RESEARCH PAPER

Received 25 July 1994
Indian J. Pharm. Sci., 1995, 57(2)pp. 71-77

Prolonged Release of Rifampicin from Internal Phase of Multiple w/o/w Emulsion Systems

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Release rates of Rifampicin encapsulated in the internal aqueous phase of w/o/w emulsion stabilized by the gelling of the external phase (w/o/gel) were measured as the function of two formulation variables - The oil phases and internal phase volume. The release rate was significantly affected by the nature of the oil phase and decreased in the order of Isopropyl myristate (IMP) > sesame oil > liquid paraffin, which was mainly due to maximum and minimum partition coefficient of drug in these phases respectively. The release rate of rifampicin from w/o/gel system was very prolonged. The release was enhanced with increasing in the volume of inner aqueous phase. These results show the utility of the w/o/gel type double emulsions for sustained release preparations and raise the possibility of control of drug release from the double emulsion system.

MULTIPLE emulsions are recent development in the field of emulsion technology and have been extensively studied from both basic and practical point of view. The use of w/o/w type double emulsions in medicine has also been investigated to prolong drug release [1], to immobilize enzyme [2], to treat overdose [3] and to obtain better immunologic adjuvants [4]. Their utilization has limited by their inherent instability. Stability can be improved by forming polymeric gel in the internal or external (continuous) aqueous phase [5]. A stabilizing film can be formed through the interfacial interaction between macromolecules such as albumin and non-ionic surfactants [6,7]. Hashida et al. [8,9] stabilized a w/o type emulsion and a w/o/w type double emulsion by gelling the inner aqueous phase and demonstrated the utility of a sphere-in- oil-in-water (s/o/w) type double emulsion as a drug carrier to lymphatics.

During last twenty years, the great value of rifampicin in the treatment of tuberculosis has been realized [10-12]. A prolonged release dosage form of rifampicin is of great importance to overcome problems of frequent dosing and to achieve patient complience.

The present investigation examines **in-vitro** release kinetics of Rifampicin under the two formulation variables; different oil phases and various phase volumes of internal aqueous phase.

EXPERIMENTAL

Materials

Rifampicin (I.P. grade) was obtained form Cadila Laboratories Ltd. The non-ionic surfactant sorbitan monooleate (Span 80) and polyoxyethylene sorbitan monooleate (Tween 80) were obtained from Loba Chemicals India Ltd. polyacrylic acid was obtained from Sigma (U.S.A.) company. The oil phases; liquid paraffin, sesame oil and isopropyl myristate were obtained from Spectrochem India Ltd. All other reagents were of either pharmacopeal grade or analytical grade. Double distilled water has been used throughout the experiments.

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Methods

The multiple emulsions were prepared by a two step emulsification procedure. The aqueous phase (phosphate buffered saline, pH 7.4) containing rifampicin 10 mg/ml was emulsified in an equal volume of oil phase containing 10% w/w sorbitan monooleate by means of magnetic stirrer (4000 r.p.m.) to produce the primary emulsion. The primary emulsion (w/o) was reemulsified in the same manner in an equal volume of phosphate buffered saline containing a surfactant mixture (Span 80/Tween 80 (3:1) (5% v/v), and polyacrylic acid (PAA) (5% w/v) to produce w/o/gel system. The final phase volume ratio was maintained at 0.5 (Fig. la,b,c).

The same method was followed for the preparation of multiple emulsions having different oil phases and various internal phase volume (Table-I).

Characterization

1. Determination of globule size — The mean globule size of the internal aqueous droplets and multiple oil droplets was determined by the microscopic method [13], using Leitz Biomed phase contrast microscope Table-II.

2. Determination of viscosity — Viscosity measurements of the multiple emulsions containing different oil phases were carried out by using a Brookfiled viscometer at room temperature using an appropriate spindle number. Each reading was taken after equilibration of the indicator dial (Table-II).

3. Determination of Emulsion stability — Stability of different formulations was evaluated by visual observation of the coalescence of globules, rate of phase volume separation (Table III) freeze thaw cycling and change in size distribution pattern over time (Fig. 2.)

4. Determination of partition coefficient — The partition coefficient of drug between oily phase (liquid paraffin, isopropyl myristate, and sesame oil) and aqueous phase (phosphate buffered saline pH 7.4) was determined by shake flask method (Table-IV).

