DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 19(15), 1965-1980 (1993)

IN VITRO - IN VIVO CORRELATION OF INDOMETHACIN RELEASE FROM PROLONGED RELEASE W/O/W MULTIPLE EMULSION SYSTEM

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ABSTRACTS

A prolonged release oral w/o/w multiple emulsion was formulated using hydroxypropyl methyl cellulose as thickening agent. The polymer when used in different proportions, controlled indomethacin release from the biphasic emulsion system. Double emulsification technique was used for formulation of the biphasic emulsion system. The stability of the emulsion was found to be inversely proportional to the drug release characteristics. The in vitro release of indomethacin followed diffusional path through the oil layer and through the polymeric oil. The in vivo release studies were carried out using rabbits as animal models. A good linear correlation was obtained between in vivo-in vitro drug release from such multiple emulsion system.

INTRODUCTION

Multiple emulsion system is an emulsion in which drops of the dispersed phases contain smaller droplets that have

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the same compositions as the external phase. Two types are possible : Water-in-oil-in-water (w/o/w) type and oil-in--water-in-oil (o/w/o) type. The potential of using controlled release multiple emulsion has been already reported by various workers (1-8). In the w/o/w multiple emulsions, the internal and external aqueous phases are separated by an oil layer and require for their formation and stability, at least two stabilising surfactants, one having a low HLB to form the primary water-in-oil emulsion and the other of higher HLB to achieve secondary emulsification. These emulsion systems have the advantages of being less viscous.

In this investigation, a prolonged release oral w/o/w multiple emulsion was formulated using suitable hydrophilic polymer, Hydroxy propyl methyl cellulose as a viscosity enhancer. This polymer when used in different proportions, was found to control drug release from the biphasic emulsion system. Indomethacin was used as the model drug. Indomethacin is a potent non-steroidal anti-inflammatory agent and inhibitor of prostaglandin synthesis. The severity of gastric side effects of indomethacin can be reduced by incorporating it in controlled release drug delivery system.

MATERIALS AND METHODS

Indomethacin, I.P. was generously supplied by Cipla Ltd., Bombay. Hydroxy propyl methyl cellulose [BDH Chemicals Ltd., Poole, England], Span 80 [Fluka Chemie A.G.,Buchs], Liquid paraffin, I.P. [S.D's Fine Chemicals Ltd., Boisar], Tween 80 [Fluka Chemie, A.G., Buchs] were commercially obtained and were used as received. The drug was passed through a U.S. standard 100 mesh sieve after drying to constant weight.

Preparation Technique

Measured amount of glass distilled water (Table 1) was warmed. Hydroxy propylmethyl cellulose (HPMC) was added to it while stirring and a sol was thus made. Six hundred mg. of dried and sieved drug was dispersed in the sol uniformly. Seventeen ml. liquid paraffin, I.P. containing 2.5% Span 80 was taken in a 50 ml beaker. The drug suspension was added into it. A primary water-in-oil emulsion was made using a vortex mixer. The primary emulsion was again re-emulsified with 45 ml aqueous phase (HPMC Sol) containing the required percent of hydroxy propyl methyl cellulose and 0.3% v/v Tween 80. During this second emulsification, the speed of vortex mixer was reduced. The prepared w/o/w multiple emulsion was transferred and kept in airtight container. The formulation batch no. ME7 (table 1) contains the drug in the outer aqueous phase. The drug was dispersed in the final HPMC sol which was used as the external aqueous phase for the second emulsion and the inner aqueous phase in this case was water.

Microscopic Observation

Formation of w/o/w multiple emulsion was confirmed by observing them under high power crossed polar microscope. The hydrodynamic characteristics of the prepared multiple emulsion droplets were checked by introducing a water soluble dye (Ponceu Red) in the innermost aqueous phase. The emulsion was suitably diluted and observed microscopically for distribution and intensity of the colour in the internal and external aqueous phase initially and at definite intervals of time.

