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# Formulation and evaluation of novel sustained release multiple emulsion containing chemotherapeutic agents

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**Abstract**: The objective of present work was to prepare and evaluate the long-term stability of W/O/W multiple emulsion with respect to the concentrations of Span 80 and Tween 80. In addition, the effect of surfactant, complexing agent, on rheological properties of emulsion was investigated. Scanning electron microscopy (SEM), zetasizer, zeta potential measurement, encapsulation efficiency and rheological properties is usually determined. It has been observed that phase separation values decreased, when increasing the concentration of Span 80, whereas Tween 80 concentration kept constant. On increasing Tween concentration, whereas Span 80 was kept constant, the phase separation was significantly increased. Hence it was selected to use high concentration of Span 80 *i.e.* 15% as lipophilic emulsifier, while low concentration of Tween 80 *i.e.* 0.1% as hydrophilic emulsifier for the further development of emulsion formulation. The stability of the formulation was assessed via microscopic observation of emulsion structure and measurement of phase separation.
**Key words:** Multiple emulsions, Span 80, Tween 80, PVP (polyvinylpyrrolidone).

## Introduction

An ideal dosage regimen in the drug therapy of any disease is the one which immediately attains the desired therapeutic concentration of drug in plasma (or at site of action) and maintains it constant, for the entire duration of treatment [1].

For many decades treatment of an acute disease or a chronic illness is done by delivery of various pharmaceutical dosage forms to the patients like tablets, capsules, pills, suppositories, creams, ointments, aerosols, and injectables, as drug carriers. There are some limitations associated with conventional dosage form. Poor patient compliance, increased chances of missing the dose of a drug with short half-life for which frequent administration is necessary. A typical peak-valley plasma concentration time profile is obtained which makes attainment of steady state concentration difficult. The fluctuating drug levels may lead to precipitation of adverse effects especially of a drug with small therapeutic index whenever overmedication occurs.

Recently, to overcome these limitations several advancement techniques for drug delivery has been developed. The primary objectives of controlled drug delivery system are to ensure safety and to improve efficacy of drugs as well as patients compliance. These techniques are capable of controlling the drug delivery rate, sustaining the duration of therapeutic activity and targeted delivery system [2].

Sustained release dosage forms are designed to achieve a prolonged therapeutic effect by continuously releasing medication over extended period of time after administration of single dose. The term

"sustained release" has become associated with those systems from which therapeutic agents may be automatically delivered at predefined rates over a long period of time. Products of this type have been formulated for oral, injectable, topical use, and inserts for placement in body cavities. In the case of injectable dosage form; this period may vary from days to month [3].

There are several approaches to achieve sustained drug delivery via parenteral route; the release is being controlled by dilution, diffusion, dissociation, and partitioning or bio erosion. The systems can be broadly classified as, Colloidal Carriers (Solutions, Disprsions, Microspheres and Microcapules, Nanoparticles and Niosomes, Liposomes, Resealed Erythrocytes), Implants and Infusion Devices.

**Multiple emulsions:** Multiple emulsions are complex systems, termed "emulsions of emulsions", i.e. the droplets of the dispersed phase contain even smaller dispersed droplets themselves. Each dispersed globule in the double emulsion forms a vesicular structure with single or multiple aqueous compartments separated from the aqueous phase by a layer of oil phase compartments [4]. Multiple emulsions are becoming popular since an additional reservoir is presented to the drug for partitioning which can effectively retard its release rate. The multiple emulsions are considered to be promising drug delivery systems by virtue of their thermodynamic stability, macroscopic homogeneity, ease of preparation and small droplet size [5].

For example in a study Etoposide phosphate and Carboplatin in combination have chosen for the treatment of small cell lung cancer. Both drugs are clinically recommended for treatment of SCLC in the following doses regimen. Carboplatin is to be given on 1st day and thereafter Etoposide is to be given on 1, 2, 3 or/and 4 and 5 day, this completes a cycle. Further up to 3 weeks no drug is recommended. The combination of these drugs has been proven to be synergistic action [6].

