in 8 h (%)
	0.5				10.5(26.1)	85.6	1425	46.6
MCC (E)	1.0	0.5	30	1:1	6.7(21.2)	89.5	1890	46.4
(% w/v)	2.0				14.2(39.0)	90.3	1550	41.3
		0.2			7.3(23.3)	87.6	1700	46.7
Tween 80 (E)	1.0	0.5	30	1:1	6.7(21.2)	89.5	1890	46.4
(% w/v)		0.8			6.1(22.5)	81.0	1900	49.5
			15		8.3(25.4)	91.3	1640	42.5
Sonication	1.0	0.5	30	1:1	6.7(21.2)	89.5	1890	46.4
time (s)			45		5.1(20.0)	76.8	1900	50.6
			60		4.4(18.9)	71.2	1950	53.9
				1:1	6.7(21.2)	89.5	1890	46.4
PV ratio	1.0	0.5	30	1:2	10.8(31.5)	90.2	1250	44.7
				1:4	3.2(39.6)	90.7	1175	40.3
CSA (O) (1%)	1.0	0.5	30	1:1	7.5(25.5)	90.5	1950	31.6
RIF (I) 0.5					7.5(25.2)	87.3	1950	24.3
(%) 1.0					7.5(25.5)	90.5	1950	31.6
1.5	1.0	0.5	30	1:1	7.4(25.5)	91.7	1950	35.7
2.0					7.4(24.9)	90.4	1950	36.8
3.0					7.5(25.3)	81.9	1950	40.2

(E) External phase
(I) Internal phase
(O) Oily phase
(pd) Polydispersity = standard deviation/mean × 100

matrix system. The secondary emulsification without Tween 80 also formed multiple emulsion due to stabilization of droplets by adsorbed MCC layer but the droplet size formed was $>50\,\mu m$. Moreover, droplets tend to coalesce upon storage. Therefore, Tween 80 was incorporated in minimum quantity required to reduce droplet size ranged below $10\,\mu m$ without affecting the stability. Increasing concentrations of Tween 80 reduced droplet size, polydispersity and yield. The viscosity was increased due to reduction in droplet size and facilitation of the MCC network formation. The release was increased due to an increase in surface area of the droplets and reduced net thickness of the liquid membrane. Increase in sonication time reduced droplet size. Sonication for a longer period than optimal or just necessary, destroyed the w/o/w emulsion droplets and consequently, the yield was decreased. The viscosity was increased upto optimum time and then decreased. The drug release also showed a sudden increase above optimum sonication time due to breaking and expulsion of droplets into the external aqueous phase. The droplet size and yield were increased with decreasing phase volume ratio and the viscosity was obviously low. The release was decreased due to high viscosity and greater control of drug diffusion by MCC network layer. Optimum parameters are shown in Table 3.

The droplet size, polydispersity and viscosity were slightly increased with the incorporation of 1% CSA in the oily phase. Increase in viscosity of oil phase retards size reduction hence improves yield. The release was remarkably lowered due to self-bodying action of CSA.

There was no effect of increasing the drug concentration in the internal phase on droplet size, polydispersity and viscosity. The yield was slightly increased. The release of rifampicin was found to increase when drug concentration in the internal phase was increased from 0.5 to 1.5%. Following this, the enhancement factor was very low and not significant. The reproducibility of results at 3% concentration was less and showed large variations. This indicates that on increasing drug concentration to a value which is much greater than its saturation solubility, the release attains an equilibrium.

Table 3: Optimized formulation and process variables

Formulation and process variables		Optimized value
MCC concentration	Internal phase	1.0% w/v
	External phase	1.0% w/v
Span 80	Oily phase	5.0% w/v
Cetostearyl alcohol	Oily phase	1.0% w/v
Tween 80	External phase	0.5% w/v
Sonication time	Primary	2.0 min
	Secondary	30 sec
Phase volume ratio	Primary emulsion	1:5
	Secondary emulsion	1:1
Rifampicin	Internal phase	2.0% w/v

Table 4: Comparison of the release profiles of rifampicin from multiple emulsions determined by methods I and II