In Vitro Drug Release Studies

Cuprophan tubing (Medicell International, London, Size 5, 20'/32' in diameter) was used as the dialysis membrane. It

TABLE - 1

Materials Required in the Formulation of W/O/W Multiple Emulsions

Batch No.[a]	Drug Suspension Total 12 ml.		Primary W/O Emulsion Total - 30 ml.(appx.)		W/O/W Multiple Emulsion Final Volume 60 ml.		
	Methanol (ml.)	HPMC (%)	Liquid Paraffin (ml.)	Span 80 (ml.)	HPMC (%)	Tween 80 (ml.)	W/O Emulsion (ml.)
ME 1	-	1.25	17	0.75	1.25	0.113	15
ME 2	-	1.0	17	0.75	1.0	0.113	15
ME 3	-	0.5	17	0.75	1.0	0.113	15
ME 4	-	0.5	17	0.75	0.5	0.113	15
ME 5	10	-	17	0.75	0.5	0.113	15
ME 6	10	-	17	0.75	0.25	0.113	15
ME 7	-	-	17	0.75	0.50 +drug	0.113	15

a - Each batch was triplicated

was washed several times with double distilled water and left soaking in distilled water overnight before use. Immediately after preparation, 15 ml of the w/o/w emulsion was pipetted to a bag made of cuprophan, double tied at each end. The cuprophan bag containing the multiple emulsion was placed in 200 ml phosphate buffer of pH 6.2 as the dialysis medium at 37±1° under stirring. Five ml aliquots were withdrawn at half an hour interval and was replenished with the fresh dialysis medium. Aliquots were suitably diluted and assayed spectrophotometrically at 320 nm in a Hitachi 200-20-UV-VIS Sphectrophotometer.

In Vivo Drug Absorption Studies in Animal Models

White male rabbits weighing 2-3 kg were used as the model animals. Three rabbits were used separately for the formulation batch and control. Indomethacin powder in the same dose was used as the control. Indomethacin was given in 10 mg/kg dose. The animals were fasted overnight prior to each experiment. An adapted needle with a bulb end was used to feed the rabbits orally. Blood samples were collected at specified time intervals and centrifuged. Plasma indomethacin concentration was determined by Hucker's method (9) with slight modification. 0.01 N NaOH solution was used instead of 0.1 N NaOH solution. The fluorescence was measured in a Shimadzu RF 540 spectro fluorometer, initiating maximum activation at 295 nm and fluorescence was observed at 380 nm. Reagents blank were run through the procedure.

RESULTS AND DISCUSSIONS

Multiple emulsions was prepared successfully by the method described. The physical stability of multiple emul-

sion was found proportional to the amount of hydroxypropyl methyl cellulose present in the internal aqueous phase and in the external aqueous phase. The increased level of HPMC produced a highly viscous multiple emulsion with a shelf life of 6 months or more. The w/o/w emulsions were instable at 10° and phase separation occured within 12 hours. The result of stability studies are summerized in table 2. The viscosity of the multiple emulsion decreased on storage at room temperature. This may be due to migration of water molecules from the inner aqueous phase to the outer aqueous phase. The journey of the water molecule in both the direction may cause thining of the oil layer which ultimately may lead to the rupture of oil layer and consequently breakdown of the multiple emulsion droplet.

The structure of a freshly prepared w/o/w multiple emulsion was observed under the microscope (figure 1). Many small droplets are seen in the inner aqueous phase. Figures 2 and 3 show the structures of ruptured w/o/w multiple emulsion droplets after 24 hours of preparation and after 15 days respectively. It may be assumed that coalescence between the inner aqueous droplets and the outer aqueous phase occured and hence the size of the inner aqueous droplets increased within the oily phase causing rupture of the emulsion droplet.

