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Research Article

Development and Evaluation of Micro Emulsion Formulations of Nebivolol for Solubility Enhancement

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Abstract

Nebivolol HCl is a newer drug of β 1-adrenergic blocker category, basically used as anti-hypertensive. It is a 3rd generation, antagonist, having NO (nitric oxide) enhancing vasodilator properties. It has 12% oral bioavailability, because of its pre systemic metabolism by the means of cytochrome P450 2D6 enzymes. Its log P value is 4.03 and 5mg is its daily dose. It is highly lipophilic drug and belongs to class BCS II, with slow dissolution. Bioavailability of any drug can be improved by avoiding its first pass metabolism and promoting solubility. Several researchers have worked on the development of ME formulations on different poor water-soluble drugs, to increase their solubility. The purpose of this study is an attempt to enhance the solubility to improve the bioavailability of nebivolol drug by developing a novel delivery system that is microemulsion (ME). ME formulations were developed using different oil, surfactant and co-surfactants in different ratio and studied on various parameters. Different preformulation tests done on received sample of Nebivolol. FTIR study was performed in order to find out any interactions between the ingredients. Based on the solubility Capmul Pg-12 was finalized oil, Tween 80 as surfactant, propylene glycol as the cosurfactant based on solubility and emulsification efficiency. Five Nebivolol ME formulations were successfully developed by use of oil, water, SA and Co-SA different ratio. Prepared formulations were studied for different properties-transmittance (%), pH, refractive index, viscosity, drug content, and solubility. It was seen that after 4 hours of diffusion, the drug released from the formulation ME5 is faster and more than that of the other i.e., $90.2 \pm 0.06\%$. It was found that ME5 was more stable and Soluble than other prepared formulations. With the better solubility the bioavailability of Nebivolol will increased and helps in faster absorption and High diffusion in systemic circulation with lower or no risk of degradation. It somehow also reduced frequent intake of drug.

Keywords: Nebivolol, micro emulsion, Ternary phase diagram, surfactant, co-surfactant.

1. INTRODUCTION:

Microemulsions (ME) basically are made up of oil, surfactant (SA) and water, with a co surfactant (Co-SA) in different ratio. This mixture is clear and stable. The resulting fluid possesses low viscosity. ME are isotropic, stable & transparent systems of droplets of immiscible liquids with diameter $>500 \text{ nm}^1$. Due to smaller droplets, they act as an excellent carrier for drugs having poor water solubility. Nebivolol HCl is a newer drug β 1-adrenergic blocker category, basically used in the management of hypertension. It is a 3rd generation, antagonist, having NO (nitric oxide) enhancing vasodilator properties. Used in the initial management of hypertension.

Peak plasma concentration achieved after its oral administration (0.5-2 h) ²⁻⁴.

It has 12% oral bioavailability, because of its pre systemic metabolism by the means of cytochrome P450 2D6 enzymes. Its log P value is 4.03 and 5mg is its daily dose. It is highly lipophilic drug and it belongs to class BCS II, with slow dissolution ⁵⁻⁶. Bioavailability of any drug can be improved by avoiding its first pass metabolism and promoting solubility.

Several researchers have worked on the development of ME formulations on different poor water-soluble drugs, in order to increase their solubility ⁷.

Current study is an attempt to increase solubility of Nebivolol. For this purpose, ME formulations were developed using different oil, surfactant and co-surfactants in different ratio and studied on various parameters.

Various preformulation studies were made in order to receive sample of Nebivolol. FTIR study was done to find out the interactions between the ingredients. Depending upon the solubility Capmul Pg-12 was selected as oil phase, Tween 80 as surfactant, propylene glycol as the co surfactant based on their solubility and emulsifying efficiency Ternary diagrams were constructed for different aspect ratio of oil, water, SA and Co-SA. Here, five Nebivolol ME formulations were successfully developed by using CapmulPg-12, water, SA and Co-SA in different ratio. Developed formulations were studied for different properties such as transmittance (%), pH, refractive index, viscosity, drug content, and solubility.

The significance of the study is to increase the solubility of Nebivolol by preparing a novel Drug delivery system of the

drug, consequently increasing its bioavailability. This development reduces the risk of drug degradation and frequent dose administration & also makes drug more stable and highly soluble that possess high degree of Diffusion and better absorption in systemic circulation in sustained and controlled manner.

Figure 1: Structure of Nebivolol¹⁶

Table 1: Composition of batches for Nebivolol ME formulations.