Time	Cumulative % drug release*		Higuchi diffusion coefficient	
(h)	Modified Method I	Method II	Slab geometry ($l/h \cdot cm^2$)	Spherical geometry ($cm^2 \cdot M/s$)
0	0	0	Method I	Method I
1	15.7	18.9		
2	22.5	27.4	$r = 0.995$	$r = 0.996$
3	26.3	34.6	$De = 2.0 \times 10^{-4}$	$De = 4.29 \times 10^{-13}$
4	30.2	39.5		
5	32.6	43.7	Method II	Method II
6	35.1	46.5		
7	37.5	49.1	$r = 0.997$	$r = 0.998$
8	38.7	52.2	$De = 3.73 \times 10^{-4}$	$De = 8.56 \times 10^{-13}$

* Mean of three determinations

Magdassi and Garti [18] modified the Higuchi model for systems with spherical geometry like multiple emulsions as:

$$3/2[1 - (1 - F)^{2/3}] - F = 3DCst/r_0^2C_0 \qquad (1)$$

where F is the fraction of drug released, D is the diffusion coefficient, Cs is drug solubility at the membrane, r_0 is the radius of the sphere and C_0 is initial drug concentration.

The right hand term was denoted as B thus:

$$B = 3DCst/r_0^2C_0 \qquad (2)$$

or

$$B = 3Det/r_0^2C_0 \qquad (3)$$

where De is the effective diffusion coefficient, $De = DCs$. Plotting B against t gives a straight line with a slope of $3De/r_0^2C_0$. A plot of B vs $1/C_0$ was found to follow power law, and variable exponent n for the time (t^n) ranged from 0.5 to 3.0 were calculated with a correlation coefficient of 0.99 to 1. This indicates the existence of a facilitated transport pathway. The parameter B for rifampicin was calculated from the release value at 8 h and the slope was calculated by plotting B against $1/C_0$. As reported earlier, the slope was found to follow power law with the exponent n value of 0.8 which indicates facilitated transport of rifampicin across the membrane.

The effect of dilution on the release profile of rifampicin was assessed by performing drug release studies [19] using method II. It was compared with method I (see 3.5.2). The results are shown in Table 4. The release determined by method II was almost 1.4 times faster. The effective diffusion coefficients were calculated by fitting release data to the Higuchi equation for slab geometry [20] and spherical geometry [18]. The regression coefficient values were very close to one showing excellent fitting to both the equations. The De value was 2.0×10^{-4} and $3.73 \times 10^{-4}\ l/h \cdot cm^2$ for slab geometry by method I and method II, respectively. While it was 4.29×10^{-13} and $8.56 \times 10^{-13}\ cm^2M/s$ for spherical geometry by the respective methods.

2.2. Stability studies

There was a slight increase in mean droplet size, polydispersity and viscosity. There was negligible creaming and phase separation of the multiple emulsion upon storage over a period of 90 d. In fact, flocculation was easily redispersible on shaking. The drug leakage was less than 6.4%. The values were slightly higher except for viscosity at room temperature (ambient conditions) than at 4 °C. When the multiple emulsion droplets were placed in a medium of high osmolarity the oil droplets containing internal aqueous phase shrink depending upon the osmolarities of the two aqueous phases and the mobility of surfactants across the liquid membrane through which water can migrate. The fresh emulsion showed rapid shrinkage and change in mean diameter because of incomplete gelling of the oil phase. After storage at ambient conditions for 90 d, less than 3% change was noted. This was due to the restricted mobility of water across the gelled liquid membrane. The multiple emulsions were also found to have

reasonably good stability towards breakdown in high speed laminar shear flow of 60 min after indicated storage periods while a fresh sample showed a gradual increase in mean droplet size over a period of 60 min. The high shear led to disturbance in adsorbed MCC layer rendering it insufficient for properly protecting the droplets against coalescence.

2.3. In vivo studies

The plasma profile and pharmacokinetic parameters are shown in the Fig. and in Table 5. The AUC of RIF-ME 600 was increased 1.4 times compared to free RIF 600 mg. The half-life was increased 4.56 times and the elimination rate was reduced by similar folds. At an another recommended dose of 450 mg the multiple emulsion (RIF-ME 450) showed almost the same (insignificant, Student's t test $p = 0.05$) AUC as that of free drug at 600 mg dose (Free RIF 600). The half-life, however, was increased by 3.65 times. The AUC and half-life were 1.4 times and 1.2 times greater for RIF-ME 600 compared to RIF-ME 450 showing dose-response relationship. The results were significant at a $p = 0.05$ level as analysed by the Student's t test. This indicate that multiple emulsions are able to prolong therapeutic plasma levels of rifampicin for 24 h in recommended doses compared to free drug.