To accomplish such cycle in one formulation for increasing patient compliance we have chosen multiple emulsion system. The multiple emulsion system has two interfaces w/o and o/w stabilized by combination of surfactants having potential for maintaining serum concentration of drugs and also reduces the side effects. They are very complex systems consist of an internal primary aqueous phase dispersed in an oily phase which is further dispersed in an external secondary continuous aqueous phase, the internal aqueous droplets can be considered as an entrapping reservoir for water soluble compounds. Drug release from the internal aqueous to external aqueous phase takes place through the oil layer of the multiple emulsion droplets which act as a water-permeable membrane under the osmotic pressure gradient. The oil layer of multiple emulsion droplets and also the hydrophobic fatty acid tails of surfactants were found to behave as a water-permeable membrane between the two aqueous phases of w/o/w multiple emulsions. The slow release of the drug in its water soluble form from within the multiple droplets is responsible for showing sustained pharmacological action. A lot of work has been done to overcome the stability problem of multiple emulsions. In practice multiple emulsion having relatively large droplets, cannot be stable on storage for long time and usually release the entrapped matter in an uncontrolled manner, therefore they have very short shelf life. The presence of two thermodynamically unstable interfaces is the major cause of instability.

The goal of these multiple emulsion colloidal carriers is to transport the drug throughout the body without exposing it to sensitive organs and tissues and then to deliver it in concentrated dosage to the target site. To a certain extent this colloidal carriers accomplish this goal. Both aqueous phases separated by oil membrane act as a semi-permeable membrane. The present work is envisaged by development of stable fine multiple emulsions assessing their long term stability [7].

## Material and Method

All chemicals and reagents were obtained commercially and all were of AR grade and used as such without any further purification. Instruments used are sonicator (Soniweld Sonicator, India Ltd), high speed homogenizer, a magnetic stirrer (Remi India Ltd.).

**Preparation of multiple emulsions:** There are basically two methods available for the preparation of multiple emulsions.

- *One step emulsification*
- *Two step emulsification*

The most common method of formulation is two-step emulsification method (Double Emulsification Technique) because this method is easy, reproducible and gives a high percentage yield. Currently, a modified two-step emulsification method was used to provide high yield and stable multiple emulsion [8].

**Optimization of multiple emulsions (W/O/W):** Different dummy batches (Non-medicated) of multiple emulsions were prepared on considering different parameters:

A. Hydrophilic lipophilic surfactant ratio
B. Stirring speed of primary and secondary emulsion
C. Stirring time of primary and secondary emulsion

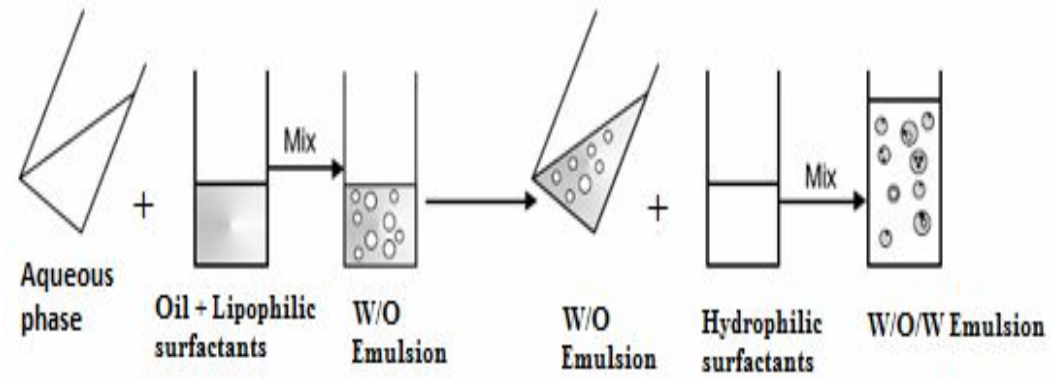


**Figure 1 Preparation of w/o/w multiple emulsions**

An aqueous solution (25ml) (an inner aqueous phase) was emulsified with an equal volume of soyabean oil containing liphophilic emulsifier Span 80 (0.1%) using a sonicator (Soniweld Sonicator, India Ltd) for 5 min. Than the stirring was done by high speed homonizer to obtain fine W/O droplets. The final W/O/W emulsion was prepared by subsequent emulsification of primary w/o (50ml) emulsion with an equal volume of an aqueous solution of hydrophilic emulsifier (Tween 8O) (0.1%), using a magnetic stirrer (Remi India Ltd.) at low speed for 5 min. The final phase volume ratio was maintained at 1:1 (Primary emulsion: external aqueous phase). The preparation of w/o/w multiple emulsions is schematically illustrated in Figure 1.

