

## Effect of Quail Egg Yolk on the Formulation and Characterisation of Self Emulsifying Drug Delivery Systems of Simvastatin

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**Abstract:** SEDDS are mixtures of oil, surfactant and co-surfactant which are emulsified in aqueous media under conditions of mild agitation and digestive motility that are encountered in the gastrointestinal (GI) tract. The aim of this study was to develop a novel formulation of SEDDS using simvastatin which is a poorly water soluble drug. The study was conducted by preparing SEDDS with moringa oleifera, tween 80 (surfactant), egg yolk (surfactant) and polyethylene glycol (co-surfactant). After screening of various vehicles (oil, surfactant and co-surfactant) based on better drug solubilising power, liquid SEDDS were formulated. Prepared formulations were characterized on the basis of self emulsification ability, emulsification time, phase separation, spontaneity of formation, viscosity. Among the 8 formulations prepared, 4 of the formulations comprising formulations A1-A4, showed very good and promising characteristics when analysed using different parameters. All the formulations recorded emulsification time below 1 min which showed a very good performance as it agreed with the study where 2 mins was used as an evaluation index by some researchers in the emulsification process. The use of quail egg yolk helped in the reduction of emulsification time drastically in the formulations. Also the quail egg yolk used reduced the viscosity of the formulation in that it helped to reduce interfacial tension of the formulations making the formulations more flowable. In drug loading, the drug was well absorbed in all the formulations with formulation A1, showing the highest drug content of 93%. In order to avoid unstable systems thermal stability test was conducted on the selected SEDDS and all the 4 formulations were found to be stable at different temperature conditions as there was no phase separation, no creaming or cracking. Cloud point determination showed that all the formulations had their cloud points above 85°C which shows that the formulations are stable at physiological temperature.

**Keywords** – characterization, formulation, Quail egg yolk, SEDDS, simvastatin

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### I. Introduction

Egg of Quail", represents in general for the oriental people an appreciated dietetic food, replacing the chicken's egg, as it is much richer in proteins and vitamins, is much easier to digest and has a therapeutic effect. The traditional Chinese and Japanese specially appreciate the pharmaceutical effect of the quail's eggs, along with the Carrot of Korea (Root of Life) and the viper's venom. The naturalist therapy, more and more approved by the modern medicine and pharmacology, recommends the natural products of vegetal and animal origin in prophylactic, curative, and preservation ends.

Self emulsifying drug delivery systems (SEDDS) have gained high publicity in the pharmaceutical industry. SEDDS emulsify spontaneously to produce fine oil in water emulsions when introduced into an aqueous phase under gentle agitation. SEDDS can be orally administered in soft or hard gelatine capsules and form fine relatively stable oil in water emulsion upon aqueous dilution [1]. SEDDS are mixtures of oils and surfactants, ideally isotropic and sometimes containing co-solvents, which emulsify spontaneously to produce fine oil-in-water emulsions when introduced into an aqueous phase under gentle agitation [2]. Self emulsifying formulations spread readily in the gastrointestinal (GI) tract and the digestive motility of the stomach and the intestine provide the agitation necessary for self emulsification. These systems advantageously present the drug in dissolved form and the small droplet size provides a large interfacial area for the drug absorption [3]. In this study quail egg yolk was used as co-surfactant to help reduce the quantity of the surfactant used because of the irritation caused by the surfactant to the gastrointestinal tract when used in large quantity. Also since the target of this work is patients with cardiovascular ailments and high cholesterol level, the use of quail egg yolk will

portend very good advantage in that the dosage of the therapeutic drug can be grossly reduced since the quail egg yolk performs similar functions and it is a natural remedy. This will reduce the side effect of the drug which has adverse side effects when taken in high dose.

## **II. Materials And Methods**

Quail egg was purchased from Sebore farm located at Mayo Belwa in Adamawa State. *Moringa oleifera* seeds were purchased at the jimeta main market in Yola Adamawa state Nigeria. The surfactant (tween 80).The drug Simvastatin was obtained as a gift from Pharmacist Vitalis Onyirioha of Ovis Pharmaceutical Limited. All other chemicals that were used were of analytical grade.

### **2.1 Pre-treatment of Materials**

Pods and shells of *moringa oleifera* were removed manually and the seed were grounded in a domestic blender. Quail egg was washed with clean water and the yolk was removed manually.

