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Simvastatin Manifold Emulsion Preparation and Evaluation: 3^2 Factorial Design ApproachArticle

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Abstract

Background: Manifold emulsions are used to increase the bioavailability of active medicinal ingredients and to provide a longer drug delivery mechanism. Hydrophobic and hydrophilic combinational surfactants are frequently used to stabilize the manifold emulsions.

Results: In order to accomplish stable manifold emulsions, critical is the ratio of these surfactants. Simvastatin (SMV) was created as a manifold emulsion in this work using a two-step emulsification process using a variety of surfactants, including tweens and spans. 3^2 factorial designs were used for optimization of particle size and drug release. The stability, percentage of drug entrapment, and *in-vitro* drug release of the various emulsions are assessed.

Conclusions: The B3 formulation offers a higher release profile than other formulations, per experiments on in vitro dissolution. As the concentration of span 60 rose, the formulation's release profile got better. In spite of SMV's poor water solubility, it has been found that different emulsions can help increase the dissolving rate and, as a result, the medication's oral bioavailability.

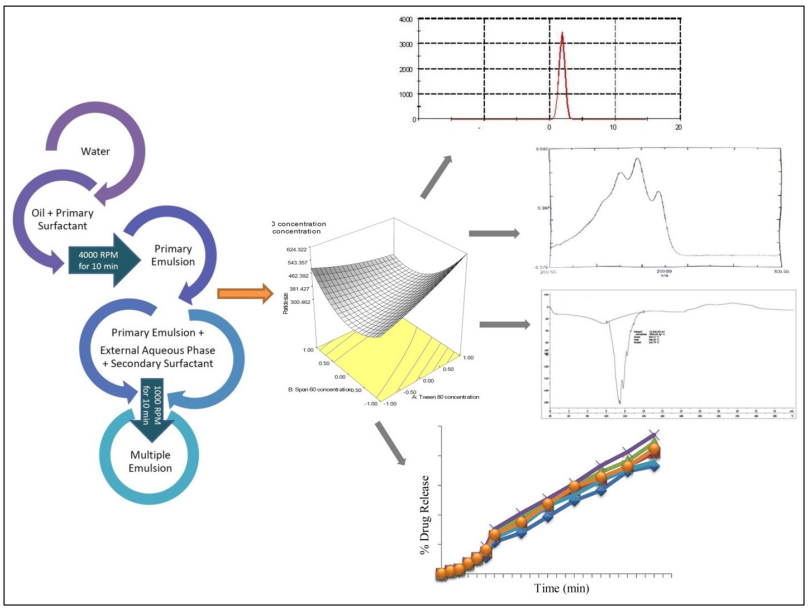
Keywords: Manifold emulsions; emulsion; surfactants; simvastatin; *in-vitro*; 3^2 factorial design; drug delivery etc.

List of Abbreviations

SMV: Simvastatin, o/w: oil-in-water, w/o: water-in-oil, W/O/W: water-in-oil-in-water, smix: surfactant-co-surfactant mixture, TLC: Thin Layer Chromatography, ANOVA: Analysis of variance, DR: drug release, CP: centipoise, DSC:

Differential Scanning Colorimetric, RH: relative humidity, ICH: International Council for Harmonization.

Graphical Abstract



Background

A colloidal dispersion of two immiscible liquids, where one phase is distributed in the other, is known as an emulsion. The two most common varieties of simple emulsions in the liquid phase are water-in-oil (W/O) and oil-in-water (O/W) emulsions [1]. The dispersed phase of manifold emulsions is a more complicated system because it is an emulsion by itself. Due to its numerous uses in the controlled release of pharmaceuticals, food formulation with reduced fat, encapsulation of active substances, and cosmetics, multiple emulsions, in particular water-oil-water (W/O/W), have attracted growing interest. W/O is frequently emulsified into the secondary aqueous phase to create W/O/W emulsions [2].

Manifold emulsions are described as emulsions that contain both o/w - oil-in-water and w/o water-in-oil emulsions at the same time. They have features that are similar to both o/w and w/o emulsions. These are pronounced as heterogeneous systems consisting one of the immiscible liquid feast in additional in the form of droplets with sizes greater than one μm. The thermodynamic stability of these two liquids that make up a system is low [3]. Because the dispersed phase's droplets contain even smaller ones, which are frequently made of a liquid that is miscible and identical to the continuous phase 3, manifold emulsions are complex systems. For the creation of manifold emulsions, both hydrophilic and lipophilic emulsifiers are utilized [4]. Manifold emulsions have been found to be promising in a variety of sectors, including separation science and pharmaceutics. Their potential biopharmaceutical uses range from enzyme mobilization to sorbent reservoirs in drug overdose treatments to adjuvant vaccines, long-acting drug delivery systems. For cosmetics, manifold emulsions were examined for their potential benefits of longer active agent release, inclusion of undesirable components and internal phase dispersion to preserve active chemical components [5]. The W/O/W - water-in-oil-in-water emulsions, often referred to as manifold emulsions, are emulsion systems in which small water droplets are stuck within larger oil droplets, which are subsequently dispersed in a continuous water phase [6]. Active compounds can release more slowly due to the presence of a reservoir phase within droplets of another phase. Manifold W/O/W emulsions made using the two-step approach include both W/O and O/W simple emulsions and need at least two emulsifiers: one to stabilize the primary W/O emulsion and one to stabilize the secondary O/W emulsion [7].