5. Measurement of drug Release — The **in vitro** drug release from multiple w/o/gel emulsions was studied by dialysis method, using cellophane tubing (spectrapore-2, sigma U.S.A.). The emulsion was taken in the dialysis bag and dialyzed against 200 ml of phosphate buffered saline (pH 7.4) at 37 ± 1°C. The dialyzing media was agitated with the help of a magnetic stirring bar on a magnetic stirrer. At appropriate intervals 5.0 ml of dialyzing fluid was withdrawn and replaced with an equal volume of fresh dialyzing fluid. The drug concentrations were analysed spectrophotometrically (Shimadzu Double Beam spectrophotometer, 150—2 UV) at 332 nm. The apparatus designed to study the release profile is shown in Fig. 3 (Table-II).

Study of the effect of internal phase volume on the **in vitro** release kinetics was conducted using batches of formulation RB- IM. 8-GT with the internal phase volume ratio ranging from 0.2 to 0.6 (Table-V).

RESULTS AND DISCUSSION

The viscosity and droplet (internal and multiple) size of different formulations manufactured under the same conditions were studied. The viscosity of different formulations RB-IM. 8- GT, RB-LS.8-GT and RB-SO.8-GT was found to 44.0, 44.5 and 45.0 CPs respectively. The men droplet diameter of the multiple oil droplets of various formulations RB-IM.8-GT, RB-LS.8-GT and RB- SO.8-GT was recorded to be 11.5 μ, 11.0 μ and 10.0 μ respectively. Whilst the internal droplet diameter was recorded to be 2.0μ, 1.8μ and 1.5μ respectively (Table-II). These multiple emulsion formulations were then studied for the effect of partition coefficient, oil phases and internal phase volume on the **in vitro** release profile of the contained drug.

Table I: Composition of Different Formulations

Formulation	Internal phase (Aqueous)	Middle oily phase	External phase (Aqueous)	Phase* volume ratio
RB-LS.8-GT	Rif.+PBS# (pH 7.4)	Liquid paraffin + span 80 (10% w/w)	Polyacrylic acid (5% w/v) + Span 80 + Tween 80 (3:1), 5% w/v in PBS (pH 7.4)	0.5
RB-IM.8-GT	Rif.+PBS (pH 7.4)	Isopropyl myristate+ Span 80 (5% w/w)	Polyacrylic acid (6% w/v) + Span 80 + Tween 80(3:1), 2% w/v in PBS (pH 7.4)	0.5
RB-50.8-GT	Rif.+PBS (pH 7.4)	Sesame oil + Span 80 (5% w/w)	Polyacrylic acid (5% w/v) + Span 80 + Tween 80 (3:1), 2% w/v in PBS (pH 7.4)	0.5

* = 0 w/o/w
# = Phosphate buffer saline

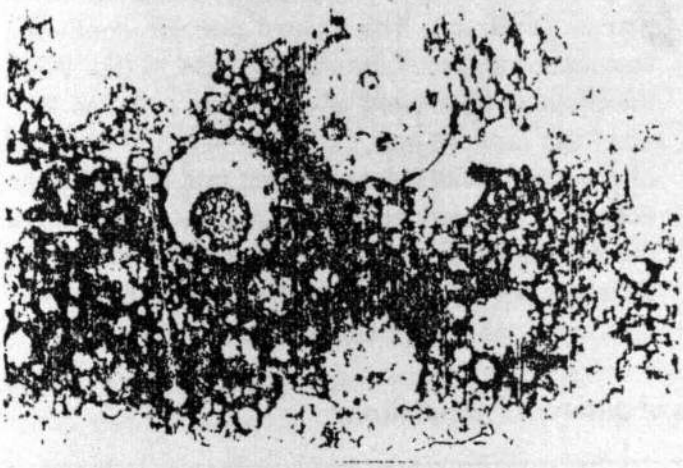

Fig. 1a: Photomicrograph of the water-isopropyl myristate-polyacrylic acid gel system (mgf = 40x)

