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Formulation And Evaluation Of Multiple Emulsion Of Valsartan

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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. Valsartan 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 Valsartan 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.
Key words: Multiple emulsions, Non-ionic surfactants, Valsartan, 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 simultaneously[1]. 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[1]. These two liquids forming a system are characterized by their low thermodynamic stability[2]. 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 [3]. 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 biopharma ceutical applications[3] include their use as adjuvant vaccines[4], as prolonged drug delivery systems[5-8], as sorbent reservoirs in drug overdose treatments[9] and in mobilization of enzymes[10-11]. 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[12-14].

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[15].

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

prepared using the 2-step method, one that has a low Hydrophile-Lipophile Balance (HLB) value to stabilize the primary W/O emulsion and one that has a high HLB value to stabilize the secondary O/W emulsion. The low-HLB surfactant is dominantly hydrophobic and is added to the oil phase. The high-HLB surfactant is dominantly hydrophilic and is added to the outer continuous aqueous phase. The concentration ratio of these two surfactants is important to obtain stable and high yields of W/O/W emulsions[16].

A unique property of W/O/W multiple emulsions com-pared to simple W/O emulsions is the diffusion of water through the oil phase because of unbalanced osmotic pressures between the internal and external aqueous phases. The oil layer acts as a membrane separating these two aqueous phases. Polar molecules dissolved in either the internal aqueous phase or the external continuous aqueous phase can pass through the oil layer by diffusion because of the concentration gradient. In the case of water this is driven by osmotic pres-sure. Molecules are often transported via micelles of hydrophobic surfactant present in the oil phase. Water diffusion causes swelling, bursting, or shrinkage of the internal aqueous droplets, affecting the stability of the multiple droplets as well as the release profiles of the active ingredients loaded in the inner dispersed aqueous phase[17].

Most cardiovascular events are attributed to high blood pressure. High blood pressure is quantitatively the largest single risk factor for premature death and disability due to its extremely high prevalence in industrialized countries. Hence, antihypertensive therapy considerably reduces the risk of developing cardiovascular complications that cause a high mortality rate in patients with hypertension [18,19].

Valsartan is a new potent, highly selective and orally active antihypertensive drug belonging to the family of angiotensin II type 1 receptor antagonists. Valsartan inhibits angiotensin II receptors, thereby relaxing blood vessels and causing them to widen, which lowers blood pressure and improves blood flow.[20,21]Valsartan is well tolerated after single and multiple dosing following single oral doses up to 400 mg and after multiple dosing[22-24] with 200 mg per day.

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 valsartan and increased oral bioavailability of valsartan in patient as compared to known solid oral dosage forms of valsartan. Development of multiple emulsions dosage formulation that have improved bioavailability to the known oral dosage forms of valsartan is challenging due to the multiplicity of challenges arising from pharmacokinetic aspects of oral drug delivery.

Valsartan has an oral bioavailability of only about 25% with a wide range of 25-40% in humans with large inter- and intra-subject variabilities. Valsartan 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 valsartan 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 valsartan with less inter and intra-subject variability is challenging.

Accordingly multiple emulsions dosage formulation of valsartan 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 valsartan"[25].

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. Valsartan 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.

EXPERIMENTAL

Physical appearance: The drug (Valsartan) 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.

Preparation of calibration curves (true wavelength approx 248 nm-255 nm)

Valsartan solution was scanned in the U.V. range of 200-400 nm using Systronic Double beam UV Visible spectrophotometer.

Determination of Wavelength of Maximum Absorbance (Λmax)

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

Preparation of Calibration Curve of Valsartan

The calibration curve was plotted between the concentration and absorbance. The calibration curve of 2-20µg/ml was carried out.

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:

$$\text{Partition Coefficient, } K = \frac{\text{Amount of drug in organic layer}}{\text{Amount of drug in aqueous layer}}$$

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.

Evaluation

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 [29].

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 247.6 nm.

The Encapsulation Efficiency was determined by following equation[5]:

$$\% EE = \frac{[\text{Total drug incorporated} - \text{Free Drug}]}{\text{Total drug}} \times 100$$

Stability tests

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

Zeta Potential

The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system. If all the particles in suspension have a large negative or positive zeta potential then they will tend to repel each other and there will be no tendency for the particles to come together. However, if the particles have low zeta potential values then there will be no force to prevent theparticles coming

together and flocculating. The general dividing line between stable and unstable suspensions is generally taken at either +30 or -30 mV. Particles with zeta potentials more positive than +30 mV or more negative than -30 mV are normally considered stable. However, if the particles have a density different form the dispersant, they will eventually sediment forming a close packed bed.

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[29].

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[29].

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 sp ectrophotometer at 247.6 nm after suitable dilution[30].

