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# FORMULATION AND EVALUATION OF MULTIPLE EMULSION OF NADALOL

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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. Nadalol 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 Nadalol by two step emulsification using different nonionic surfactants, Tweens & Spans, and evaluate for stability, percentage drug entrapment, in vitro drug release. The study concluded that stable multiple emulsion with high entrapment efficiency can be prepared by two step emulsification method using Spans40, 60, 80 as primary emulsifier and Tween80 as secondary emulsifier.

**KEYWORDS:** Multiple emulsions, Non-ionic surfactants, Bioavailability, Stability.

## 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 simultaneously1. They combine the properties of both w/o and o/w emulsions. These have been described as heterogeneous systems of one immiscible liquid dispersed in another in the form of droplets, which usually have diameters greater than 1 μm. These two liquids forming a system are characterized by their low thermodynamic stability. Multiple emulsions are very complex systems as the drops of dispersed phase themselves contain even smaller droplets, which normally consist of a liquid miscible and in most cases identical with the continuous phase. Both hydrophilic and lipophilic emulsifiers are used for the formation of multiple emulsions. Multiple emulsions were determined to be promising in many fields, particularly in pharmaceutics and in separation science. Their potential biopharmaceutical applications include their use as adjuvant vaccines, as prolonged drug delivery systems5-8, as sorbent reservoirs in drug overdose treatments and in mobilization of enzymes. Multiple emulsions were also investigated for cosmetics for their potential advantages of prolonged release of active agent, incorporation of incompatible materials and protection of active ingredients by dispersion in internal phase. Also water-in-oil-in-water (W/O/W) multiple emulsions are emulsion systems where small water droplets are en-trapped within larger oil droplets that in turn are dispersed in a continuous water phase. Because of the presence of a reservoir phase inside droplets of another phase that can be used to prolong release of active ingredients. Multiple W/O/W emulsions contain both W/O and O/W simple emulsions and require at least 2 emulsifiers to be present in the system when The development of multiple emulsion dosage formulation of certain active ingredients is challenging. When formulating multiple emulsions dosage formulations, the objective is to provide an increased release of Nadalol and increased oral bioavailability of Nadalol in patient as compared to known solid oral dosage forms of Nadalol. Development of multiple emulsions dosage formulation that have improved bioavailability to the known oral dosage forms of Nadalol is challenging due to the multiplicity of challenges arising from pharmacokinetic aspects of oral drug delivery. Nadalol has an oral bioavailability of only about 25% with a wide range of 25-40% in humans with large inter- and intra-subject variability's. Nadalol also has pH dependent solubility whereby it ranges from very slightly soluble in an acidic environment to soluble in a neutral environment of gastrointestinal tract. The permeability of Nadalol is low 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 Nadalol with less inter and intra subject variability is challenging. Accordingly multiple emulsions dosage formulation of Nadalol which has enhanced release and bioavailability properties with less inter and intra subject variability would be desirable. Thus the aim of the present study is to "formulate and evaluate the multiple emulsion of Nadalol".

## MATERIALS AND METHODS

Span 40 purchased from Hymedia laboratories, span 60 and span 80 from Merch laboratories, and Tween 80 is also from Merch laboratories, heavy paraffin oil from Rankem. Nadalol drug from span 40, 60, 80, and Tween are used as an surfactant Emulsifying agent, nonionic surfactant. Paraffin oil is used as an emollient, lubricant.

### Method of Preparation
Multiple emulsions were prepared by two step emulsification process: a) Preparation of primary emulsification; b) Secondary emulsification 26-28.

**Primary emulsification**: 10 ml of distilled water containing 25 mg of drug was gradually added to 14 ml of oil phase containing primary emulsifier (Span40, Span60, and Span 80) and 25mg of drug with continuous stirring at 5000 rpm for 5 minutes. It gives the primary emulsion.

**Secondary emulsification**: 20 ml of viscous primary emulsion was emulsified further with an external aqueous phase containing secondary emulsifier (Tween80) and 50 mg drug with continuous stirring at 1000 rpm for 10 min. All the formulations were prepared by following the same procedure. Effect of primary emulsifier was observed by evaluating several formulations.

