DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 21(7), 869-878 (1995)

PROLONGED RELEASE MULTIPLE EMULSION BASED SYSTEM BEARING RIFAMPICIN : IN VITRO CHARACTERISATION

S. Nakhare* and S.P. Vyas

Department of Pharmaceutical Sciences,
Dr.Harisingh Gour Vishwavidyalaya,
SAGAR (M.P.) 470 003 India

ABSTRACT

Multiple emulsions containing rifampicin were prepared and evaluated for in vitro characterisation. The effect of pH of internal and external aqueous phase on in vitro release profile of rifampicin from multiple emulsions were studied. The partition coefficient of rifampicin between internal aqueous phase (at variable pH) and liquid paraffin (oily phase) was estimated and its effect on the release profile was elucidated.

INTRODUCTION

Water/oil/water multiple emulsion (w/o/w emulsions) have many potential applications in the fields of medicine, cosmetics [1,2] and foods [3]. The potential medicinal application include multiple emulsions as carriers for lymphatic drug delivery [4] controlled and prolonged drug release [5,6] and a reservoir to absorb drug overdose [7].

During last years the great value of rifampicin in the treatment of tuberculosis has been realized [8,9]. The conventional dosage form requires frequent administration to the patient and thus mode of therapeutics is inconvenient. It is appreciated that a prolonged release dosage form of rifampicin would reduce this problem to a great extent.

A w/o/w emulsion is likely to be the more effective dosage form. Since the extra partitioning step with the drug initially expected to affect the drug release profile. The survey of literature shows that no substantial work has been done on drug release characteristics of w/o/w emulsions, particularly under the influence of different pH of internal aqueous phase and external aqueous phase as well as the presence of organic solvent (methanol)in internal aqueous phase. Therefore these parameters were undertaken in the present study.

MATERIALS

Rifampicin (I.P. grade) a gift sample from Cadila Laboratories Ltd. The lipophilic surfactant sorbitan monooleate (Span 80) and hydrophilic surfactant, polyoxyethylene sorbitan monooleate (Tween 80) supplied by Loba Chemicals Ltd. Liquid paraffin, methanol, potassium chloride, citric acid, disodium hydrogen phosphate and all other chemicals were of either pharmacopeal or analytical grade. Double distilled water has been used throughout the experiments.

METHOD

w/o/w emulsion was prepared employing two steps emulsification procedure [10]. The composition of different formulations are shown in Table-1 [Fig. 1].

CHARACTERISATION

The multiple emulsions were characterised for droplet size and viscosity. The influence of partition coefficient and pH of internal and external aqueous phase on in vitro release profile was studied.

Droplet Size : The prepared multiple emulsions were diluted 1:100 with an external phase and size of droplets were measured by microscopic method [11], using Leitz Biomed Phase Contrast Microscope (Table-2).

Viscosity : The vescosity of different formulations were determined using Brookfeild viscometer at room temperature (Table-2).

Partition Coefficient : The release of drug from internal aqueous phase of w/o/w emulsion depends on the nature of the drug, pH of internal and external

TABLE 1

Composition of Different Formulations

Formulations	Internal phase (Aqueous)	Middle oily phase	External Aqueous phase	Phase volume ratio (w/o/w)
RB-LS.8-BT	Rif + PBS(pH 7.4) (1%)	Liquid paraffin+Span 80*	PBS(pH 7.4)+Tween 80·	0.5
RBM-LS.8-BT	Rif + PBS(pH 7.4) + methanol (1%)	Liquid paraffin+Span 80	PBS(pH 7.4)+Tween 80	0.5
RB_4-LS.8-B_7T	Rif + McIB(pH 4) (1%)	Liquid paraffin+Span 80	McIB(pH 7)+Tween 80	0.5
RB_5-LS.8-B_7T	Rif + McIB(pH 5) (1%)	Liquid paraffin+Span 80	McIB(pH 7)+Tween 80	0.5
RB_7-LS.8-B_7T	Rif+McIB(pH 7) (1%)	Liquid paraffin+Span 80	McIB(pH 7)+Tween 80	0.5
RB_8-LS.8-B_7T	Rif+McIB(pH 8) (1%)	Liquid paraffin+Span 80	McIB(pH 7)+Tween 80)	0.5
RB_7-LS.8-B_4T	Rif+McIB(pH 7) (1%)	Liquid paraffin+Span 80	McIB(pH 4)+Tween 80	0.5
RB_7-LS.8-B_6T	Rif+McIB(pH 7) (1%)	Liquid paraffin+Span 80	McIB(pH 6)+Tween 80	0.5
RB_7-LS.8-B_8T	Rif+McIB(pH 7) (1%)	Liquid paraffin+Span 80	McIB(pH 8)+Tween 80	0.5
RB_8-LS.8-B_8T	Rif+McIB(pH 8) (1%)	Liquid paraffin+Span 80	McIB(pH 8)+Tween 80	0.5