RESULT AND DISCUSSION

The objective of present work is to development and evaluation of multiple emulsion of valsartan 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. UV maxima of drug were found out by using Systronic UV spectrophotometer.

Physical appearance: The drug (Valsartan) powder was examined for its organoleptic properties like colour and odour. And it was observed that Valsartan 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). Solubility data shown in **table 2.**
Melting point determination: Melting point of Valsartan was found at 115±2 ºC.

Preparation of calibration curve:
Valsartan solution was scanned in the U.V. range of 200-400 nm using Systronic UV Visible spectrophotometer. The spectrophotometric method of analysis of Valsartan at λ_{max} 247.6 nm was found to be reproducible and highly sensitive. The standard curves of Valsartan were prepared in Methanol and Phosphate buffer solution (pH 6.8), at λ_{max} 247.6 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 **given in table 3.**

Preparation of Calibration Curve of Valsartan 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 'r^2' values were calculated as 0.995 as shown **in table no. and figure no.2.**

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 Valsartan also revealed its hydrophobic nature which is given **in table 4.**

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 5.**

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 40^0C (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[31].

Liquefaction
No liquefaction was observed in the primary emulsion at all storage conditions. For the multiple emulsion, while no liquefaction was observed in the samples kept at 8^0C (in refrigerator) and 25^0C (in oven) during 28 days, slight liquefaction was observed in the samples kept at 40^0C (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[32-33].

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 **8 & 9** and photographs are shown in **Figure 6.** Globule sizes of emulsion systems can be determined by light microscope[34], laser diffraction methods[35], electron microscope[35-37] or by coulter counter. Light microscope was used in this study. The increase or decrease in globule sizes indicates instability[1,3.] 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. Mean globule size of the freshly prepared formulation was reported in **figure7 and 8.**

Microscopic examination
The microscopical images of various formulations are as shown in **figure 4.**

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 5.**

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 9,10,11,12,13.**

Zeta potential
The report of zeta potential is shown in **fig. 6.**

Figure 1: Simple dissolution cell

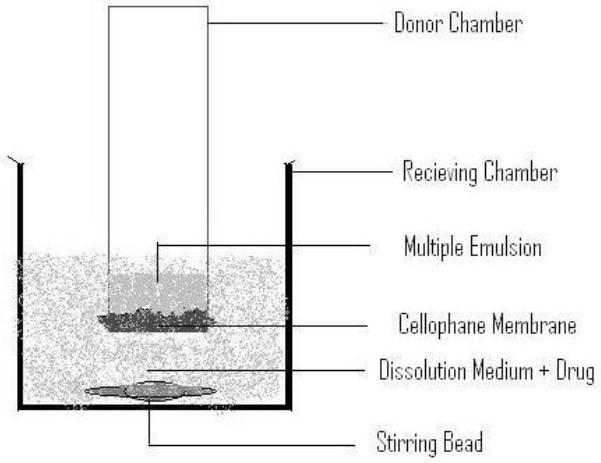


Table 1: List of various ingredients

Formulation	Trial Batch	X1 (mg)	X2 (gm)	X3 (gm)	X4 (gm)	X5 (ml)	X6 (ml)	X7 (ml)
F1	1	100	0.6	-	-	1	14	30
F2	2	100	-	0.6	-	1	14	30
F3	3	100	-	-	0.6	1	14	30

X1-Drug, X2- Span 40, X3- Span 60,X4- Span 80, X5- Tween 80, X6- Liquid Paraffin, X7- Phosphate buffer (6.8).

Table 2: Solubility of Valsartan in different solvents

S. No.	Solvent	Solubility
1.	Phosphate buffer 6.8	Soluble
2.	Methanol	Freely Soluble
3.	Ethanol	Freely soluble
4.	Acetonitrile	Soluble
5.	Water	Sparingly Soluble

Table 3: Standard Curve of Valsartan in Phosphate buffer (pH 6.8) at 247.6 nm

S. No.	Drug Conc. (µg/ml)	Absorbance	Statistical Parameters
1.	10	0.241	Correlation coefficient- **r** = 0.995 Slope **m** = 0.017 Intercept **c** = -0.034 Equation of Line- y = 0.017 x -0.034
2.	20	0.391	
3.	30	0.571	
4.	40	0.732	
5.	50	0.891	

