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Formulation Development and Evaluation of Febuxostate Nanoemulsion Emulsion for the Treatment of Gout

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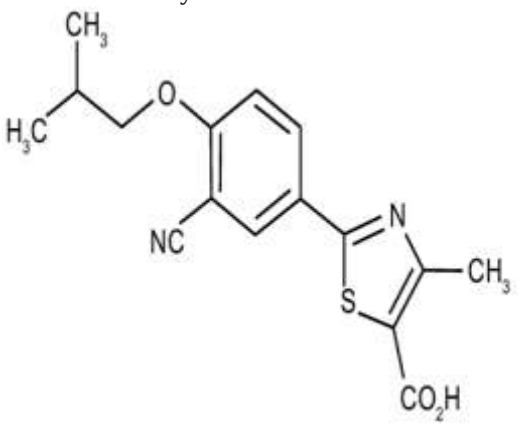
ABSTRACT: The aim of present research was to design and develop Nanoemulsion of Febuxostst for solubility enhancement. Febuxostat is a non-purine selective inhibitor of xanthine oxidase. It belongs to BCS class II i.e. poorly soluble and highly permeable drug. Due to its poor solubility, it is incompletely absorbed after oral dosing and bioavailability varies among individuals. Therefore, to overcome these shortcomings nanoemulsions have been designed. Nanoemulsion was formulated by high speed homogenization technique using isopropyl myristate as oil, tween 80 and span 80 were selected as surfactant.The formulations were evaluated for droplet size, zeta potential, drug content. The optimized formulation contains droplet size 358.5nm and zeta potential -29.1mv. In-vitro dissolution study of nanoemulsion showed 42.37 % release within 6hrs. Hence, it is concluded that nanoemulsion enhances the solubility of Febuxostat.

Keywords: Febuxostat, Nano emulsion, isopropyl myristate, zeta potential.

I. INTRODUCTION[1-10]

Febuxostat denoted as FBX is a non purine selective inhibitor of xanthine oxidase/xanthine reductase. The chemical name of FBX is 2-[3-cyano-4-(2-methyl propoxy) phenyl]-4-methyl1, 3-thiazole-5-carboxylic acid.



Chemical structure of Febuxostat

It is indicated for the long-term management of hyperuricemia in patients with gout. It belongs to BCS class II with low solubility and high permeability. Because of low solubility the bioavailability of the drug is hampered and it also undergoes enzymatic degradation in intestine as well as in liver. Food interferes with the absorption of drug and decreases the Cmax to 38-49%. Thus, it has undesirable dissolution profile and poor bioavailability following oral administration. Poor water soluble drugs present significant challenges during dosage form designing due to their inadequate solubilization in digestive fluids. Most of the newly discovered drugs receive little or no aqueous solubility as a challenge for the successful formulation development and commercialization of new drugs in the pharmaceutical industry. The bioavailability of a drug is a function of dissolution rate of the drug which is controlled by the surface area of the drug. In the category of poorly soluble drugs the change in surface area of the drug will show considerable changes in the solubility and dissolution of the drug. Febuxostat belongs to BCS class II i.e. poorly soluble and highly permeable drug. Due to poor solubility, it is incompletely absorbed after oral dosing and bioavailability varies among individuals. To overcome these shortcomings novel drug delivery system (NDDS) plays a crucial role. Nano emulsions have been widely used especially in dermatology. They are capable to incorporate a variety of hydrophilic and hydrophobic drugs, to enhance the accumulation of drug at the administration site and to reduce side effects. They are considered to be in the range of 100 nm to 1000nm. Various effects such as surface area and area to volume ratio and many other physical properties get magnified when reduced to nanoscale. Most of the current research works in almost all technical and biomedical fields is based on nano size. Nano emulsions are thermodynamically stable transparent (translucent) dispersions of oil and water stabilized by an

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interfacial film of surfactant molecules having a droplet size of less than 1000 nm. The optically clear and low-viscous formulation with enhanced solubility and minimum droplet size diameter would pose a definite promise in improving the significance of poorly soluble drug. So, the objective of the present research work was to formulate Nano emulsion of Febuxostat for improving the solubility and bioavailability of drug.

II. MATERIAL AND METHODS:

Materials

Febuxostat was generously gifted by Sun Pharma Mumbai, isopropyl myristate, span 80, tween 80 were procured from SD fine chemicals and all other chemicals and solvents were of analytical grade.

Methods

Determination of organoleptic properties

The physical identification of Febuxostat was done by checking its physical appearance i.e. colour, nature and physical state. Weighed quantity of Febuxostat as drug was taken and viewed in well illuminated place.

Determination of Melting point

Melting point of the drug was determined by using capillary method. Drug was filled into capillary tube by sealing its one end at the height of 3 mm from the closed end. The capillary was introduced into the digital melting point apparatus and the point at which the drug starts melting was noted until the entire samples get melted.

Identification of drug by FTIR

Fourier transforms infrared spectral spectroscopy (FTIR) the pure drug was mixed with IR grade potassium bromide in a ratio of (1:100) and pellets were prepared by applying 10 metric ton of pressure in shimadzu hydrophilic press. The pellets were then scanned over range of 4000-400 cm-1 in FTIR spectrometer. FTIR spectrum of Febuxostat showed the presence of the peaks which complies with the reference spectra.

Preparation of Standard Calibration Curve of Febuxostat

10 mg of drug (Febuxostat) was accurately weighed from calibrated digital weighing balance and was transferred to 100 ml volumetric flask. Small quantity of methanol was added to dissolve the drug. The volume was made up to 100 ml using methanol to prepare stock solution of 100 µg/ml. From the stock solution 0.2, 0.4, 0.6, 0.8, 1.0 ml of solution was pipetted into 10 ml volumetric flasks and volume was made up to 10 ml to form concentrations of 2, 4, 6, 8, 10 µg/ml with phosphate buffer. The absorbance was measured with the help of UV Spectrophotometer at 318 nm by taking phosphate buffer as reference solution. All the studies were done in triplicate (n=3) with the same instrument.

Determination of solubility of various solvents (oil, surfactants)

In this excess amount of drug(Febuxostat) was taken and dissolved in various excipients used in the study. The solutions were sonicated for 1hr at room temperature and maintained at 25℃ for 48 hrs on an orbital shaker Orchid, Mumbai. Then this was filtered through a 0.22µm nylon membrane filter. These were suitably diluted and analyzed, spectrophotometrically (UV/Vis spectrophotometer, Elico), for the dissolved drug at 318 nm. All trials were performed in triplicate.