The attentiveness ratio of these binary surfactants is crucial for producing steady and in height yields of W/O/W emulsions. A distinguishing characteristic of W/O/W manifold emulsions in comparison to simple W/O emulsions is the diffusion of water through the oil phase as a result of inconsistencies in the osmotic pressures between the internal and external aqueous phases [7,8]. The two watery phases are separated from one another by the oil layer. Polar molecules dispersed in either the internal discontinuous aqueous phase or the external continuous aqueous phase can diffuse through the oil layer due to the concentration gradient. Osmotic pressure is the primary force at work in the case of water. Molecules are commonly transported by hydrophobic surfactant-containing micelles that are present in the oil phase [9]. By causing the internal aqueous droplets to inflate, rupture, or shrink, water diffusion influences both the steadiness stability of the manifold droplets and the release silhouettes of the active chemicals placed in the inner scattered aqueous phase [10]. The majority of cardiovascular incidents are caused by high blood pressure. In height blood pressure is the most significant jeopardy factor for early mortality and disability due to its extraordinarily high prevalence in many developed nations [11]. As a result, antihypertensive medication lowers the risk of cardiovascular problems, which are associated with a high mortality rate in hypertensive patients [12]. SMV is a unique antihypertensive mediated drug that belongs to the angiotensin II type I receptor antagonist family membership. It is a strong, highly selective, and orally active antihypertensive agent. SMV blocks angiotensin II receptors, causing blood vessels to relax and broaden, lowering blood pressure and improving blood flow. SMV is well tolerated after oral doses of up to 400 mg given once, twice, or three times day, as well as after successive doses of 200 mg [13]. It's difficult to come up with multiple emulsion dose formulations for some active components. The goal of producing manifold emulsions dosage formulations is to deliver enhanced SMV release and oral bioavailability in patients as equated to recognized solid oral SMV dosage forms. Due to the numerous obstacles deriving from pharmacokinetic features of oral drug administration, developing several emulsions prescribed amount prepared formulation design that have enhanced bioavailability to the existing oral dosage forms of SMV is difficult [14]. SMV has a poor oral bioavailability of about 25% in humans, ranging from 25 to 40%, and exhibiting significant inter- and intra-subject variability. Additionally, the solubility of SMV is pH-dependent, ranging from hardly solvable in an acidic surroundings atmosphere to soluble in the neutral gastrointestinal tract environment [15].

Because SMV permeability is low and pH dependant, it declines when the environment in the gastr-ointestinal tract changes from acidic to neutral. These complex biopharmaceutical propertiesmake it challenging to design a more releaseable and bioavailable dose form of SMV with lower inter- and intra-subject variability. For the optimization of particle size and drug release, we have used 32 factorial designs. The independent variables were selected as tween 80 concentrations and span 60 concentrations. Therefore, the ideal multi-emulsion SMV dosage formulation would have enhanced release and absorption properties as well as less inter- and intra-subject variability. The objective of the current work is to describe and evaluate the multidimensional SMV emulsion. [16].

Materials and Methods

The SMV used as active pharmaceutical ingredient which is purchased from Vesta Pharma Chem Pvt. Ltd. Surat (Gujarat) and other required exploited materials and solvents were found of analytical status.

Pre-formulation study The drug sample was identified by consuming a UV-spectrophotometer, and the resulting spectrum was compared to the SMV reference standard spectrum. SMV calibration curves in pure water, phosphate buffer pH 6.8, and methanol with 0.05 percent acetic acid, with evidence of Beer's law compliance [16].

Thin layer chromatography was used to investigate drug-excipient interactions. A precoated silica gel aluminium plate 60 F_{254} (10 cm x 20 cm, layer thickness 0.2 mm, E-merck, Darmstad, Germany) used as the stationary phase. These plates were used for research and then prewashed in methanol. The mobile phase was composed of ethyl acetate, toluene, and methanol (6:2:2 v/v/v). As visualizing agent iodine fumes were used. The washed ampoules were filled with physical mixes of medication and excipients, which were then sealed. In the stability room, the sealed ampoules were stored at 37.50°C for the 28 livings days. After that, the Rf

values of plain drugs and mixes were calculated [17].

The dosing range is 5 to 80 mg per day, taken orally in one dose in the evening. A maximum of 80 mg (10ml) per day should be taken in one dose in the evening. Only if they have not achieved their treatment objectives on inferior doses and the beneficial welfares are likely to outweigh the dangers should patients with unadorned hypercholesterolemia and in height jeopardy of cardiovascular issues take the 80 mg (10ml) dose. The 20 mg/ 5 ml product should be used for doses of 20 mg or less [18].

Formulation and Development

Phase Diagram Preparation and Manifold emulsion Formulations - The aqueous titration technique was secondhanded to develop the diagram of pseudo ternary phase, which contain oil-in this case, light liquid paraffin-smix (surfactant-co-surfactant mixture)-Span 60 and Tween 80-and double-distilled water-to obtain the constituents and their concentration ranges, which can lead to a significant amount of manifold emulsion existence area. Tween 80 and Span 60 were used to combine the surfactant and cosurfactant in predetermined weight ratios (1:3, 1:2, 1:1.7, 1:1.5, 1:1.6, and 1:1.7). Then, at room temperature, each surfactant and cosurfactant mixture's aliquots (Smix) were mixed with oil at 25°C [19].

Preparation of Manifold emulsions - Manifold emulsions were equipped by the twofold step emulsification progression, their diagrammatic representation depicted in figure1.

Primary emulsification: With constant stirring at 4000 r/min for 10 min, 3 ml of distilled water containing 24 mg of drug was gradually added to 12 ml of oil phase containing 66 mg of drug and primary emulsifier (Span 60).

Secondary emulsification: With constant stirring at 1000 revolutions per minute for 10 minutes, 15 ml of a viscous primary emulsion was further emulsified with an external aqueous phase containing secondary emulsifier (Tween 80) and 150 mg of medication [8,20].

Formulation composition of manifold emulsions - Formulation of manifold emulsions proceed with following components their description defined in Table 1.

Batch no	Tween 80 concentration (µg/mL)	Span 60 concentration(µg/mL)	Particle size (nm)	Drug release (%)
F1	-1	1	466	89
F2	-1	0	302	90
F3	0	1	450	91
F4	0	-1	369	79
F5	0	0	355	88
F6	0	0	367	86
F7	1	1	388	79
F8	1	0	298	84
F9	1	-1	698	81
F10	-1	-1	412	80
F11	0	0	300	86
F12	0	0	360	92

F13	0	0	315	89
Coded levels				
Independent variable	Low level (-1)	Medium level (0)	High level (+1)	
X2= Span 60 concentration	1.5	3	4.5	
X1= Tween 80 concentration	1.00	2.00	3.00	