Figure 2: calibration curve of valsartan in phosphate buffer 6.8

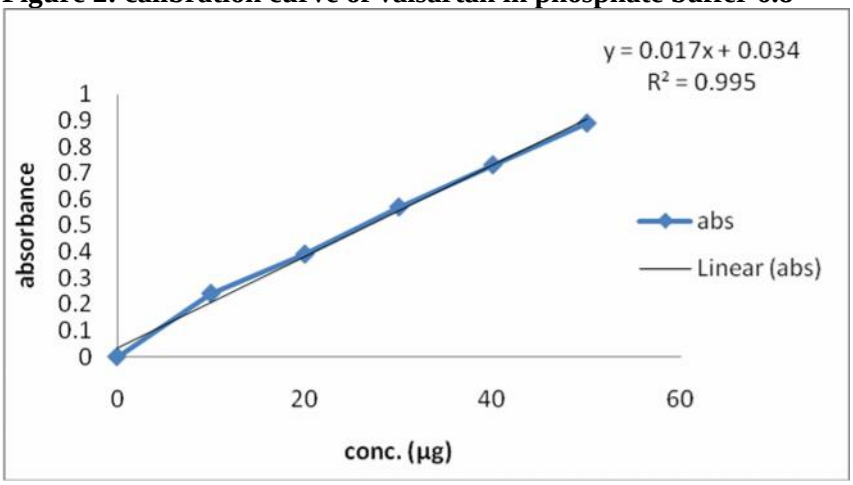
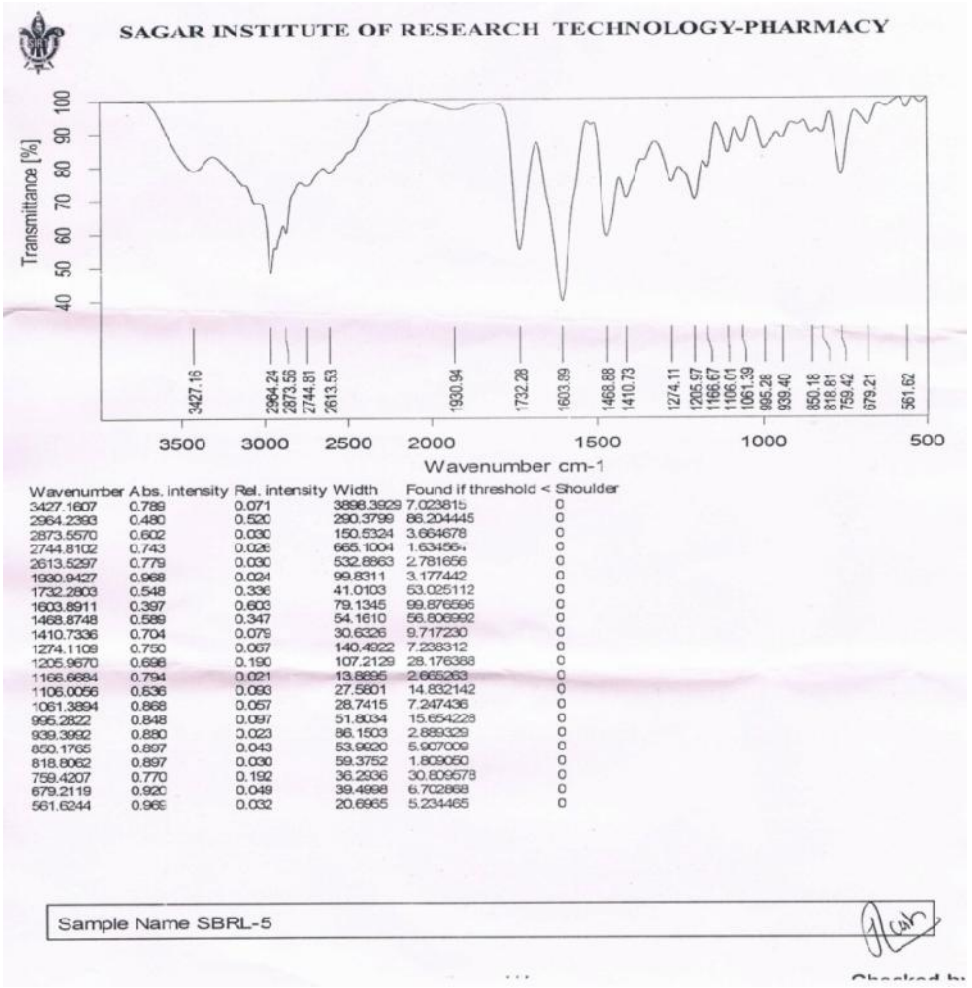
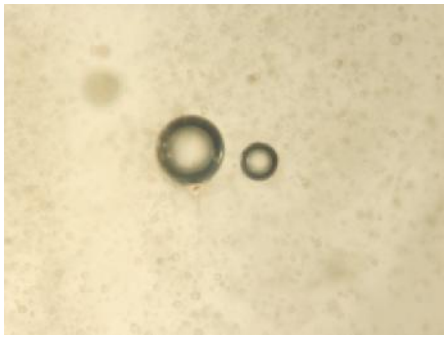