PREPARATION OF NANOEMULSIONS:

NanoemulsionofFebuxostatwaspreparedusingsolventevaporationfollowedby probesonication emulsification technique. In detail, the aqueous phase was prepared by dispersingCaptex 200 P (1.71 mL) & Egg lecithin (1.77 mL) in distilled water with continuous stirring.The organic phase was prepared by adding different amounts of Tween 80 into Captex oil.The drug wasdissolvedin this oil and surfactantmixture with continuous stirring.Theaqueous phase and the oil phase were heated separately at 60-70°C with stirring.The oilphasewasthenhomogenizedintoaqueousphaseusingahigh-speedhomogenizer(Ultraturrax, IKA-T23) at 10,000 rpm for 20 min at 50-60°C. Then emulsion was transferredtoprobesonicatorapparatusandthetemperaturewasmaintainedbetween25°Cduring[1].

Table 1:Thecoded andactualvaluesofthevariablesusedinthefullFactorialdesignofFBX-NEs

IndependentVariables	Actualandcodedvalues	
	Low(-1)	High (+1)
X_1=Conc.ofOil	0.5	1
X_2=Conc.ofS-mix	0.5	1

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Optimizationoftheingredientsusingafullfactorial design

Tableisthe3^2fullfactorialdesignwiththecontentoflipid(X_1),polymer(X_2)asindependent variables or PS, ZP, PDI, and EE as responses. The result of the experimentaldesign was analyzed using Statistical software Design-Expert® Version 7.0.0. Each responsecoefficientwas studied forits statistical significance at a95 %confidencelevel.TheP-valueof probe > F‟ less the 0.05 indicates that model terms are significant and greater than 0.05indicates that model terms as insignificant and should be removed from analysis to generate thereduced model. All independent variables, their levels along with actual and coded values ofthese variables are shown in Whereas mean particle size of FBX-NEs use as(Y_1), ZP as (Y_2),PDI as (Y_3) & %EE as Y_4were selected as response parameter as the dependent variables.Usingthisdesign,wewereabletochoosethebestmodelamongthelinear,two-factorinteraction model and quadratic model due to the analysis of variance (ANOVA) F-value byusing Statistical software Design-Expert® Version 7.0.0 was employed for statistical analysisandgraphplotting.Theeffectofindependentvariablesontheresponseswascalculatedby

Table 2: Formulationsof NEsloadedwithFBXby3^2simple fullfactorial design

Run	X_1(mL)	X_2(mL)
F1	2.50(+1)	2.25(-1)
F2	1.50(+1)	2.25(+1)
F3	2.00(-1)	2.00(+1)
F4	1.50(+1)	2.25(-1)
F5	1.50(-1)	1.75(-1)
F6	2.00(+1)	1.75(-1)
F7	1.50(-1)	2.00(-1)
F8	2.50(+1)	1.75(+1)
F9	2.50(+1)	2.00(-1)

X_1 (mL) = Oil (high level-1.0, low level-0.5), X_2 (mL) = S-mix (surfactant & co-surfactant)concentration(highlevel-1.0,low level-0.5).

Characterization of Nanoemulsion

Characterization of nano-emulsions is of most importance in order to ensure the production of emulsions which fall within the desired droplet size range,viscosity and charge and are stable with time. Several techniques have been developed to characterize emulsions such as particle size analysis, polydispersity index and zeta potential determination, differential scanning calorimetry. Some of these methods will be highlighted below.

1. Thermodynamic stability studies: The formulations were subjected to different thermodynamic stability tests.

a) Heating cooling cycle: Three cycles between the temperature 4˚C and 45˚C with storage at each temperature not less than 48 hrs was studied. Those formulations, which were stable at these temperatures, were subjected to centrifugation test.

b) Centrifugation: formulations which were stable in the above test were centrifuged at 3600 rpm for 30min. Those formulations that did not show any

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phase separation were taken for freeze thaw stress test.

c) Freeze thaw cycle: Between – 18℃ and +25℃ three freeze thaw cycles with storage at each temperature for not less than 48 h was done for the formulations.

2. Drug content:in this 2 ml of Nano emulsion was taken in 10 ml volumentric flask and the volume was made up to 10 ml using methanol. 1ml of stock solution was diluted to 10 ml with phosphate buffer pH 6.0 phosphate buffer which was further diluted to give a final concentration of 10 µg/ml (10ppm) solution. Percent drug content was calculated spectrophotometrically at 318 nm.

3. Particle size determination: Particle size of emulsion can be determined using several techniques. Some of the major techniques are hydrodynamic chromatography, photon correlation spectroscopy, spectroturbidimetry, field flow fractionation, sensing zone, electron microscopy and sedimentation.

4. Zeta Potential: Determination Zeta potential is a measurement of surface potential. The magnitude of zeta potential gives an indication of potential stability of an emulsion. Zeta potential is an important parameters in determining the stability of an emulsion and other colloidal dispersion, zeta potential larger than about 25mV is typically required to stabilize a colloidal system. Zeta potential is determined by a number of factors, such as the particle surface charge density, the concentration of counter ions in the solution, solvent polarity and temperature. Zeta potential can be determined using the Malvern Zeta sizer or the Nicomp particle sizer. Zeta potential is determined by electrophoretic light scattering (ELS).The smoluchowski equation can be used to compute the zeta potential from electrokinetic mobility µ. $\mu=\zeta\varepsilon/\eta$…….equation. Where ε is the permittivity and η the viscosity of the liquid used

5. Dissolution studies of Nanoemulsions: Dissolution studies for febuxostat Nano emulsions were performed in pH 6 phosphate buffer using USP dissolution test apparatus with a paddle stirrer. The paddles were allowed to rotate at a speed of 75 rpm. The dissolution medium was maintained at a temperature of 37±0.5℃ and the samples were withdrawn for every 1hr.The volume of withdrawal samples were replaced by fresh dissolution medium in order to keep the volume of dissolution medium constant.Then the withdrawal samples were checked for absorbance at 318 nm using UV-Visible spectrophotometer.

III. RESULTS AND DISCUSSIONS:

Physical appearance: Physical appearance of febuxostat

Test	Specification	Observation
Nature	Amorphous	Amorphous
Color	White	White
Physical state	Solid powder	Solid powder

Melting point: analysis Melting range of febuxostat was found to be 238-239℃.

Identification of drug by FTIR: FouriertransformedinfraredspectraofFBXwastakenby usingtheKBrdiskmethod. The obtained IR spectra of FBX given in Fig. Observed peaks of the FBX areshown in Table. which are similar to the standard IR spectra of drug reported in theliterature.