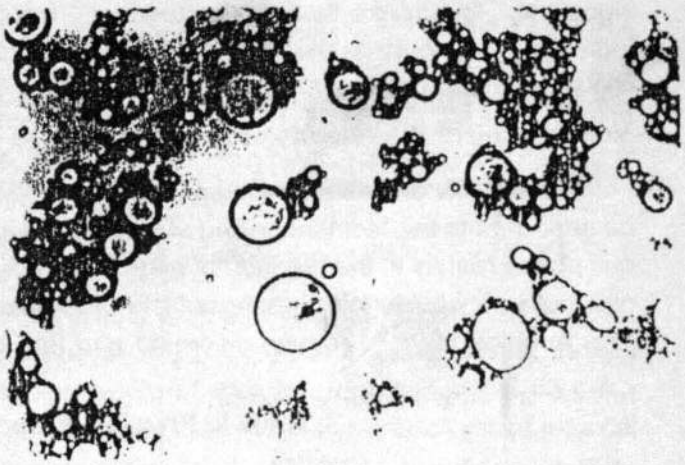

Fig. 1b: Photomicrograph of the water-liquid paraffin-polyacrylic acid gel-system (mgf = 40x)

Stability Studies — Table III represents the visually measured percentage of phases separation of different formulations. As it is clear from the data that separation of phases proceeded slowly and only a small amount of oil was separated from the total emulsion system.

Fig. 2 shows the effect of aging on the droplet size distribution analysis of different emulsion systems. All formulations exhibited flocculation (microscopic observation) of the globules but did not show coalescene nor any increase in globule diameter. The mean droplet diameter was not significantly dif-

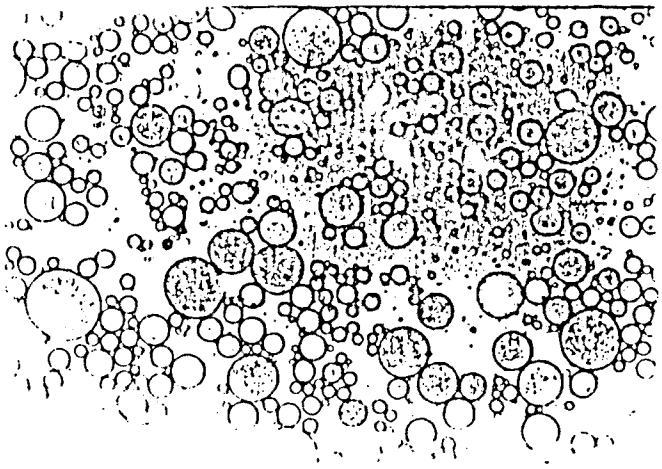

Fig. 1c: Photomicrograph of the water-sesame oil-polyacrylic acid gel system (mgf = 40x)

ferent from the initial value. These different emulsion systems were easily reconstituted by shaking them vigorously. The freeze-thaw cycle study for 1 month indicates that there is no phase separation, only slight creaming has been observed.

The stability of these formulations could possibly be attributed to the fact that gelling of external aqueous phase results in the creation of hydrophilic polymer network surrounding each multiple oil droplet. This enables them to remain discreate and free of collisions. The second possible reason is that the formation of gel in external aqueous phase, increases the viscosity of the formulations, resulting in the decrease of globule size and hence the possibility of coalescene is reduced. The other possible reason may also be the negligible density different between two phases that is - primary/internal phase (w/o primary emulsion) and external aqueous phase (gel).

Partition coefficients

In multiple emulsion system, the drug is available for the absorption after two step partitioning phenomenon. The first partitioning of drug between internal aqueous phase and middle organic phase and second partitioning between middle organic phase and external aqueous phase. Therefore partition coefficient of drug (in Ist step) is very important and has been determined by taking different oil phases (Table-IV). The highest partition coefficient was recorded for the formulation RIM. (4.08). While the partition coefficient of other two systems RLP and RSO are 0.95 and 1.25 respectively. This could presumably attributed to the fact that highest partitioning in the presence of isopropyl myristate is mainly due to its surfactant (solubilizing) like characteristic and hence the large amount of drug presents in oily phase for second partitioning.