**A. Optimization of Span 80 and Tween 80 concentration:** For optimization of Span 80 and Tween 80 concentration, stirring speed 1500 rpm for 30 minutes for primary emulsion, 600 rpm for 5 minutes for secondary emulsion and phase volume ratio (1:1:1) were kept constant, while Span 80 and Tween 80 contents were varied at different weight percent ratio.

## Results and Discussions:

**A. % Phase separation:** Formulations (E1 to E10) were stored for one week and observed the % phase separation, which was determined by following equation

$$\% \text{ Phase Separation} = \frac{100\ (V_{sep})}{\text{Vol. of batch}} / [(V_1 + V_2) / (V_1 + V_2 + V_O)]$$

$V_1$, $V_2$, $V_0$ represents the volume of inner aqueous, dispersion phase and middle oil phase respectively.AS shown in Table1.

**Table 1 Optimization of Span 80 and Tween 80 concentration**

| Batches | Span80, % (wt/v) | Tween 80, % (wt/v) | % phase separation | Stability |
|---|---|---|---|---|
| E1 | 0.1 | 0.1 | 64 | Water and oil |
| E2 | 1 | 0.1 | 56 | Water and oil |
| E3 | 5 | 0.1 | 48 | W/O/W and oil |
| E4 | 10 | 0.1 | 41.3 | W/O/W and oil |
| **E5** | **15** | **0.1** | **25.3** | **W/O/W and oil** |
| E6 | 20 | 0.1 | 17.3 | W/O/W and oil |
| E7 | 25 | 0.1 | 10.6 | W/O/W and oil |
| E8 | 15 | 0.5 | 37.3 | Water, simple o/w |
| E9 | 15 | 1 | 46.6 | Water, simple o/w |
| E10 | 15 | 1.5 | 56 | Simple o/w |

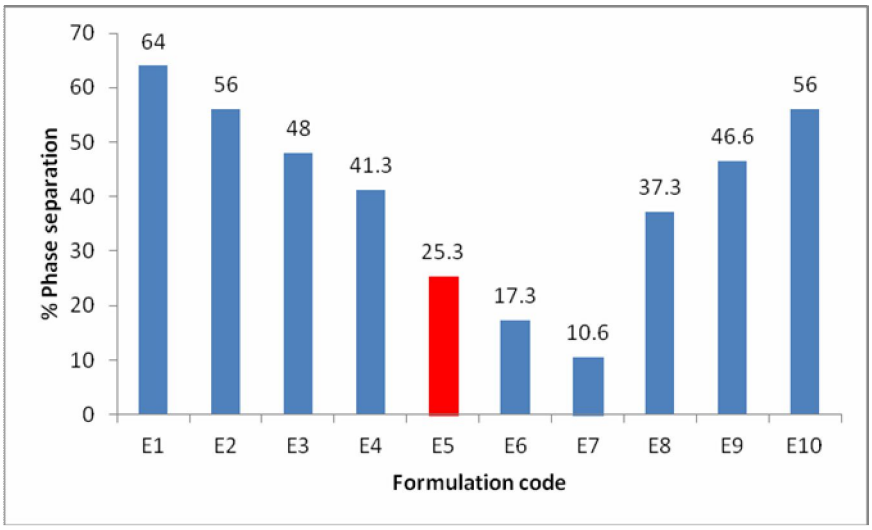


**Figure 2 % phase separation of E1 to E10 formulation**

**Table 1** and **Figure 2** shows the phase separation (%) values of various multiple emulsion formulations (E1 to E10) developed by varying the concentration of emulsifying agents.

