Section: Research Paper

DESIGN AND CHARACTERIZATION OF TOPIROXOSTAT NANOEMULSION GEL

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Abstract:
The aim of present research was to design and develop Nanoemulsion of Topiroxostat for solubility enhancement. Topiroxostat 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 Topiroxostat.

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

INTRODUCTION

Topiroxostat denoted as TPX is a non purine selective inhibitor of xanthine oxidase/xanthine reductase. The chemical name of TPX is 4-(5-pyridin-4-yl-1*H*-1,2,4-triazol-3-yl) pyridine-2-carbonitrile.

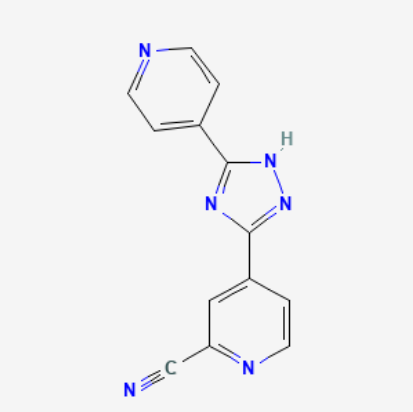


Fig 1: Chemical structure of Topiroxostat

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.

Topiroxostat 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 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 drug1-10. So, the objective of the present research work was to formulate Nano emulsion of Topiroxostat for improving the solubility and bioavailability of drug.

MATERIAL AND METHODS

Materials

Topiroxostat 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 Topiroxostat was done by checking its physical appearance i.e. colour, nature and physical state. Weighed quantity of Topiroxostat 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 Topiroxostat showed the presence of the peaks which complies with the reference spectra.

Preparation of Standard Calibration Curve of Topiroxostat

10 mg of drug (Topiroxostat) 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(Topiroxostat) 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:

The nanoemulsions are prepared by high speed homogenization technique. In this the homogeneous organic solution composed of oil (isopropyl myristate) and a lipophilic surfactant (span 80) and drug (topiroxostat). The homogeneous aqueous phase was formed by water, and hydrophilic surfactant (Tween 80). The aqueous phase was added in the organic phase under constant homogenization. Then o/w emulsion was formed. The stirring was maintained to let the system reach equilibrium.

Table 1: Composition of Topiroxostat Nano emulsion

S.No	Formulations	Drug (topiroxostat) in mg	Isopropyl myristate in ml	Span 80 In ml	Tween 80 In ml	Distilled water In ml
1	TPXN1	40	3	1	1	10-15
2	TPXN2	40	3	2	1	10-15
3	TPXN3	40	3	3	2	10-15
4	TPXN4	40	3	3	3	15-20
5	TPXN5	40	3	3	4	15-20

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 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 parameter 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 topiroxostat 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.

RESULTS AND DISCUSSIONS

Physical appearance

Table 2: Physical appearance of topiroxostat

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

Melting point analysis

Melting range of topiroxostat was found to be 238-239℃. Identification of drug by FTIR. Identification of topiroxostat was carried out using Fourier Transform Infra-Red Spectroscopy (FTIR), Infra-red spectra of topiroxostat were determined using FTIR (S PECTRUM Rx1: shimadzu) using potassium bromide method. The baseline correction was done by scanning potassium bromide pellets over a range of 400-4000 cm-1. Then the pellets containing potassium bromide and topiroxostat mixture and excipients were scanned and data were interpreted.