Table II: Physical Properties of Different Formulations

Formulation	Viscosity (CPS)	*Mean Droplet diameter (μg)		Cumulative percentage drug released* in 6 hrs
		Internal droplets	Multiple oil droplets	
RB-IM.8-GT	44.0	2.0	11.5	22.0
RB-LS.8-GT	44.5	1.8	11.0	14.0
RB-SO.8-GT	45.0	1.5	10.0	16.0

* = Mean of three observations

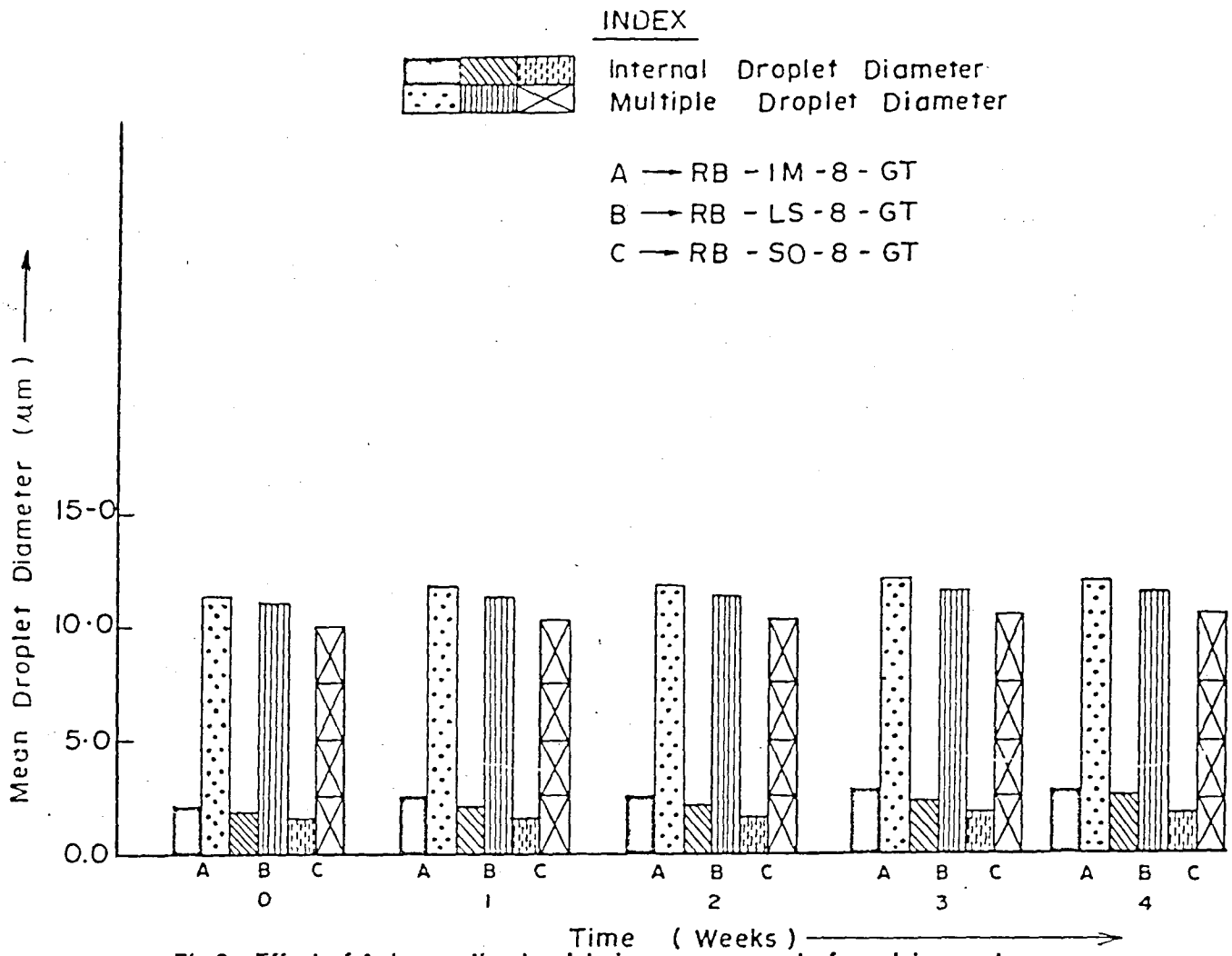

Fig.2 - Effect of Aging on the droplet size measurement of emulsion system.

The presence of surfactant increases the partition coefficient as it may act as a carrier and solubilizing agent for the present drug (Table-IV).