Code	S _{mix} ratio	% w/w composition		
		% Oil	% S _{mix}	% Water
ME1	1:1	30	60	10
ME2	1:2	60	35	5
ME3	1:3	35	60	10
ME4	2:1	50	40	10
ME5	3:1	40	55	5

2. METHOD

2.1 Materials Used: Nebivolol Hydrochloride is received from Cadila pharmaceuticals, Gujarat. Capmul Pg-12 was obtained from CDH, Delhi. Castor Oil, linseed oil, Soyabean oil, Peanut oil, Castor oil are received from Jindal refinery, Uttarakhand. Tween-80, Tween-60, Span-40 and others are brought from sweta scientific, Lucknow.

2.2 Preparation:

Pseudo- Ternary phase diagrams (PTPD):

PTPD Diagrams were plotted using water titration method. For the ME area under plot and estimation of the resulting possibility of formed ME formulations with selected oil, SA, and Co-SA. The amount of SA and Co-SA used were 1:1, 2:1 and 3:1, while 5 % oil was used in exact amount. These ingredients were admixed with oil, to get different ratio of weight i. e., 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9. Add water drop wise with stirring through a magnetic stirrer. A constant temperature was maintained until a homogeneous dispersion or solution starts appearing.

Figure 2: Pseudo Ternary phase diagram¹⁷⁻¹⁹

Physical appearance can be observed by visual evaluation after adding each drop. At end point the titration was declared to be completed where, the resulting solution was converted into a transparent one. The total amount of water, pre-owned to make this formulation turbid was noted. The total % of each phase was determined. The same process

was acquired for the other SA and Co-SA ratio. All the SA and Co-SA ratio come UP with dotted area in the PTPD⁸.

Preparation of Nebivolol loaded ME formulations

Total Five ME formulations were formulated through water titration method. Nebivolol (100) mg was blended with selected oil (Capmul Pg-12), SA (Tween-80) and Co-SA (Propylene glycol). Addition of water was done gradually through stirring. Finally, crystal clear ME formulations were attained.

2.3 Preformulation Studies:

Physical Appearance: Examine the authenticity of the received Nebivolol gift sample.

M.P. determination

Melting point of Nebivolol was determined by the means of Thiels tube method. Three hundred ml of paraffin was poured to Thiels tube, and sealed with the flame. Melting temperature of Nebivolol was recognized using thermometer⁹.

FTIR analysis

Fourier Transform Infra Red analysis (FTIR) was used to consider drug-excipient interaction by detecting the samples in the range of 400-4000 cm⁻¹. The pure drug was combined with surfactant, co-surfactant and oil and the mixture was analyzed. Spectral comparison was done with FTIR of pure drug to eliminate the possibility of important functional groups of the drug that interacts with the excipients¹⁰.

Determination of λ_{max}

10 mg of Nebivolol was placed in 2 volumetric flasks of 100ml. Buffer of pH 7.4 is used as the diluents to make stock solution A. Exact ten ml was removed from it and transferred to 100ml volumetric flask (mention stock solution B). Sample from stock solution B was used and detected under UV Spectrophotometer. 250 nm was the point where λ_{max} was obtained¹¹.

Figure 3: FTIR Spectra of Nebivolol

Figure 4: FTIR Spectra of Nebivolol with capmul-pg12, tween-80 & PEG

Standard curve

Exact 2.38 g di-sodium hyd. Phosphate (Na_2HPO_4) was alloyed with 0.19 g KH_2PO_4 with .0 g NaCl to get 1000ml total volume. Exact 10 mg Nebivolol was lay hold and dissolved in buffer to obtain 1000 $\mu\text{g}/\text{ml}$ concentration. 1 ml sample of it is dilute with 10 ml buffer (known to be stock solution). Different concentration were prepared from this stock solution and detected by UV spectrophotometer at 638.2 nm^{12} .

Table 2: Nebivolol standard curve (Medium= buffer, pH 7.4)

S.N.	Concentration ($\mu\text{g}/\text{ml}$)	Absorbance
1	0	0
2	2	0.025 $\pm$ 0.08
3	3	0.0471 $\pm$ 0.09
4	4	0.052 $\pm$ 0.03
5	5	0.066 $\pm$ 0.11
6	6	0.084 $\pm$ 0.04
7	7	0.091 $\pm$ 0.08
8	8	0.114 $\pm$ 0.05
9	9	0.125 $\pm$ 0.08
10	10	0.136 $\pm$ 0.06

Figure 5: Standard Curve of Nebivolol at pH 7.

Selection of the oil phase

Maximum solubility of Nebivolol was considered to be obtained using different oils. Oils like castor oil, soybean oil, Kollisolv GTA, MCT, and Capmul Pg-12 were used for solubility consideration. Capmul Pg-12 was selected as the oil phase¹³ due to High solubility.