It has been observed that phase separation values decreased when increasing the concentration of Span 80 from 0.1% to 25% (E1-E7), whereas Tween 80 concentration kept constant (0.1%). Formulation E-5 exhibited minimum phase separation *i.e.* 25.3% and further E-6 and E-7 also have minimum phase separation but there was much increase in viscosity, if primary emulsion becomes more viscous there was difficulty in redispersion. Therefore E5 having Span 80 concentrations of 15% was selected as optimum formulation. The Tween concentration was increased from 0.5 – 1.5% (E8 –E10), whereas 15% Span 80 was kept constant. When increasing the concentration of Tween 80, the phase separation was also increased from 37.3 to 56%.

Hence it was selected to use 0.1% Tween 80 as hydrophilic emulsifier for the further development of emulsion formulation.

**B. Optimization of stirring speed for primary and secondary emulsion:** In order to determine the effect of stirring speed for primary and secondary emulsion formulations (E5.1 to E5.13), all other variables i.e. 15% w/v Span 80 and 0.1% w/v Tween 80 concentration, stirring time and phase volume ratio were kept constant.

**Evaluation:** The formulations (E5.1 - E5.13) were kept at room temperature for 1 week to check their stability in term of phase separation.

**Table 2** and **Figure 3** shows the phase separation (%) values of various multiple emulsion formulations (E5.1 to E5.13) developed by varying stirring speeds (rpm).

It has been observed that phase separation values decreased when increasing the stirring speed of primary emulsion from 1500 to 4500 rpm (E5.1 to E5.7), whereas stirring speed of secondary emulsion were kept constant (600 rpm). Formulation **E16** exhibited minimum phase separation i.e. 16 %, thus E16 having stirring speed 4000 for primary emulsion was selected as optimum formulation.

The stirring speed of secondary emulsion was increased from 400 – 650 rpm (E5.8 –E5.13), whereas speed of primary emulsion 4000 rpm was kept constant. When increasing the stirring speed, the phase separation was decreased from 36.3 to 21.5 %. The batch E5.12 (600 rpm) exhibited minimum phase separation i.e. 16.2 %.

Hence it was selected to use stirring speed 600 rpm for secondary emulsion for the further development of emulsion formulation.

**Table 2 Optimization of stirring speed for primary and secondary emulsion**

| Batches | Stirring speed for primary emulsion for 30 min. | Stirring speed for secondary emulsion for 5 min. | % Phase separation |
|---|---|---|---|
| E5.1 | 1500 | 600 | 50.6 |
| E5.2 | 2000 | 600 | 38.6 |
| E5.3 | 2500 | 600 | 28 |
| E5.4 | 3000 | 600 | 25.3 |
| E5.5 | 3500 | 600 | 22 |
| **E5.6** | **4000** | **600** | **16** |
| E5.7 | 4500 | 600 | 20 |
| E5.8 | 4000 | 400 | 36.3 |
| E5.9 | 4000 | 450 | 30.6 |
| E5.10 | 4000 | 500 | 25.2 |
| E5.11 | 4000 | 550 | 20 |
| **E5.12** | **4000** | **600** | **16.2** |
| E5.13 | 4000 | 650 | 21.5 |