#### **2.1. Preparation of egg yolk solution**

The solution was prepared by dispersing 0.5 g of quail egg yolk in 100cm<sup>3</sup> of distilled water in a volumetric flask and made up to the mark. This was used as the stock solution.

#### **2.2. Formulation design of SEDDS**

A total of 8 samples were formulated comprising:

A total of 8 formulations A1-A8 comprising Tween 80, Egg yolk solution, moringa oleifera seed oil (MSO), Polyethylene glycol (PEG 200)

The produced blends were then stirred continuously and left to equilibrate for 24 h at ambient temperature to obtain a homogenous isotropic mixture. The SEDDS formulations were stored at ambient temperature until further use.

## **III. Characterisation of SEDDS**

### **2.3. Determination of Emulsification time**

The emulsification time i.e the time required for the pre-concentrates to form micro emulsion upon dilution were monitored by visual observation. The emulsification time of SEEDS were determined by dispersing formulation in 500ml of purified water at 37°C in USP dissolution apparatus type II (paddle type) at 50rpm.

### **2.4. Solubility of drug**

The solubility of the drug in the oil (MSO), egg yolk, tween 80 was determined by dissolving an excess amount of the drug in 500 mg of the oil, surfactants, co-surfactant and egg yolk in stoppered vials. The mixtures were continuously stirred using vortex mixer for 10 min and kept at 37°C ± 0.5°C in a water bath shaker for 72 h to attain equilibrium. The equilibrated samples were centrifuged (3000rpm for 15 min) and supernatant were filtered through 0.45µm membrane filter and diluted with mobile phase. Drug content was quantified using ultraviolet-visible (UV-VIS) spectrophotometer at 296nm [4].

### **2.5. Screening of components**

Screening of egg yolk and tween 80 was done on the basis of percent transmittance. Emulsification ability of the surfactants were assessed by adding 300 mg to MSO. The mixture will be gently heated to 40-45°C for 30 s to achieve homogenization. Out of this mixture, 50 mg will be weighed and diluted up to 50 cm<sup>3</sup> with double distilled water to yield fine emulsion. The resulting mixture will be observed visually for the relative turbidity. The emulsion will be allowed to stand for 2 h and transmittance will be assessed by UV-VIS spectrophotometer at 638nm, using double distilled water as blank.

## **IV. Preparation of self-emulsifying drug delivery systems (SEDDS)**

The amount of oil, surfactant and co-surfactant to be taken were decided on the basis of microemulsification region in the ternary phase diagram or on visual observations made on the SEDD formulations. The formulations that showed clear transparent appearance, were completely miscible, did not show any phase separation, were homogeneous, were selected for preparation with simvastatin,. Simvastatin was accurately weighed into screw-capped glass vials and dissolved in oil. The mixture was warmed in a water bath at 37°C. Surfactant and co-surfactant were added to the mixture and stirred for 10 min using a magnetic bar. The formulations was further be sonicated at 45°C for 15 min. Four formulations (A1-A4) with different concentrations of oil, surfactant and co-surfactant, each containing simvastatin at a final loading of 40 mg and 20 mg drug was prepared [5].

**V. Physicochemical characteristics of self-emulsifying drug delivery system**

**5.1. Drug content**

Self-emulsifying drug delivery system containing simvastatin was added in 50 mL volumetric flask containing methanol and mixed well with shaking and was sonicated for 10-15 min. 0.1 mL of this solution was diluted with 25 cm<sup>3</sup> fresh methanol and the percentage drug content was determined using UV-spectrophotometer at λ<sub>max</sub> 296 nm [6]

**5.2. Thermal stability studies of SEDDS**

Each formulation of SEDD containing different ratios of quail egg and tween 80 was taken in a test tube and diluted with 10ml of distilled water at 37°C. The thermal stability study was determined by carrying out heating cooling cycle, centrifugation test and freeze thaw cycle.

**5.3. Heating cooling cycle**

Six cycles between refrigeration temperatures of 4°C and 45°C, with storage at each temperature for not less than 48 h was studied. The stable formulations were subjected to centrifugation test.

**5.4. Centrifugation test**

Passed SMEDDS were centrifuged at 3500 rpm for 30 min using digital centrifuge. If SMEDDS did not show any phase separation, it will be taken for freeze-thaw stress test.