The in vitro release of indomethacin from w/o/w multiple emulsion is inversely proportional to the concentration of the polymer in both the aqueous phases (figure 4). An increased content of the polymer produced a highly stable emulsion but drug release from such emulsion was very poor. On the other hand, emulsion stability was hampered when the polymer content was lowered, but the drug release rate improved. The release of drug from the outer

TABLE - 2

Results of Stability Studies of Controlled Release Multiple Emulsion at Room Temperature[a]

Multiple Emulsion Batch No.	Time of Observation - '+' indicates - starting of phase separation '-' indicates - absence of phase separation							
	1 Hour	8 Hours	24 Hours	7 Days	15 Days	1 Month	3 Months	6 Months
ME 1	-	-	-	-	-	-	-	-
ME 2	-	-	-	-	-	-	+	+
ME 3	-	-	-	-	-	+	+	+
ME 4	-	-	-	+	+	+	+	+
ME 5	-	-	-	+	+	+	+	+
ME 6	-	-	-	+	+	+	+	+
ME 7	-	-	-	+	+	+	+	+

a - Average of triplicate studies

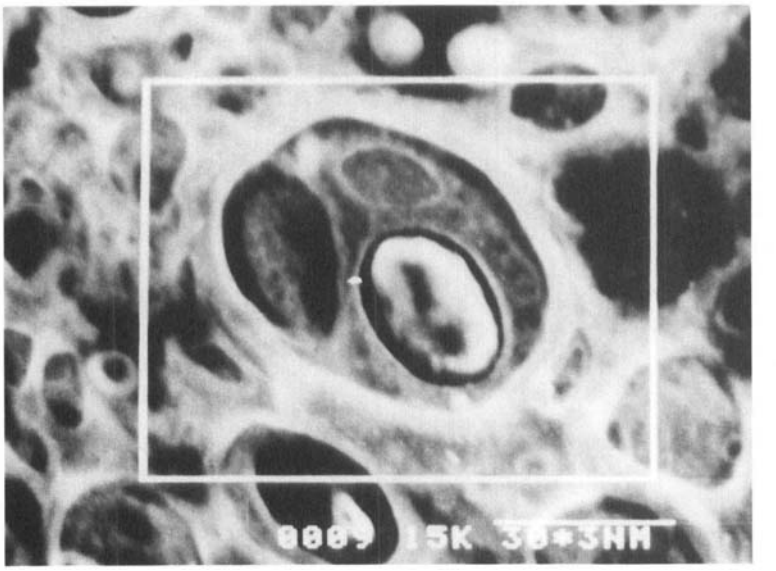

Figure 1. Scanning Electron Micrograph of Freshly Prepared w/o/w Multiple Emulsion Batch No. ME 5, Magnification x750.

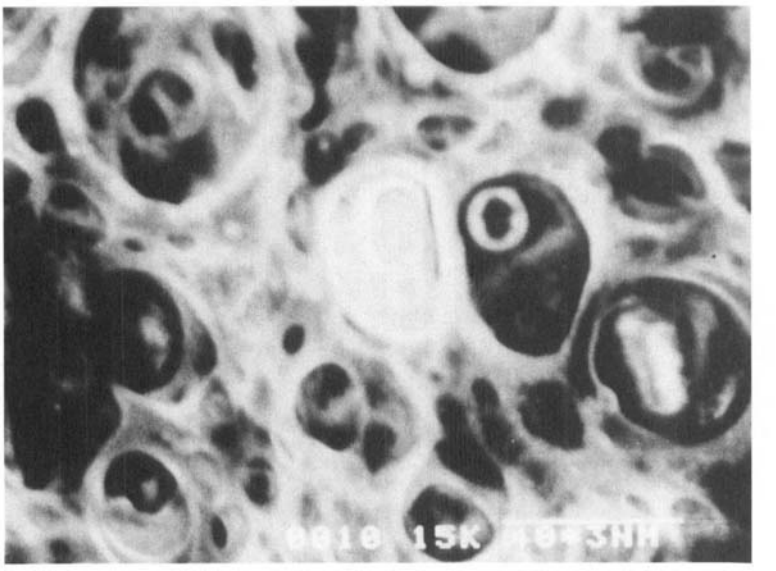

Figure 2. Scanning Electron Micrograph of W/O/W Multiple Emulsion after 24 hours of Preparation, Batch No. ME 5, Magnification x750