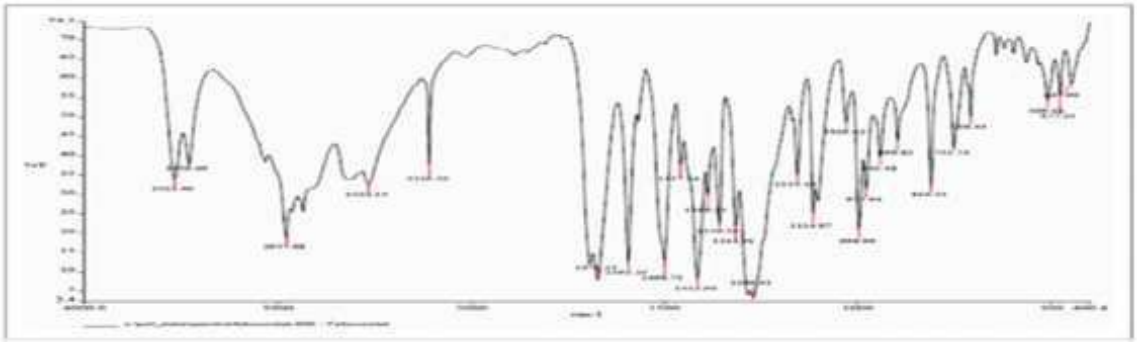


Fig 2: Infra red spectra of topiroxostat Interpretation of IR spectra

Wave number(cm-1)	Functional groups
2250-2220	C=N stretching
2500-3000	O-H stretching
1680-1820	C=O

Calibration curve of Topiroxostat

S.no	Concentration (µg/ml)	Absorbance
1	2	0.129
2	4	0.246
3	6	0.357
4	8	0.473
5	10	0.613

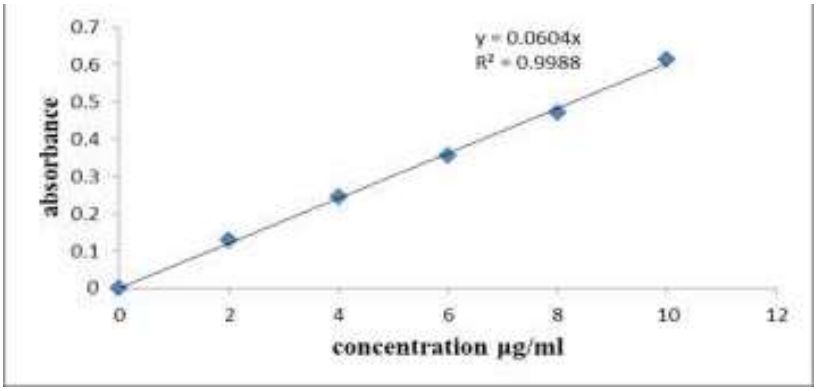
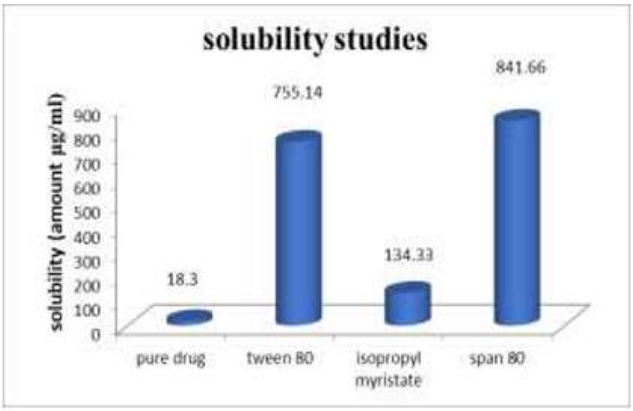


Fig 3: calibration curve of topiroxostat

Solubility studies

Table : Solubility studies

Oil and surfactants	Concentration of drug dissolved in (µg/ml)
Water	18.3
Tween 80	755.14
Isopropyl myristate	134.33
Span 80	841.66



CHARACTERIZATION OF NANO EMULSIONS

1. **Thermodynamic stability studies**

A)**Heating cooling cycle**

	No.of cycles exposed			
Formulation	0	1	2	3
TPXN1	√	√	×	-
TPXN2	√	×	-	-
TPXN3	√	√	×	-
TPXN4	√	√	√	√
TPXN5	√	√	√	×

- All the formulations were subjected to three heating and cooling cycles (4 to 45℃). The response of formulations for heating and cooling was recorded. Among all TPXN4 has shown better results i.e.., this formulation could able to sustain up to 3 cycles of heating and cooling.

B) **Centrifugation**

	Time exposed to centrifuge		
Formulations	0min	15min	30min
FBXN1	√	×	-
FBXN2	√	×	-
FBXN3	√	√	×
FBXN4	√	√	√
FBXN5	√	√	×

All the prepared formulations were subjected to centrifugation at 3600 rpm for 30 min and at each 15 min the samples were visually observed for any physical instability. Among all the prepared formulations TPXN4 was able to retain its physical stability towards centrifugation for 30 min at 3600 rpm.