Figure 3: FTIR of Valsartan

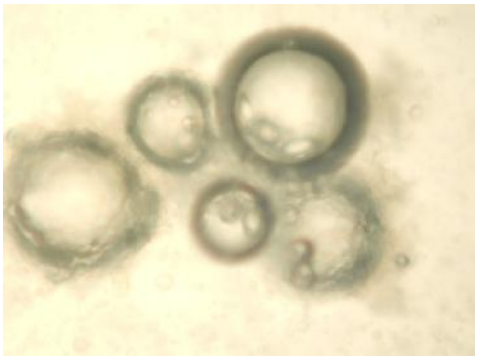


Wavenumber	Abs. intensity	Rel. intensity	Width	Found if threshold <	Shoulder
3427.1607	0.789	0.071	3898.3929	7.023815	0
2964.2393	0.480	0.520	290.3799	86.204445	0
2873.5570	0.602	0.030	150.5324	3.664678	0
2744.8102	0.743	0.026	665.1004	1.634564	0
2613.5297	0.779	0.030	532.8863	2.781656	0
1930.9427	0.968	0.024	99.8311	3.177442	0
1732.2803	0.548	0.336	41.0103	53.025112	0
1603.8911	0.397	0.603	79.1345	99.876596	0
1468.8748	0.589	0.347	54.1610	56.806992	0
1410.7336	0.704	0.079	30.6326	9.717230	0
1274.1109	0.750	0.057	140.4922	7.238312	0
1205.9670	0.698	0.190	107.2129	28.176388	0
1166.6684	0.794	0.021	13.8695	2.665263	0
1106.0056	0.636	0.093	27.5801	14.832142	0
1061.3894	0.868	0.057	28.7415	7.247436	0
995.2822	0.848	0.097	51.8034	15.654228	0
939.3992	0.880	0.023	86.1503	2.889329	0
850.1765	0.897	0.043	53.9920	5.907009	0
818.8062	0.897	0.030	59.3752	1.809050	0
759.4207	0.770	0.192	36.2936	30.809578	0
679.2119	0.920	0.049	39.4998	6.702868	0
561.6244	0.969	0.032	20.6965	5.234465	0

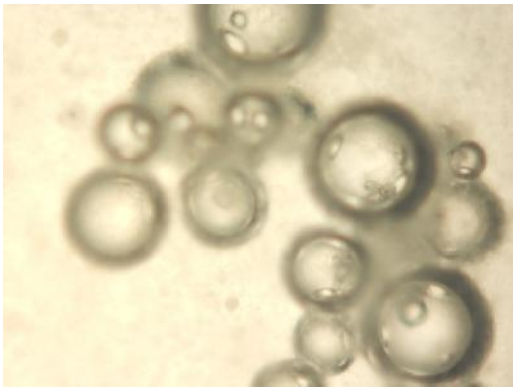
Figure 4: Microscopic image of F1, F2, F3.



(F1)



(F2)



(F3)

Table 4: Partition coefficient values of Valsartan

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

Figure 5: showing entrapement efficiency

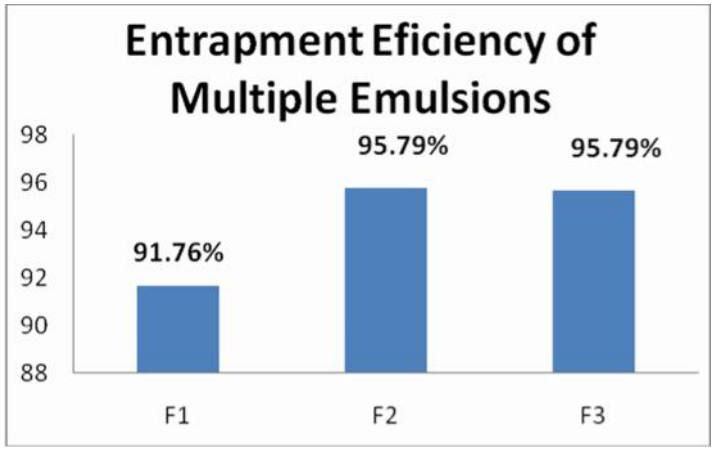


Table 5: Important band frequencies in IR spectrum of valsartan

Peak (cm^{-1})	**Groups**	**Observed peak value(cm^{-1})**
3000-2950	Aromatic cyclic enes	2964
1750-1700	CO group of acid	1732
1650-1600	Quinolines	1603
1450-1400	Carbonyl group	1410
1300-1250	Hydroxyl group	1274

Table 6: Organoleptic characteristics:

Organoleptic characteristics of the primary and multiple emulsions formulated are presented in Table 6.

