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DEVELOPMENT OF STABLE MULTIPLE EMULSION OF ATORVASTATIN

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ABSTRACT : Multiple emulsions have been proposed to have numerous uses including their use for enhancement of bioavailability or as a prolonged drug delivery system. But the inherent instability of this system needs to be overcome before they find potential application in pharmaceuticals. Multiple emulsions are often stabilized using a combination of hydrophilic and hydrophobic surfactants. The ratio of these surfactants is important in achieving stable multiple emulsions. Atorvastatin was selected as a model drug to study the potential of multiple emulsion to improve bioavailability with the hypothesis that improvement of drug release profile will reflect the enhancement of bioavailability of the drug. The objective of this study was to prepare multiple emulsion of Atorvastatin by two step emulsification using nonionic surfactants, and evaluate for stability, percentage drug entrapment, in-vitro & ex-vivo drug release. Different formulation variables like type & proportion of primary & secondary emulsifier and phase volume ration of internal phase:external phase; and process variables like speed & time of stirring during primary & secondary emulsification were optimized to get stable multiple emulsion with high entrapment efficiency. The study concluded that stable multiple emulsion with high entrapment efficiency can be prepared by two step emulsification method using Span60 as primary and Tween80 as secondary emulsifier at 30:70 phase volume ratio of internal phase:external phase with optimized speed of stirring at 5000 r/min for 10 mins for primary emulsification and 1500 r/min for 7 mins for secondary emulsification.

Key words: Multiple Emulsion, Nonionic Surfactants, Atorvastatin, Rat Ileum

INTRODUCTION

Water-in-oil-in-water (W/O/W) multiple emulsions are polydisperse vesicular systems in which the dispersed oil drops contain even smaller dispersed droplets, which generally consist of a liquid identical to the continuous phase (Florence et al. 1982, De Luca et al. 1991). Because of the presence of a reservoir phase inside droplets of another phase that can be used to prolong release of active ingredients, multiple emulsions find many applications in industries such as pharmaceuticals and cosmetics (Matsumoto S, et. al., 1976). The most common method for the preparation of multiple emulsions is the two step emulsification method using a homogenizer or high-speed laboratory mixer (Yazan Y. et. al 1993). Water-in-oil-in-water (w/o/w) multiple emulsions have many potential applications in various fields, such as pharmaceutics, cosmetics and food (Omotosho et al. 1990, Cunha et al. 1998), taking advantage of the liquid membranes of the oil phase. Several properties of W/O/W multiple emulsions make them particularly attractive in cosmetics as well as in the pharmaceutical field: the good capacity to entrap active substances (Mishra et al. 1990), the protective effect towards substances that may undergo degradation (Gallarate et al. 1999a, Gallarate et al. 1999b), the possibility of introducing non-compatible substances in two aqueous compartments of the same product, the prolonged release of drugs or active substances (Raynal et al. 1993, Denine et al. 1996, Magdassi et al. 1986).

Much effort has been devoted so far to the study for application of w/o/w emulsions to practical use, however multiple emulsions are unstable with respect to creaming and they are rather coarse and drugs targeting becomes difficult (Khopade et al. 2000). W/o/w emulsions are thermodynamically unstable, which causes various problems during their storage, such as leakage of the content from the inner aqueous phase, flocculation of the droplets, phase separation, and so on. (Florence et al. 1985) suggested three methods to overcome these problems: use of a high viscous oil to prevent or suppress the diffusion of the component, polymerization of surfactant molecules adsorbed on the interface, and gelation of the oil and/or aqueous phases of the emulsions. Blending of surfactants (Frenkel et al. 1983) or adsorption of albumin (Omotosho et al. 1989) or poly (acrylic acid) (Cole et al. 1997) on the interface has also been proposed for the stabilization. However, the effective method has not been accomplished yet.