Table 1: Formulation composition of emulsion

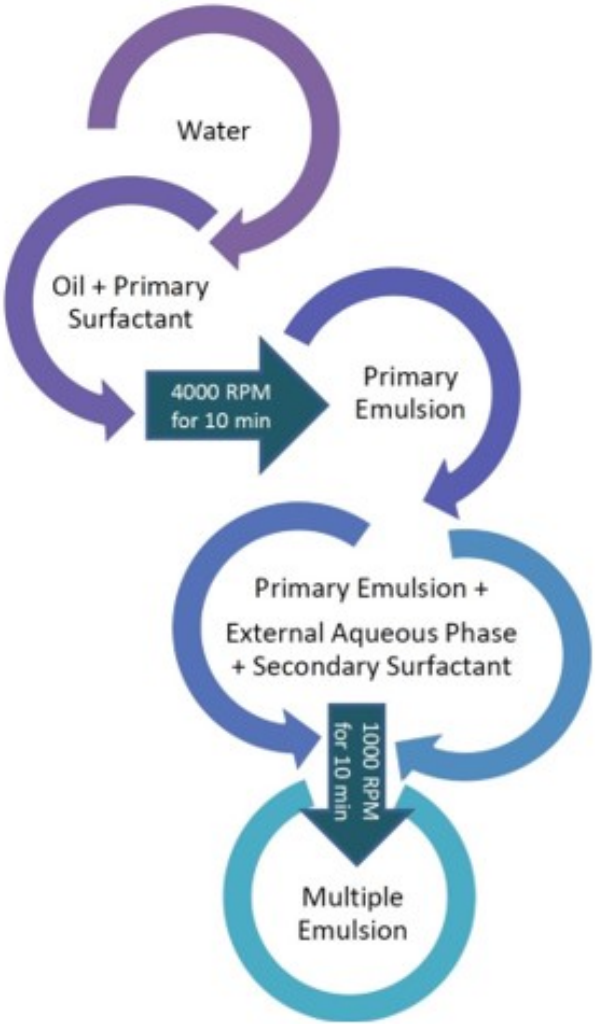


Figure 1: Flow chart of formulation of manifold emulsions

Evaluation of Manifold emulsions – Evaluation of the manifold emulsions proceeded with pH identification, particle size, viscosity, centrifugation, entrapment efficiency study, *In vitro* drug diffusion studies, zeta potential, differential scanning calorimeter and stability study with same parameters [9, 21 - 25].

Optimization

The Minneapolis-based Stat- Ease Inc.'s DESIGN-EXPERT software was used to carry out the statistical experimental inquiry. The impact of independent factors on answers was found and optimized using the 32 (three level, two factor) response surface approach. The independent variables chosen as low (1), medium (0), and high (+1) values were the stirring rate/speed (X 1) and

concentration of span 60 (X 2). The dependent factors were determined to be the percentages of particle size (Y 1) and DR (Y 2). The statistical setup for the selected dependent and independent variables is shown in Table 1. The impact of independent factors (x 1, x 2) on dependent variables is described by the subsequent equation (Y 1, Y 2) [26,27].

$$Y = \beta_0 + \beta_1 x_1 + \beta_1 x_1 + \beta_3 x_1 x_2 + \beta_3 x_1 x_2 + \beta_4 x_1^2 + \beta_5 x_2^2 \ldots\ldots\ldots\ldots (1)$$

Where,

Y is stand for the response, β_0 is stand for the intercept and $\beta_1 - \beta_5$ is for regression coefficients. x_1, x_2 are for individual effects. $x_1 x_2$ are for the interaction effect and are x_1^2, x_2^2 represent quadratic effects. The significance of the model was evaluated at $P<0.05$ level using One-way ANOVA. [25]

Results

UV-spectroscopy, IR spectroscopy, and melting point measurement were used to identify the substance. The melting point of SMV was determined using the capillary method, revealing a melting with the range of 135-138 °C. In UV-spectroscopy after scanning with a spectrophotometer, the solution of SMV was found to have maximum absorption at 238 nm, which was described as max in the literature. As a result, the drug sample obtained complies with the reference spectra as per figure 2.

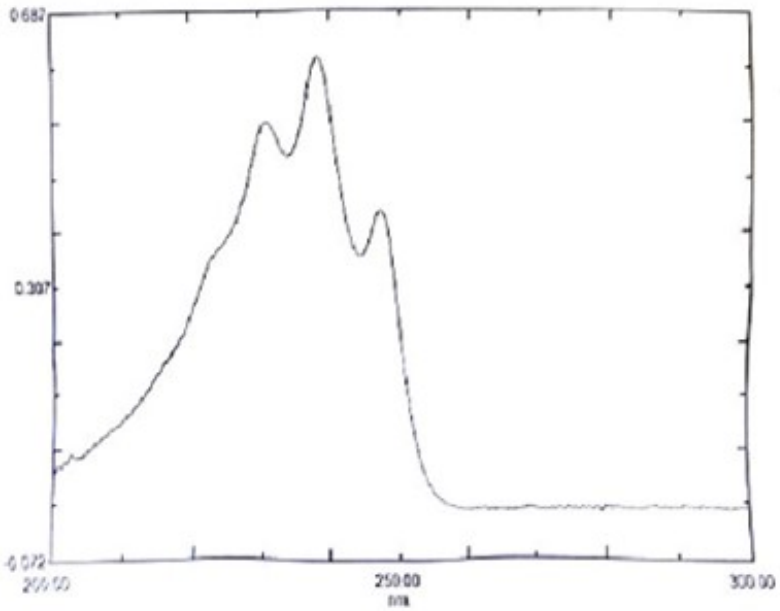


Figure 2: UV-spectrum of SMV

Calibration curve of SMV: Calibration curve of proceeded SMV in water at concentrations of 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL, 10 µg/mL, 12 µg/mL, 14 µg/mL, and 16 µg/mL, with absorbance values of 0.158, 0.210, 0.376, 0.488, 0.624, 0.748, 0.863, and 0.9. Figure 3 shows the calibration curve of SMV in distilled water. The standard calibration curve of SMV in pH 6.8 phosphate buffer followed the same steps as the concentration in distilled water, with absorbance values of 0.140, 0.260, 0.349, 0.490, 0.620, 0.732, 0.849, and 0.945, as shown in figure 4.

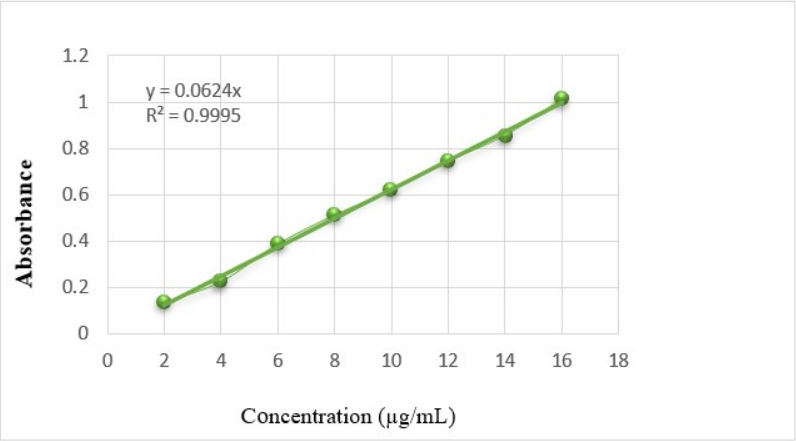


Figure 3: Calibration curve of SMV in distilled water.