**Physical appearance:** The drug (Nadolol) 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 10 mg of drug sample in 10 ml of solvent as water, methanol, ethanol, acetonitrile, pH buffer 6.8 in small test tubes and well solubilized by shaking.

**Melting point determination:** 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. When the drug was packed 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.

### Determination of partition coefficient
25mg of drug and 25 ml of distilled water and 25 ml of methanol was taen in the separating funnel the separating funnel were shaken for 2 hrs in a wrist action shaker for equilibration. And was allowed to stand for 1hrs, then the two phases were separated and the amount of the drug in aqueous phase as well as in lipophilic phase was analysed spectrophotometrically. The partition coefficient of the drug in both the phases was calculated by using formula:

### Fourier-Transform Infra Red spectroscopy (FTIR)
The IR spectrum of drug substance was authenticated using IR spectroscopy. The presence of characteristic peaks associated with specific structural characteristics of the drug molecule was noted.

### Globule size
In this study, globule sizes of the multiple emulsions prepared were determined using light microscope fitted with a digital camera for the freshly prepared emulsions and for the emulsions
kept at different conditions for 28 days.

### Entrapment efficiency 30
Percentage Entrapment Efficiency (% EE) was determined by taking freshly prepared W/O/W multiple emulsions and immediately centrifuged at 4000 rpm for 10 min. Then 1ml of the aqueous phase (the lower layer) was precisely withdrawn through 2 ml hypodermic syringe and diluted properly with phosphate buffer 6.8. The solution was filtered with a Millipore filter (0.22 mm in pore size) and drug content was analyzed on UV spectrophotometer at 267.6 nm.

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

### Microscopic tests
Multiple emulsions were analyzed under the microscope to confirm the multiple characters. A drop of multiple emulsion was placed on the glass slide, diluted with water and covered by a glass cover. A drop of immersion oil was placed on the cover slide and observed under the microscope.

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

**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-2.7 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 250 ml vessel containing 100 ml of PBS pH 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 predetermined time intervals and the drug content was determined on UV spectrophotometer at 267.6 nm after suitable dilution.

## RESULTS AND DISCUSSION
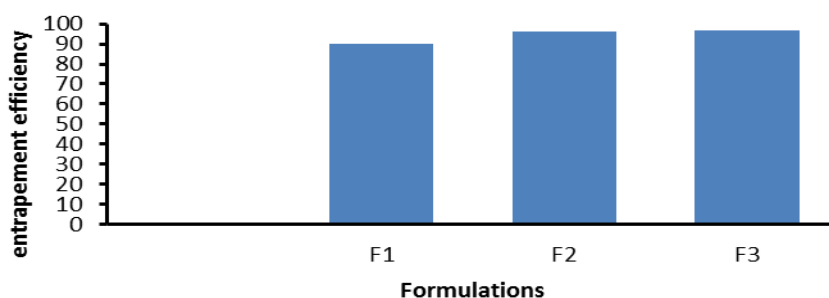
**Table 1: List of various ingredients.**

| Formulation | X1 | X2 | X3 | X4 | X5 | X6 | X7 |
|---|---|---|---|---|---|---|---|
| F1 | 100 | 0.6 | --- | -- | 1 | 40 | 30 |
| F2 | 100 | -- | 0.6 | -- | 1 | 40 | 30 |
| F3 | 100 | -- | -- | 0.6 | 1 | 40 | 30 |

**Table 2: Solubility of Nadolol in different solvents.**

| S.No | Solvents | Solubility |
|---|---|---|
| 1 | Phosphate buffer 6.8 | Soluble |
| 2 | Methanol | Freely Soluble |
| 3 | Ethanol | Freely Soluble |
| 4 | Acetonitrile | Soluble |
| 5 | Water | Sparingly Soluble |

**Table 3: Partition coefficient values of Nadolol.**

| S.No | Solvent system | Partition Coefficient |
|---|---|---|
| 1 | n-Octanol/Distilled water | 4.4 |



