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Research Article

FORMULATION AND EVALUATION OF NIFEDIPINE MULTIPLE EMULSIONS

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ABSTRACT

Multiple emulsions are used to enhance bioavailability of various drugs and as a prolong drug delivery system. Multiple emulsions often stabilized using a combination of hydrophilic and hydrophobic surfactants. The ratio of these surfactants is important in achieving stable multiple emulsions. In this study, multiple emulsion of Nifedipine was prepared by two step emulsification method using different surfactants such as tweens and spans. The multiple emulsion is evaluated for stability, percentage drug entrapment and in -vitro drug release. FT-IR studies indicated that there is no chemical interaction in the mixtures. The in-vitro dissolution studies have shown that F3 formulation has higher release profile as compare to other formulation. As the concentration of span 40 increases, the release profile of the formulation was improved. Hence it is concluded that the multiple emulsions are useful for the improvement of dissolution rate and thereby oral bioavailability of poorly water soluble drug Nifedipine.

Keywords: Bioavailability, surfactant, stability, multiple emulsions, Nifedipine, Span 40, etc.

INTRODUCTION

Multiple emulsions are defined as emulsions in which both types of emulsions i.e. water-in –oil (w/o) and oil –in-water (o/w) exist simultaneously.Multiple emulsion require at least two emulsifiers to be present in the system when prepare using the two step method, one that has a low hydrophilic- lipophilic balance value to stabilize the primary w/o emulsion and one that has a high HLB value to stabilize the secondary/w emulsion. The low HLB surfactants dominantly hydrophobic and is added to the oil phase.[1].

Multiple emulsions finds wide range of applications in controlled or sustained drug delivery, targeted delivery, taste masking, bioavailability enhancement, enzyme immobilization etc.[2,3]Numerous investigations were to describe the effects of different food-grade components (e.g. lipid phases, emulsifiers, electrolytes, biopolymers, sugars) on the stability of multiple emulsions (W/O/W and O/W/O). In addition to the emulsion composition, the stability of such systems also depends heavily on the emulsion matrix, influenced by the dispersing methods used[4,5]. Multiple emulsions may prove useful for the creation of food emulsions with improved physicochemical properties or for the development of novel delivery systems[6].

For the application of W/O/W emulsions as drug delivery systems it is important to prepare a very stable W/O/W emulsion in which countless submicron water droplets are encapsulated. Multiple emulsion stability is correlated with the interfacial film strength (measured by interfacial elasticity) of the hydrophobic surfactant at the mineral oil/external continuous aqueous phase interface. The formation of the meta stable dimpled structures and the long-term stability of the multiple emulsions were dependent on the osmotic pressure of the inner droplets.[7,8]

Micro fluidic technique to generate highly controlled multiple emulsions. The high degree of control and scalability afforded by this method makes it a flexible and promising route for engineering designer emulsions and

microcapsules with multiphase structures.[9]Moreover, its generality will enable fabrication of novel materials containing complex internal structures.[10]

A stable multiple emulsions containing a skin anti-aging agent and using paraffin oil. Vitamin C, was incorporated into the inner aqueous phase of water-in-oil-in-water (w/o/w) multiple emulsion at a concentration of 1%.[11]

Most cardiovascular events are attributed to high blood pressure. High blood pressure is quantitavily the largest single risk factor for premature death, henceantihypertensive therapy considerably reduces the risk of developing cardiovascular complications that cause a high mortality rate. Nifedipine is a new potent, highly selective and orally active antihypertensive drug belonging to the family of angiotensin II type 1 receptors antagonists. Nifedipine inhibits angiotensin II receptors, there by relaxing blood vessels and causing them to widen, which lowers blood pressures and increase blood flow. Nifedipine is a calcium channel blocking agent, it is widely used in the treatment of angina pectoris and in the management of hypertension. Chemically it is 4-(2-nitrophenyl)-2,6-dimethyl-3,5-dicarbomethoxy- 1,4-dihydropyridine.