Rif - Rifampicin; PBS-Phosphate buffer saline; McIB - McIl vaine buffer series

* Span 80 = 30%; ·Tween 80 = 0.5%

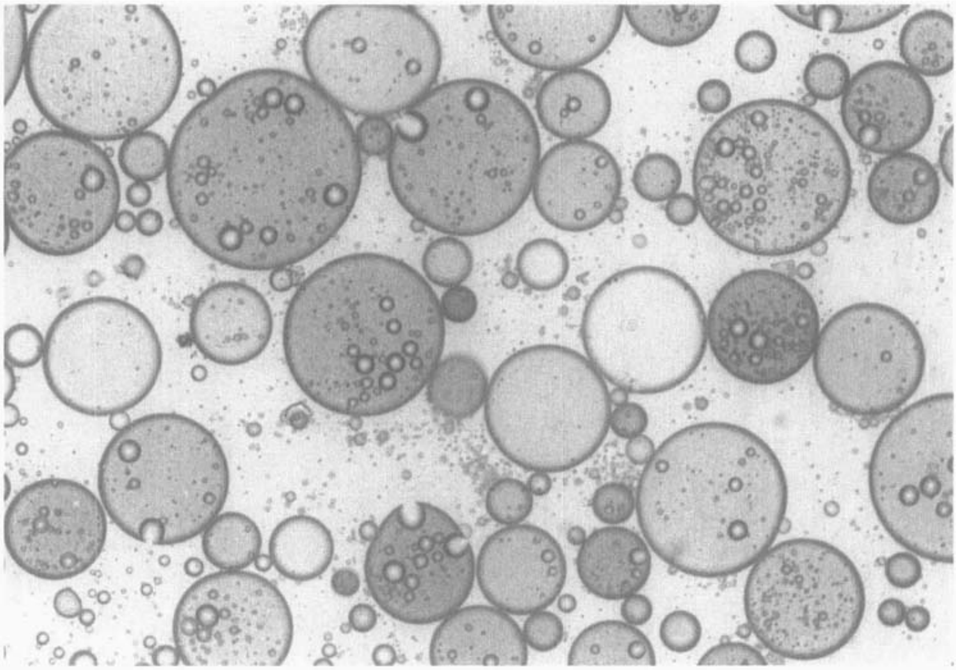

FIGURE 1

Photomicrograph of Multiple Emulsion (w/o/w)
Immediately after the Preparation (x 400)

aqueous phase and partition coefficient of drug [12] between internal aqueous phase and oily phase. The partition coefficient of rifampicin was determined by Shake flask method (Table 3).

In order to observe the effect of pH of aqueous phase on partition coefficient of drug a series of McIlvaine buffer was used (Table-4).

In-vitro Release Profile : The in-vitro release profiles of drug from multiple w/o/w emulsion were studied by dialysis method using cellophane tubing (Sigma, U.S.A.). The w/o/w emulsion was taken in the dialysis bag and dialyzed against 200 ml of isotonic buffer saline (pH 7.4) at 37±1°C while the receptor fluid agitated with the help of a magnetic stirrer. At appropriate intervals 5.0 ml of receptor fluid was

TABLE 2

Viscosity and Droplet size of Different Formulations

Formulations	Viscosity (Cps)	Multiple droplets diameter(μm)
RB-LS.8-BT	31.0	15.0 ± 2.0
RBM-LS.8-BT	32.0	14.0 ± 1.0
RB_4-LS.8-B_7T	32.0	16.0 ± 2.0
RB_6-LS.8-B_7T	30.0	15.0 ± 1.0
RB_7LS.8-B_7T	30.0	15.0 ± 2.0
RB_8-LS.8-B_7T	32.0	16.0 ± 1.0
RB_7-LS.8-B_4T	30.0	14.0 ± 2.0
RB_7-LS.8-B_6T	31.0	16.0 ± 1.0
RB_7-LS.8-B_8T	30.0	15.0 ± 2.0
RB_8-LS.8-B_8T	30.5	14.0 ± 1.0

TABLE 3

Effect of Surfactant and Nature of Aqueous Phase on Partition Coefficient

Formulation	Aqueous phase	Organic/oily phase	Partition* coefficient (K)
BLP	Rif + PBS (pH 7.4)	Liquid paraffin	0.95
BMLP	Rif + PBS (pH 7.4) + methanol	Liquid paraffin	1.18
BLPS	Rif + PBS (pH 7.4)	Liquid paraffin + span 80	1.09
BMLPS	Rif + PBS (pH 7.4) + methanol	Liquid paraffin + span 80	1.66

* Mean of three observations; PBS-Phosphate buffer saline.