**Figure 1: showing entrapement efficiency.**

**Table 4: Organoleptic characteristics Organoleptic characteristics of the primary and multiple emulsions formulated are presented in Table.**

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

- = No change; + = slight change; W = white; YW = yellowish-white; ++ = more change A = 8oC; B = 250C; C= 400C (in oven) (n = 3).
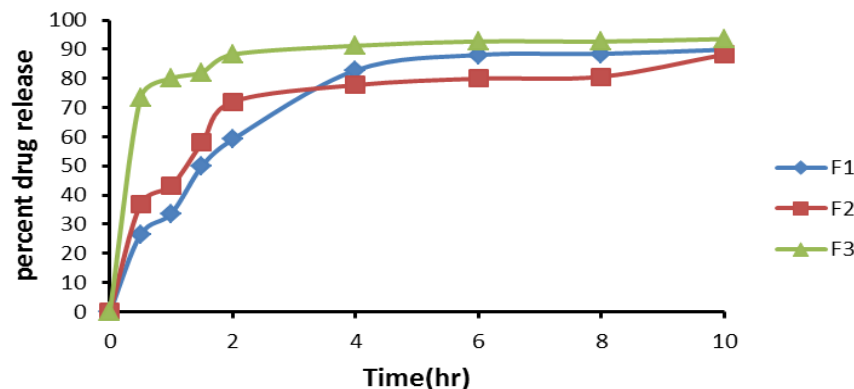
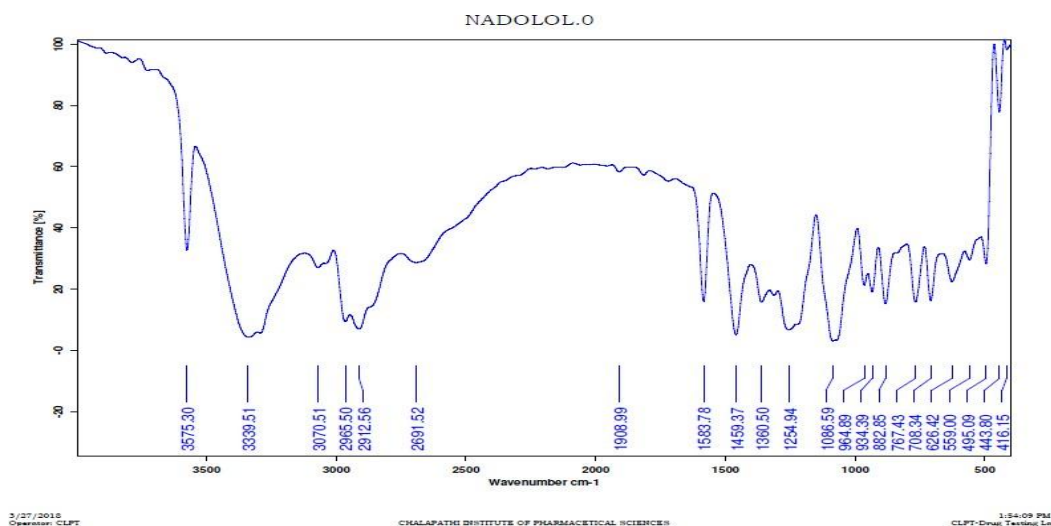**Figure 2: In-vitro drug release of multiple emulsions.**



**Fig. 3: FTIR graph of Nadolol.**

## DISCUSSION

**Physical appearance:** The drug (Nadolol) powder was examined for its organoleptic properties like colour and odour. And it was observed that Nadolol was whitish crytalline powder.

**Solubility study**
The sample was qualitatively tested for its solubility in various solvents. It was determined by taking 10 mg of drug sample in 10 ml 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.(IP 2003).

**Melting point determination:** Melting point of Nadolol was found at 115±2 ºC.

**Preparation of calibration curve**
Nadolol solution was scanned in the U.V. range of 200-400 nm using lab india UV Visible spectrophotometer. The spectrophotometric method of analysis of Nadolol at max 267.6 nm was found to be reproducible and highly sensitive. The standard curves of Nadolol were prepared in Methanol and Phosphate buffer solution (pH 6.8), at max 267.6 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.