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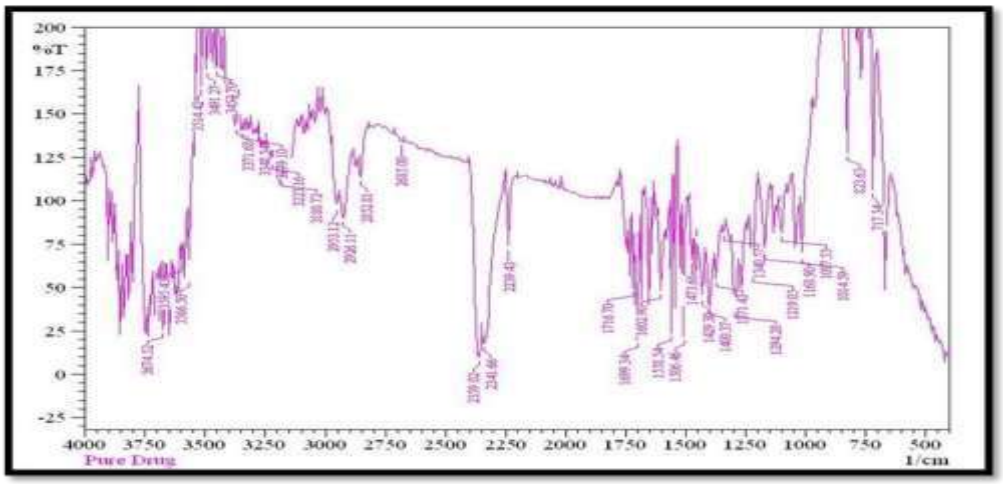

Table1:ObservedpeakofFBX

Range	Observedpeakes	Functional Group
3300-2500	2953.12	COOH
2260-2222	2239.43	C=N
1780-1650	1699.34	C=O
1400-1600	823.63	Thiazolering
1275-1200	1219.05	Alkylarylerher

STANDARDCALIBRATIONCURVE

Standardcalibration curveofFebuxostat(FBX)inmethanol

Graph of absorbance Vs concentration was plotted and found to be linear over the range of 2-12 µg/ml indicating its compliance with Beer‟s and Lambert‟s law. Results are shown inTable8.4andFig.

Table3:Standardcalibration curveof(FBX)in methanol

Concentration(µg/mL)	Absorbance
0	0.000
2	0.123
4	0.282
6	0.422
8	0.545
10	0.791
12	0.123

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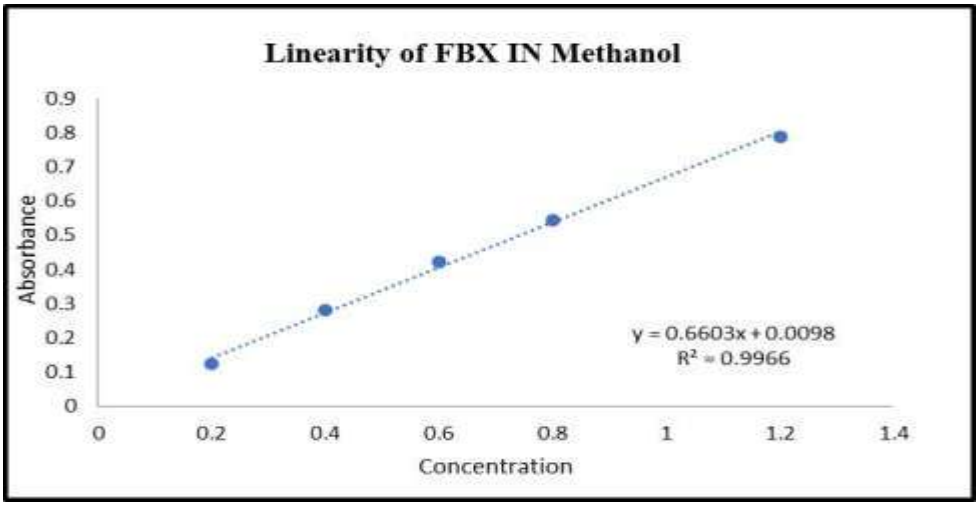

Figure3:Standardcalibration curveofFebuxostat(FBX)inmethanol

SOLUBILITYSTUDY:

Solubility is the property of a solid, liquid, or gaseous chemical substance called solute whichis to be dissolved in a solid, liquid, or gaseous solvent to form a homogeneous solution of thesolute in the solvent. The solubility of a substance fundamentally depends on the solvent usedas well as on temperature and pressure. The extent of the solubility of a substance in a specificsolventismeasuredasthesaturationconcentration whereaddingmoresolutedoesnotincrease its concentration in the solution. The solubility of Febuxostat (FBX) was assessed bytheshakeflaskmethod.Briefly,anexcessamountofFebuxostatwas addedto

1 mL of each sample (oil/surfactant) in a test tube and vertex up to 10 to 15 min at the vertexand then stay for 12 hrs. After that sample was centrifuged at 10,000 rpm for 10 min to settleundissolved drugs. An aliquot of the supernatant was suitably diluted with methanol and theconcentration of Febuxostat was determined spectrophotometrically The solubility data wasreportedinTable 12 andsolubilityofFebuxostatindifferentsolventwas shownin Fig.4.

a) PseudoternaryPhaseDiagram:

Preparationof phase diagramsthepseudoternary phasediagramsof oil,surfactant,co-surfactant, and water was developed using the aqueous titration method to obtain the NEregion. Furthermore, to optimize the ratio of surfactant and co-surfactant, different ratios ofSmix (1:1, 2:1, 3:1, and 4:1) were taken. For each, phase diagram, the ratio of oil to the Smixwerevariedas1:9,1:8,1:7,1:6,1:5,1:3.5,1:3,1:2, 2:8,3:7,4:6,5:5,6:4,7:3,8:2and9:1.

