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PREPARTION AND OPTIMIZATION OF NANOEMULSION FORMULATIONS OF ANTIHYPERTENSIVE DRUG CARVEDILOL

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Abstract
The aim of the present study was focused on the development of nanoemulsion of carvedilol, an antihypertensive drug, to be administered through oral route. Twelvenanoemulsionformulations of carvedilol containing different oily phases and different proportions of surfactant-Tween 80 and co-surfactant- PEG 400 were prepared by ultrasonication method and after preliminary evaluation four formulations (F1, F2, F3& F4) were selected for further study. Prepared carvedilol containing nanoemulsions were evaluated for-droplet size and shape through Transmission Electron Microscopy, drug contents and *in-vitro* drug release patterns through dissolution studies. The entrapment efficiency for various formulations was found to be between 61.02±0.231% to 96.57±0.212%. The drug showed better release rate in comparison to conventional dosage form. All the formulations showed better results in terms of stability. Among the four formulations the best results were found with F1 formulation of carvedilol which gave the highest release of drug 31.28±3.46% after 1 hr and 88.41±2.72% after 24 hrs. The droplet size range in optimized formulation F1, F2, F3 and F4 was found to be between 20.76 to 107.38nm. The droplets were uniform and spherical in shape. It can be concluded that for oral administration of carvedilol a better solubility and bioavailability can be achieved by use of nanoemulsion formulation of the drug which otherwise has poor solubility and bioavailability.

Keywords: Carvedilol, Nanoemulsion, *S*mix ratio, Transmission Electron Microscopy, Ultrasonication

INTRODUCTION

Carvedilol is a beta-blocker antihypertensive drug. It is a non-selective, cardiac beta-blocker with peripheral vasodilating effects.[(1)]Beta-blocker category drugs affect the heart and blood circulation through arteries and veins. Carvedilol is used to treat heart failure and hypertension. American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Rhythm Society guideline recommend carvedilol as a beta-blocker of choice for heart failure with reduced ejection fraction (HFrEF).[(2)] Carvedilol is an oral medication dosed twice a day for therapy in immediate-release form or once-daily dosing in a controlled release form.

Carvedilol exhibits very low bioavailability because of its poor aqueous solubility.[(3, 4)]Further Carvedilol being a weakly basic BCS class II drug, exhibits complex pattern of solubility in GI tract during transition from stomach to intestine due to pH gradient of GI. Carvedilol has a basic pKa 7.8 and acidic pKa 15. It dissolves well in the acidic media of stomach but may precipitate at basic pH of small intestine.[(5)]Stillhart et alfound that 78.2–91.8% of carvedilol gets precipitatedeither in a crystalline form or amorphous form during digestive transition conditions.[(6)]

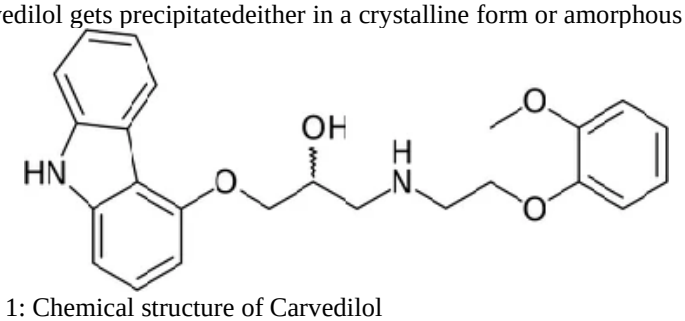


Fig. 1: Chemical structure of Carvedilol

In recent years much attention has been given towards developing alternative drug delivery, particularly onnanoscale,for improvement in the bioavailability of poorly water soluble or hydrophobic compounds which generally leads to greater efficacy.[(7)]A nanoemulsion is considered to be thermodynamically or kinetically stable liquid dispersion of an oil phase and a water phase, in combination with surfactant. The dispersion phase typically comprises of small particles or droplets, with a size range of 5-200nm, and has very low oil/water interfacial tension.Reducing droplet sizes to the nanoscale leads to some very interesting physical properties, such as optical transparency and unusual elastic behavior. As the droplets are in nano size, nanoemulsionsdo have increased interfacial areas that effect the transport properties of the drug which is a very important factor, particularly in sustained and targeted drug delivery.[(8)] Plasma concentration profiles and bioavailability are more reproducible when the drug is administered in the form ofnanoemulsion formulation.[(9)]Nanoemulsions have a much higher