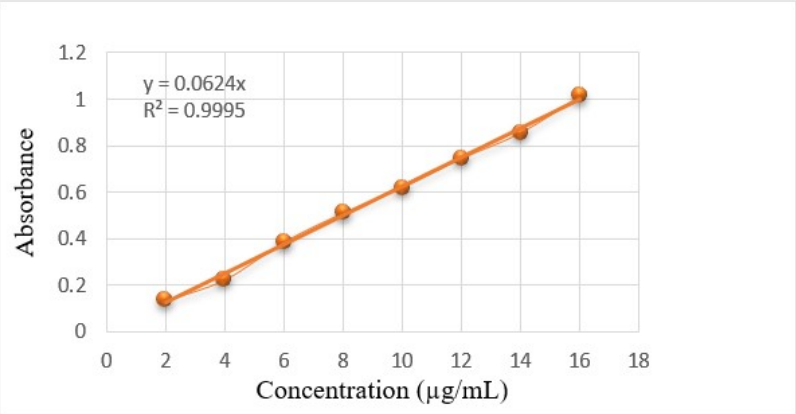


Figure 4: Calibration curve of SMV in pH 6.8 phosphate buffer

IR Spectroscopy Study

Simvastatin in its purest form exhibits distinct peaks in its FT-IR spectrum as shown in fig 5, at 3554 cm^{-1} for the stretch vibration of the OH, 2968 cm^{-1} and 2929 cm^{-1} for the stretch vibration of the CH, and 1267 cm^{-1} and 1724 cm^{-1} for the stretch vibration of the C-O and C=O carbonyl functional groups.

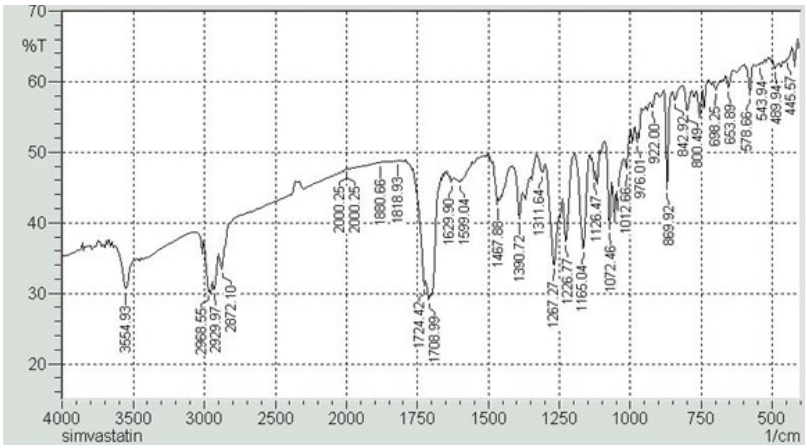


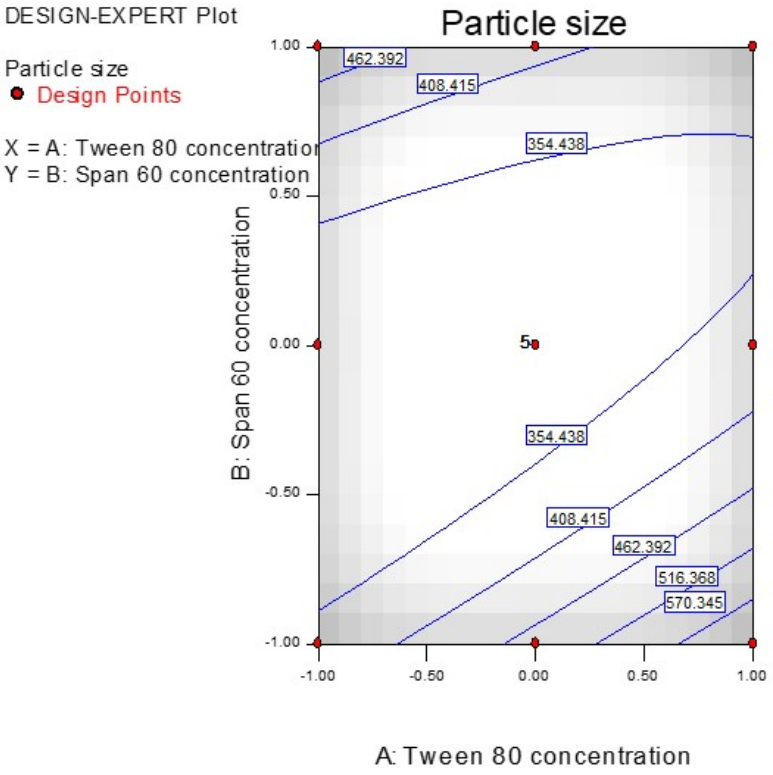
Figure 5: FTIR spectrum of simvastatin (SMV)