**5.5. Freeze thawing**

Freeze thawing was employed to evaluate the stability of formulations. The formulations were subjected to 3-4hrs of freeze thaw cycles, which included freezing at – 4°C for 24hrs followed by thawing at 40°C for 24hrs.

**5.6. Viscosity determination**

20 g of each of formulation of SMEDDS were weighed and transferred to beaker and the viscosity of the formulation was determined with the help of Brookfield Viscometer DV-E model spindle no 6, at 10 rpm for 5 min and the corresponding dial reading on the viscometer were noted.

**5.7. Cloud point measurement**

The SEDDS formulations were diluted with distilled water in the ratio of 1:250, placed in a water bath and their temperature was increased gradually. Cloud point was measured as the temperature at which there was a sudden appearance of cloudiness visually studied.

**6.0. Results and discussion**

**6.1. Formulation of self emulsifying drug delivery system (SEDDS)**

Different formulations of SEDDS were produced. 8 different samples of SEDDS formulations were produced comprising:8 formulations A1-A8 comprising Tween 80, Egg yolk solution, MSO, Polyethylene glycol (PEG 200)

**6. 2. Table 1:** Requirements for the formulation of optimised SEDDS

Formulations	MSO in ml	Tween 80 in ml	Egg yolk in ml	PEG 200 in ml
A1	1	4	4	1
A2	1	3.5	3.5	2
A3	1	3	3	3
A4	1	2.5	2.5	4
A5	1	2	2	5
A6	1	1.5	1.5	6
A7	1	1	1	7
A8	1	0.5	0.5	8

**6.3. Screening study**

**6.3.1. Table 2:** Data showing solubility of simvastatin in each of the components

Vehicle	Solubility of symvastatin
PEG 200	97.8
Tween 80	72.1
MSO	62.9
Quail egg yolk	60.8

Drug loading per formulation is a critical design factor which can be dependent on its solubility in various formulation components. Drug may be solubilized in the oily core and/or on the interface of these structures, so the selected vehicles should have a good solubilizing power to the drug. As a pre-formulation step, drug solubility in different components was determined visually till no drug crystals were detected indicating

that it was dissolved. The selected oil has fair fluidity, proper self-emulsification properties and is efficiently digested. The surfactants have similar high solubilizing properties with those of Labrasol and curcumin [7].

Regarding the co-surfactant (PEG-200) according to earlier report, [8], the co-surfactant can lower the interfacial tension of the surfactant in microemulsions resulting in a more flexible and dynamic layer. The drug in this energy rich system can diffuse across the flexible interfacial surfactant film between the phases: a thermodynamic process that increases partitioning and diffusion. It can decrease the fluidity of SEDDS, enhance drug incorporation into the SEDDS, improves self-emulsification properties and possesses penetration enhancement effect [9]. It can also reduce the required amount of surfactant [10]. The self-emulsifying formulation consisted of MSO (oil), tween 80 (surfactant), quail egg yolk solution (surfactant), PEG 200 (co-surfactant). Each showed a clear and monophasic liquid at ambient temperature when introduced to an aqueous phase. This is important because good solvent property is desired in order to allow presentation of the drug in solution. Tween 80 is highly hydrophilic due to the presence of polyoxyethylene chain in the molecule of tween [5].

**6.4. Preparation and assessment of self- emulsification of SEDDS**

SEDDS were prepared and their self-emulsifying properties were visually observed. These systems should be a clear, monophasic liquid when introduced into aqueous medium and should have good solubilizing properties to present the drug in a solution. Also individual components should have good miscibility with each other to produce a stable formulation. The visual grading of the process of self-emulsification upon dilution is shown below

**6.5. Assessment of self- emulsification of SEDDS formulations**

**Table 3**

Formulation	Mutual Miscibility	Spontaneity of formulation	Homogeneity	Dispersibility	Appearance	Overall performance
A1	Miscible	Good	Good	Good	Transparent	Good
A2	Miscible	Good	Good	Good	Transparent	Good
A3	Miscible	Good	Good	Good	Transparent	Good
A4	Miscible	Good	Good	Good	Transparent	Good
A5	Miscible	Good	Poor	Poor	Milky	Poor
A6	Miscible	Good	Moderate	Moderate	Milky	Good
A7	Miscible	Good	Moderate	Moderate	Milky	Good
A8	Miscible	Good	Poor	Poor	Milky	Poor