Waterwasaddeddrop-wisetoeachOil–Smixmixtureundervigorousmixing. Based on the above results, Captex 200 P, Tween-80 as a surfactant, and Lipoid E 80 ascosurfactant was chosen as candidates in preparation of NE. These excipients were also foundmiscible with each other. The pseudo-ternary phase figures of several S-mix ratio is presentedin Fig 10. Tween 80 and Lipoid E80 were selected to obtain the S-mix. The colored spotsrepresent the NE area. Four pseudo-ternary phase systems were created, in which the Smixwas made at different mass ratios (1:1, 2:1, 3:1, and 4:1). Fig. 8.8 represents that Smix 1:1has a minimum NE area, whereas S-mix 2:1 created the maximum NE area. As we decreasethe concentration of surfactant, from Smix 4:1 to Smix 2:1, a higher NE area was observed. Itmight be due to the higher HLB value of Tween-80 (HLB-15), and with an addition in thequantity of tween-80, the HLB of the system also increases. For the development of ternaryphase diagrams,the Smixratiobelow4:1wasnotselectedbecauseaswedecreasedthisratio NE region also decreased. Furthermore, the S-mix ratio above 2:1 was also not selectedbecause it also produces a ternary phase diagram with a lesser NE region. Based on thepseudo-ternary phase diagram, a 2:1 ratio of Tween 80 and Lipoid E 80 was confirmed owingto itsmore oil solubilizing potential.For the optimization purpose, the “minimum” quantityof

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Smix was applied as a constraint on the remaining five NEs. Based on the constraintapplied,itwasfoundtobetheoptimizedformulationthatwasfurthersubjectedtocharacterization.

Table4:TernaryphaseaqueousTitration resultsinS-mixRatio1:1

a)S-mix(1:1)						
Sr no.	Volof oil	VolofSmix	Volofwater	water(%w/w) w1	S-mix(%w/w) w2	Oil(%w/w) w3
1	0.1	0.9	1.5	29.18	35.01	3.57
2	0.2	0.8	1.8	46.89	43.51	9.5
3	0.3	0.7	2.3	56.76	36.48	9.07
4	0.4	0.6	2	51	28	21
5	0.5	0.5	5	33.69	4.3	61.99
6	0.6	0.4	0.3	13	55	31
7	0.7	0.3	0.4	16.4	60.95	22.63
8	0.8	0.2	0.3	12.71	72.03	15.25
9	0.9	0.1	0.2	8.73	83.4	7.86

Table5:TernaryPhaseaqueous titration resultsin smix2:1

b)Smix(2:1)						
Sr no.	Vol.of oil	Volof Smix	Volof water	Water (%w/w) w1	Smix(%w/w) w2	Oil(%w/w) w3
1	0.1	0.9	6.5	95.72	1.62	2.65
2	0.2	0.8	5.5	79.64	4.42	15.92
3	0.3	0.7	5	74.07	21.93	36.31
4	0.4	0.6	1	33.33	42.33	24.33
5	0.5	0.5	0.6	37.97	4.05	58.22
6	0.6	0.4	0.3	13.33	37.77	48.88
7	0.7	0.3	0.2	9.47	29.85	60.66
8	0.8	0.2	0.2	9.56	20.09	70.33
9	0.9	0.1	0.1	4.71	10.04	79.23

Table6:TernaryphaseaqueoustitrationinS-mix(3:1)

c)Smix(3:1)

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Srno.	Vol ofoil	Vol ofSmix	Vol ofwater	Water (%w/w)w1	Smix(%w/w)w2	Oil (%w/w)w3
1	0.1	0.9	5	70.52	26.93	2.53
2	0.2	0.8	0.8	27.53	59.4	12.48
3	0.3	0.7	0.4	22.22	65.77	12
4	0.4	0.6	0.3	16.66	52.91	30.41
5	0.5	0.5	0.3	12.19	63	28.04
6	0.6	0.4	0.5	13.33	48.88	7.3
7	0.7	0.3	0.2	20.74	26.14	53.11
8	0.8	0.2	0.2	9.56	20.09	70.33
9	0.9	0.1	0.2	9.7	10.19	80.09

Table7Ternaryphaseaqueoustitrationresultssmix ratio4:1

d)Smix(4:1)						
Srno.	Volofoil	VolofSmix	Volofwater	Water(%w/w)w1	Smix(%w/w)w2	Oil(%w/w)w3
1	0.1	0.9	3.5	62.61	34.16	3.22
2	0.2	0.8	0.2	8.84	75.22	15.92
3	0.3	0.7	0.6	25.53	62.97	11.48
4	0.4	0.6	0.5	20	50.8	29.2
5	0.5	0.5	0.3	13.15	46.66	40.35
6	0.6	0.4	0.4	20.2	42.92	36.86
7	0.7	0.3	0.4	17.31	27.27	55.41
8	0.8	0.2	0.3	12.76	17.02	7.21
9	0.9	0.1	0.2	9.75	10.24	80.47

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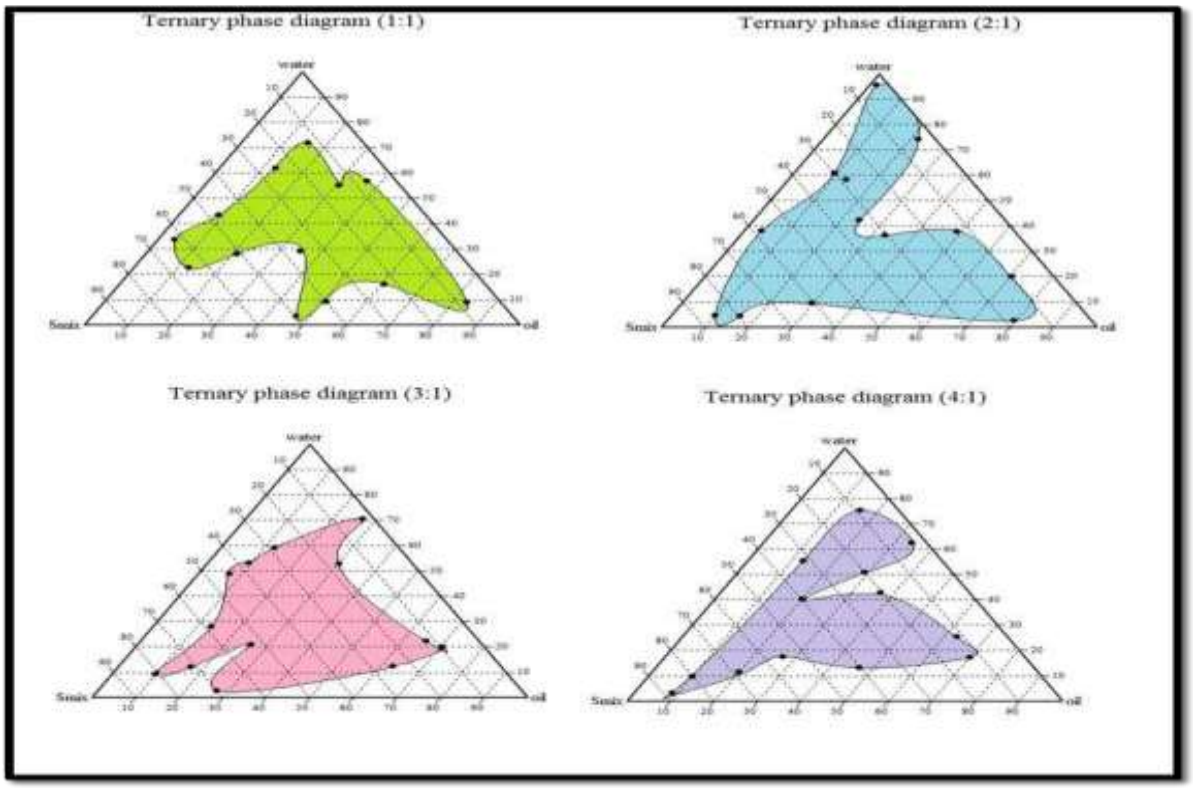

Figure4:Pseudo-ternaryphasediagramsofdifferentS-mixratios.