Selection of SA and Co-SA

The solubility of Nebivolol was determined in SA and Co-SA. The efficiency of emulsifying of SA and Co-SA was determined in order to know their capability of emulsification in selected oil. Nebivolol was admix in equal ratio of SA, and diluted properly and examined for transmittance at 638 nm by using UV-Vis spectrophotometer. The effortless formation of preparation was detected by inverting the volumetric flask, which is used to obtain uniform emulsion. Similarly, transmittance of Co-SA and Nebivolol was evaluated. The main observations were determined which of them was able to produce more clearly ME formulations at the minimum concentration¹⁴.

Solubility analysis

Weigh 10 gm oil in a beaker, alloy with 100 mg Nebivolol. They were mixed properly by using stirring through a magnetic stirrer. After complete dissolution of Nebivolol,

Again add 10mg Nebivolol further in the same way. The addition process was continued until, a saturated solution is achieved. The resulting Final solution was checked by using UV-spectrophotometer at 250 nm. Similarly, the solubility of Nebivolol is estimated in SA and Co-SA¹⁵.

Figure 6: Solubility of Nebivolol in different oils

2.4 Evaluation parameters for ME of Nebivolol

A) General physical characteristics

• Percentage Transmittance:

It was determined by using UV spectrophotometer (638 nm). It lies within the range of 98.57 ± 0.09 to $99.42 \pm 0.23\%$ for all the ME formulations prepared with Nebivolol which confirms good transparency nature of formulations.

Table 3: Emulsification efficiency with surfactants and selected oil (Capmul Pg-12).

Surfactant	% Transmittance	HLB Value
Tween-60	85.147 ± 0.0172	14.9
Tween-80	88.127 ± 0.0241	15
Labrasol	74.271 ± 0.0218	14

• pH determination:

For the ME formulations of Nebivolol, the pH value lies in between 3.44 ± 0.08 to 4.12 ± 0.09 .

Figure 7: Comparative pH values of micro emulsion formulations.

• Drug Content:

For the prepared ME formulations of Nebivolol, the % drug content lies in 98.37 ± 0.08 to 99.61 ± 0.03 .

• Viscosity:

The Viscosity of formulated ME formulations of Nebivolol, lies in between 64.23 ± 2.1 to 70.56 ± 5.77 %.

B) In-Vitro Drug release.

• Kinetic modeling for ME formulations :

In current study PCP disso Version 2 software was used in for the estimation of drug release pattern. Different Models for the release kinetic profile are shown in Table 9. First kinetic order and Korsmeyerpeppas models were used for plotting of kinetic data. Drug Release was monitored through diffusion process.

• Drug release kinetic data analysis:

Drug release data was evaluated through PCP disso software for the kinetic models. First order kinetics along with Peppas and Korsmeyer model were studied.

Table: 4 Kinetic release Data

Batch	Kinetic model	Parameters
ME1	Peppas and Korsmeyer	$R = 0.965$, $K_1 = 4.234$, $n = 0.750$
ME2	Peppas and Korsmeyer	$R = 0.974$, $K_1 = 3.147$, $n = 0.854$
ME3	First order	$R = 0.952$, $K_1 = 5.61$, $n = 0.750$
ME4	Peppas and Korsmeyer	$R = 0.934$, $K_1 = -0.070$
ME5	Peppas and Korsmeyer	$R = 0.963$, $K_1 = 6.812$, $n = 0.772$

- **Drug release studies:**

After 4 hours of diffusion it was observed that the drug released from the formulation ME5 is faster and more than that of the other ratios i.e., $90.2 \pm 0.06\%$.

Table 5: Evaluation parameters of prepared Nebivolol micro emulsion formulations

Batch	Transmittance (%)	pH	Refractive index	Viscosity (cp)	Drug content (%)	Solubility mg/ml
ME1	99.3 ± 0.08	3.56 ± 0.08	1.3548 ± 0.007	64.23 ± 2.1	98.37 ± 0.08	27.87 ± 0.08
ME2	99.27 ± 0.11	3.76 ± 0.12	1.3420 ± 0.008	65.46 ± 3.7	99.32 ± 0.34	28.87 ± 0.11
ME3	99.42 ± 0.23	3.82 ± 0.07	1.3718 ± 0.004	70.56 ± 5.77	99.61 ± 0.03	29.87 ± 0.08
ME4	98.57 ± 0.09	3.44 ± 0.08	1.3620 ± 0.008	68.43 ± 3.34	99.52 ± 0.02	25.87 ± 0.09
ME5	98.63 ± 0.21	4.12 ± 0.09	1.3518 ± 0.016	69.36 ± 4.74	98.43 ± 0.04	31.87 ± 0.07

3. RESULTS AND DISCUSSION

1) Transmittance

Transparency of formulated ME formulations of Nebivolol were detected by using U.V. Spectrophotometer to determine % transmittance at 638 nm. As regards blank is concerned, distilled water was used as blank.