Atorvastatin, a synthetic HMG Co-A Reductase inhibitor, is widely used in treatment of primary hypercholesterolemia and dyslipidemia. Atorvastatin is indicated as adjunctive therapy to diet for the treatment of patients with elevated serum triglyceride levels (Fredrickson Type IV). Oral bioavailability of Atorvastatin is very low (Only 14%) due to its presystemic clearance in gastrointestinal mucosa and first pass hepatic metabolism. However, very few studies have been reported for enhancement of bioavailability of poorly water soluble drugs by formulating as multiple emulsions.

The present study is based on the hypothesis that improvement of in vitro as well as ex vivo (using rat intestines) dissolution profile will reflect the enhancement of bioavailability of the drug.

MATERIALS

Atorvastatin was obtained as a gift sample from Ranbaxy Research Centre (Bhaddi, Himachalpradesh), India. Atorvastatin tablet (STORVAS FC 20MG) was purchased from market. Paraffin oil (light), Span20, Span60, Span80, Span85, Tween20, Tween60 and Tween80 were purchased from Loba Chem (Mumbai, India). Atorvastatin was obtained as a gift sample from Ranbaxy Research Centre (Bhaddi, Himachalpradesh). Phosphate Buffer Saline pH 7.4 (PBS pH 7.4) was prepared as described in the Indian Pharmacopoeia (1996) and necessary chemicals were obtained from the Loba Chem (Mumbai, India). All chemicals and reagents were of AR grade and used as such without any further purification.

METHODS

Preparation of Multiple Emulsion

Multiple emulsions were prepared by two step emulsification process: a) Preparation of primary emulsification; b) Secondary emulsification. (Florence et al. 1982, Hideaki et al. 2000)

Primary emulsification: 12ml of distilled water containing 24mg of drug was gradually added to 28ml of oil phase containing primary emulsifier (Span20, Span60, Span80 or Span85) and 56mg of drug with continuous stirring at 5000 r/min for 5 min. Different variables like, type of primary emulsifier (table 1), concentration of emulsifier (table 2), speed of rotation & time of rotation (table 3) and volume fraction of internal phase:external phase (table 4) were optimized as recorded in tables.

Secondary emulsification: 30ml viscous primary emulsion was emulsified further with an external aqueous phase containing secondary emulsifier (Tween20, Tween60 or Tween80) and 140 mg drug with continuous stirring at 1000 r/min for 10 min. Different variables like, type of secondary emulsifier (table 5), concentration of emulsifier (table 6), speed of rotation & time of rotation (table 7) and volume fraction of internal phase:external phase (table 8) were optimized as recorded in tables.

Evaluation of Multiple Emulsion

Globule size: Microscopic analysis was performed on optical immersion microscope (Olympus BS 40) in order to measure globule size and multiple natures of all formulations. The oil droplet size was measured by dynamic laser light scattering technique using particle size analyzer (Malvern Mastersizer 2000).
Entrapment efficiency (Khopade et al. 2000): Percentage Entrapment Efficiency (% EE) was determined by taking freshly prepared W/O/W multiple emulsions and immediately centrifuged at 4000 r/min for 10 min. Then 1ml of the aqueous phase (the lower layer) was precisely withdrawn through 2 ml hypodermic syringe and diluted properly with 0.1N HCl. The solution was filtered with a Millipore filter (0.22 µm in pore size) and drug content was analyzed on UV spectrophotometer at 240 nm. The Encapsulation Efficiency was determined by following equation:

% EE = [(Total drug incorporated –Free Drug)/ Total drug] X 100

In vitro drug release study: The in vitro drug release study was carried out on a simple dissolution cell using cellophane membrane (thickness-200µm, breaking strength-2.7 kgf/cm). Prior to release studies, the cellophane membrane was soaked in distilled water for 6 hours, washed frequently 4 times by changing distilled water, then immersed in 5% v/v glycerol solution for at least 60 min and washed finally with 5 portions of distilled water. 15 ml freshly prepared multiple emulsion was added to donor chamber, made up of a hollow glass tube (2.5 cm in diameter and 10 cm in length) and membrane was tied on bottom end of the tube with a nylon string. This tube was dipped into 1000 ml vessel containing 900 ml of PBS pH 7.4 and was stirred at 75 r/min osn a magnetic stirrer and maintained at 37 °C which acted as receiving chamber. Aliquots of 1ml were collected from receiving chamber at predetermined time intervals and the drug content was determined on UV spectrophotometer at 240 nm after suitable dilution.