Time	Liquefaction			Color			Phase separation			Centrifugation		
	A	B	C	A	B	C	A	B	C	A	B	C
0 hr	-	-	-	W	W	W	-	-	-	-	-	-
1 hr	-	-	-	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 = $8^{o}C$; B = $25^{0}C$; C = $40^{0}C$ (in oven) (n = 3).

Table 7: showing Invitro release Study:

Time (Hr)	**Cumulative % of Drug Release**		
	F1	**F2**	**F3**
1	7.44	7.72	8.14
2	13.65	13.39	15.09
3	20.15	19.74	23.85
4	28.50	26.81	33.58
5	37.44	35.34	43.36
6	47.05	45.04	53.22
7	57.19	55.34	63.96
8	68.16	66.06	75.01

Figure 6: Zeta potential Report

Zeta Potential Report

Sample Details

Sample Name:	F-3 ZETA 1		
SOP Name:	mansettings.dat		
General Notes:			
File Name:	F-3 ZETA.dts	**Dispersant Name:**	Water
Record Number:	1	**Dispersant RI:**	1.330
Date and Time:	Tuesday, August 21, 2012 5:1...	**Viscosity (cP):**	0.8872
		Dispersant Dielectric Constant:	78.5

System

Temperature (°C):	25.0	**Zeta Runs:**	13
Count Rate (kcps):	94.9	**Measurement Position (mm):**	2.00
Cell Description:	Clear disposable zeta cell	**Attenuator:**	7

Results

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-38.5	**Peak 1:**	-39.1	96.5	4.04
Zeta Deviation (mV):	5.16	**Peak 2:**	-20.4	3.5	2.37
Conductivity (mS/cm):	0.138	**Peak 3:**	0.00	0.0	0.00
Result quality	Good				

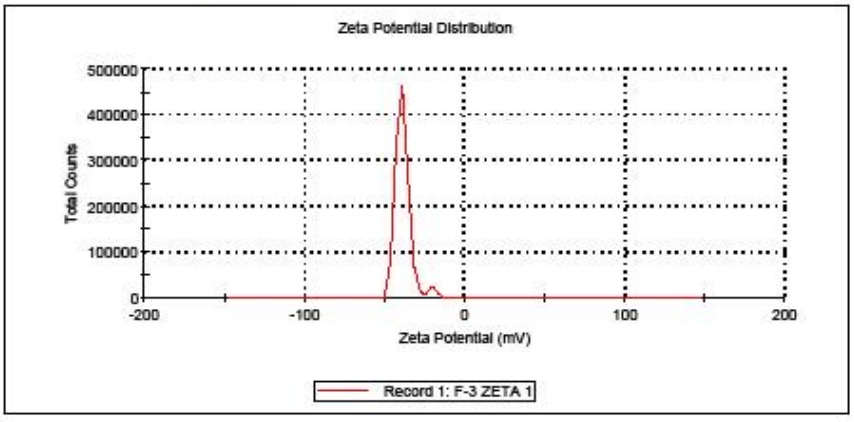
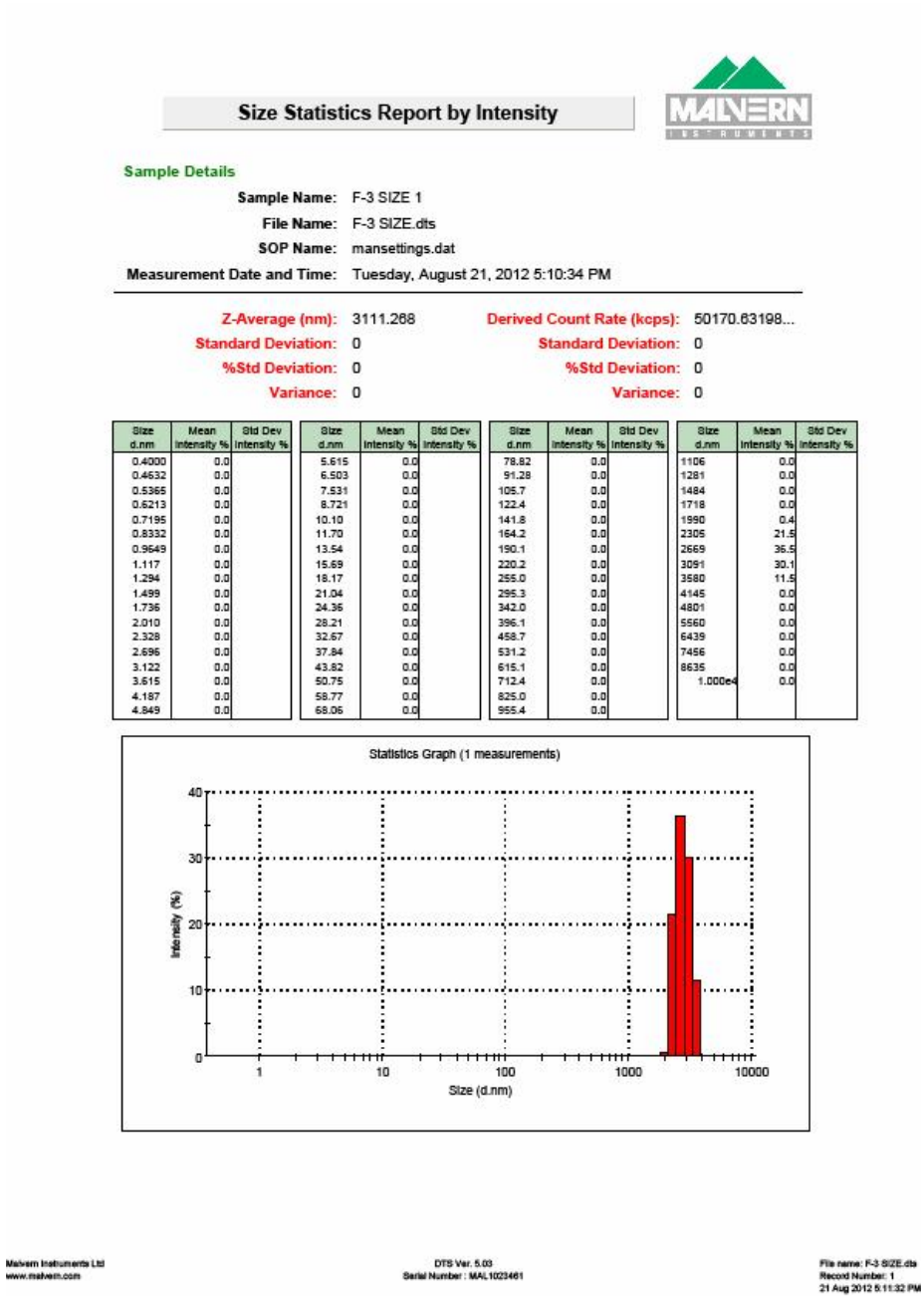