C) **Freeze thaw cycle**

Formulation	0	1	2	3
TPXN1	√	×	-	-
TPXN2	√	×	-	-
TPXN3	√	√	×	-
TPXN4	√	√	√	√
TPXN5	√	√	√	×

- All the prepared formulations were subjected to three freeze thaw cycles (-18 to +25℃). The response of the formulation for freeze thaw cycle was recorded. Among all TPXN4 has shown better results i.e.., other formulations could not sustain up to 3 cycles.

2. **Assay of Topiroxostat Nano Emulsion**

Table: Percent drug content of Nano emulsion formulation

S.NO	**Nano Emulsion Formulation**	**Percent drug content**
1	TPXN1	80.26
2	TPXN2	83.23
3	TPXN3	88.4
4	TPXN4	100
5	TPXN5	96.2

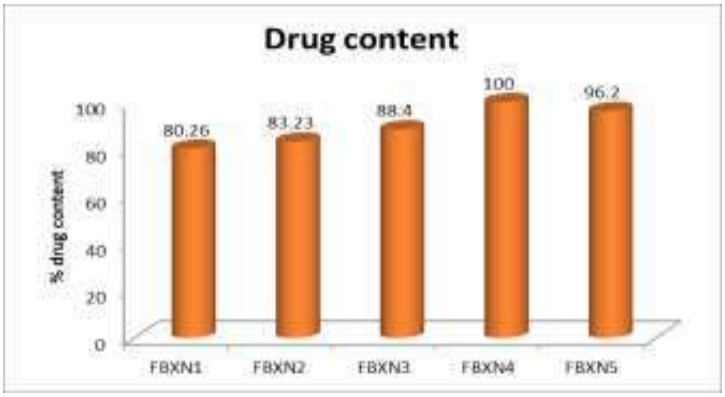


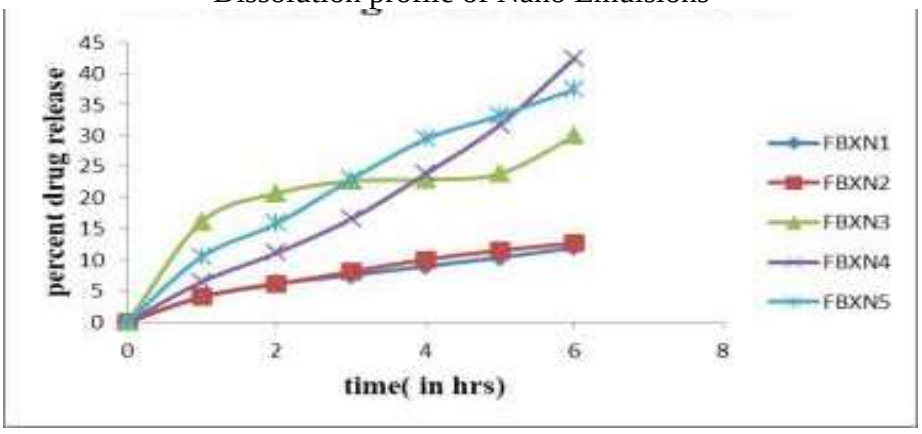
Fig: Percent drug content of Nano emulsion formulations

The drug content of Nano Emulsion is mentioned in the table. The drug content was calculated for all the prepared formulations and the values range from 80.26% to 100%. Among all TPXN4 gave maximum drug content value of 100%

3. **Dissolution profile**

Time (in hrs)	Percent drug release (n=3) of nano emulsions				
	TPXN1	TPXN2	TPXN3	TPXN4	TPXN5
1	4.01	4.08	16.16	6.48	10.37
2	6.18	6.07	20.7	11.1	15.86
3	7.5	8.062	22.68	16.57	22.95
4	8.96	10.05	22.87	23.81	29.4
5	10.38	11.51	23.88	31.72	33.22
6	11.96	12.71	29.92	42.37	37.42