TABLE 4

Effect of pH on partition Coefficient

Formulation	Aqueous phase	Organic phase	Partition* Coefficient (K)
B_4LP	Rif + McIB (pH 4.0)	Liquid paraffin	0.38
B_5LP	Rif + McIB (pH 5.0)	Liquid paraffin	0.50
B_6LP	Rif + McIB (pH 6.0)	Liquid paraffin	0.92
B_7LP	Rif + McIB (pH 7.0)	Liquid paraffin	1.30
$B_{7.4}LP$	Rif + McIB (pH 7.4)	Liquid paraffin	1.10
B_8LP	Rif + McIB (pH 8.0)	Liquid paraffin	0.25

* Mean of three observations;
McIB - McIlvaine buffer series was used.

withdrawn and replaced with 5.0 ml of fresh receptor fluid. The drug concentration was analysed spectrophotometrically (Shimadzu Double Beam Spectrophotometer, 150-02 UV) at 255 nm

RESULTS AND DISCUSSION

The droplet size and viscosity of different formulations prepared under same conditions were studied. The mean droplet size of the emulsions were recorded to be in the range of 15-18 μ. The viscosity of multiple emulsion formulations was nearly the same i.e. recorded to be in the range of 30.0-32.0 cps for all the formulations (Table-2). These multiple emulsion formulations were then studied for the effect of partition coefficient and pH of the internal and external aqueous phase on the *in-vitro* release profile of the contained drug.

Partition Coefficient

In multiple emulsion system the drug is available for the absorption after two step partitioning phenomenon [13]. Therefore, the effect of pH of internal and external aqueous phase and nature of oily/organic phase on drug release profile was studied. The partition coefficient of different products are recorded in Table-3. The partition coefficient of BLP, BMLP, BLPS, BMLPS was found to be 0.95, 1.18, 1.09, 1.66 respectively. The higher partition coefficient measured in case of BMLP (1.18) and BMLPS (1.66) could presumably be attributed to the incorporation of methanol in the internal phase of the respective formulation. The methanol being to some extent as a common solvent for both the phases i.e. water as well as liquid paraffin thus as a consequence could have increased the quantity of drug in organic phase.

The presence of surfactant increase the partition coefficient, as it may act as carrier for the drug (BLP = 0.95 and BLPS = 1.09).

The effect of pH on partition coefficient (K) of drug was also studied and is shown in Table-4. It was found to increase with increasing the pH from 4 to 7 and at pH 7, maximum value was obtained. The K values recorded for various system are $B_4LP = 0.38$, $B_5LP = 0.50$, $B_6LP = 0.92$ and $B_7LP = 1.30$ respectively; beyond pH 7 the K then declined ($B_{7.4}LP = 1.10$) and $B_8LP = 0.25$). This could possibly be attributed to the fact that at the pH 7 drug exists in the unionised state while at acidic and basic pH the drug is extensively ionized and hence maximum and minimum partition coefficient values were recorded respectively.

<u>In-vitro Release Studies</u>

The results of in vitro release profile of rifampicin from freshly prepared formulations are shown in Table-5.

In vitro release of rifampicin was noted increases, in the presence of methanol (Table-5). The cumulative percentage drug released for RB-LS.8-BT and RBM-LS.8-BT was recorded to be 40.0 $\pm$ 3% and 45 $\pm$ 2% respectively.This may be due to the increased partition coefficient of drug between internal aqueous phase and intermediate oil phase.