A1-A4 showed good miscibility, spontaneous formation. The mixtures were homogeneous and dispersed well. They all formed transparent mixture and are accepted as SEDD/SMEED

Samples A6-A7 showed moderate performance because they were miscible, spontaneous , homogeneous but appeared milky and there was no phase separation. Samples A5-A8 gave very poor formulation because they phase separated, were not well dispersed. In all, four optimized SEDDS formulations from sample A were accepted for further investigation comprising A1, A2, A3 and A4

**6.5.1. Selected optimized SEDDS formulations for further studies**

Sample A: A1-A4 Comprising tween 80, quail egg yolk, MSO, and PEG-200

**6.6. Emulsification time and viscosity**

The rate of emulsification is an important index for the assessment of the efficiency of emulsification [11], that is the SEDDS should disperse completely and quickly when subjected to aqueous dilution under mild agitation. The result of the emulsification times and viscosities obtained for the selected formulations for the different *Moringa*/quail egg/tween 80/PEG-200 compositions is presented in table 6

**6.7. Table 4: Evaluation parameters for the optimised SEDDS**

Formulations	Oil/surfactant/Co-Surfactant proportion	Mean EM (sec)	Dynamic viscosity
A1	10/80/10	20	0.0335
A2	10/70/20	15	0.0329
A3	10/60/30	9	0.0276
A4	10/50/40	4	0.1873

The shortest emulsification time of 3sec was recorded for formulations B8, the longest emulsification time of 20 sec was recorded for formulation A1. Also all the formulations recorded emulsification time below 1 min. This result shows a very good performance of all the selected SEDDS formulations as it agrees with the study where 2 mins was used as an evaluation index by some researchers in the emulsification process [6]. A trend observed from the result above showed a noticeable increase in emulsification time as the surfactant concentration increases. Also the co-surfactant used helped to reduce the interfacial tension of the surfactant

thereby reducing the emulsification time to below 1 min. In the work of [12], they used only oil and surfactant without a co-surfactant and obtained highest emulsification time of 1 min and 54 sec which was attributed to effect of the surfactant on the formulation due to its high viscosity. Looking at viscosities in table 4, some solvents like tween 80 have a high viscosity [13] and in this study, its use as a surfactant with a gradual increase in its concentration in the various SEDDS formulations resulted in incremental viscosities and corresponding increase in emulsification time recorded. Also quail egg solution used in addition to the reduced concentration of tween 80 might also have contributed to the lower time of the emulsification process and also the low values of viscosities for the formulations A3 and A4

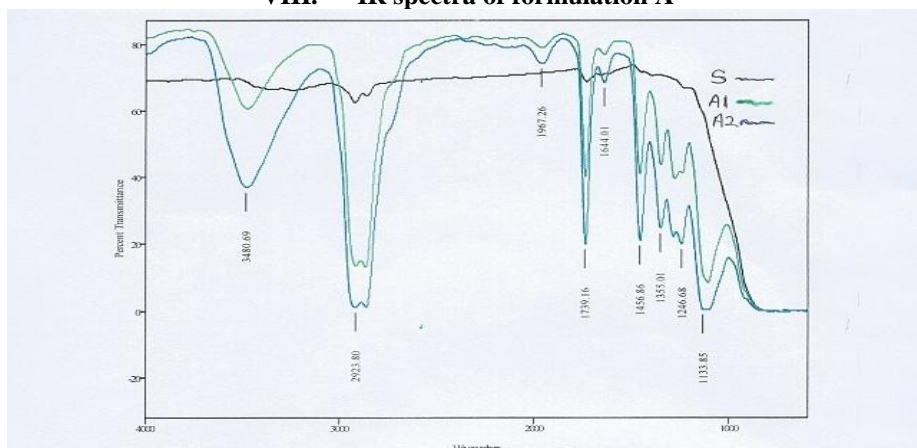
**VII. Solubility of drug in oil, surfactant and co-surfactant**

The oil, surfactant and co-surfactants were screened on the basis of maximum drug solubility. This is important in the case of oral formulation development because of other components in solubilisation of target dose which could lead to risk of drug precipitation in the GI lumen due to lowering of solvent capacity. The MSO showed good solubility of the drug as there was no precipitation of the drug. PEG and quail egg solution also showed good solubility as there was no precipitate. Drug loading is a key factor for the selection of the suitable formulation. A good balance between drug loading and efficient emulsification is required. In this case, the simvastatin can be said to have a good wetting property. The co-surfactant could be said to have migrated to the oil/water interface and form a mixed surfactant/co-surfactant film. The co-surfactant causes a transitory lowering of the interfacial tension during the formation of the dispersion and thus allow the drug to be solubilised.