ParticlesizeandPolydispersityindexofFBX-NE

The major objective of using general optimal design was to determine the levels of the twofactors i.e. oil and surfactant concentration (1.71 mL) (X_1) and Surfactant, Co-surfactantConcentration(1.77mL)(X_2)whichproducestheNEs withminimum particle size andmaximum entrapment efficiency. The particle size of the NEs is a crucial factor because itdetermines the rate and extent of drug release as well as drug absorption. The smaller dropletsize provides a larger interfacial surface area for drug absorption. Also, it was suggested thatthe smaller droplet size permit a faster release rate. Also, it has been reported that the smallerparticle size of the nanoemulsion droplets may lead to more rapid absorption and improvebioavailability.Also,PDImeasuresthewidth ofparticlesizedistribution.IfPDIislowerthan 0.1, it might be associated with high homogeneity in the particle population, whereashigh PDI values suggest a broad size distribution. The particle size and polydispersity index(PDI) of the fabricated batches were in the range of 92.2 to 79.2 nm, and 0.383 to 0.316respectively.Ithasbeenpreviouslyreportedthatseveralparticlesofmeandiameteraround 0.3 and 1.0 µm are preferably absorbed by the payer"s patches (which drains its content intothe lymphatic system) in comparison to particles of 3.0 µm. the particle size obtained in thepresent study is within the size range (92.2 to 79.2 nm) required for efficient lymphaticuptake, therefore, it may be expected that the size is acceptable and would not be a limitingfactorinthelymphaticuptake of thepreparednanoemulsions.Sizes ofFBX-NEs,withdifferent percentages of Captex 200 P, Tween 80, and Lipoid E 80 are shown along with thePolydispersity index (PDI)in Table 21. The particle size and Polydispersity index (PDI) ofthe optimized FBX-NEs was found to be 74.88 nm and 0.308 respectively as seen in Figure5.

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Table5:Particlesize,PDI,Zetapotentialand%EEofFebuxostatloaded NEs

Run	X_1(mL)	X_2(mL)	PS Y_1 (nm)	ZP Y_2	PDI Y_3	EE Y_4(%)
F1	2.50(+1)	2.25(-1)	90.1	-32.2	0.323	82.5
F2	1.50(+1)	2.25(+1)	85.2	-32.5	0.332	78.2
F3	2.00(-1)	2.00(+1)	79.2	-26.3	0.358	85.3
F4	1.50(+1)	2.25(-1)	87.1	-25.6	0.316	86.2
F5	1.50(-1)	1.75(-1)	86.4	-29.1	0.334	84.3
F6	2.00(+1)	1.75(-1)	88.5	-28.3	0.359	87.6
F7	1.50(-1)	2.00(-1)	80.4	-29.6	0.345	79.7
F8	2.50(+1)	1.75(+1)	92.2	-35.2	0.383	74.2
F9	2.50(+1)	2.00(-1)	81.2	-32.1	0.312	75.5

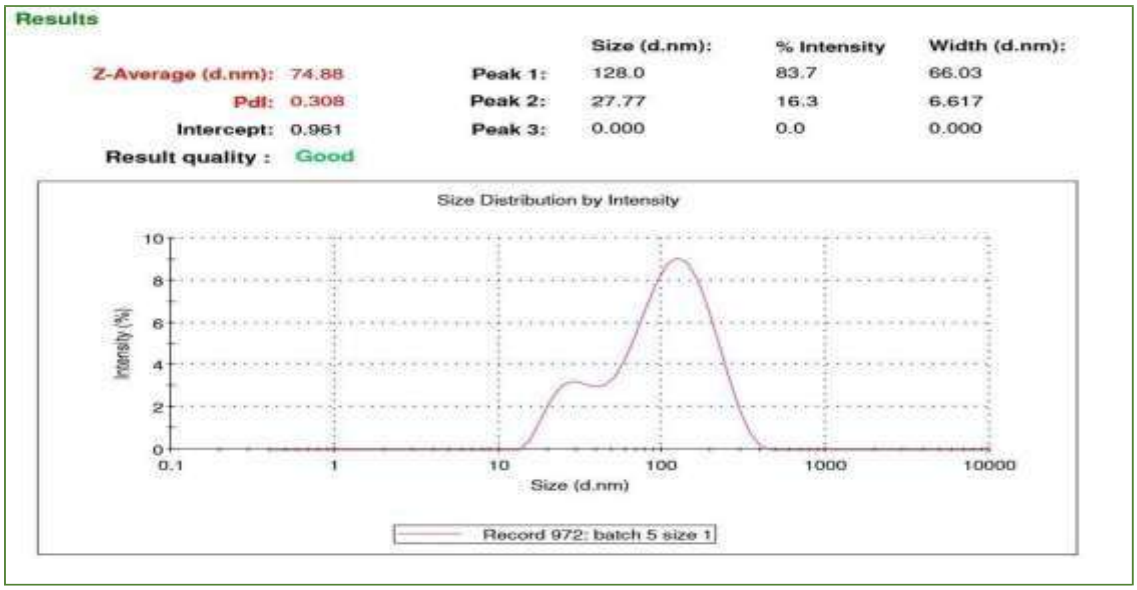

Figure6:Dropletdiametersizegraphofoptimizedformulation

ConductivityTest

The o/w nanoemulsion passes the conductivity test due to water in the continuous phase while w/onanoemulsion vice-versa. The resultantnanoemulsion isof o/w type as optimized nanoemulsionshowntheconductivitywith avalueof167.66 ±0.57mS(mean ±SD,n=3).