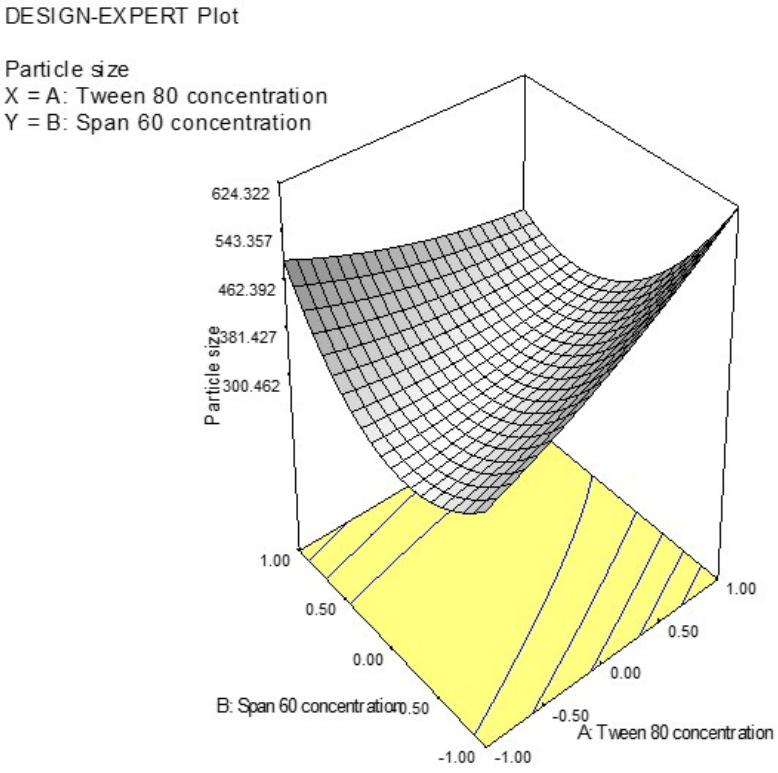
Drug and Polymer Interaction Study

Thin Layer Chromatography (TLC) – SMV: SMV + Tween 80 – 0.32, SMV + Span 60 – 0.29, and SMV + LLP – 0.31 were discovered utilizing TLC and Rf values of SMV – 0.30, SMV + Tween 80 – 0.32, SMV + Span 60 – 0.29, and SMV + LLP – 0.31. SMV has a standard Rf value of 0.3 + 0.02 as documented in the literature.

Pseudo-ternary Phase diagram study: For phase behavior, the graphic is really informative as the pseudo-ternary phase diagrams for manifold emulsion with the surfactant and co-surfactant ratios elucidates the area of manifold emulsion zones changes as the ratio of surfactant to co-surfactant changes in the ternary phase diagram. When the surfactant to co-surfactant ratio was altered, the area of manifold emulsions did not vary.

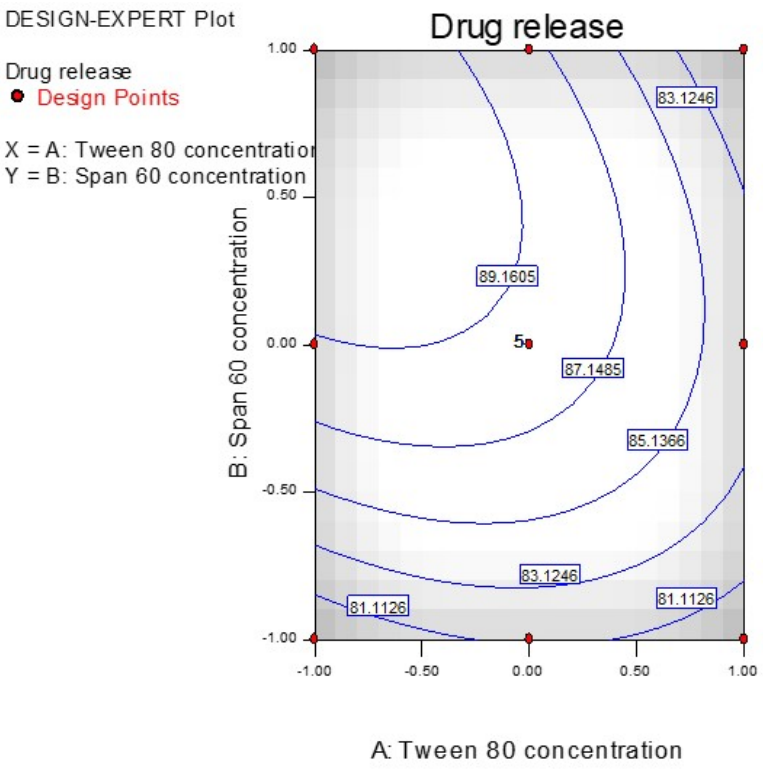
Optimization

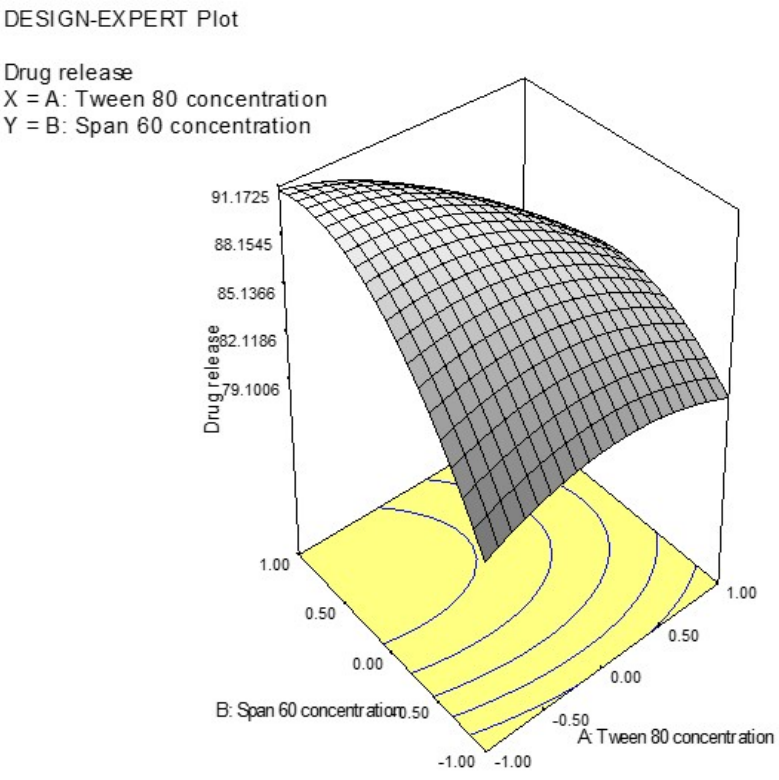




(b)

Figure 5: 2D contour plot (a) and 3D response surface plot (b) for particle size





(b)

Figure 6: 2D contour plot (a) and 3D response surface plot (b) for Drug release

To find the impacts of sovereign factors (X_1, X_2) on dependent variables (Y_1, Y_2), a 3^2reply surface approach stayed used. The effects of an independent variable were examined using 2D (figures 5a, 6a) and 3D counters plots (figure 5b, 6b). When attempting to comprehend the primary impacts and interactions of the independent variables, the three-dimensional (3D)

response surface graph is quite obliging. In all 13 experimental runs, which are listed in table 1, the particle size ranged from 298.2-698.54 nm and the DR ranged from 79.8-92.45 percent in 45 minutes. Contour plot and polynomial equations are developed in order to study the mathematical relationship between the dependent variable and the independent variables. For the quadratic model for both Y1 and Y2 responses, correlation coefficient (R^2) value was 0.7669 and 0.8833, respectively, it is signifying good fit (shown in **Table 2**). For Particle size (Y1) and DR (Y2) response following equations was obtained.