**7.1. Table 5:** Formulation of SEDDS with simvastatin

Formulation	Drug (simvastatin)	MSO in ml	Tween 80 in ml	Quail egg yolk in ml	PEG 200 in ml
A1	40	10	40	40	10
A2	20	10	35	35	20
A3	40	10	30	30	30
A4	20	10	25	25	40

**VIII. IR spectra of formulation A**



**Fig 1:** IR spectra of formulation A comprising S (simvastatin), A1 (MSO+Tween 80+Quail egg yolk+PEG 200), A2 (MSO+Tween 80+Quail egg yolk+PEG 200+simvastatin)

The IR spectra of the formulations showed that the drug was well absorbed by the formulations and there was no chemical interaction between the drug and the various formulations. Looking at the various peaks there was no additional or different functional group found and there was no change in the spectra of the different formulations.

**8.1. Drug content**

**8.1.1. Table 6:** Data showing percent drug content of the formulations

Formulations	Abs of drug	Abs of formulations	Percentage (%) drug Content
A1	0.432	0.401	93
A2	0.432	0.326	75
A3	0.432	0.342	79
A4	0.432	0.335	77

In all the formulations the drug was well absorbed in all formulations with formulation A1 showing the highest drug content of 93%. The formulation has the maximum drug loading and also absorbed maximally. The good solubility of the drug in the surfactant, co-surfactant and oil together with proper S/CoS/oil combination may be the causes of the high drug loading in the above formulations.

### **8.2. Thermal stability test**

All the four (4) samples passed the thermal stability test. These formulations were used for further studies. The thermal stability study was designed to identify and avoid the unstable systems. SMEDDS are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant and co-surfactant, with no phase separation, no creaming or cracking. It is the thermal stability which differentiates nano or microemulsion from emulsions that have kinetic stability and will eventually phase separate [14].

### **8.3. Cloud point determination of self emulsifying drug delivery system**

Cloud point is that temperature that there is sudden appearance of cloudiness visually. All the 8 formulations had their cloud points higher than 85<sup>0</sup>C which shows that these formulations are stable at physiological temperature. Other formulations had their cloud points below 50<sup>0</sup>C which showed that there was phase separation below that temperature. This may be due to drug precipitation [14].

## **IX. Conclusion**

Self emulsifying drug delivery system is a vital tool in overcoming the formulation difficulties and improving the oral bioavailability of hydrophobic/lipophilic drug. The oral delivery of hydrophobic drug can be made possible by SEDDSs, which have been shown to substantially improve oral bioavailability with future development of this technology. SEDDSs will continue to enable novel applications in drug delivery and solve problems associated with the delivery of poorly soluble drugs. In this study SEDDS/SMEDDS formulations were successfully formulated for oral administration. From the above it can be safely concluded that the self emulsifying drug delivery system of simvastatin with quail egg yolk as an additional surfactant showed good and promising performance.

### **9.1. Recommendation**

Studies in this work have shown that the use of moringa oleifera oil extract can be safely and effectively used in the formulation of self emulsifying drug delivery system using simvastatin as the therapeutic drug. Also it has been established that using quail egg yolk can be advantageous as it can help in the reduction of the quantity of the drug used as both perform similar function as anti-cholesterol agent. Simvastatin as a synthetic drug may have adverse side effect when taken in large dose. So the addition of quail egg yolk as an additional surfactant will help in the reduction of the quantity of the drug used during formulation. However, it is recommended that:-

In vitro drug release studies should be performed to determine the rate of drug release from the formulation.

Controlled drug release studies should also be conducted using a suitable polymeric material to control the rate and duration of the release of the drug to avoid the case of the drug being released at the same time or in uncontrolled manner which can be harmful to the system.

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