surface area and free energy than conventional emulsion/macro emulsion that make them an effective transport system.[10]The small droplet size prevents any flocculation of the droplet and their coalescence. That's why system remains dispersed with no separation.[11]Nanoemulsion can be formulated in variety of formulations such as foams, creams, liquids, and sprays.[12]It also provides protection from hydrolysis and oxidation as drug in oil phase in O/W since drug in nanoemulsion is not exposed to attack by water and air.[13]Nanoemulsions have been reported to make the plasma concentration profiles and bioavailability of drugs more reproducible.[14]Nanoemulsionsare now being used in most form of drug delivery systems, namely topical, ocular, intravenous, intranasal and oral delivery.

MATERIALS AND METHODS

Chemicals: Carvedilol API was procured from Yarrow chem. Products Mumbai. Oleic acid, IPM,Tween 80, PEG 400, Hydrochloric acid, Methanol &Potassium dihydrogen orthophosphate, NaOHwere procured from the chemical store of department of Pharmacy, M.J.P. Rohilkhand University, Bareilly; place of this piece of research work.

METHODS

Before preparation and evaluation of nanoemulsions containing API- carvedilol, preformulation studies on various physico-chemical properties of procured drug was done along with the chemical authenticationthrough physical appearance, melting point determination and FT-IR spectra.

Organoleptic properties of drug

As per Indian pharmacopoeia drug sample was physically characterized on the basis of color, odor and taste. All these parameters were than compared with standard drug.

Melting point determination

Melting point of the drug sample was determined by capillary tube method. In this method small amount of drug was filled in the three separated capillaries sealed at one end. The capillary tubes were placed in the melting point apparatus (Macro scientific works). The temperature was gradually increased and the temperature, at which samples started to melt,was noted down. This procedure was repeated three times and average melting point was taken on record.

FT-IR spectrum of Carvedilol drug

FT-IR spectra of pure drug Carvedilol was carried out to check the authenticity of drug.

Determination of carvedilol Solubility in oily phases, surfactants & co-surfactants

For the determination of solubilities of carvedilol in various available phases (oleic acid, IPM, 1:1 blend of oleic acid&IPM, castor oil, Tween 80, PEG 400, propylene glycol, span 20)proposed to be used for preparing nanoemulsions,the excess amount of drug was transferred in 5 ml of each phaseseparately and placed on isothermal shaker water bath at 37±0.5ºC for 72 hoursso as to facilitate the dissolving of drug through infringementof stagnant layers by shaker. After 72 hrs samples were filtered through Whatman filter paper to remove undissolved drug. 1 ml of each sample was suitably diluted with methanol and then with distilled water and analyzed for the amount of drug present in solution through UV Visible spectrophotometer at λ max285nm.

Method of preparation of nanoemulsion

Nanoemulsions of carvedilol were prepared by ultrasonication method as described by Tang and Sivankur[15]with minor modifications. In this method preselected quantity of the drug (carvedilol) was separatelydissolved in accurately measured quantity of each oily phase. On the basis of solubility studies 3chosen oily phaseswere- oleic acid, IPM and 1:1 blend of these two(OA & IPM). Chosen surfactant was Tween 80 and co-surfactant PEG400. For different formulations varying quantities of co-surfactant PEG400 were taken in beaker and mixed with drug containing oily phase under cont

inuous stirring. That's how oil phase was prepared. For the preparation of aqueous phase Tween 80 was dissolved in distilled water. Aqueous phase was placed on the magnetic stirrer and then the oil phase was added to it drop wise with continuous stirring 500 rpm for 15 minutes. The solution was then ultrasonicated for 20 min.