From the above experimental evidence it can be concluded that the multiple emulsion was stable and showed prolonged drug dynamics in blood when given orally. Studies with combination of first line drugs in the same system are underway.

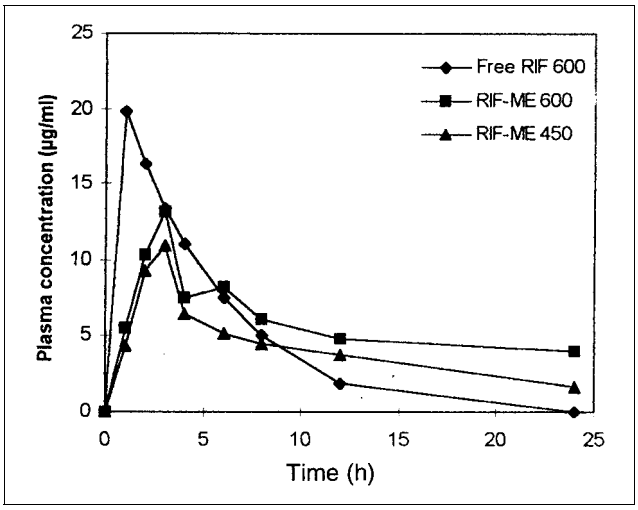

Fig.: Plasma profile of rifampicin from various multiple emulsion formulations

Table 5: Pharmacokinetic parameters

Parameter	Free RIF	RIF-ME 600	RIF-ME 450
AUC ($\mu g \cdot ml^{-1} \cdot h$)	99.98	137.55	97.35
K_{el} (h^{-1})	0.194	0.046	0.057
$t_{1/2}$ (h)	3.30	15.06	12.05
T_{max} (h)	1.0	3.0	3.0
C_{max} (µg/ml)	19.79	13.19	10.97

3. Experimental

3.1. Materials

Microcrystalline cellulose (MCC, Avicel RC 591, FMC Corporation, USA), Span-80, Tween-80 (Qualigens, Bombay, India), liquid paraffin (Paras chemicals, Pune, India), Cetostearyl alcohol (Loba Chemie, Bombay, India) were used. Ingredients of buffer solutions were of A.R. grade. Distilled water was used in all experiments.

3.2. Preparation of multiple emulsions

Multiple emulsions were prepared by an improved two step method [10, 11]. Each step consisted of two substeps: mechanical stirring and sonication. The internal aqueous phase was added to the oil phase by stirring at 3500 rpm for 10 min. This emulsion was sonicated using a probe sonicator (Imeco Sonifiers, India) for 2 min and placed in a water bath at 70 °C. The viscous primary emulsion formed was emulsified again in an external aqueous phase at 1500 rpm for 2 min followed by sonication for 30 s. The temperature was maintained at 70 °C during second emulsification. The emulsion was stirred on a magnetic stirrer for 10 min with gradual cooling and kept in a refrigerator at 4 °C until the temperature was equilibriated. The effect of a particular variable was studied by varying its values keeping other values same. CSA was however omitted, during optimization studies.

3.3. Formulation optimization

3.3.1. Primary emulsification

The primary emulsion was prepared with 0.5%, 1% and 2% MCC in the internal phase and with 0%, 1%, 5% and 10% Span-80 in the oily phase. The emulsion was optimized on the basis of adsorption of MCC and coalescence of droplets under the light microscope and the viscosity of primary emulsion formed. Sonication time for the primary emulsion was varied from 1, 2, 3, 4 min and the phase volume ratio (w/o) was kept as 1:2, 1:3, 1:5, 1:7 and 1:9. These parameters were optimized on the basis of size reduction, flowability and viscosity.

3.3.2. Secondary emulsification

The primary emulsion was emulsified in the presence of 0.5%, 1% and 2% MCC without Tween 80. Keeping 1% MCC in the external phase constant, concentration of Tween-80 was varied as 0.2%, 0.5% and 0.8%. The stability, size, viscosity and yield of the multiple emulsion were determined. The sonication time was varied as 15, 30, 45 and 60 s and the phase volume ratio [(w/o):w] was kept at 1:1, 1:2 and 1:4. The optimum parameters were selected on the basis of size, viscosity, yield and release in 8 h.

3.3.3. Oily phase

CSA in 1% concentration was incorporated in the oily phase with the aid of heat and the effect on size, yield, viscosity and drug release were studied.