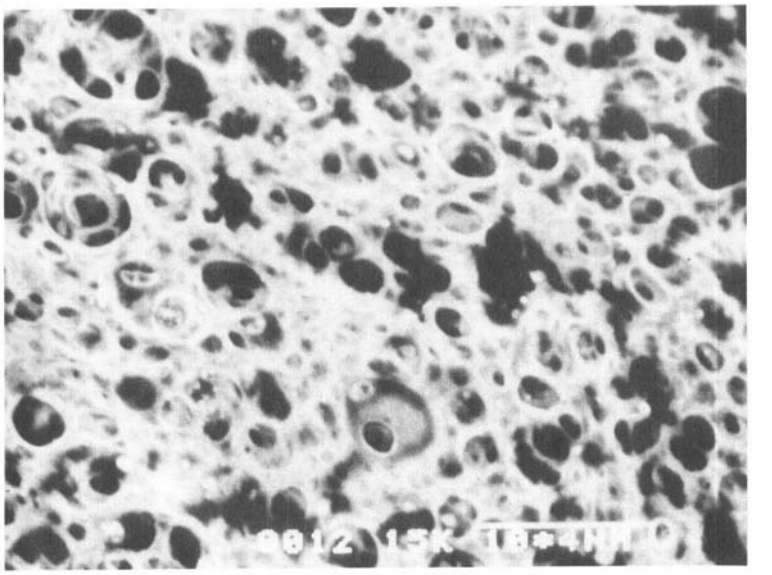

Figure 3. Scanning Electron Micrograph: W/O/W Multiple Emulsion after 15 days of preparation, Batch No. ME 5, Magnification x500.

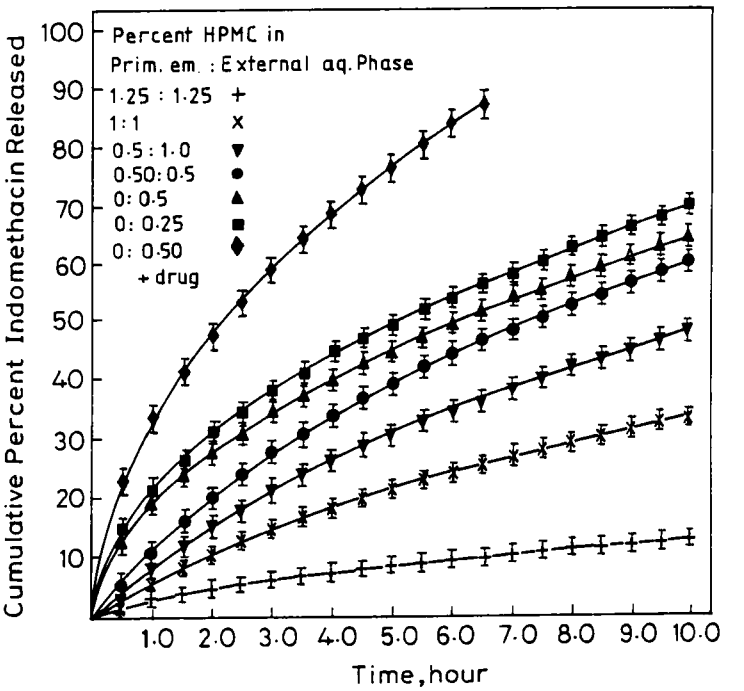

Figure 4. Release Profile of Indomethacin from Controlled Release Multiple Emulsion Systems.