From batch F, average droplet size increases which could be attributed to the interfacialdisruption elicited by enhanced water penetration into the oil droplets mediated by the highsurfactant concentration and leading to the ejection of oil droplets into the aqueous phase.Varying polydispersity values show thatgooddroplet distribution had occurred in somebatches while in some droplet distribution i.e. PDI > 0.2 which is quiet, not suitable fornanoemulsion. Moreover, it can be seen that formulation F12 had required. Amount of drug-loaded in nanoemulsion passing thermodynamicstability test. So, the formulation F wasconsidered to be optimized as the droplet size (± 0 nm) (mean ± SD, n=3) was minimum as acomparisonwithotherformulation.The polydispersity indexof formulationFislowesthaving a value of ± (mean ± SD, n=3). Since the diameter of the dispersed oil droplets ofthe optimized nanoemulsion (F12) was much smaller than the 100 nm, such droplets areconsidered to be suitable for intranasal administration to achieve

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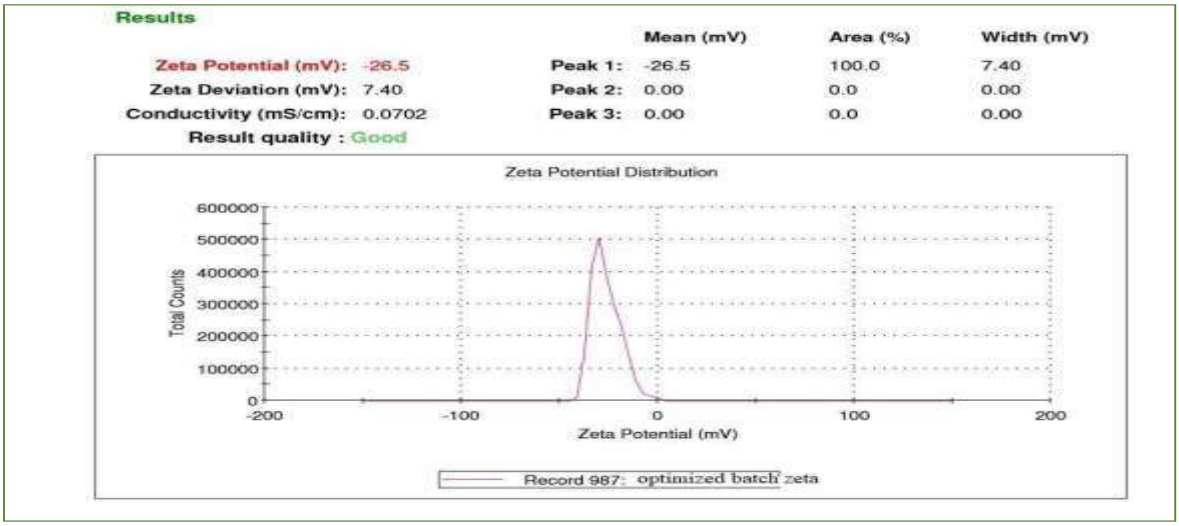
brain targeting. The dropletsize of the nanoemulsion is an important factor as this determines the rate and extent of drugreleaseaswellas absorption.

Zetapotentialdetermination

The zeta-potential of the optimized FBX-NEs was found to be -26.5 as seen in Figure 8.16. Itpossessesnegative surface chargesdue tothe negatively chargedTween80.Also,bothsurfactant (Tween 80) and co-surfactant (Lipoid E 80-Egg Lecithin) were also negativelycharged. It is currently admitted that zeta potentials under -30 mV, are optimum and less than(-60) mV, are required for full electrostatic stabilization.Electrostatic surfactants are knowntoimpartfavorableelectricalpotentialtonanoparticlesleadingtohigherzetapotential.However,higher concentrationsofthesesurfactantsdecreasethezetapotentialofnanoparticles due to a reduction in the thickness of the diffuse layer. Consequently, ithasbeenproposedthattoimpartpracticalelectricalpropertiesontonanoemulsions,theelectrostatic surfactants should be used in conjunction with stearic surfactants. In general,particles could be dispersed stably when the absolute value of zeta potential is above 30 mVdue to the electric repulsion between particles. We found zeta potential values of the NEs inthe range of 26.2- to -25.6 mV. This implies that the FBX-NEs prepared using solventevaporation followed by probe sonication emulsification method would be a physically stablesystem.

Figure7:Zetapotentialgraphofoptimized formulation



RefractiveIndex

The refractive index value for the optimized formulation was found to be 1.43 ± 0.23 (mean ±SD, n=3) which was compared with that of the water 1.44 ± 0.10 (mean ± SD, n=3), nosignificant difference was found between both the values. The refractive index data prove thetransparencyofthesystem.

pHDetermination

The pH of optimized nanoemulsion was determined with digital pH meter in the triplicatemanneranditfound6.7± 0.589to,7.2 ±0.487 which isrequiredfororaldrugdelivery.

Centrifugalstressmeasurement

1 mL aliquot of nanoemulsion was subjected to centrifugation at 12000 rpm for 10 min. thecreaming volume of each nanoemulsion was calculated by using formula and formulationwerecompleted.NocreamingofCracking ofnanoemulsionfoundvisuallyafterstudy.

DeterminationofViscosity

The viscosity of optimized nanoemulsion (F) was found 0.0159 ± 0.003 Pas (mean ± SD,n=3). They are Non-Newtonian Liquids (viscosity decreases when the shear rate or shearstress increases) having Low viscosity values ensure easy handling and packing. Therefore,theviscosityofFBX-NEshowed pseudo-plasticbehaviour.

DrugContentbyusingUVspectroscopy

The drug content of the optimizedbatch was found to 87.23 % ± 0.896 (mean ± SD,

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n=3).The optimum concentration of oil, surfactant, and co-surfactant was essential for maximumdrug loading in theformulation togive maximum drug content of optimizedbatch. Allformulationvariablesshowedasignificanteffect ondrug content.

Entrapmentefficiency(EE%)oftheFBX-NEs.

9 different batches of FBXNEs were preparedby varying the lipid concentrations.Theamount of drug was kept constant for all the batches. The entrapment efficiency of all thebatchesrangesfrom74.2%to86.6%respectively.Suchahighvalueofentrapmentefficiency may be due to the highly lipophilicnature of the drug FBX andits high solubilityinCaptex200P.Allvalues depictedin **Table 5.**

In-VitroStudy:

The in-vitro release study for FBX-NE was performed by the dialysis bag technique. In detailnanoemulsion containing 40mg drug was taken for the study. The study was conducted for12 hr (2 hr in SGF and rest in SIF). The results were compared with the drug release fromFebuxostat suspension (control) prepared using 0.4% w/v methylcellulose and the kinetics ofdrugreleasemechanismwasstudied.TheFBX-NEandplainsuspensionweremonitoredfor2 hrs followed by SIF for 10 hrs. The result is shown in figure 18. From plain suspension,therewas15.38%drugreleasein4hrsandbyendof8hrs,therewasonly29.77%release.In the case of drug release from nanoemulsion formulation, there was a 69.86 % drug releasein 8 hrs and 93.61% by the end of 12 hrs. The Highest R^2 value 0.9945 values were obtainedinZeroorderKineticmode.