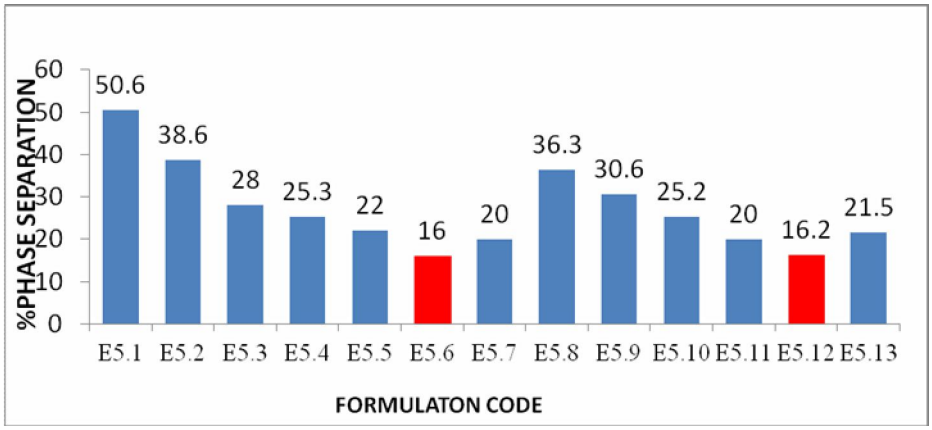


**Figure 3 % phase separation of E5.1 to E5.13 formulations**

**C. Optimization of stirring time for primary and secondary emulsion:** For optimization of stirring time for primary and secondary emulsion, formulations (E5.6.1 to E5.6.8), all other variables i.e. 15% w/v Span 80 and 0.1% w/v Tween 80 concentration, stirring speed 4000 and 600 rpm for primary and secondary emulsion respectively and phase volume ratio were kept constant.

The formulations (E5.6.1 – E5.6.8) were kept at room temperature for 1 week to check their stability in term of phase separation.

Table 3 and Figure 4 shows the phase separation (%) values of various multiple emulsion formulations (E5.6.1 to E5.6.8) developed by varying stirring time.

It has been observed that phase separation values decreased, when decreasing the stirring time of primary emulsion from 35 to 20 min. (E5.6.1– E5.6.4), whereas stirring time of secondary emulsion was kept constant (5 min.). Formulation E5.6.3 exhibited minimum phase separation i.e. 14.8 %. Therefore E5.6.3 having stirring time 25 min. for primary emulsion was selected as optimum formulation.

The stirring time of secondary emulsion was decreased from 6 – 2 min. (E5.6.5 – E5.6.8), whereas time of primary emulsion 25 min. was kept constant. When decreasing the stirring time, the phase separation was decreased. The batch E5.6.7 (3 min.) exhibited no phase separation.

Hence it was selected to use stirring time 3 min. of secondary emulsion for the multiple emulsion formulations.

**Optimized parameters:**

- Span 80 concentrations (15%)
- Tween 80 concentration (0.1%)
- Stirring speed for primary & secondary emulsion (4000 & 600 rpm)
- Stirring time for primary & secondary emulsion (25 & 3 min)

**Table 3 Optimization of stirring time for primary and secondary emulsion**

| Batches | Stirring time for primary emulsion (min.) | Stirring time for secondary emulsion (min.) | % Phase separation |
|---|---|---|---|
| E5.6.1 | 35 | 5 | 19.3 |
| E5.6.2 | 30 | 5 | 16.3 |
| E5.6.3 | **25** | **5** | **14.8** |
| E5.6.4 | 20 | 5 | 18.4 |
| E5.6.5 | 25 | 6 | 17.5 |
| E5.6.6 | 25 | 5 | 14.6 |
| E5.6.7 | **25** | **3** | **0** |
| E5.6.8 | 25 | 2 | 4 |