2) Refractive index:

For the formulated ME formulations of Nebivolol, the refractive index lies in between 1.3420 ± 0.008 to 1.3718 ± 0.004 .

3) Solubility:

A solubility profile indicates that the sample received by the company was Nebivolol. The solubility of Nebivolol was estimated in various oils to select the one having high solubility. Capmul Pg-12 has shown maximum solubility, thus selected as the oil to formulate ME formulations. Similarly, Tween 80 was selected as SA, Propylene glycol as the Co-SA based on their solubility and emulsification efficiency.

4) Drug Content

The drug content formulated ME formulations of Nebivolol was determined by dissolving 1 ml (=10mg) in methanol (10ml). The measurement of Drug Content is done using UV spectrophotometer at 250 nm¹⁶. ME5 was found to be with highest drug content.

Figure 8: Comparative % drug content of micro emulsion formulations.

5) In-Vitro Drug release:

For estimating in-Vitro release Franz diffusion Cell (20ml) was used. The prepared ME formulations of Nebivolol undergoes diffusion process through Franz diffusion Cell, with a capacity of 20ml. 20 ml Buffer (pH7.4) is filled in receptor chamber. Donor compartment holding Nebivolol ME formulations is attached with Cellophane membrane. All the samples were examined using UV spectrophotometer at 250nm. Data collected shows Formulation with Capmul-pg12 have better in-vitro drug release in given span of time.

Table 6: In vitro study of prepared Nebivolol micro emulsion formulations

(min)	Percentage drug released $\pm$ SD ^a				
	ME1	ME2	ME3	ME4	ME5
0	0	0	0	0	0
20	6.4 ± 0.08	6.8 ± 0.15	11.5 ± 0.29	19.2 ± 0.08	21.8 ± 0.53
40	11.36 ± 0.01	12.8 ± 0.47	19.6 ± 0.18	30.7 ± 0.09	33.5 ± 0.32
60	16.9 ± 0.07	21.5 ± 0.25	29.7 ± 0.17	35.5 ± 0.11	41.6 ± 0.28
80	23.3 ± 0.05	27.4 ± 0.55	37.4 ± 0.18	40.6 ± 0.15	47.2 ± 0.50
100	32.43 ± 0.13	35.6 ± 0.26	42.9 ± 0.33	48.2 ± 0.21	54.3 ± 0.48
120	38.64 ± 0.17	41.1 ± 0.34	51.2 ± 0.33	53.5 ± 0.08	62.6 ± 0.35
140	41.68 ± 0.08	47.1 ± 0.21	57.7 ± 0.09	66.3 ± 0.07	69.7 ± 0.32
160	46.41 ± 0.09	53.8 ± 0.15	62.6 ± 0.08	70.2 ± 0.15	77.2 ± 0.15
180	47.15 ± 0.11	54.8 ± 0.32	68.3 ± 0.12	75.4 ± 0.21	82.3 ± 0.09
200	49.56 ± 0.14	55.7 ± 0.09	69.4 ± 0.21	79.09 ± 0.35	84.8 ± 0.08
220	50.49 ± 0.06	58.4 ± 0.11	72.8 ± 0.06	81.5 ± 0.06	86.1 ± 0.05
240	53.41 ± 0.08	60.2 ± 0.23	75.43 ± 0.08	83.6 ± 0.09	90.2 ± 0.06

Mean $\pm$ SD, N=3

Figure 9: Different release models for Nebivolol micro emulsion formulations.

4. CONCLUSION:

Preformulations and evaluation studies shows Nebivolol solubility has been enhanced using Capmul-Pg12. Total five ME formulations were developed in current study.

ME formulations of ME5 batch was found to be optimum, on the basis of performed tests. After the incorporation of the drug, the microemulsion systems remained stable and optically clear showing no phase separation. The solubility of the drug was confirmed using conductivity measurements which indicated that the drug may be present at the interface of the oil and aqueous phases. UV-visible spectroscopic studies indicated that the system was optically clear. We can conclude that our microemulsion system helps increase the solubility

Of the hydrophobic drug with the help of hydrophobic component of microemulsion and lipophilic part of the surfactant. ME showed higher *in vitro* drug release when compared with commercially available drug. Hence, on the basis of developed formulation characterization and spectroscopic studies it may be concluded that the ME formulation can be employed to improve the bioavailability of a poorly soluble drug like Nebivolol using a suitable & compatible medium.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

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