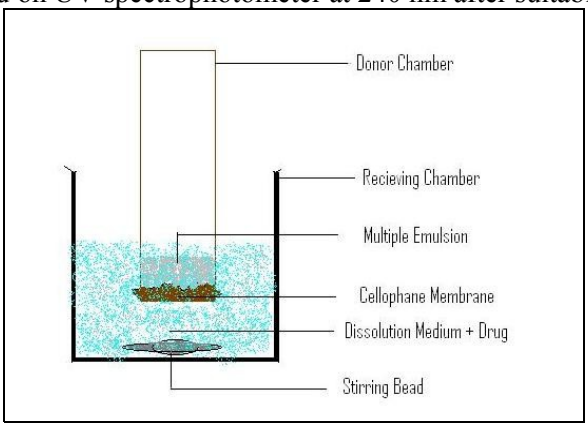

Figure 1. Figure shows simple dissolution cell

Ex vivo drug release study: Drug release study was performed on rat ileum by following perfusion method. The special apparatus (Figure 2) consist of 'U' shaped glass tube having 1cm inner diameter with cannulated cut on upper half arm of the 'U' tube. Healthy and untreated rat was sacrificed; ileum was collected and immediately dipped into beaker containing freshly prepared phosphate buffer pH 7.4. The ileum was washed properly with PBS pH7.4 and cleaned for any fecal material, tissues or veins by pressing squeezing the ileum smoothly. The ileum was cut for approximately 7cm of length and marked at 1cm away from both the ends. The ileum was gripped on both the ends of apparatus (A & B) and fixed with adhesive tap. The lower end of the ileum was closed by a thread and approximately 5ml of multiple emulsion was filled in ileum. Then a thread was tied at the upper mark of ileum to close it.

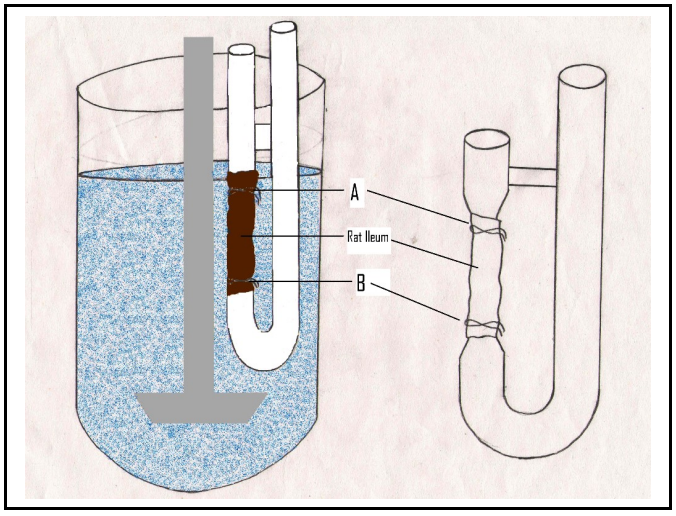

Figure 2. Figure shows apparatus for the ex vivo study using rat ileum

After placement of ileum on the perfusion apparatus, the apparatus was dipped into the dissolution media (Phosphate buffer, pH 7.4) in the dissolution vessel and the dissolution study was performed at 50 r/min and 37 ± 0.5 ˚C. The data of dissolution study was recorded in table 7.

Statistical analysis: Each value was expressed as the mean of three consecutive observations. For group comparisons, the one-way layout ANOVA with duplication was applied. Significant differences of the mean values were evaluated by student's unpaired t-test. p value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Multiple emulsions were prepared by two step emulsification process: a) Preparation of primary emulsification; b) Secondary emulsification. During primary emulsification different surfactants like Span20, Span60, Span80 and Span85 were tried at 10% concentration while keeping all other parameters like, stirring speed (5000 r/min), stirring time (5 min) and volume fraction of internal phase:external phase (30:70) constant.