Nifedipine has an oral bioavailability of only about 25% with a wide range of 25-40% in humans with large inter and intra subject variability. Nifedipine also has pH dependent solubility whereby it ranges from very slightly soluble in an acidic environment to soluble in a neutral environment of gastro intestinal tract. The permeability of Nifedipine is low under and also pH dependent where it decreases as environmental pH increases from acidic to neutral pH values in the gastro intestinal tract .As a result of these complex biopharmaceutical properties, development of a more releasable and bioavailable dosage form of Nifedipine with less inter and intra subject variability is challenging.[12] It is reported to have a short biological half-life of 2 to 5 hrs. It is lipophilic in nature with log Pvalue of 3 in the n-octanol/water system. Hence Nifedipine was chosen as the drugof choice in the present investigation. Accordingly, multiple emulsions dosage formulations of Nifedipine, which has enhanced release and bioavailability properties with less, inter and intra subject variability would be desirable.[13] Thus, the aim of the present study is to formulate and evaluate the multiple emulsion of Nifedipine.

MATERIALS AND METHODS

Nifedipine drug, Span 40, Tween 80, Liquid paraffin, buffer solution (pH-6.8) Distilled water were used to prepare multiple emulsion.

Methodology

A) Drug related studies

Physical appearance

The drug (Nifedipine) powder was examined for its organoleptic properties like colour and odour.

Solubility study

The sample was qualitatively tested for its solubility in various solvents. It was determined by taking 10mg of drug sample in 10ml of solvent as water, methanol, ethanol, acetonitrile, p^H 6.8 in small test tubes and well solubilized by shaking.

Melting point

The melting point was determined by the capillary method using digital melting point apparatus. The capillary tube was fused and filled by pressing the open end gently into pure drug sample and packed by tapping the bottom of the capillary on a hard surface so that the drug packed down into the bottom of the tube, the tube was placed into the slot of the apparatus, the apparatus was started and the temperature was noted at which the drug melt.

Preparation of calibration curves

Nifedipine solution was scanned in the u.v range of 200 – 400 nm using systronic double beam u.v visible spectrophotometer.

Determination of wavelength of maximum absorbance (λ max)

10mg of drug was weighed accurately and transferred to 10ml of volumetric flask. Then phosphate buffer 6.8 (suitable solvent) was added to dissolve the drug completely. The volume was made up to 10ml with solvent. The prepared sample was 1000 µg/ml. 1ml of above solution was then transferred to another 100ml volumetric flask and diluted it up to the mark with phosphate buffer 6.8. This sample was 10µg/ml.

Preparation of calibration curve of Nifedipine

The calibration curve was plotted between the concentration and absorbance. The concentrations ranging from 1-25ug/ml were used to construct calibration curve.

Determination of partition coefficient

25mg of drug and 25ml of distilled water and 25ml of methanol was taken in the separating funnel. The separating funnel was shaken for 2hours in for equilibration. And was allowed to stand for 1hour, then the two phases were separated and the amount of the drug in aqueous phase as well as in lipophilic phase was analyzed spectrophotometrically. The partition coefficient of the drug in both the phases was calculated by using formula

Partition coefficient, K=

$$\frac{\text{amount of drug in the organic layer}}{\text{amountofdruginaqueouslayer}}$$

B) Method of preparation

Multiple emulsion were prepared by two step emulsification process 1) Preparation of primary emulsification 2) Secondary emulsification.

Primary emulsification

10ml of distilled water containing 25mg of drug was gradually added to 14ml of oil phase containing primary emulsifier (span 40) and 25mg of drug with continuous stirring at 5000 rpm for 5minutes. It gives the primary emulsion.

Secondary emulsification

20ml of viscous primary emulsion was emulsified further with an external aqueous phase containing secondary emulsifier (tween 80) and 50mg drug with continuous stirring at 1000rpm for 10 minutes. All the formulations were prepared by following the same procedure. Effect of primary emulsifier was observed by evaluating several formulations (Table 1).

C) Evaluation

1. Microscopic study

Multiple emulsions were analyzed under microscope with 100X magnification, using immersion oil.In this study, globule sizes of the multiple emulsions prepared was observed for 28 days.