Selection of Surfactant andCo-surfactant ratio (*S*mixratio)

For optimization of proportion of surfactant and co-surfactant concentrations,nanoemulsion dispersion formulations were prepared containingdefinite amount of different oily phases (oleic acid, IPM and 1:1 Blend of oleic acid&IPM) with differentproportionsof surfactant (Tween 80) and co-surfactants (PEG400). These *S*mix ratios were taken in increasing concentration of cosurfactant with respect to surfactant and decreasing concentration of surfactant with respect to cosurfactant so as to study the comprehensive effect of *S*mix ratioon formation of nanoemulsion.

Compositions of these formulations have been given in table 1, 2 and 3. These nanoemulsion dispersions were then screened on the basis of physical appearance,viscosity and %entrapment efficiency.

Table 1: Formulations of nanoemulsion dispersion for optimization of *S*mix ratiofor oleic acid:

Dispersion Code	Drug (Carvedilol) mg	Oleic acid (in ml)	*S*mix ratio	Surfactant (Tween 80) (in ml)	Co-surfactant (PEG) (in ml)	Water upto 100 ml
A1	31.25	5	4:1	8	2	85
A2	31.25	5	3:2	6	4	85
A3	31.25	5	2:3	4	6	85
A4	31.25	5	1:4	2	8	85

Table 2: Formulations of nanoemulsion dispersion for optimization of *S*mix ratio for IPM:

Dispersion Code	Drug (Carvedilol) mg	Isopropyl Myristate (in ml)	S-mix ratio	Surfactant (Tween 80) (in ml)	Co-surfactant (PEG) (in ml)	Water upto 100 ml
B1	31.25	5	4:1	8	2	85
B2	31.25	5	3:2	6	4	85
B3	31.25	5	2:3	4	6	85
B4	31.25	5	1:4	2	8	85

Table 3: Formulations of nanoemulsion dispersion for optimization of *S*mix ratiofor 1:1 blend of OA &IPM:

Dispersion Code	Drug (Carvedilol) mg	1:1 Blend OA+IPM (in ml)	S-mix ratio	Surfactant (Tween 80) (in ml)	Co-surfactant (PEG) (in ml)	Water upto 100 ml
C1	31.25	5	4:1	8	2	85
C2	31.25	5	3:2	6	4	85
C3	31.25	5	2:3	4	6	85
C4	31.25	5	1:4	2	8	85

Evaluation of Prepared Nanoemulsion formulations of carvedilol

Physical appearance

The physical appearance was simply determined by shaking the formulations and theninspected visually for transparency/turbidity.

Viscosity [16]

The viscosity of the formulations was determined by observing the meniscus of nanoemulsion in a test tube containing the formulation after inclining the test tube. Under this method, the flow velocity of formulation along the wall of the tube was noted.Less viscous formulations flow fast and continuously, like propylene glycol, whereas intermediateviscous formulations flow slowly; and high-viscosity formulations flow very slowly or practically no flow as do the gels.

Entrapment efficiency

For separating un-entrapped drug,nanoemulsion dispersions (10ml) wereseparately subjected to centrifugation for 30 min at 3500 rpm. The obtained supernatant was taken and diluted to 5ml with methanol and then suitably diluted with distilled water. The resultant solution was analyzed by UV spectroscopy at λ max. The percentage of drug entrapped was calculated by the formula-

$EE\% = [(Ct-Cf)/Ct] \times 100\%$

Where, Ct= concentration of total drug
Cf= concentration of un-entrapped drug

Screening of nanoemulsion formulation

On the basis of transparency and entrapment efficiency,4nanoemulsion formulations of carvedilol (among 12 prepared formulations- containing different oily phases and different proportions of surfactant-cosurfactant mix)A2, A3, C2 and C3 were selected for further study and were re-named as FI, F2, F3 and F4. The coding and compostion of selected nanoemulsion formulations are given in table no. 4.