Table 1. Table shows data for selection of primary emulsifier

Batches	Types of Surfactant	Concentration of Surfactant	Stability (globule size, µm) Initial	after 4 weeks	% Entrapment Efficiency
B_1	Span 20	10%	Not formed	-	-
B_2	Span 60	10%	5.41	5.79	95.10%
B_3	Span 80	10%	8.94	12.87	84.36%
B_4	Span 85	10%	13.82	24.23	53.93%

n=3; values shown above are mean of three observations

Data recorded in table 1 clearly revealed that Span 20 did not form emulsion, Span60, Span 80 and Span 85 formed emulsion with good drug entrapment. But based on stability Span 60 was selected as a primary emulsifier as it gave highest entrapment efficiency and very good stability. Various proportions of Span60 were tried and data was recorded in table 2.

Table 2. Table shows data for optimization of concentration of primary emulsifier

Batches	Concentration of Surfactant	Stability (globule size, µm)		% Entrapment Efficiency
		Initial	after 4 weeks	
B_5	5%	9.25	12.86	45.75%
B_6	8%	7.81	9.55	62.32%
B_7	12%	5.40	6.53	83.11%
B_2	10%	5.41	6.52	95.10%

n=3; values shown above are mean of three observations

It was concluded from table 2 that Span60 formed emulsion with very good stability at 10% and 12% but the entrapment efficiency was higher at 10%; hence this batch was carried for further optimization of speed and time of stirring as recorded in table3.

Table 3. Table shows data for optimization of speed and time of stirring for primary emulsification

Batches	Speed of Stirring (r/min)	Time of Stirring (min)	Stability (globule size, µm)		% Entrapment Efficiency
			Initial	after 4 weeks	
B_8	4000	5	6.14	7.97	91.83%
B_9	6000	5	5.03	6.89	92.33%
B_2	5000	5	5.41	6.52	95.10%
B_{10}	5000	7	4.38	5.22	93.28%
B_{11}	5000	10	3.12	3.49	97.54%
B_{12}	5000	12	3.10	3.81	94.48%

n=3; values shown above are mean of three observations

In all previous batches, speed of stirring and time of stirring were kept constant at 5000 r/min and 5 min Based on the entrapment efficiency and stability data recorded table 3, 5000 r/min was selected as optimized speed of stirring and 10 min as optimum time of stirring. This batch (Batch B_{11}) was further exposed to different volume fraction ratio of internal phase to external phase and data was recorded in table 4.

Table 4. Table shows data for optimization of volume fraction in primary emulsion

Batches	Phase Volume Ratio	Volume of IP & EP*	Stability (globule size, µm)		% Entrapment Efficiency
			Initial	after 4 weeks	
B_{11}	30:70	12ml:28ml	3.12	3.49	97.54%
B_{13}	40:60	16ml:24ml	4.98	7.64	85.05%
B_{14}	25:75	10ml:30ml	2.97	3.25	88.39%

n=3; values shown above are mean of three observations

Based on the observation of table 4, Batch B_{11} was selected as optimized primary emulsion and was carried forward for optimization of secondary emulsification.

During secondary emulsification different non ionic surfactants like, Tween20, Tween60 and Tween80 were tried as secondary emulsifier at 10% concentration keeping all the other parameters like, stirring speed (1000 r/min), stirring time (10 min) and volume fraction of internal phase:external phase (30:70) constant.