2. Entrapment efficiency

Freshly prepared W/O/W multiple emulsions was taken and immediately centrifuged at 4000rpm for 10minutes. Then 1ml of the aqueous phase (the lower layer) was withdrawn through 2ml hypodermic syringe and diluted properly with phosphate buffer 6.8. The solution was filtered with a Millipore filter (0.22mm in pore size) and drug content was analyzed on U.V spectrophotometer at 370 nm. The encapsulation efficiency was determined by following equation

$$\%EE = \frac{[\text{total drug in corporated} - \text{free drug}]}{totaldrug} \times 100$$

Stability tests

Stability tests were performed at different storage conditions for both primary and multiple emulsions. The tests were performed on samples kept at 8 ± 0.1 °c(in refrigerator), 25±0.1°c(in oven), 40±0.1°c(in oven)and 40±0.1°c at 75% relative humidity(RH) (in stability cabin).

Organoleptic characteristics

Organoleptic characteristics (i.e. colour, liquefaction and phase separation) of both primary and multiple emulsions kept at different storage conditions were noted at various intervals , i.e. 0h, 1h, 1 day, 3 days, 7days, 14 days, 21days and 28 days for 28 days.

P^H determination

The p^H value of the freshly prepared emulsions and the emulsions kept at different conditions were determined by a digital p^H meter. P^H measurements were repeated for multiple emulsions after 1, 3, 7, 14, 21, 28 days for preparation.

FT-IR Studies

FT-IR was performed on TENSOR 27T-IR Spectrometer with 0.4cm^{-1} Resolution [Apodised] KBrBeamsplitter (BRUKER OPTICS, Germany). Nifedipine, Tween 80, Span 40 and a mixture of Nifedipine, Tween 80, and Span 40 in a ratio of 1:1:1:1 was analyzed.

In vitro drug release study

The in vitro drug release study was carried out on a simple dissolution cell using cellophane membrane (thickness – 200mm, breaking strength -2.7kgf/cm). Initially, the cellophane membrane was soaked in distilled water for 6 hours, washed 3 to 4 times by changing distilled water, then immersed in 5% v/v glycerol solution for at least 60minutes and washed finally with 5 portions of distilled water. 15ml freshly prepared multiple emulsion was added to donor chamber , made up of a hollow glass tube (2.5 cm in diameter and 10cm in length) and membrane was tied on bottom end of the tube with a nylon string. This tube was dipped into 250 ml vessel containing 100ml of PBS p^H 6.8 and was stirred at 100 rpm on a magnetic stirrer and maintained at 37°c, which acted as receiving chamber. Aliquots of 1ml were collected from receiving chamber at pre-determinedtime intervals and the drug content was determined on UV spectrophotometer at 247.6nm after suitable dilution.

RESULTS AND DISCUSSION

The drug (Nifedipine) powder was examined for its organoleptic properties like colour and odour. And it was observed that Nifedipine was whitish crystalline powder .The sample was qualitatively tested for its solubility in various solvents. It was determined by taking 10mg of drug sample in 10ml of solvent as water, methanol, ethanol, acetonitrile, PH buffer 6.8 in small test tubes and well solubilized by shaking. Solubility study in different solvents at room temperature revealed that it is soluble in, ethanol, methanol, phosphate buffer 6.8 and it is sparingly soluble in water (I.P 2003) (Table 2). Melting point of Nifedipine was found at 172±2°C. Partition coefficient value of Nifedipine was found to be 3.17 in Octanol/water system.

Preparation of calibration curve

Nifedipine solution was scanned in the u.v range of 200-400nm using systronic u.v-visible spectrophotometer. The Spectrophotometric method of analysis of Nifedipine at λmax 370 nm was found to be reproducible and highly sensitive. The standard curves of Nifedipine were prepared in methanol and phosphate buffer solution (PH 6.8), at λmax 370 nm. The data were regressed to obtain the straight line. The correlation coefficient greater than 0.99 was observed in all the cases, which indicated that, the drug follows Beer-Lambert's law in the concentration range of 10mg/10ml (Table 3).

Preparation of calibration curve of Nifedipine in phosphate buffer 6.8

The standard solutions of Nifedipine was subsequently diluted with PH 6.8 phosphate buffer containing 1% sodium lauryl sulphate to obtain series of dilutions containing 5,10,15,20 and 25µg/ml of Nifedipine in solution. The absorbance of the above solution measured in U.V spectrophotometer. The absorbance values were plotted against concentration of drug (Figure 2).