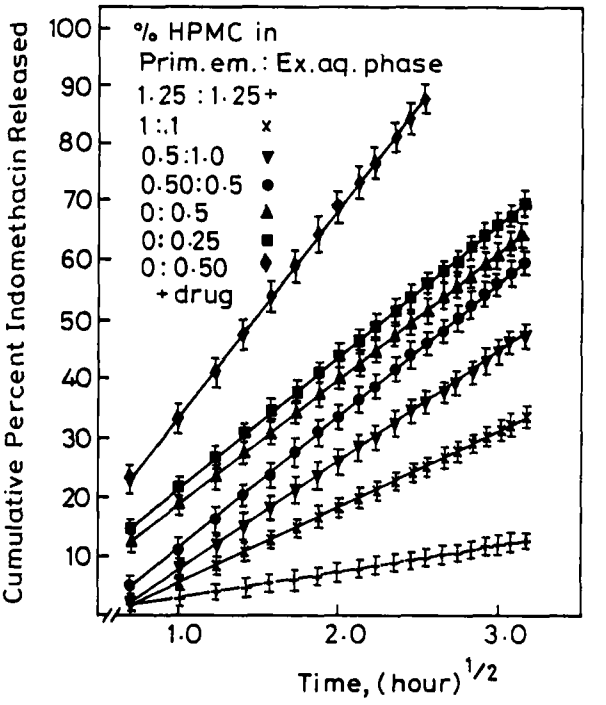

Figure 5. Drug Release Versus Square Root Time Plot of Indomethacin Incorporated Controlled Release Multiple Emulsion System.

aqueous phase (for batch no. ME 7) was much faster than rest of the formulation batches. Comparing the release pattern of other batches of multiple emulsion (ME 1 to ME 6) with the batch no. ME 7, it may be concluded that the rate limiting step in the in vitro release of drug from the prepared multiple emulsion was the transport through the oil phase and through the polymeric dispersion, rather than the transport through the dialysis membrane.

A linear relationship was observed between the amount of drug released and the square root of time (figure 5). This suggests that the drug release through the oil layer and polymers may be diffusional in nature. The release

TABLE - 3

Summary of In Vitro Drug Release Kinetics of Indomethacin Loaded W/O/W Type Multiple Emulsions

Batch No. of Multiple Emulsion	% HPMC in Prim. em. : ext. phase	$t_{50\%}$[a] (hr.)	First Order Rate Constant[a] ($hr.^{-1}$)	Higuchi Constant[a] ($mg.min.^{-\frac{1}{2}}$)	Weibull Equation[a] (Shape Parameter)
ME 1	1.25 : 1.25	*	-	0.327	-
ME 2	1.0 : 1.0	*	-	0.865	-
ME 3	0.5 : 1.0	*	-	1.127	-
ME 4	0.5 : 0.5	7.5	0.0924	1.557	1.0095
ME 5	0 : 0.5	6.2	0.112	1.924	1.0120
ME 6	0 : 0.25	5.1	0.136	2.123	1.0127
ME 7	0 : 0.5 Drug in ext. phase	2.2	0.315	3.331	1.0079

* Release was so slow that 50% release of drug did not occur within 10 hours

a - Average of triplicate batches

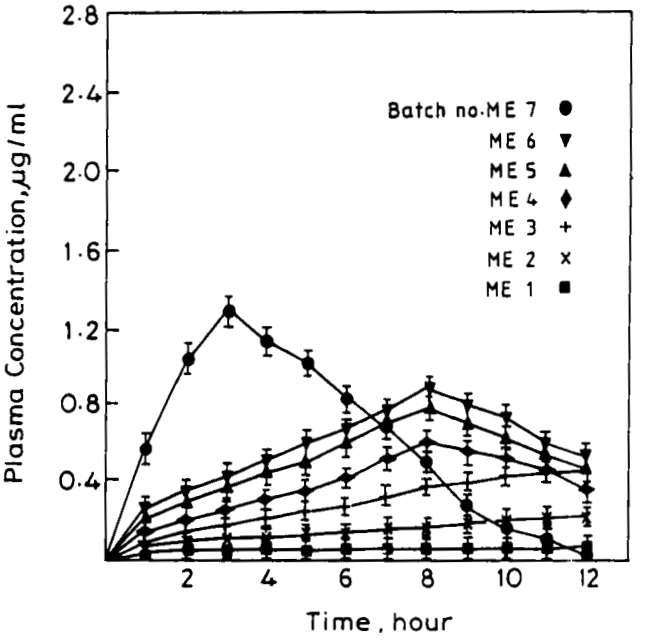

Figure 6. Mean Concentration of Indomethacin after Oral Administration of W/O/W Type Multiple Emulsion.