Table 4: Optimized formulations of Carvedilol nanoemulsion-

Formu-lation code	Drug (mg)	Oily phase (5 ml)	S-mix ratio	Surfactant (Tween 80) (in ml)	Co-surfactant (PEG) (in ml)	Water upto 100 ml
F1	31.25	Oliec Acid	3:2	6	4	85
F2	31.25	Oliec Acid	2:3	4	6	85
F3	31.25	1:1 Blend (OA+IPM)	3:2	6	4	85
F4	31.25	1:1 Blend (OA+IPM)	2:3	4	6	85

Evaluation ofSelectedNanoemulsion formulations

Physical appearance
The prepared nanoemulsion preparations were examined visually for their color, clarity, homogeneity and presence of any foreign dust particles.

pH of Nanoemulsion
The pH of the nanoemulsion preparations were determined using digital pH meter. 5ml of nanoemulsionwithout dilution was used for determination of pH.

Drug content
Carvedilol from the nanoemulsion was extracted by dissolving it in acetonitrile and then diluted with distilled water. Carvedilol contents were analyzed by UV spectroscopy at λ max. The percentage of drug content present in the prepared formulations were calculated by the formula-
%Drug content= [Contents of drug found / Contentclaimedin formulation] X 100%

Transmission electron microscopy (TEM)
Nanoemulsion dispersion morphology and size analysis was done by Transmission Electron Microscopy. TEM was performed at SAIF lab AIIMS, New Delhi. The prepared samples were firstly subjected to negatively stained phosphotungstic acid solution and transferred to a carbon coated grid. Excess sample was removed by filter paper and dried at room temperature. After the preparation of grid microphotographs were taken.

***In vitro* drug release**
The *in vitro* dissolution studieswere performed using USP dissolution test apparatus-Type II in 500 ml of dissolution media [3, 4]-1:1 mixture of acetonitrile& phosphate buffer 7.4. RPM was maintained at 50 and temperature at 37±0.5ºC. 5 ml of formulatednanoemulsion which contains a single dose 3.125 mg of drug (Carvedilol) was kept in a dialysis bag, made by treated cellophane membrane,andhanged in the place of basket. At various time intervals (1,2,3,4,5,6, and 24 h) media samples (10ml) were withdrawn and the withdrawn quantity of dissolution media was immediately replaced with fresh media. The samples were filtered, diluted and analyzed for the amount of drug present using UV spectrophotometer at λmax 285 nm.

RESULTS& DISCUSSION

The results ofpreformulation studies of the drug were found to be as follows-

Physical appearance

Color: Pure white
Odor: Odorless
Taste: Tasteless
Appearance: Powdered form

Melting Point
Melting point of the drug Carvedilol was found to be 114.3±0.23ºC.The normal range is 114-115ºC.

Solubility
The solubilities of Carvedilol in different oily phases, surfactants and co-surfactants were determined. The solubility of carvedilol was found to be highest in oleic acid (28.24±5.62mg/ml) among all tested oily phases and highest

inPEG 400 (31.26±6.14) among tested co-surfactants. It was found to be 17.89±3.29 in Tween 80. Estimated solubilities of carvedilol in different phases are given in table no. 5

S. No	Oils	Solubility mg/ml
1.	Oleic acid	28.24±5.62
2.	Isopropyl myristrate	0.82±2.25
3.	Oleic acid: IPM (1:1)	15.28±9.32
4.	Castor oil	10.35±2.84
5.	Tween 80	17.89±3.29
6.	PEG 400	31.26±6.14
7.	Propylene glycol	19.23±2.61
8.	Span 20	07.56±1.56

Table 5: solubility of Carvedilol in various phases

FTIR spectrum

The FTIR spectrum of pure carvedilol,as depicted in figure no. 2, showed the peaks at wavelengths (cm^{-1}) - 3342.75 (Aliphatic Secondary amine, NH stretch), 1629.9 (Secondary amine NH bend), 1174.69 (Secondary amine, CN stretch), 1346.36(Aromatic Secondary amine, CN stretch), 3450 (Aromatic Secondary amine, NH stretch), 3203.87(Hydroxy group, Hbonded OH stretch), 1097.53 (Secondary alcohol, C-O stretch), 1303.92 (Primary or secondary, OH in-plane bend), 617.24(Alcohol, OH out-ofplane bend), 2820 (Methoxy (CH3-O-), C-H stretch), 1253.77 (Aromatic ethers, Aryl-O stretch), 2922.25 (Methylene C-H asymmetric stretch), 2847.03(Methylene C-H symmetric stretch),1448.66 (Methylene C-H bend),1606.76, 1587.47 and1502.6 (Aromatic ring stretch(C=C)), 3090 (Aromatic C-H stretch), 1213.27 (Aromatic C-H in-plane bend), 748.41(Aromatic C-H out-of-plane bend).These peaks were corresponding with the described one in standard spectrum of drug carvedilol which confirms the purityof procured drug.