Evaluation

1. Organoleptic Properties

Colour

Freshly prepared primary emulsion was yellowish white in colour there was no change in colour at different storage conditions. This shows the primary emulsion was stable at different storage conditions up to 28days. Freshly prepared multiple emulsions was yellowish white in colour.

Liquefaction

No liquefaction was observed in the primary emulsions at all storage conditions. For the multiple emulsions while no liquefaction observed in the sample kept at 8°c (in refrigerator) and 25°c (in oven) during 28days slight liquefaction was observed in the samples kept at 40°c in oven 28th day. Liquefaction is the sign of instability it may be attributed to the passage of water from the internal phase to external phase.

Phase separation

In the case of primary emulsions, no phase separation was observed in any of samples. This indicates that primary emulsion was stable at all storage conditions at 28days. For the multiple emulsions, no phase separation was seen in the samples kept at all storage conditions, except slight phase separation beginning on 28thday (Table 4).

2. Microscopic examination

Upon microscopic analyses, multiple emulsion systems were photographed (100X) randomly and some of those photographs are given. (Figure.3). Globules sizes of the multiple emulsions kept at different storage conditions were observed.Light microscope was used in this study. The increase or decrease in globule sizes indicates instability.

3. pH values

The pH determination was carried out on digital pH meter. The multiple emulsions has a neutral pH, which was desirable for administration. Also they do not produce irritation(ranged from 7.0 to 7.3 at different storage conditions).

4. Entrapment efficiency

The entrapment efficiency of all the three formulations was good and comparable. (Figure.4).

5. FT-IR spectra

The FTIR spectrum of pure Nifedipine (Fig. 4) showed the characteristic peaks at wave numbers of3320 cm–1 due to >N–H stretching (>N– H of pyridine), at 1680 cm–1 due to (N=O)asymmetric stretching (Aryl-NO2), at 1220 cm–1 is due to (N=O)2 symmetric stretching (Aryl-NO2) and at 1520 cm–1 due to asymmetric carboxylate anion confirming the drug structure. Similarly, in the mixture of Nifedipine,Tween 80, and Span 40 the resulting infrared spectra had the characteristic peaks of Nifedipine.This indicated that there is no chemical interaction in the mixtures (Figure 5).

6. In vitro drug release

The result indicates that F3 formulation has higher release profile as compare to other formulations (Figure 6). As the concentration of span 40 increases, the release profile of the formulation was increased (Table 5).

CONCLUSION

Multiple emulsions have been proposed to have numerous uses including their use for enhancement of bioavailability or as a prolonged drug delivery system.Nifedipine has low bioavailability. Few studies have been reported for enhancement of Bioavailability of poorly water soluble drugs by formulating as multiple emulsions. The objective of present work is to development and evaluation of multiple emulsion of Nifedipine for oral drug delivery.The investigations presented lead us to conclude that the multiple emulsions of Nifedipine is prepared using liquid paraffin and non-ionic surfactants like Tween80, different concentrations of span40 by two step emulsification methods. Freshly prepared primary emulsion was yellowishwhite in colour, liquefaction, phase separation are at various storage conditions were studied. Organoleptic characteristics of the primary and multiple emulsions formulated are reported. The microscopical images of various formulations are reported. The multiple emulsion of Nifedipine can be used to reduce dosefrequency, decrease side effect and improved patient compliance.

Table 1: List of various ingredients

Formulation	Trial batch	Drug (mg)	span 40(ml)	Tween 80 (ml)	Liquid paraffin (ml)	phosphate buffer (6.8)(ml)
F1	1	100	0.5	1	14	15
F2	2	100	1	1	14	15
F3	3	100	1.5	1	14	15

Table 2: Solubility of Nifedipine in different solvents

S.No	Solvent	Solubility
1	Phosphate buffer 6.8	soluble
2	acetone	soluble
3	Ethanol	sparingly soluble
4	chloroform	soluble
5	Water	insoluble

Table 3: Standard Curve of Nifedipine in Phosphate buffer (pH 6.8)

S.No	Drug Conc. (µg/ml)	absorbance	Statistical parameters
1	5	0.215	Correlation coefficient = 0.995 Slope m = 0.018 Intercept c = -0.036 Equation of Line y= 0.018 x -0.036
2	10	0.395	
3	15	0.563	
4	20	0.752	
5	25	0.874	