$$Y1 = +322.72 + 34x_1 - 29.17x_2 + 18.97x_1^2 + 128.47x_2^2 - 91x_1x_2 \quad (3)$$

$$Y2 = +88.41 - 2.50x_1 + 3.17x_2 - 1.95x_1^2 - 3.95x_2^2 - 2.75x_1x_2 \quad (4)$$

Positive and negative numbers represent the synergistic and antagonistic effects, respectively, in the equations above. Table 3 provides the ANOVA for models Y_1 and Y_2 response. For Particle size (Y_1), quadratic equation predicts that, it was affected by the independent variables x1, x_1^2, x_2^2 has synergistic effect as well as (3) factor x2,and x1x2 has antagonistic effect. Similarly, in drug release (Y_2) response, it also signifies quadratic equation, it was affected by the independent factors x2 has positive effect and factors x1, x_1^2, x_2^2 and x1x2 has negative effect. P <0.05 was considered significant for the effects of these independent factors on drug release (DR) and particle size (nm). Both the models were significant at *F* values of 4.60 and 5.71 at P <0.05. The diagnostic case statistics for different response variables are shown in Table 4 together with actual, expected, and residual values. The prediction error was obtained by comparing the experimental value that resulted to the value that was predicted. Since there was less of a discrepancy between the actual and projected values, the model was found to be strongly fit.

Source	Std.Dev.	R-Squared	AdjustedR-Squared	PredictedR-Squared	Remarks
Y1					
Linear	112.1196	0.087407	-0.09511	-1.17546	
2FI	101.4255	0.327875	0.103833	-2.43108	
Quadratic	67.72433	0.766922	0.600438	-0.9003	Suggested
Cubic	52.26425	0.90085	0.76204	-7.54165	
Y2					
Linear	4.038596	0.374533	0.24944	-0.34085	
2FI	3.842056	0.490536	0.320715	-1.24316	
Quadratic	2.706876	0.803312	0.66282	-0.15177	Suggested
Cubic	2.3003	0.898543	0.756503	0.122813	

Table 2: Summary of results of regression analysis for responses *Y*1 and *Y*2

Source	Sum ofSquares	DF	MeanSquare	F Value	Prob > F	Remarks
Y1						
Model	105642.2	5	21128.44	4.606574	0.0352	significant
x1	6936	1	6936	1.512236	0.2585	
x2	5104.167	1	5104.167	1.112847	0.3265	
x12	993.4319	1	993.4319	0.216595	0.6558	
x22	45580.79	1	45580.79	9.93785	0.0161	
x1x2	33124	1	33124	7.221932	0.0312	

Y2						
Model	209.479	5	41.8958	5.717865	0.0204	significant
x1	37.5	1	37.5	5.117934	0.0581	
x2	60.16667	1	60.16667	8.21144	0.0241	
x12	10.48358	1	10.48358	1.43078	0.2706	
x22	43.05501	1	43.05501	5.876071	0.0458	
x1x2	30.25	1	30.25	4.128467	0.0817	

Table 3: ANOVA of models for *Y*1 and *Y*2

Batch No.	ActualValue	PredictedValue	Residual	Batch No.	ActualValue	PredictedValue	Residual
Y1				Y2			
F1	412	374.3218	37.67816	F1	80	79.10057	0.899425
F2	369	480.3563	-111.356	F2	79	81.29885	-2.29885
F3	698	624.3218	73.67816	F3	81	79.60057	1.399425
F4	302	307.6897	-5.68966	F4	90	88.96552	1.034483
F5	367	322.7241	44.27586	F5	86	88.41379	-2.41379
F6	298	375.6897	-77.6897	F6	84	83.96552	0.034483
F7	466	497.9885	-31.9885	F7	89	90.93391	-1.93391
F8	450	422.023	27.97701	F8	91	87.63218	3.367816
F9	388	383.9885	4.011494	F9	79	80.43391	-1.43391
F10	360	322.7241	37.27586	F10	92	88.41379	3.586207
F11	355	322.7241	32.27586	F11	88	88.41379	-0.41379
F12	300	322.7241	-22.7241	F12	86	88.41379	-2.41379
F13	315	322.7241	-7.72414	F13	89	88.41379	0.586207

Table 4: Diagnostics case statistics for various response variables

Characterization of Manifold Emulsions: As per literature, the pH of several emulsions was found to be in the range of 4.5 to 6.5. The optimized formulation P4 had a pH of 5.3. Photon correlation spectroscopy was used to quantify particle size with a Malvern particle size counter, which can measure size by intensity. To acquire the optimal scattering intensity for size analysis, roughly 0.1 ml manifold emulsions were added to 10 ml double distilled water. The improved manifold emulsion (P4) had an average particle size of 300.7 d. nm which shown in figure 7.

			Size(d.nm):	%Intensity	Width(d.nm):
Z-Average(d.nm):	300.7	Peak1:	320.8	100.0	88.77
PdI:	0.129	Peak2:	0.000	0.0	0.000
Intercept	0.940	Peak3:	0.000	0.0	0.000

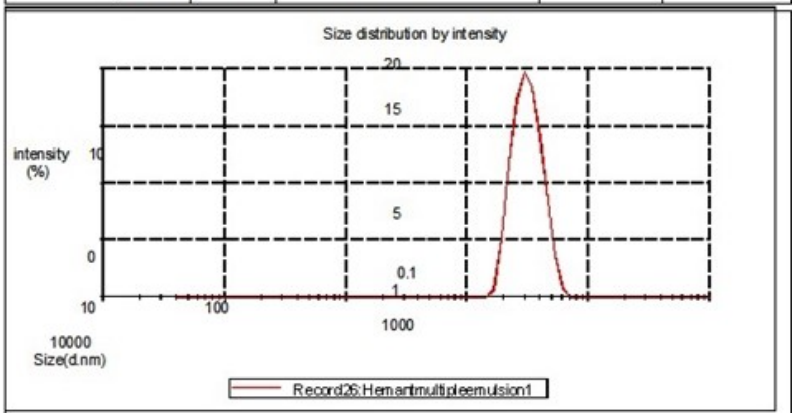


Figure 7: Particle size of SMV manifold emulsion (P4)

Zeta Potential: Zeta potential of P4 was -19.4 mv. The negative Zeta potential indicates that droplets of manifold emulsions having no charge that is system is stable. Zeta potential was determined by using Zetasizer. There was no charge on particles, so no flocculation of particles has been observed such a thing responsible to leading the longer shelf life of the SMV manifold emulsion. The manifold emulsion was stable and the results of zeta potential are shown in figure 8.