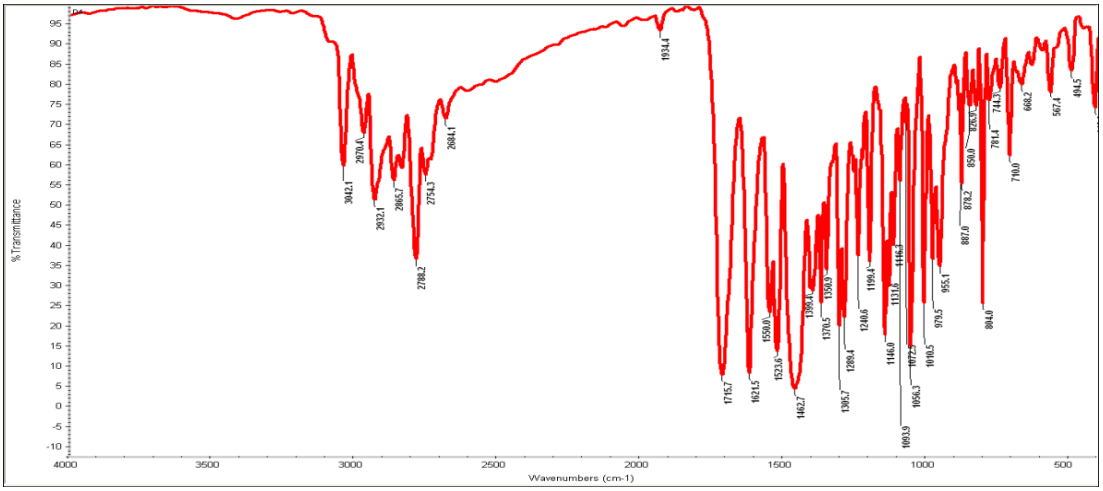


Fig. 2: FT-IR spectrum of drug Carvedilol

SCREENING OF PREPARED NANOEMULSION FORMULATIONS

For the selection of nanoemulsion formulations for detailed studies, oil and Smix were optimized on the basis of physical appearance, viscosity and %entrapment efficiency.

Physical appearance

Physical appearance of all the 12 formulations has been given in table no. 6. On the basis of physical appearance only transparent formulation were selected.

Viscosity

The viscosity of all the formulations, as determined through the method described in methodology, has been given in table no. 6.

Entrapment efficiency

Entrapment efficiency of nanoemulsion dispersions(A1, A2, A3, A4, B1, B2, B3, B4, C1, C2, C3 and C4 as shown in table 1, 2 and 3)were determined by method described in methodology. The results are given in table no. 6.

Nanoemulsion code	Oily phasae	S mix Ratio	Physical appearance	Viscocity	%Entrapment Efficiency
A1	Oliec Acid	4:1	Transparent	High	80.55±0.651
A2	Oliec Acid	3:2	Transparent	Intermediate	96.57±0.212
A3	Oliec Acid	2:3	Transparent	Intermediate	94.18±0.223
A4	Oliec Acid	1:4	Translucent	Low	74.11±0.123
B1	IPM	4:1	Transparent	Intermediate	66.55±1.02
B2	IPM	3:2	Translucent	Intermediate	73.11±0.23
B3	IPM	2:3	Translucent	Low	75.23±0.645
B4	IPM	1:4	Hazy	Low	72.01±0.774
C1	1:1 Blend OA+IPM	4:1	Transparent	High	61.02±0.231
C2	1:1 Blend OA+IPM	3:2	Transparent	Intermediate	85.45±0.03
C3	1:1 Blend OA+IPM	2:3	Transparent	Low	84.22±0.316
C4	1:1 Blend OA+IPM	1:4	Translucent	Low	71.66±0.26