constants as calculated, are presented in table 3. Figure 6 represents the plasma level curves of the controlled release multiple emulsion system and indomethacin drug powder. The absorption of indomethacin from conventional capsule containing powdered drug was very rapid and the peak plasma concentration was high. But the prepared multiple emulsion produced flatenned peak showing prolonged drug action over 12 hour study. The maximum plasma concentration (C_{max}) and the time required to reach C_{max} (t_{max}) were recorded for the formulation. The area under the curve (AUC) from zero to final sampling time (AUC_{o-t}) was calculated using the trapezoidal rule (10). The results of some of the batches of prepared w/o/w multiple emulsion ME 1, ME 2, ME 3 in table 4) were discarded because of

TABLE - 4

Pharmacokinetic Parameters[a] of Indomethacin Loaded W/O/W Type Multiple Emulsion System after Oral Administration in Rabbits

Batch No. of Multiple Emulsion	% HPMC in Prim. em. : ext. phase	C_{max} (µg/ml)	t_{max} (hr.)	AUC (µg. hr/ml)	F_{rel} (%)
ME 4	0.5 : 0.5	0.61	8	4.71	52.62
ME 5	0. : 0.5	0.78	8	6.24	78.99
ME 6	0 : 0.25	0.89	8	7.17	90.76
ME 7	0 : 0.5	1.29	3	7.86	99.49
	+ Drug in ext. phase				

a - Average of six observations

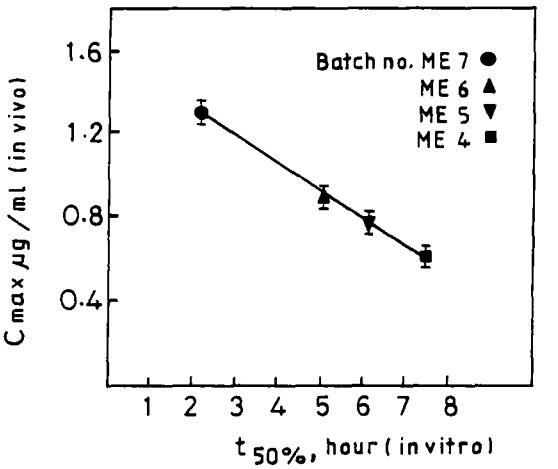

Figure 7.

low plasma concentration attained from those formulation batches. The effective therapeutic level of indomethacin, 0.5 µg/ml could not be attained from them (figure 6). A correlation between the in vitro dissolution studies and in vivo animal experiments was established by plotting $t_{50\%}$, the time taken to release 50% of indomethacin in vitro, versus C_{max} in vivo (figure 7). A linear plot suggests a good correlationship between them.

Thus it was found that the hydroxypropylmethyl cellulose was successfully used not only as a viscosity inducing agent, but also as a controlling agent of indomethacin release from the w/o/w multiple emulsion system. Though w/o/w type multiple emulsions were prepared with great success, the stability of the formulation was of concern. Stability was found to be inversely proportional to the release rate of drug. The drug release profile of a highly stable w/o/w type multiple emulsion (ME 1) was poor. Main emphasis of this work was focussed on obtaining an optimum

release pattern of drug from the prepared multiple emulsions and hence formulations were designed accordingly.

In conclusion it may be said that a good control was successfully achieved over the release rate of indomethacin from the prepared w/o/w multiple emulsions using hydroxy propyl methyl cellulose as the drug release rate controlling polymer. The polymer loaded w/o/w multiple emulsion can be successfully used as a controlled release oral liquid drug delivery system.

ACKNOWLEDGEMENT

The research work was carried out by the grants provided by University Grants Commission, India. Indomethacin was generously supplied by Cipla Limited, Bombay, India.

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