Table 5. Table shows data for selection of secondary emulsifier

Batches	Types of Surfactant	Concentration of Surfactant	Stability (globule size, µm)		% Entrapment Efficiency
			Initial	after 4 weeks	
B_{15}	Tween 65	10%	20.74	22.32	70.13%
B_{16}	Tween 80	10%	18.12	21.81	71.21%
B_{17}	Tween 85	10%	18.49	21.55	81.12%

n=3; values shown above are mean of three observations

Tween 80 at 10% concentration, showing better drug entrapment and good stability as secondary emulsifier hence, was selected as secondary emulsifier and was tried with higher concentrations to get maximum drug entrapment with enhanced stability.

Table 6. Table shows data for optimization of concentration of secondary emulsifier

Batches	Concentration of (Tween 85)	Stability (globule size, µm)		% Eentr/minent Efficiency
		Initial	after 4 weeks	
B_{17}	10 %	18.49	21.55	81.12%
B_{18}	12 %	14.58	19.53	83.25%
B_{19}	14 %	13.89	17.12	87.14%
B_{20}	16 %	13.42	16.83	88.10%
B_{21}	18 %	12.23	16.24	85.73%

n=3; values shown above are mean of three observations

Table 6 revealed that Tween 80 shows maximum drug entrapment and good stability at 16% concentration. After optimization of secondary emulsifier, speed of stirring and time of stirring were optimized as recorded in table 7.

Table 7. Table shows data for optimization of speed and time of rotation for secondary emulsification

Batches	Speed of Stirring (r/min)	Time of Stirring (min)	Stability (globule size, µm)		% Entrapment Efficiency
			Initial	after 4 weeks	
B_{20}	1000	10	13.42	16.83	88.10%
B_{22}	1000	7	14.98	17.46	85.51%
B_{23}	1000	12	10.51	13.85	88.39%
B_{24}	1200	5	11.28	15.87	85.48%
B_{25}	1200	10	9.54	11.42	89.57%
B_{26}	1500	5	8.24	9.87	89.57%
B_{27}	1500	7	6.35	7.05	91.42%
B_{28}	1500	10	6.89	8.56	88.81%

n=3; values shown above are mean of three observations

1000 r/min stirring speed was showing good drug entrapment but not sufficient even with longer duration of time and produced larger globules with questionable stability. Hence higher stirring speed was tried with variable time duration as recorded in table 7, where 1500 r/min & 7 min were found to be optimum speed and time of stirring respectively. After optimization of different variables, volume fraction was also optimized and recorded in table 8.

Table 8. Table shows data for optimization of volume fraction in secondary emulsion

Batches	Phase Volume Ratio	Volume of PE & SE*	Stability (globule size, µm)		% Entrapment Efficiency
			Initial	after 4 weeks	
B_{27}	30:70	30ml:70ml	6.35	7.04	91.42%
B_{29}	40:60	40ml:60ml	8.89	10.87	94.25%
B_{30}	25:75	25ml:75ml	5.82	6.80	74.91%

n=3; values shown above are mean of three observations; PE & SE=primary emulsion & secondary emulsion

Increase in volume fraction of internal phase led to increase in drug entrapment but the stability was compromised, and with decreased volume fraction of internal phase stability was increased but drug entrapment was compromised. Hence volume fraction of internal phase:external phase ratio was kept at 30:70.

After optimization of all the above mentioned variables, the optimized batch was prepared repeatedly to check the reproducibility. Optimized values of different variables were summarized in a table below.

Table 9. Table shows final batch with optimized parameters

PARAMETER	OPTIMIZED VALUE	PARAMETER	OPTIMIZED VALUE
Primary emulsification		**Secondary emulsification**	
Internal phase (Aqueous phase)		*Internal phase (Primary emulsion)*	
Drug	24 mg	Primary emulsion	30 ml
Water…up to	12 ml		
External phase (Oil phase)		*External phase* (Aqueous phase)	
Span60 (10 % W/V)	4 gm	Tween80 (16 %W/V)	16 gm
Drug	56 mg	Drug	140 mg
Liquid paraffin…up to	28 ml	Water… up to	70 ml
Process parameters		*Process parameters*	
Speed of rotation	5000 r/min	Speed of rotation	1500 r/min
Time of rotation	10 min	Time of rotation	7 min

Note= each 15 ml of multiple emulsion contains 30 mg of drug

The optimized batch was evaluated for in vitro & ex vivo dissolution study and compared with marketed tablet under same experimental conditions and the data was recorded as a chart in figures below.