Table 6: Effect of *S*mix ratio on physical appearance, viscosity and entrapment efficiency

On the basis of physical appearance, viscosity and % entrapment efficiency- less viscous and more transparent formulations with high %entrapment efficiencyfour formulations (A2, A3, C2 and C3),out of 12 prepared formulations, were selected for further study and were named as F1, F2, F3 and F4.

EVALUATION OF SELECTED NANOEMULSION FORMULATIONS

Physical appearance

The prepared nanoemulsion preparations were examined visually for their color, clarity, homogeneity and presence of any foreign dust particles. All the four selected formulations were transparent, homogeneous and free from any foreign particle.

pH of nanoemulsion

The pH of the nanoemulsions determined using digital pH meter were found to be as given in table no. 7.

Table 7: pH of Carvedilol nanoemulsion formulations

Sl.No.	Formulation no.	pH
1.	F1	6.54±0.95
2.	F2	6.92±0.50
3.	F3	6.71±0.63
4.	F4	6.65±0.23

***Each value represents mean S.D. of three observations**

Drug contents

The drug contents in selected formulations were found to be as shown in table no. 8.

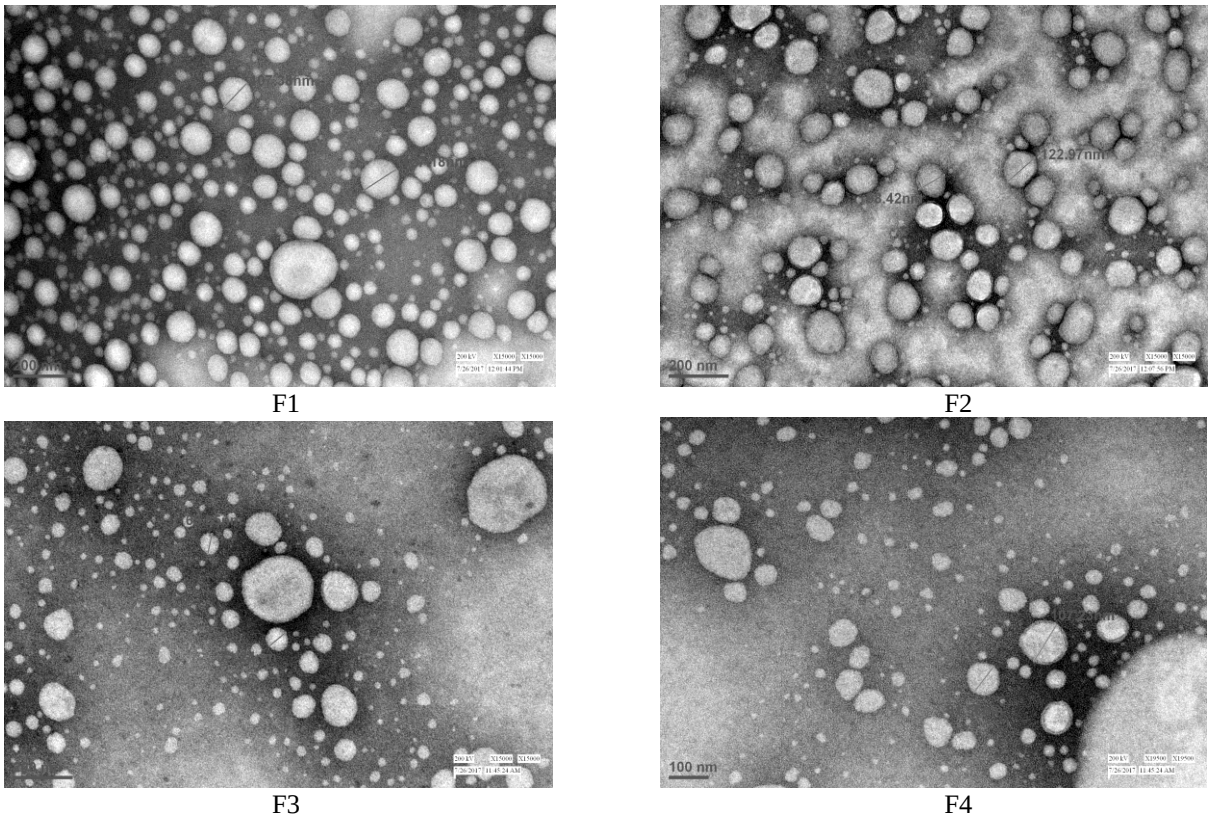
Table 8: Drug contents of nanoemulsion formulations.