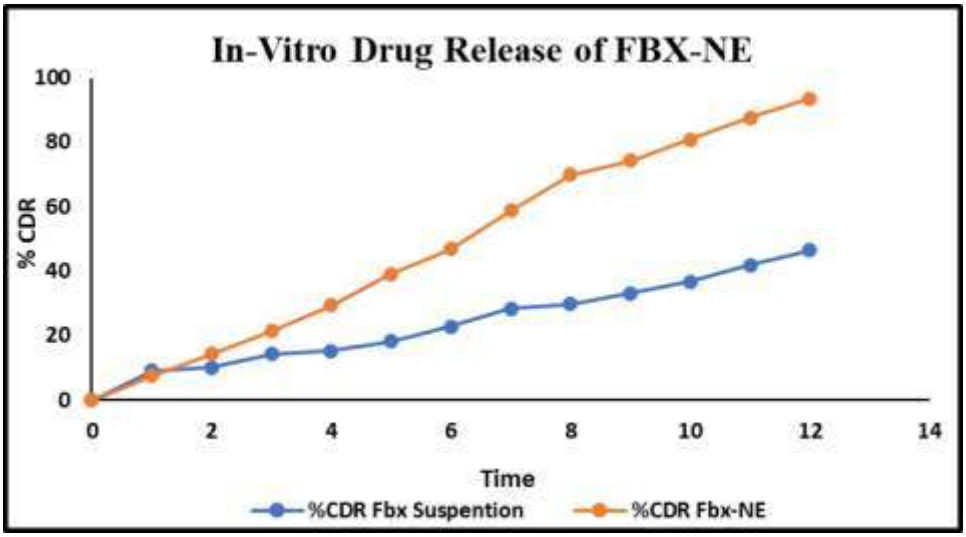

Figure9:In-vitrodrug releaseofFBX-NE

Table8:In-vitroreleaseprofileofoptimizedbatch

SrNo	Time(h)	%CDR
0	0	0
1	1	7.54464
2	2	14.3899
3	3	21.3883
4	4	29.3518
5	5	39.1312
6	6	46.8148
7	7	58.8293
8	8	69.8659
9	9	74.1967
10	10	80.7628
11	11	87.6083

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12	12	93.6155

A) Drugreleasekinetics

To study release kinetics, in vitro diffusion data obtained from optimized formulation wereappliedforvariouskineticsmodelviz.Zeroorder,firstorder,Higuchirelease,andkorsemeyer-Peppas equation. Results are shown in Table 8.22. The zero order plot for batchNEshowedinfigure8.19.TheR^2valuewasgreater forzeroorderkineticplotascomparedto other (First order, Higuchi) hence the nanoemulsion formulation follows zero order kineticdrugreleaseprofilei.e.thedrugreleaserateisindependentofitsconcentration.Thepharmaceutical dosage forms following this profile release the same amount of drug by a unitof time and it is the ideal method of drug release to achieve a pharmacological prolongedaction.Toconfirmtheexactmechanismofdrugreleasekineticsfromnanoemulsionformulation the data were fitted according to Korsmeyer-Peppas equation. When n takes thevalue of 0.5, it indicates the diffusion control drug release and for the value 1.0, it indicatesswelling control drug release. Values of n between 0.5 to1.0 can be regarded as indicatesbothphenomena(Anamoloustransport).The korsemeye-peppasplotforbatchFBX-NEshowed in figure 8.22. The value of n in case of batch FBX-NE nanoemulsion formulationwas suggested that the release of moxifloxacin from the nanoemulsion formulation followedtheAnamoloustransportmechanismshowsdiffusionhadanessentialroleindrugrelease.

Batch Code	Zeroorder (R^2)	Firstorder (R^2)	Higuchi (R^2)	Korsemere-papas(R^2)	Bestfitted model
FBX-NE	**0.9945**	**0.7584**	**0.9151**	**0.8704**	**Zeroorder**

Table8.22Kineticmodelfittingofnanoemulsionformulation

AFM(AtomicForceMicroscopy)

To determine the morphological properties and to confirm data obtained on droplet size andPDI by PCS. AFM was performed using AutoProbe CP- Research SPM (TM Microscopes-Bruker) with 90 µm large area scanners. Formulations were diluted with ultra-pure water 500times(V/V),10µLofdilutedsamplewasplacedoncircularmicaanddriedinvacuum.Duetothenatureofthe samples,noncontactmodewasapplied.AFMmeasurementswereperformed in air, using noncontact probes Bruker Phosphorous doped silicon Tap300, with Alreflective coating and symmetric tip. Driving frequency of the cantilever was about 300 kHz.Both topography and "error signal" AFM imageswere taken and later analysed. The 3DimageofAFM resultwas shown in fig.9.

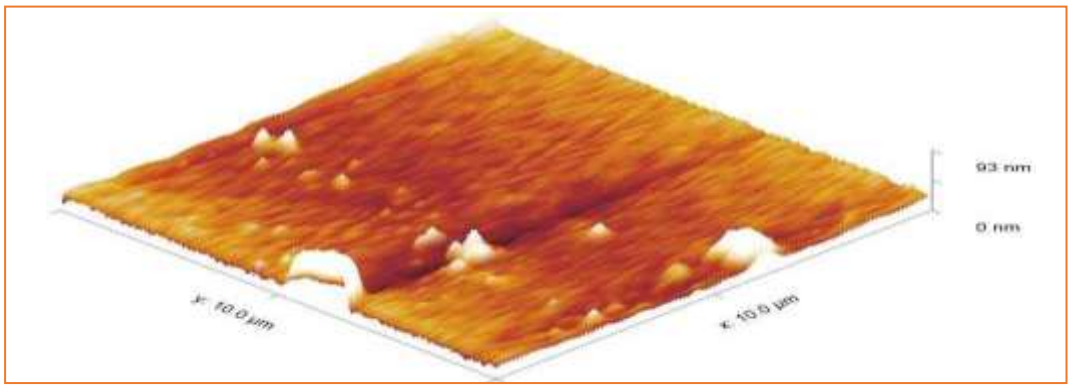

Figure9:AtomicForceMicroscopyofoptimized FBX-NEs

HR-TEM(HighResolutionTransmissionElectronMicroscopy):

High-resolutiontransmissionelectronmicroscopy(HR-TEM)HR-TEMimagingofoptimized FBX -NE was performed to observe the shape and size of the particle. The samplewas prepared by mounting a single drop of FBX-NE dispersion on the carbon-coated coppergrid which was stained by the

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phosphotungstic acid (2 wt% negative stain) and uranyl acetate(1 wt% positive stain). After staining the sample was allowed to air dry for a few mins underroom temperature and the image was captured by TEM (Jeol/JEM 2100) at a voltage 200 kV.The TEM imaging (Fig.9) showed the nanoparticles were a nearly spherical shape with asmooth surface that appeared as a blackish spot. This study concluded with particle size wellinagreementwithDLSmeasurementwithuniform distributionofparticles.