Figure 7: showing size statistics report



Size Statistics Report by Intensity

Sample Details

Sample Name: F-3 SIZE 1
File Name: F-3 SIZE.dts
SOP Name: mansettings.dat
Measurement Date and Time: Tuesday, August 21, 2012 5:10:34 PM

Z-Average (nm): 3111.268
Standard Deviation: 0
%Std Deviation: 0
Variance: 0

Derived Count Rate (kcps): 50170.63198...
Standard Deviation: 0
%Std Deviation: 0
Variance: 0

Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %
0.4000	0.0		5.615	0.0		78.82	0.0		1106	0.0	
0.4632	0.0		6.503	0.0		91.28	0.0		1281	0.0	
0.5365	0.0		7.531	0.0		105.7	0.0		1484	0.0	
0.6213	0.0		8.721	0.0		122.4	0.0		1718	0.0	
0.7195	0.0		10.10	0.0		141.8	0.0		1990	0.4	
0.8332	0.0		11.70	0.0		164.2	0.0		2305	21.5	
0.9649	0.0		13.54	0.0		190.1	0.0		2669	36.5	
1.117	0.0		15.69	0.0		220.2	0.0		3091	30.1	
1.294	0.0		18.17	0.0		255.0	0.0		3580	11.5	
1.499	0.0		21.04	0.0		295.3	0.0		4145	0.0	
1.736	0.0		24.36	0.0		342.0	0.0		4801	0.0	
2.010	0.0		28.21	0.0		396.1	0.0		5560	0.0	
2.328	0.0		32.67	0.0		458.7	0.0		6439	0.0	
2.696	0.0		37.84	0.0		531.2	0.0		7456	0.0	
3.122	0.0		43.82	0.0		615.1	0.0		8635	0.0	
3.615	0.0		50.75	0.0		712.4	0.0		1.000e4	0.0	
4.187	0.0		58.77	0.0		825.0	0.0				
4.849	0.0		68.06	0.0		955.4	0.0				