Table 1. Table shows data of in vitro and ex vivo dissolution study

Time	in vitro dissolution study		ex vivo dissolution study	
(min)	CPR of ME	CPR of Tablet	CPR of ME	CPR of Suspension
0	0	0	0	0
15	05.67	04.34	05.10	03.70
30	11.83	09.05	09.5	07.00
45	16.23	11.85	13.23	09.68
60	20.84	15.56	16.11	11.69
120	47.20	35.11	33.43	24.34
180	70.60	53.54	50.51	38.29
240	94.43	65.31	68.79	52.56
300	100.40	75.26	99.81	70.25

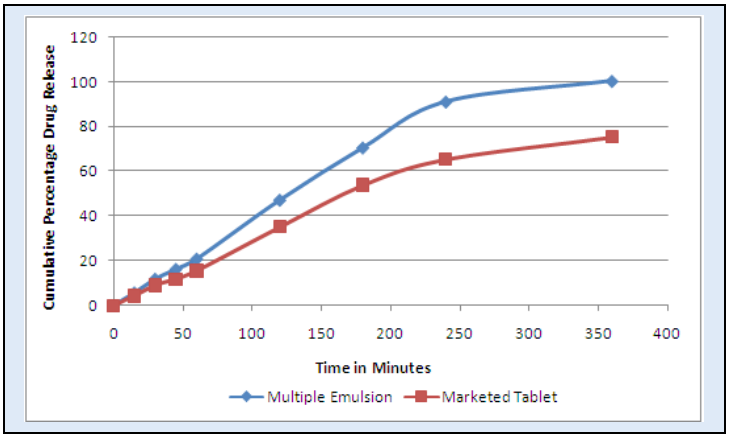

Figure 3. Comparison of in vitro dissolution study of Atorvastatin from Multiple emulsion and Tablet

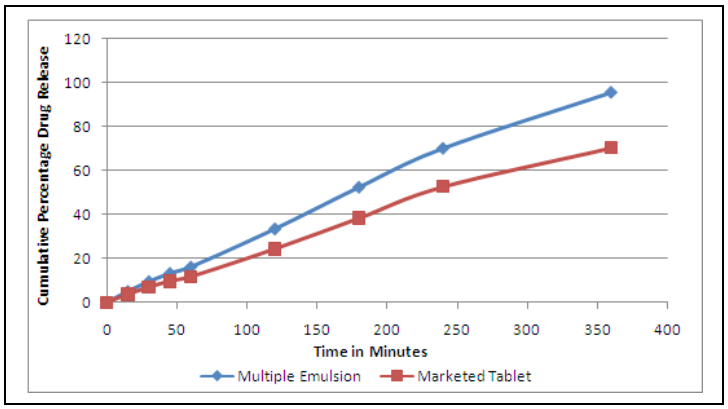

Figure 4. . Comparison of ex vivo dissolution study of Atorvastatin from Multiple emulsion and Tablet

The figure 3 suggests nearly zero order release profile for multiple emulsion where as tablet shows similar profile during initial half time but becomes saturated in later half. In ex vivo dissolution study, nearly zero release profile was observed from both multiple emulsion and tablets. Comparison of data by F test reveals that multiple emulsion shows significantly higher release of drug as compared to tablets.

CONCLUSION

In the present study, multiple emulsion of Atorvastatin was prepared using different non ionic surfactants by two step emulsification. The main purpose was to develop stable multiple emulsion with higher entrapment efficiency. The study revealed that multiple emulsion can be optimized for good stability and higher entrapment efficiency by optimizing different formulation variables like type & proportion of primary & secondary emulsifier and phase volume ration of internal phase:external phase; and process variables like speed & time of stirring during primary & secondary emulsification.

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