In vitro Release studies

(1) Effect of oil phase — The release profile of drug from freshly prepared formulations having the different oil phase shown in Table II. It was observed that high cumulative percentage drug released was obtained from the formulation RB- IM.8-GT (22.0%). Whilst from the formulations RB-SO.8-GT and RB-LS.8-GT the cumulative percentage drug released was 16.0% and 14.0% recorded respectively. This was mainly due to maximum and minimum partitioning of drug in different oil phases.

(2) Effect of phase volume Ratio — In order to observe the effect of internal phase volume on *in-vitro* release profile of drug the formulation RB-IM.8-GT was selected. Table-V shows the effect of internal phase volume on cumulative percentage of drug release, keeping other formulation parameters at their optimum value. It was found as the volume of internal aqueous phase increased the cumulative percentage drug released was enhanced. It is suggested that enhancement of release may result the thining of the oily phase (equation 1).

$$\bar{\Delta} = [\bar{D}^3/\theta\ w/o)^{1/3} - \bar{D}]/2$$

$\bar{\Delta}$ = Thickness of oil layer,

0w/o= Internal phase volume ratio.

Table-III: Effect of Aging on the Percentage of Phase Separation

Time in weeks	Percentage of Phase separation		
	RB-IM.8-GT	RB-SO.8-GT	RB-LP.8-GT
0	0	0	0
1	0.95%	1.0%	1.10%
2	1.20%	1.25%	1.30%
3	1.50%	1.80%	1.65%
4	2.50%	2.0%	2.40%

Table-IV: Partition Coefficient of Rifampicin in the Presence of Different Oil Phases

Formulation	Aqueous phase	Organic phase	Partition coefficient(K)*	
			Absence of surfactant	Presence of surfactant
RLP	Rif.+PBS (pH 7.4)	Liquid paraffin	0.95	1.09
RSO	Rif.+PBS (pH 7.4)	Sesame Oil	1.25	1.70
RIM	Rif.+PBS (pH 7.4)	Isopropyl myristate	4.08	5.12

* = Mean of three observations.

Table-V: Effect of Internal Phase Volume on Cumulative Percentage Drug Release

S. No.	Internal phase volume ratio	Cumulative percentage drug release in 6 hrs (Mean* + s.e.m.)
1.	0.2	10.0 ± 2%
2.	0.3	13.0 ± 1%
3.	0.33	15.0 ± 4%
4.	0.40	18.0 ± 2%
5	0.50	22.0 ± 3%
6.	0.55	26.0 ± 1%
7.	0.60	30.0 ± 4%

* = Mean of three observations.

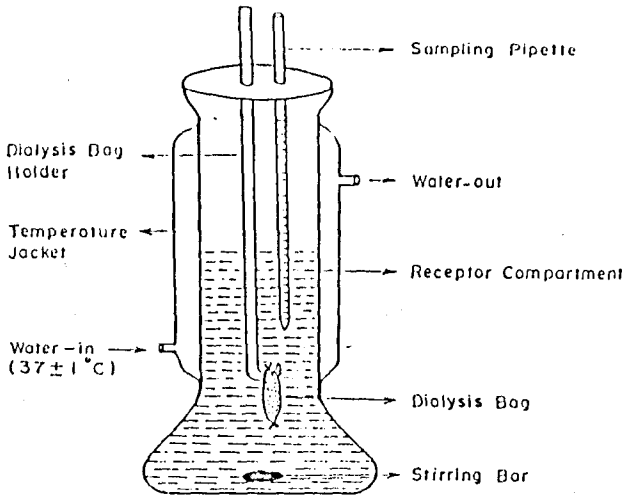

Fig.1: In-vitro Release Assembly

Thickness of the layer is inversely proportional to the internal phase volume. Another possible reason is that increasing the phase volume may result the decrease in the yield of w/o/w double emulsion at 2nd step of emulsification.

It is concluded that all parameters such as oil phases, internal phase volume and partition coefficient of drug affect the **in vitro** release profile of drug (Rifampicin). Multiple emulsion system bearing rifampicin could successfully be used in release modification and holds promise to be used in the development of long acting rifampicin parenteral system.

ACKNOWLEDGEMENT

We are grateful to Cadila Laboratories Ltd. India for providing gift sample of Rifampicin and Council of Scientific and Industrial Research India for financial aid.

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