S. No	Formulation code	Drug contents in %
1.	F1	95.32±1.23
2.	F2	94.45±0.99
3.	F3	91.23±1.52
4.	F4	90.27±3.62

***Each value represents mean S.D. of three observations**

Transmission electron microscopy (TEM)

TEM was done at 200KV and various magnifications of X15000, X19500, X29000 and X43000 revealed the morphology of nanoemulsion dispersion. The droplet size was found to be between the range of **20.76-107.38nm** as shown in figure no. 3.



F1 F2

F3 F4

Fig. 3: TEM of nanoemulsion dispersions

***In-vitro* drug release**

In-vitro dissolution studies were performed to understand the release pattern of drug from 4 selected formulationsout of 12. Release patterns were also compared with that of API and conventional tablet formulation having same amount of drug. Data of cumulative drug release in % is given in table no. 9. The graph plotted between %cumulative drug release and time is given in figure no. 4.

Table 9: In-vitro drug release of Carvedilol nanoemulsion formulation

	Cumulative drug release (%)					
Time (hr)	**F1**	**F2**	**F3**	**F4**	**Tablet**	**API**
0	0	0	0	0	0	0
1	31.28±3.46	26.81±2.71	22.49±1.23	21.65±0.52	12.4±1.41	15.68±0.9
2	43.56±0.86	35.43±1.72	28.16±0.98	26.56±4.32	19.25±0.93	24.7±1.07
3	52.48±1.72	41.02±3.70	35.10±2.33	32.26±1.51	24.98±0.69	29.5±2.5
4	61.27±0.07	45.28±1.00	40.99±1.52	38.59±5.32	30.57±0.90	35.8±2.0
5	68.78±2.47	50.84±1.90	50.36±0.63	47.26±1.84	37.58±0.88	44.1±2.6
6	79.55±1.65	59.33±1.37	56.33±2.32	54.96±3.51	42.30±1.81	50.15±1.9
24	88.41±2.72	82.98±1.99	73.28±1.02	71.88±2.64	55.01±0.88	65.4±1.6

***Each value represents mean S.D. of three observations**

After 1 hr the % release was found to be36.28±3.46, 26.81±2.71, 22.49±1.23, and 21.65±0.52 % for F1, F2, F3 and F4 nanoemulsion formulationsrespectively whereas release was only 12.4±1.41% and 10.68±0.9%for tablet and API. All the four formulations of nanoemulsion showed much better initial release as compared to conventional tablet and API. T_{50} was found in 2-3 hours for F1 whereas4-5 hours for F2&F3 and 5-6 hours for F4& API. Tablet of carvedilol failed to release 50% drug even after 6 hrs and $T_{50}$was present somewhere in between 6 to 24 hrs, may be in 6-7 hrs as may be extrapolated by release pattern from 0 to 6 hrs.

After 6 hours %drug release was found to be 79.55±1.65 % for F1, 59.33±1.37 % for F2, 56.33±2.32 % for F3, 54.96±3.51 % for F4 whereas only 42.30±1.81 for conventional tablet and 50.15±1.9 % drug release forAPI having

the same amount of drug. The comparative release rates from all fournanoemulsion formulations, conventional tablet and API were in the following order-
F1>F2>F3>F4>API >conventional tablet

Experimental data clearly shows that the release of the drug carvedilol can be influenced by formulating it into a nanoemulsion preparation using different amounts of different surfactants and co-surfactants. Comparison of release rates of all the four preparednanoemulsion formulations with that of marketed conventional tablet and API revealed that all the Carvedilol nanoemulsion formulations have better release rates and among these four formulations, it was formulation F1 which has come up as optimized nanoemulsion formulation of this experimental work.