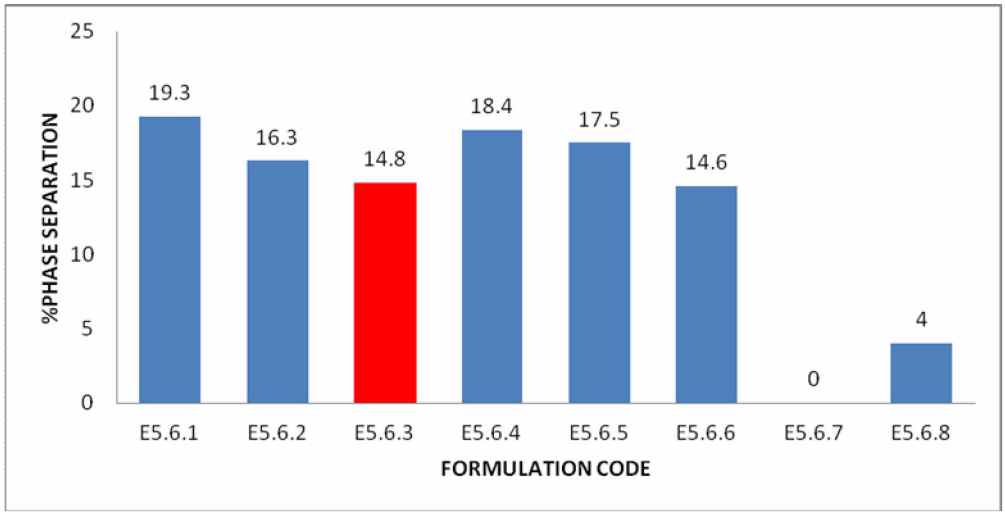


**Figure 4 % phase separation of E5.6.1 to E5.6.8 formulations**

**Table 4 Optimization of PVP concentration**

| Batches code | PVP (%) | Phase separation (%) | Stability in days at room temp. in term of phase separation |
|---|---|---|---|
| F2a | 0.5 | 2.6 | 6 |
| **F2b** | **1** | **0** | **Stable** |
| F2C | 1.5 | 2.1 | 4 |

**Table 5 Optimization of PVA concentration**

| Batches code | PVA (%) | Phase separation (%) | Stability in days at room temp. in term of phase separation |
|---|---|---|---|
| F3a | 0.5 | 4 | 3 |
| **F3b** | **1** | **1.73** | **10** |
| F3C | 1.5 | 4.8 | 2 |

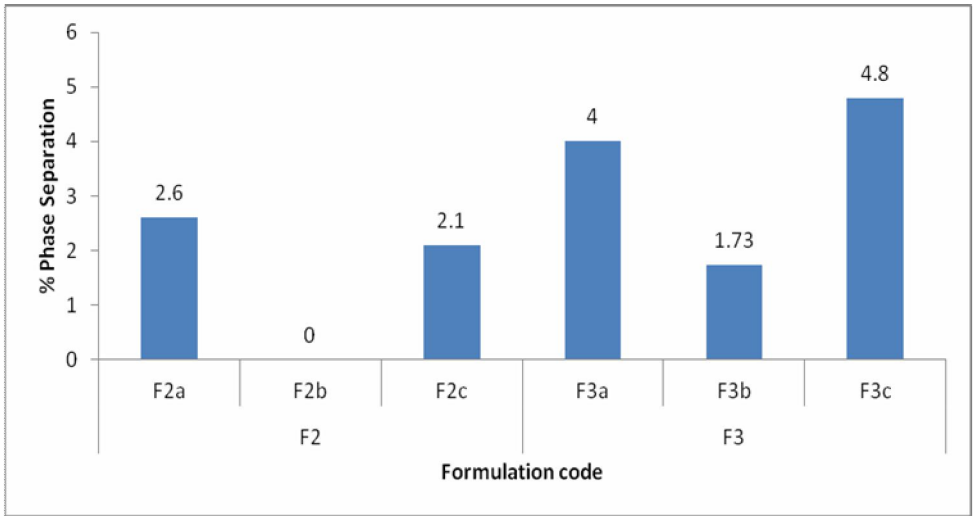


**Figure 5 % phase separation of F2 and F3 formulations**

**Incorporation of complexing agent/polymer into formulation and Stability Profile:-** After the drug incorporation in multiple emulsion, we had selected the two complexing agents as stabilizer, namely [9]

- Poly vinyl pyrrolidone (PVP)
- Poly vinyl alcohol (PVA)

Different batches of PVP and PVA were prepared at different concentration (0.5 to 1.5%) and were optimized in term of phase separation. From the results obtained in Table 4 and Table 5 polyvinyl pyrrolidone was selected (1%) in both internal and external aqueous phase as complexing agent.

## Conclusion:

The proposed multiple emulsion system showed excellent stability over the time with

- Span 80 concentrations (15%)
- Tween 80 concentration (0.1%)
- Stirring speed for primary & secondary emulsion (4000 & 600 rpm)
- Stirring time for primary & secondary emulsion (25 & 3 min)

The formulation complied with the requirements of small particle size, and stable drug-carrier. The present investigation is seminal however; elaborative studies and clinical trials are warranted to assess real potential of the developed carrier systems.

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