**Preparation of Calibration Curve of Nadolol in phosphate buffer 6.8**
The calibration curve was plotted between the concentration and absorbance. The calibration curve of 10mg/10ml was carried out. The slope and intercept of the calibration curve were 0.017and 0.034 respectively. The correlation coefficient 'r2' values were calculated as 0.999.

**Determination of partition coefficient**
Partition coefficient studies are carried out to find out extent of drug transfer in the aqueous and the other non aqueous layer. This phenomenon usually is done to obtain the drug concentration in the either layer. Partition coefficient value of Nadolol also revealed its hydrophobic nature.

**Fourier-Transform Infra Red spectroscopy (FTIR)**
The IR spectrum of drug substance was authenticated using IR spectroscopy. The presence of characteristic peaks associated with specific structural characteristics of the drug molecule was noted. Various peaks of the drug are shown in figure 3**.**

**Color**
Freshly prepared primary emulsion was creamy white in color. There was no change in color at different storage conditions. This shows that primary emulsion was stable at different storage conditions up to 28 days. Freshly prepared multiple emulsion was white in color. There was little change in color of samples kept at 400C (in oven) the color became yellowish white. The change in color appeared on the 21st day and persisted up to 28 days. The change in color at the end of the observation period may be due to the oily phase separation which is promoted at elevated temperatures.

**Liquefaction**
No liquefaction was observed in the primary emulsion at all storage conditions. For the multiple emulsion, while no liquefaction was observed in them samples kept at 80C (in refrigerator) and 250C (in oven) during 28 days, slight liquefaction was observed in the samples kept at 400C (in oven) on 21st day. Liquefaction is the sign of instability; it may be attributed to the passage of water from the internal phase to external phase as described by many researchers.

**Phase separation**
In the case of primary emulsion, no phase separation was observed in any of samples. This indicates that primary emulsion was stable at all storage conditions for 28 days. For the multiple emulsions, no phase separation was seen in the samples kept at all storage conditions, except slight phase separation beginning on the 21st day.

**Globule size**
Globules sizes of the multiple emulsions kept at different storage conditions are represented Graphically in Figure F1,F2,F3 and photographs**.** Globule sizes of emulsion systems can be determined by light microscope34, laser diffraction methods, electron microscope or by coulter counter. Light microscope was used in this study. The increase or decrease in globule sizes indicates instability1, 3. The multiple droplets may coalesce with the other oil drops, internal aqueous droplets may be expelled individually, more than one drop may be expelled, internal globules may coalesce before being expelled out resulting in the shrinkage of internal droplets or water may pass from the external phase to the internal aqueous phase resulting in the swelling of internal droplets followed by complete rupture of droplets.

**PH values**
pH values of skin range between 5 and 6, and 5.5 are considered to be the average pH of the skin. Therefore, the formulations intended for dermal application should have a pH value around this range.

**Entrapment efficiency**
The entrapement efficiency data was shown in figure 2.

**In vitro drug release**
The result indicate more release of F3 formulation will be higher release profile as compare to other formulation and data was shown in in figures 2.

**CONCLUSION**
The objective of present work is to development and evaluation of multiple emulsion of Nadolol for oral drug delivery. The drug Preformulation studies were carried out like FTIR studies to find out that the various functional groups are same as the standard drug and it was found that was no interaction between drug and surfactant. Organoleptic characteristics of the primary and multiple emulsions formulated are presented in Table 1. Freshly prepared primary emulsion was creamy white in color, liquefaction, phase separation are presented in Table 6. The microscopical images of various formulations are as follows Figure 4. The investigations presented lead us to conclude that the multiple emulsions prepared using Nadolol and non-ionic surfactants like Tween80, span40, span60, span80 by two step emulsification methods. In vitro release profile was applied on various kinetic models in order like zero order first order, equation.

To find out the mechanism of drug release from multiple emulsions and the best fit with highest regression correlation coefficient was found with zero order the rate constants are calculated from the slop of respective plots the release mechanism of multiple emulsion. Nadolol which thereby reduce dose frequency, decrease side effect and improved patient compliance.

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