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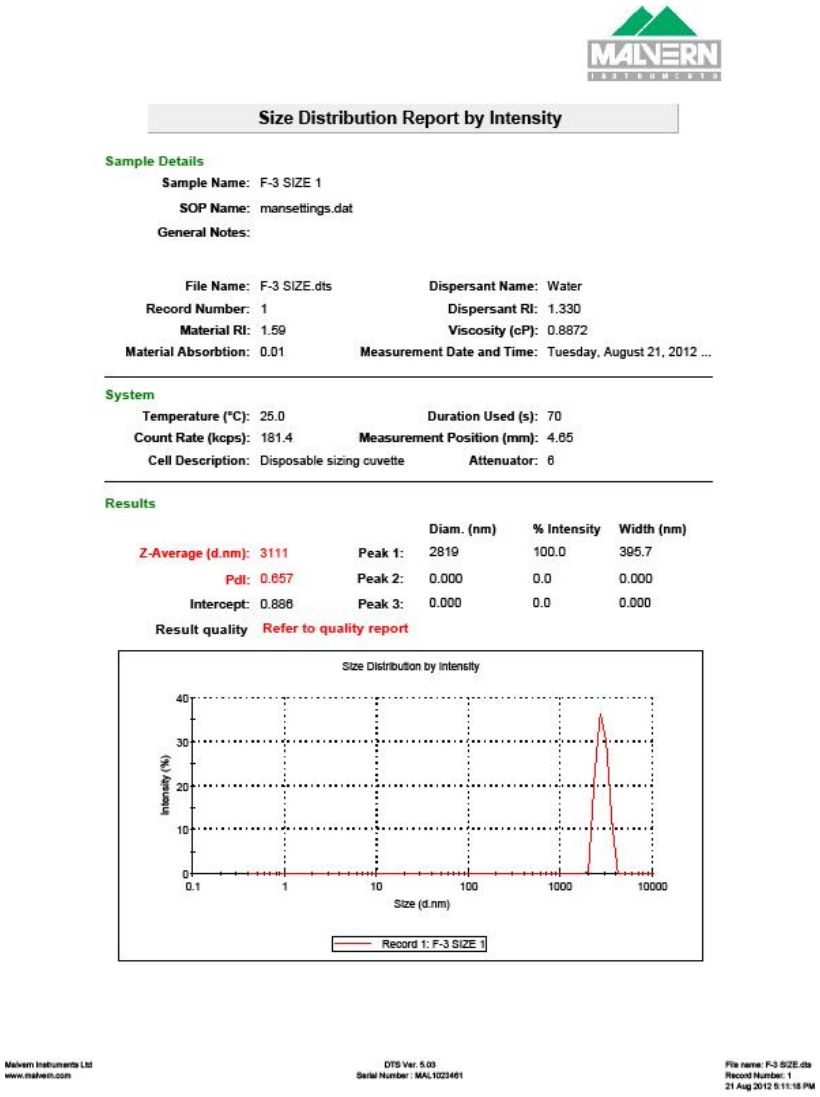
File name: F-3 SIZE.dts
Record Number: 1
21 Aug 2012 5:11:32 PM

Figure 8: size distribution report



Size Distribution Report by Intensity

Sample Details

Sample Name: F-3 SIZE 1
SOP Name: mansettings.dat
General Notes:

File Name: F-3 SIZE.dts
Record Number: 1
Material RI: 1.59
Material Absorbtion: 0.01
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Measurement Date and Time: Tuesday, August 21, 2012 ...

System

Temperature (°C): 25.0
Count Rate (kcps): 181.4
Cell Description: Disposable sizing cuvette
Duration Used (s): 70
Measurement Position (mm): 4.65
Attenuator: 6

Results

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 3111	Peak 1:	2819	100.0	395.7
PdI: 0.657	Peak 2:	0.000	0.0	0.000
Intercept: 0.886	Peak 3:	0.000	0.0	0.000

Result quality: Refer to quality report

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Figure 9: In-vitro drug release of multiple emulsions