Table 6: Stability studies

Evaluation Parameter	4 °C						Room temperature					
	Fresh	7	15	30	60	90	Fresh	7	15	30	60	90
Droplet size (µm)	–	7.5	7.5	7.5	7.6	7.8	7.4	7.5	7.6	7.8	7.8	8.2
Viscosity (cps)	–	2000	2050	2100	2150	2175	1950	2000	2050	2050	2150	2150
Drug leakage (%)	0	1.5	1.6	2.2	2.5	3.7	0	1.7	1.9	2.9	3.3	6.4
Phase separation (%)	0	nil	nil	nil	1.0	1.0	0	nil	nil	1.0	1.0	1.0
Creaming (v.i.)	0	nil	nil	floc	floc	floc	0	nil	nil	floc	floc	floc
Osmotic stress (%)	nsc	nsc	nsc	nsc	nsc	nsc	5.0	3.2	3.1	nsc	nsc	nsc
Shear stress (%)	–	–	–	–	–	–	3.0	2.0	2.0	1.0	2.0	1.0

floc: Flocculation; nsc: No significant change; v.i.: Visual inspection
(a): % decrease in area of hysteresis loop (shear rate vs shear stress plot)

3.3.4. Effect of drug concentration in internal phase

Formulations containing 0.5, 1, 1.5, 2, 3% drug in the internal phase were prepared and the effect on size, yield, viscosity and drug release were studied.

3.4. Physical parameters

The droplet size was measured photomicrographically under a Nikon HFX Labophot microscope, Japan. Yield was determined by centrifugation method. Rheological studies were done using a Bookefield viscometer (Brookefield Eng. Labs., Stoughton, U.S.A.) using an appropriate spindle number [10].

3.5. Drug release studies [19]

3.5.1. Method I

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 Corp. USA) tied at both ends. It was placed in a rotary basket and rotated in a 900 ml of phosphate buffer (pH 7.4) at 100 rpm maintained at 37 ± 0.1 °C. The analysis was made spectrophotometrically at 334 nm on a Shimadzu UV 1601 spectrophotometer. The method was repeated with formulations containing 0.5, 1, 1.5, 2, 3% drug in the internal phase.

3.5.2. Method II

Ten ml of the selected w/o/w emulsion were dispersed in 880 ml of donor medium (PBS pH 7.4). A seamless dialysis tubing (Spectrapore, Sigma USA) containing 20 ml of receptor phase was immersed in the donor phase. The drug concentration dialysed into the acceptor phase was analysed periodically as before. The release data were fitted to the Higuchi equation for slab [20] and spherical geometry [18].

3.6. Stability studies

The percentage of phase separation, change in mean droplet size, polydispersity and percent drug leakage and viscosity were monitored periodically at 4 °C and ambient conditions upon storage. The accelerated stability was determined by subjecting fresh and stored multiple emulsion to osmotic and orthokinetic stress. The multiple emulsion was added to equal volume of 1.2% NaCl for applying osmotic stress. For orthokinetic stress, continuous shear flow at an average shear rate of $500\ s^{-1}$ in a viscometer for 60 min was applied to the multiple emulsion. The change in droplet size was determined in both cases.

3.7. In vivo studies

Plasma concentrations of the drug were determined to assess the bioavailability of the selected preparations. The formulations containing rifampicin were administered orally to six healthy male volunteers aged between 24 and 28 years (mean 25.6 years) and weighing between 52 and 65 kg (mean 56 kg). Written consent was taken from each volunteer after the object and procedure of the trial had been clearly explained to them. Their clinical examination revealed no abnormalities. A total crossover study was conducted. Three formulations were tested containing 600 mg free rifampicin (Free RIF-600), a multiple emulsion equivalent to 600 mg (RIF-ME 600) and 450 mg (RIF-ME 450) rifampicin. A formulation was administered to each subject with 100 ml of water following an overnight fast. The volunteers continued fasting for another 4 h after the dose was given. They were given standard diet at 2, 6 and 12 h post dosing. The use of stimulants like tea, coffee and other beverages was forbidden. Blood samples (2 ml) were collected at 0, 1, 2, 3, 4, 6, 8, 12, 24 h intervals and stored at 4 °C until analysis. Following a washout period of 15 d, the volunteers recieved another formulation and the series of samples were taken following the same schedule as described. The plasma concentration of rifampicin was estimated by the method reported by Jamaluddin et al. [21].

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