			Mean(mV)	Area(%)	Width(mV)
ZetaPotential(mV):	-19.4	Peak1:	18.8	100.0	4.29
ZetaDeviation(mV):	90.6	Peak2:	0.00	0.0	0.00
Conductivity(mS/cm):	0.138	Peak3:	0.00	0.0	0.00

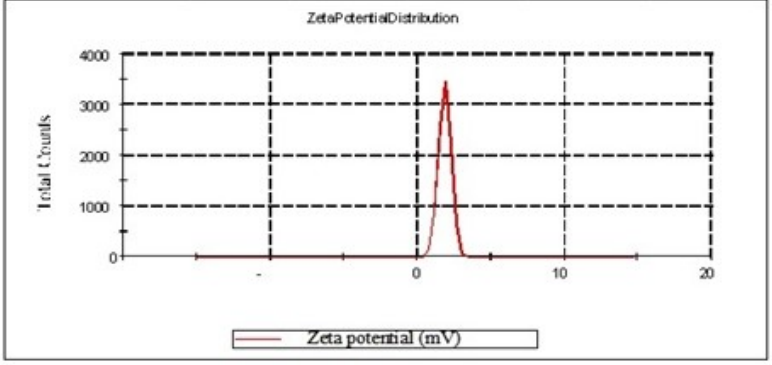


Figure 8: Zeta potential of SMV manifold emulsion (P4)

Viscosity: Such rheological property of the multiple emulsions was evaluated by BROOKFIELD-DV-II+ pro viscometer using spindle 00 UL adaptor at 25 ± 0.5 °C, at 5 rpm. The optimized formulation's viscosity was determined and for all formulations was found to be less than 1560 centipoise (cP). For P1 and P2 have lower viscosity than P3 and P4, because P3 and P4 have higher surfactant concentrations than P1 and P2. Tween 80 and Span 60, for example, were exceedingly viscous surfactants. Tween 80 and Span 60 had viscosities of 970-1080 CP and 425 CP, correspondingly. Because the concentration of surfactants in P5 and P6 is higher than in P3 and P4, the viscosity is higher. Lower viscosity is a desirable attribute of various emulsions, as seen by the viscosity results are as for P1-1525, P2-1530, P3-1533, P4-1540, P5-1548 and P6-1553 cP respectively.

Entrapment Efficiency: For determination of entrapment efficiency, freshly prepared W/O/W multiple emulsions were

centrifuged at 4000 r/min for 10 minutes to calculate percent entrapment efficiency (%EE). Then, using a 2 ml hypodermic needle, 1 mL of the aqueous phase (the bottom layer) was accurately removed and diluted with phosphate buffer 7.4 as directed. The solution was filtered through a millipore filter (0.22 mm pore size) and the drug content was measured at 238 nm using a UV spectrophotometer. The system was observed microscopically for appearance. After centrifugation was performed entrapment efficiency. The entrapment efficiency of formulation batches are as P1-80.66%, P2-97.98%, P3-98.36%, P4-99.2%, P5-87.34% and P6-89.44%. The P4 Batch formulation has highest entrapment efficiency. This type of entrapment efficiency determination is highly beneficial for content estimation in the creation of multiple emulsions. The following equation was used to calculate the encapsulation efficiency.

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

***In vitro* drug release studies:** *In-vitro* diffusion testing of several emulsions was done using a diffusion cell of Franz with a 2.0 cm diameter and a 16 ml capacity. A dialysis membrane with a molecular weight cutoff range of 12000–14000 kDa was utilized as the diffusion membrane. Pieces of dialysis membrane were soaked in phosphate buffer pH 6.6 for 24 hours before to the experiment. The dialysis membrane was put atop the diffusion cell, which was filled with phosphate buffer pH 6.6. The temperature was kept at 37 °C. The numerous emulsion equivalents to 10 mg of simvastatin were deposited in the donor chamber after a 20-minute pre-incubation period. Samples were taken out of the receptor compartment for 4 hours at a time and replaced with the same quantity of new phosphate buffer solution, then measured at 238 nm with a spectrophotometer. The results show that the B4 formulation has a better release profile than the others. The release profile of the formulation was raised as the concentration of span 60 increased, as seen in figure 9.

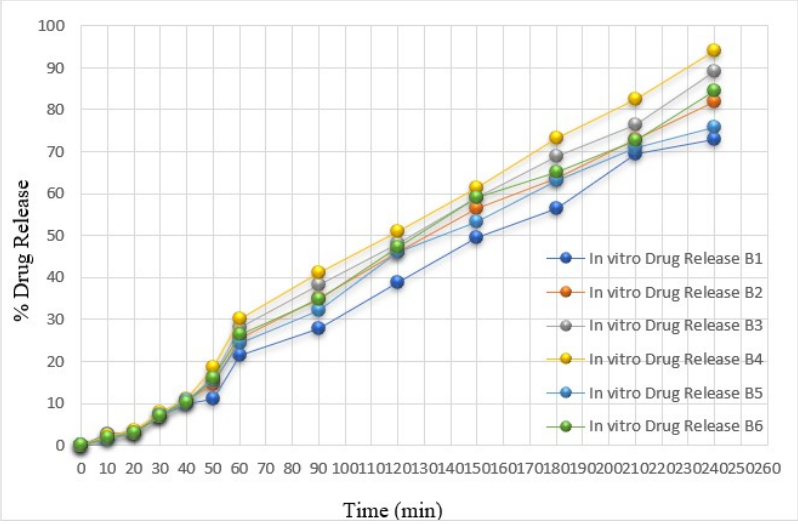


Figure 9: *In vitro* drug release studies of SMV manifold emulsions

Differential Scanning Colorimetric Analysis: In a nitrogen atmosphere, differential scanning calorimetry (DSC) of the medication, simvastatin, and mixing of all constituents in simvastatin multiple emulsion was performed by heating the sample from 300°C to 4000°C at a rate of 100°C/min. Thermogram revealed that there was no interaction between the medication and the surfactant. Differential Scanning Colorimetric (DSC) of drug, SMV and mixture of all ingredients in SMV manifold emulsion were carried out by heating the sample from 30°C to 400°C, at heating rate of 10°C /min. in a nitrogen environment. Thermogram obtained was observed that no interaction has occurred between drug and the surfactant and the results are showed in figure 10.