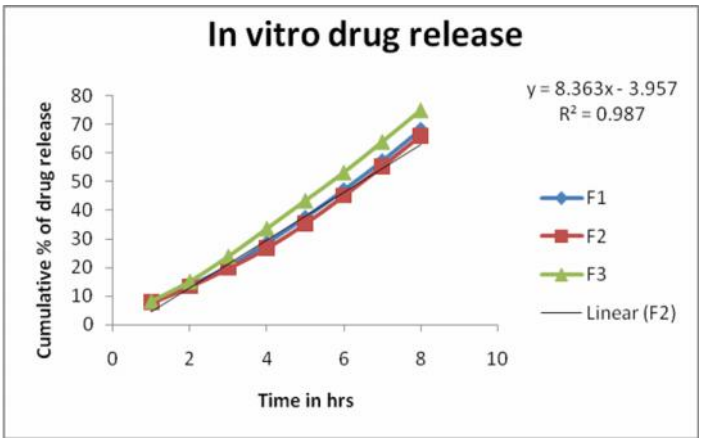


Figure 10: showing Zero order release kinetics of F1, F2, F3

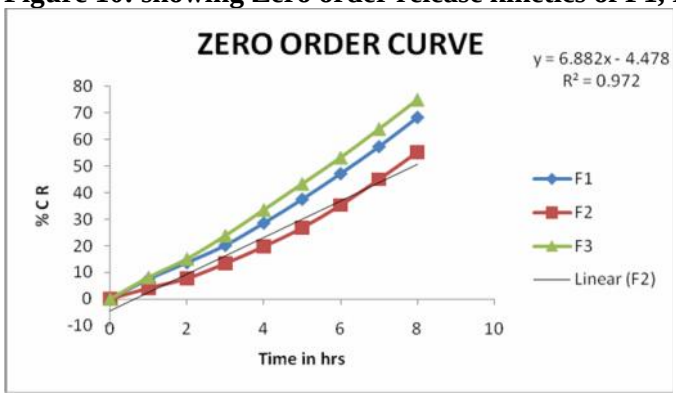


Figure 11: showing First order release kinetics of F1, F2, F3

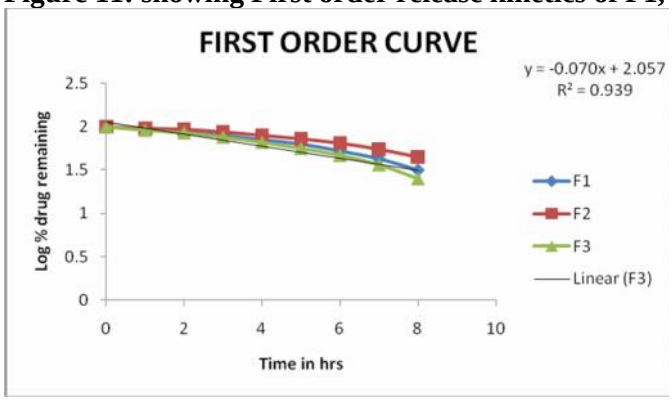


Figure 12: showing Higuchi release kinetics of F1, F2, F3.

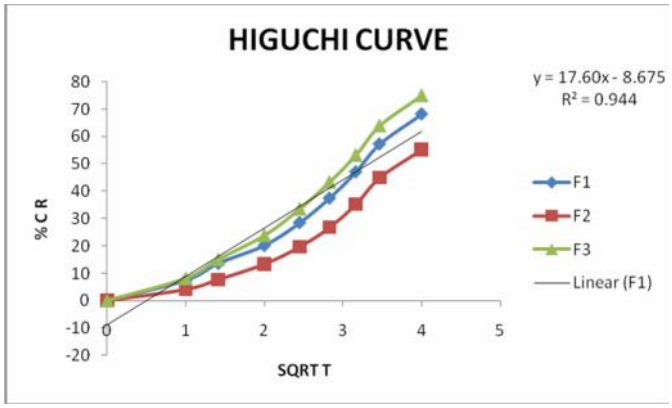
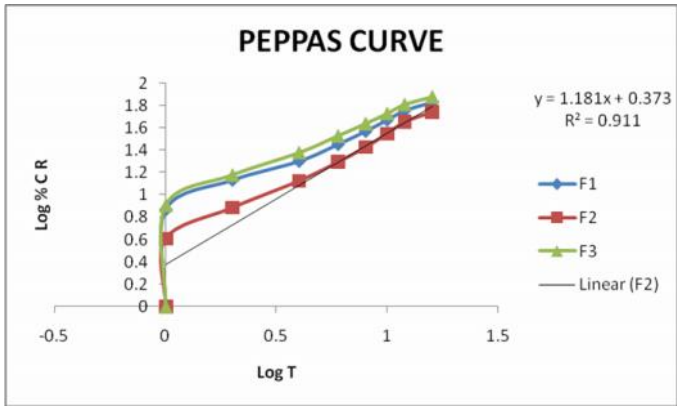


Figure 13: showing Peppas release kinetics of F1, F2, F3.



SUMMARY AND CONCLUSION

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. Valsartan has low bioavailability; few studies have been reported for enhancement of Bioavailability of poorly water soluble drugs by formulating as multiple emulsions. The present study is based on the hypothesis that improvement of in vitro as well as ex vivo dissolution profile will reflect the enhancement of bioavailability of the drug.

The objective of present work is to development and evaluation of multiple emulsion of valsartan 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 6.

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 valsartan and non-ionic surfactants like Tween80, span40, span60, span80 by two step emulsification methods.

In vitro release profile was applied on various kinetic model in order like zero order first order, Higuchi equation, and Peppas equation. To find out the mechanism of drug release from multiple emulsions. 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. Valsartan which thereby reduce dose frequency, decrease side effect and improved patient compliance.

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