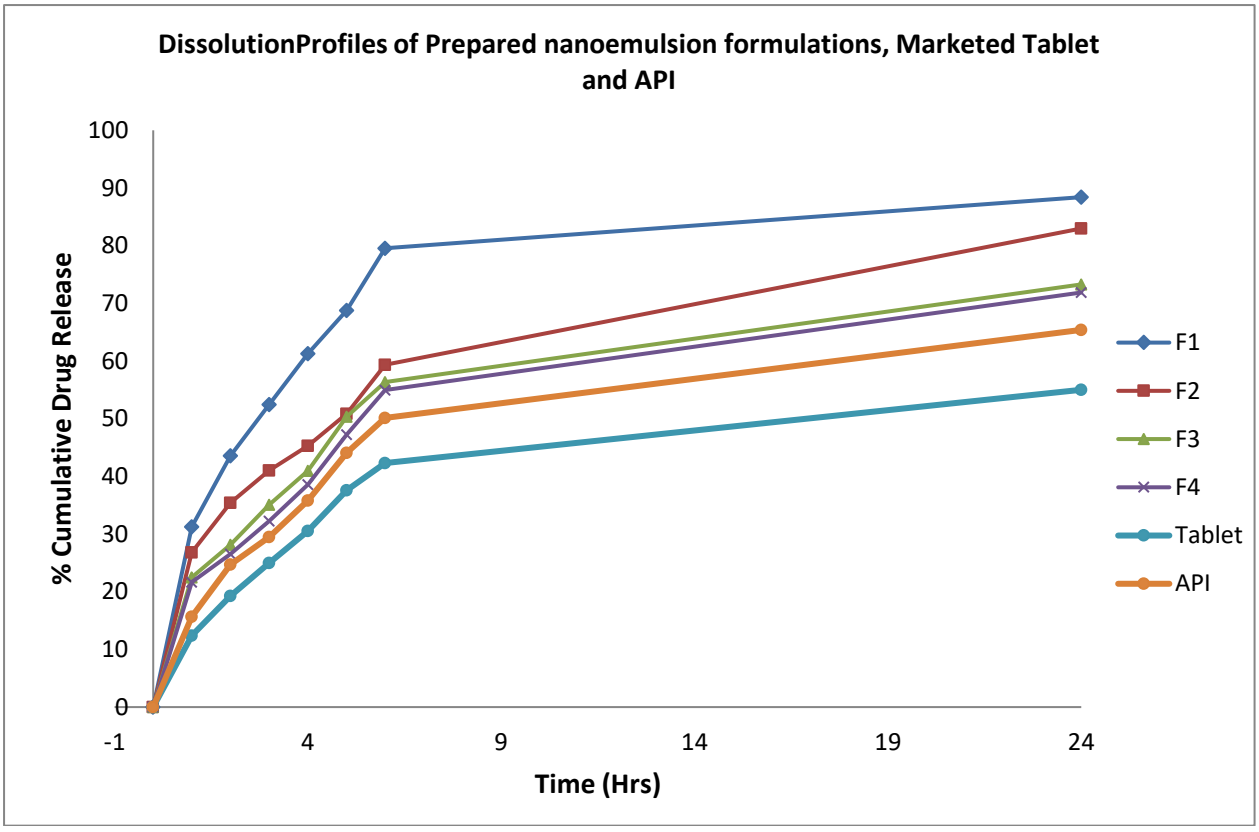


Fig. 4: DissolutionProfiles of Prepared nanoemulsion formulations, Marketed Tablet and API

Abbreviations:

PEG= Polyethylene Glycol; IPM= Isopropyl Myristate; OA= Oleic Acid; RPM= Rounds Per Minute.

CONFLICT OF INTEREST: None

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