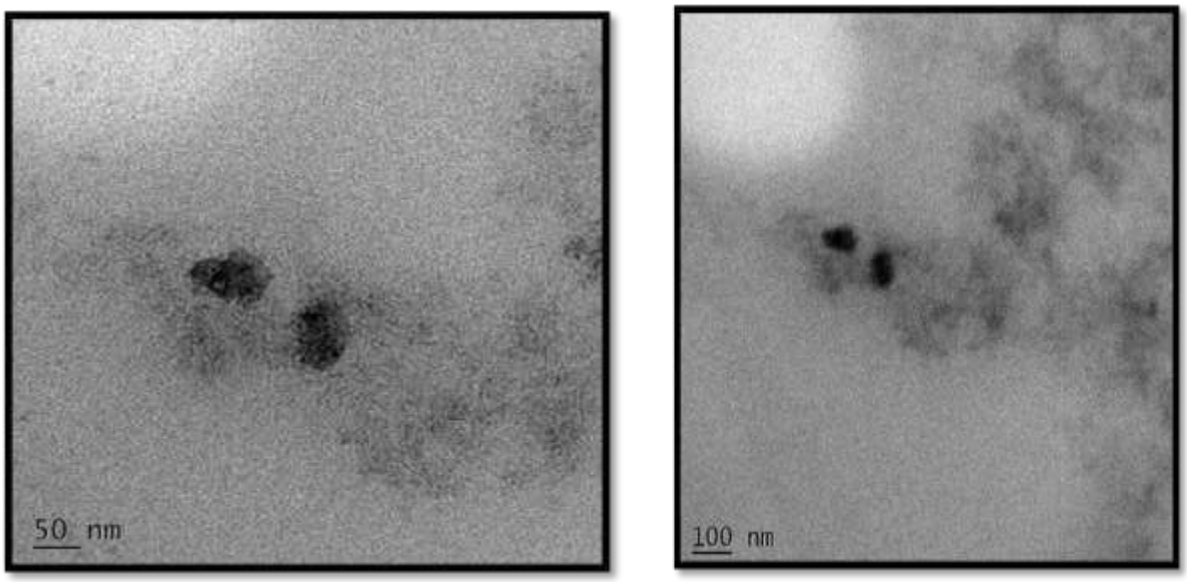

Figure10 HR-TEMimagesofFBX-NE

Accelerated Stability Studies

Formulation showing optimum droplet diameter, PDI, and drug content (FBX) were selectedfor stability studies. According to ICH guidelines, optimized formulation was stored at 25 ±2℃, 60 ± 5% RH. Formulations were evaluated at periodical intervals of three months forvariousevaluationparameterswhichshowthatallresultsareinacceptablelimits.Theoptimizedbatchshowsthat formulationcanbestored wellatroomtemperature(25^0C)

Table10:Stabilitystudies

Parameter	**Measurementon1st month**		**Measurement after 2nd month**		**Measurementmon after 3rd th**	
	4°C	25°C	4°C	25°C	4°C	25°C
Particlesize	42.23±1.00	43.56±1.03	46.23±0.637	47.23±1.56	49.66±0.262	50.26±0.83
PDI	0.126±0.025	0.158±0.039	0.178±0.012	0.159±0.051	0.259±0.077	0.289±0.014
Zetapotential	-12.09±0.031	-13.03±0.559	-12.82±0.327	-12.82±0.244	-13.87±0.502	-13.59±0.350
Entrapment	90.6±0.236	85.66±0.15	77.69±0.891	89.76±	91.00±1.250	79.66±0.8

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efficiency	8	0.789	97	

IV. CONCLUSION

Febuxostat(FBX)isanoveldrugthatisdevelopedforthetreatmentofgoutandhyperuricemia. FBX is a weak acid (PKa 3.42) which is practically insoluble in water and thesolubility was about 12.9 µg/ml in the water at 37 °C. The bioavailability of FBX is low andthe half-life is about 5-8 hr. FBX is classified as a Biopharmaceutics Classification System(BCS) II drug due to its low solubility and high permeability. Hence, there is a need forformulating the Nanoparticles of the Febuxostat drug with suitable excipients and with fewercomplications. Due to low water solubility FBX is having poor bioavailability.Here weincrease the solubility of FBX with incorporating in various oils and itfound higherinCaptex 200 P oil and compatible with tween 80 and egg lecithin as a surfactant. The oil,surfactant, and co-surfactant concentration S-mix concentration was decided by plotting thepseudoternaryphasediagram.

Full factorial design used for the optimization of the nanoemulsion batch with preparing 9bathesofformulationforfurtheroptimizationofthebatch.

For them to achieve higher bioavailability, the dissolution rate of the drug was enhanced bythesynthesisofFBX-NEsusingprobesonicationemulsificationtechnique.TheCumulative % drug release concerning time was found to 93.61% for the optimized batch. From the invitro study, it became evident that enhancement in the bioavailability of FBX observed afterdrugloadedwithnanoemulsionformulation.The microscopicstudyindicatedasmoothsurface and uniform size distribution. From the stability study of the formulation,itwasfound that the particle size and EE were not changed significantly. NEs were found to bestablefor 3months at25± 2^{o}C and 60± 5%RH.

The full factorial design proved to be successful in predicting the optimized formulation ofFBX-NEs, based on particle size, Zeta potential, and entrapment efficiency. The optimizedformulation produced nanoparticles with 74 nm in size with a narrow size distribution (PDIof 0.308), the zeta potential of -26.5 mV, and entrapment efficiency of 87%. In vitro releaseprofiles of NEs indicated a burst release for the first 2 hrs followed by prolonged releaseprofileforFBX untilabout12hrsormore.withtheNEformulation.

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