Table 4: Organoleptic properties of multiple emulsions

Time	Liquefaction			Color			Phase separation			Centrifugation		
	A	B	C	A	B	C	A	B	C	A	B	C
0hr	-	-	-	yw	yw	yw	-	-	-	-	-	-
1hr	-	-	-	yw	yw	yw	-	-	-	-	-	-
24hr	-	-	-	yw	yw	yw	-	-	-	-	-	-
72hr	-	-	-	yw	yw	yw	-	-	-	-	-	-
7days	-	-	-	yw	yw	yw	-	-	-	-	-	-
14days	-	-	-	yw	yw	yw	-	+	-	+	+	+
21days	-	-	-	yw	yw	yw	-	+	+	+	+	+
28days	-	+	+	yw	yw	yw	-	+	+	+	+	+

= no change; + =slight change; yw =yellowish white; ++ =no more change;
A=8°C; B=25°C; C=40°C (in oven) (n=3)

Table 5: Invitro drug release of multiple emulsions

Time	F1	F2	F3
0	0	0	0
1	10.12	13.34	15.33
2	20.55	24.85	23.09
3	28.71	34.08	35.91
4	37.19	45.04	46.04
5	46.77	54.36	56.12
6	59.89	64.84	66.41
7	64.12	73.15	74.84
8	71.34	79.19	84.93

(a) (b)

Fig. 1: Preparation of multiple emulsions

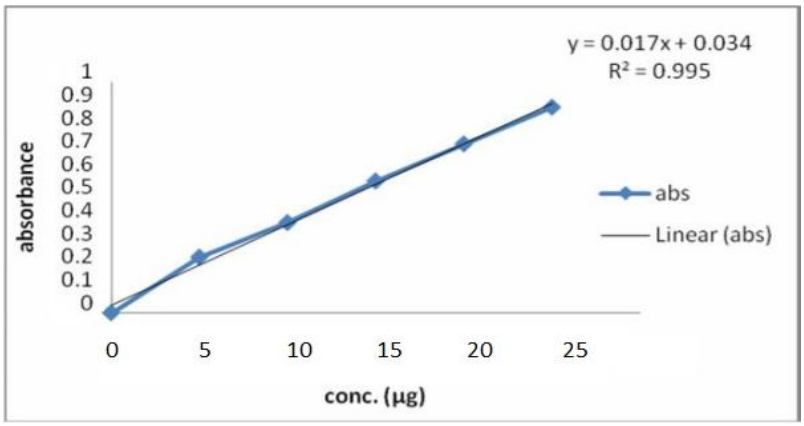
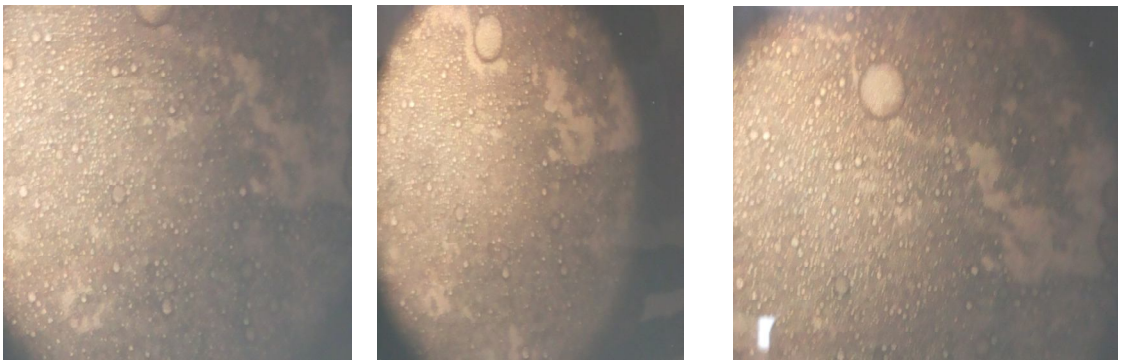


Fig. 2: calibration curve of Nifedipine in phosphate buffer 6.8



(a) (b) (C)