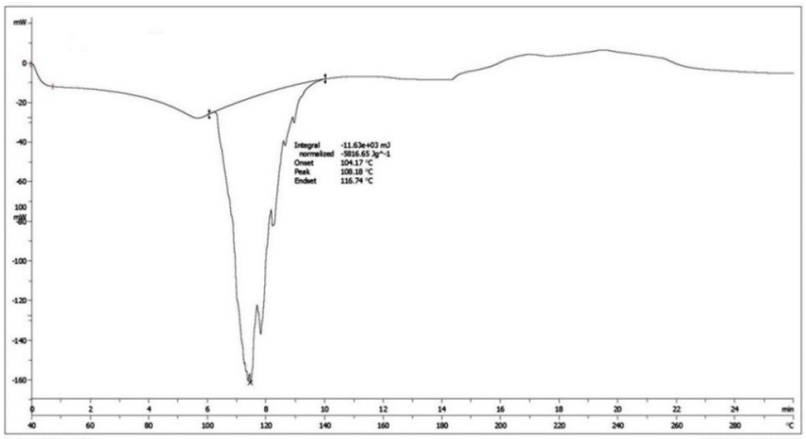


Figure 10: Differential Scanning Colorimetric (DSC) of drug

Stability Study

For stability testing, formulas with the best particle size, microscopic appearance, entrapment efficiency, and *in vitro* drug release studies were chosen. The selected formulation of batch - P4 was stored for 90 days at 40°C and 75 % relative humidity (RH) by rendering to the International Council for Harmonization (ICH) recommendations. The particle size, microscopic appearance, entrapment efficiency, and *in vitro* drug release study of the formulations were all examined at one-month intervals. The evaluation parameters do not indicate any significant differences and are all within acceptable ranges, as shown in table 5.

Formulation Code	Parameters	Storage Time			
		0 Month	1 Month	2 Month	3 Month
P4 Batch	Particle size (d. nm)	300.4	300.9	300.6	300.2
	Microscopic appearance	Clear with translucent appearance	Clear with translucent appearance	Clear with translucent appearance	Clear with translucent appearance
	Entrapment efficiency	99.09	99.02	99.22	99.04
	In vitro drug release study	97.16	97.10	97.11	97.13

Table 5: Evaluation parameter of stability batch after 3 months

***In Vitro* Release Study of Manifold Emulsion**: Under the same experimental settings, the improved batch was assessed for *in vitro* dissolution research, and the results were documented in table 6 and figure 11.

Time (min)	*In vitro* release study of manifold emulsion
0	0
10	2.48
20	3.55
30	7.97
40	11.03
50	18.39

60	30.22
90	42.01
120	50.82
150	61.2
180	73.22

Table 6: Table shows data of *in vitro* dissolution study

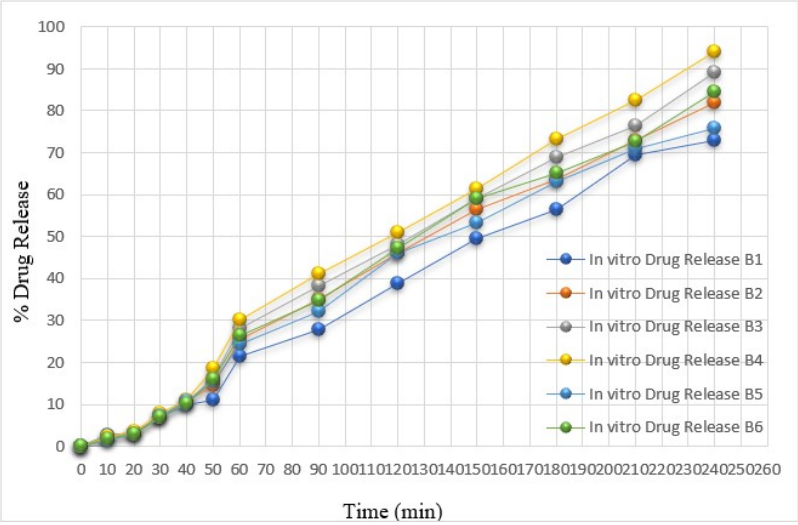


Figure 11: *In vitro* dissolution study of manifold emulsion.

Discussion

The Rf value of SMV was 0.31, and various mixtures of drug and polymers showed no significant differences when compared to the conventional Rf value of the medicine. So, according to the TLC investigation, there was no interaction between the medication and the surfactant.

Zeta potential: The negative Zeta potential indicates that droplets of manifold emulsions having no charge that is system is stable. There was no charge on particles, so no flocculation of particles has been observed.

Viscosity: Lower viscosity is a desirable attribute of various emulsions, as seen by the viscosity results are as for P1-1525, P2-1530, P3-1533, P4-1540, P5-1548 and P6-1553 cP respectively.

Entrapment Efficiency: The P4 Batch formulation has highest entrapment efficiency. This type of entrapment efficiency determination is highly beneficial for content estimation in the creation of multiple emulsions.

***In Vitro* Drug Release Studies:** The release profile of the formulation was raised as the concentration of span 60 increased.

Differential Scanning Colorimetric Analysis: Thermogram obtained was observed that no interaction has occurred between drug and the surfactant.

Stability Study: The evaluation parameters do not indicate any significant differences and are all within acceptable ranges. As per the *in vitro* release study of manifold emulsion shows significantly prolong release of drug as in 180 min. manifold emulsion able to release the drug up to 73.19%.

Conclusions

The liquid paraffin, Tween 80, and Span 60 can be used to create a stable manifold emulsion, which has successfully improved SMV's ability to dissolve. These three substances can also be used as options for oils, surfactants, and co-surfactants. The creation of the ideal mixture of oil, surfactant, and co-surfactant for the creation of a stable manifold emulsion has been demonstrated to be an essential step. Using an in vitro release study, we assess the safety, efficiency, and quality with potency of the SMV manifold emulsion. As a result, it is possible to decrease the frequency of dosages, adverse effects, and improve patient compliance.

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