The release profile of drug from different formulations having the aqueous phase of different pH were represented in Table-5. It was observed that

TABLE 5

Cumulative Percentage Release of Rifampicin from Different Formulations

Formulations	Cumulative percentage release in 6 hrs (Mean $\pm$ s.e.m.)
RB-LS.8-BT	40.0 $\pm$ 3%
RBM-LS.8-BT	45.0 $\pm$ 2%
RB_4-LS.8-B_7T	28.0 $\pm$ 2%
RB_6-LS.8-B_7T	35.0 $\pm$ 2%
RB_7-LS.8-B_7T	52.0 $\pm$ 2%
RB_8-LS.8-B_7T	20.0 $\pm$ 1%
RB_7-LS.8-B_4T	37.0 $\pm$ 1%
RB_7-LS.8-B_6T	42.0 $\pm$ 2%
RB_7-LS.8-B_8T	25.0 $\pm$ 1%
RB_8-LS.8-B_8T	15.0 $\pm$ 2%

the high cumulative percentage release was obtained from the RB_7-LS.8-B_7T formulation (52.0 $\pm$ 2%) while from the formulation RB_4-LS.8-B_7T, RB_6-LS.8-B_7T and RB_8-LS.8-B_7T, the cumulative percentage released was 28.0$\pm$2%, 35.0$\pm$2% and 20.0$\pm$1% recorded respectively. This was due to the maximum and minimum partition coefficient of drug at these particular pH and hence the large and small quantity of drug is available for second partitioning step respectively which affects the total release pattern of drug.

In order to observe the effect of pH of external aqueous phase on in vitro release profile of drug the formulation RB_7-LS.8-B_7T was selected. The release profiles of different formulations shown in Table-5. The maximum cumulative percentage drug released was recorded in the case of RB_7-LS.8-B_7T (52.0 $\pm$ 2%) formulation. Both internal and external phases were maintained at pH 7 and highest partitioning (in Ist and IInd step) of drug at this particular pH was observed. The cumulative percentage drug released from the formulations viz. RB_7-LS.8-B_4T, RB_7-LS.8-B_6T, RB_7-LS.8-B_8T were 37.0±1%, 42.0±2% and 25.0±1% respectively. This was due to the pH depending partitioning of drug in second step (partition of drug between liquid paraffin and external aqueous phase).

From the above discussion it is confirm that pH of both aqueous phases greatly influenced the release profile of drug. In order to confirm this statement formulation RB_8-LS.8-B_8T was prepared. The lowest cumulative percentage drug release was obtained (15.0±2%). This was mainly due to the minimum partitioning of drug in both steps. Hence the multiple emulsion in which both aqueous phases maintained at pH-8 gave maximum prolongation of drug in comparison to other formulations.

CONCLUSIONS

It is thus concluded that all the parameters i.e. pH of internal and external aqueous phase, nature of organic phase and partition coefficient of the drug at the different pH affects the *in-vitro* release profile of rifampicin.

It is inferred from the study that multiple emulsion holds promise to be used as long acting parenteral system. The *in-vivo* evaluation study on rifampicin multiple emulsions are under progress in this laboratory.

ACKNOWLEDGEMENT

The author* is thankful to Council of Scientific and Industrial Research India for financial aid and Cadila Laboratories Ltd. India for providing gift sample of rifampicin.

REFERENCES

1. T.J. Lin, H. Kurihara and H. Ohta, **J. Soc. Cosm. Chem.**, <u>26</u>, 121 (1975).

2. M. De Luca, J.L. Grossiord, J.M. Medard, C.Vaution and M. Seiller, **Cosmetics and Toiletries**, <u>105</u>, 65 (1990).

3. Y. Takahashi, **Yukagaku**, <u>35</u>, 880 (1986).

4. S. Fukushima, M. Nishida and M. Nakano, **Chem. Pharm. Bull.**, <u>35</u>, 375 (1987).

5. J.K. Pandit, B. Mishra and B. Chand, **Ind. J. Pharm. Sci.**, <u>49</u>, 103 (1987).

6. B. Mishra and J.K. Pandit, **J. Controlled Release**, <u>14</u>, 53 (1990).

7. Y. Morimoto, K. Sugibayashi, Y. Yamaguchi and Y. Kato, **Chem.Pharm.Bull.,** **27**, 3188 (1979).

8. International Colloguim, Brostel, Germany May 1968 in Antibiotica et. **Chemotherapia,** **16**, 316 (1970).

9. European symposium on Rifampicin, Pellenberg, Belgium. **Acta Tubere Pneumol,** **60,** 249, 588 (1969).

10. S. Matsumoto, Y. Kita and D. Yonezawa, **J.Colloid Interface Sci.,** **57**, 353 (1976).

11. M.J. Groves and D.C. Freshwater, **57(8)**, 1273 (1968).

12. A.T. Florence and D. Whitehill, **Int. J. Pharma.,** **111**, 277 (1982).

13. S. Nakhare and S.P. Vyas, **Die Pharmazie** (In press) (1994).