Fig. 3: Microscopic images of multiple emulsions

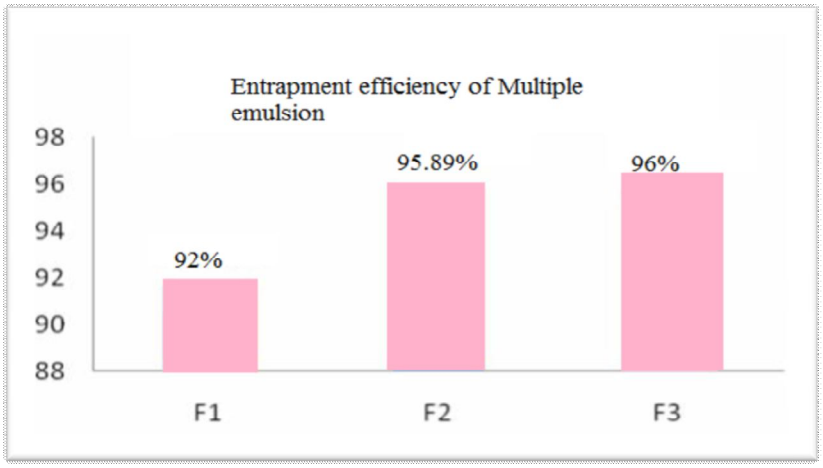


Fig. 4: Entrapment efficiency of multiple emulsions

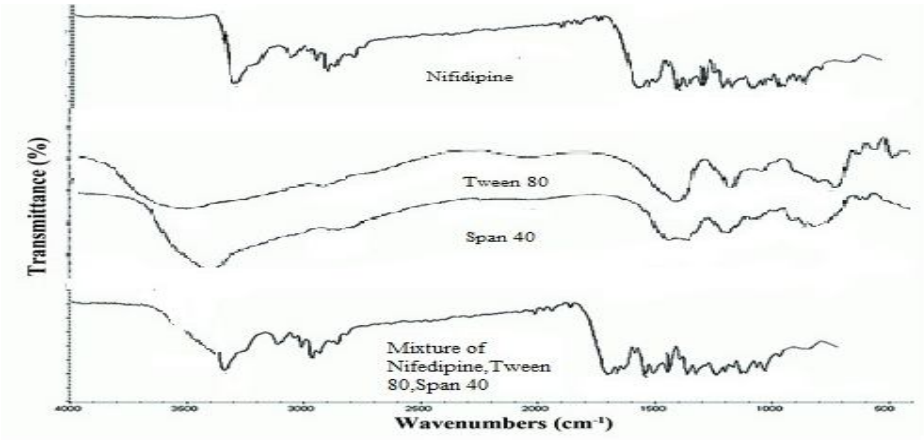


Fig. 5: FTIR spectra of (a) Nifedipine, (b) Tween 80, (c) Span 40, (d) Nifedipine, Tween 80, Span 40 mixture in the ratio of 1:1:1

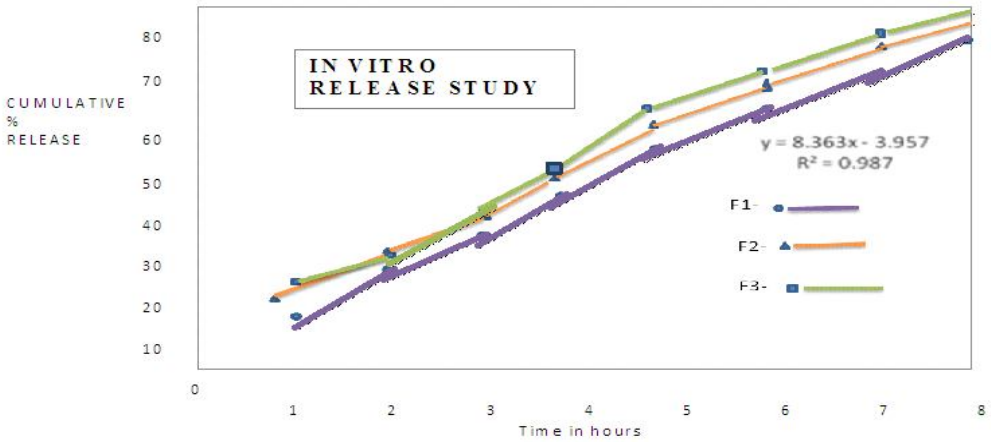


Fig. 6: In-vitro drug release of multiple emulsions

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