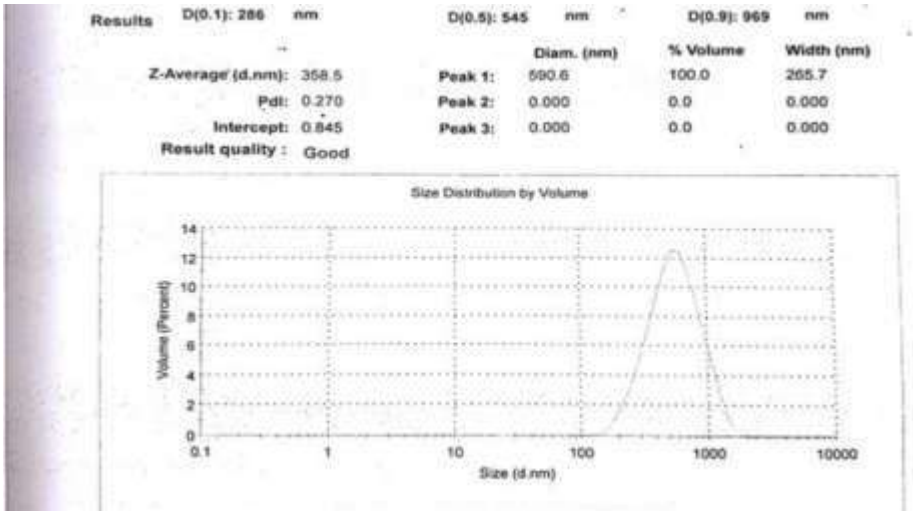
Dissolution profile of Nano Emulsions



Dissolution profile of Nano Emulsions

In-vitro drug release of all the prepared Nano emulsions were carried out in phosphate buffer of pH 6.0. The percent drug release was calculated for all the prepared formulations and the values ranged from 11.96% to 42.37%. Among all TPXN4 gave maximum drug release of 42.37% in 6 hours, hence this was selected as the optimized formulation and further analysis was done. The results of heating and cooling, centrifugation, freeze and thaw, drug content and drug release studies showed that among all the prepared formulations TPXN4 has shown better results. So this formulation was taken for further instrumental analysis.

4. **Particle size distribution of Nano emulsions**



Particle size of TPXN4

- The globule size of TPXN4 is 358.5nm.

5. **Zeta potential**

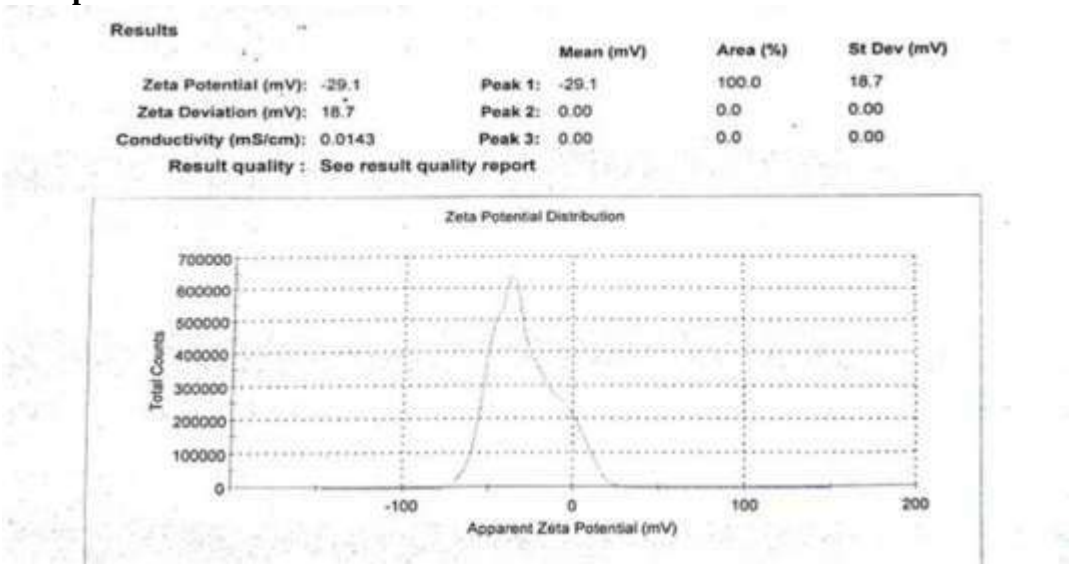
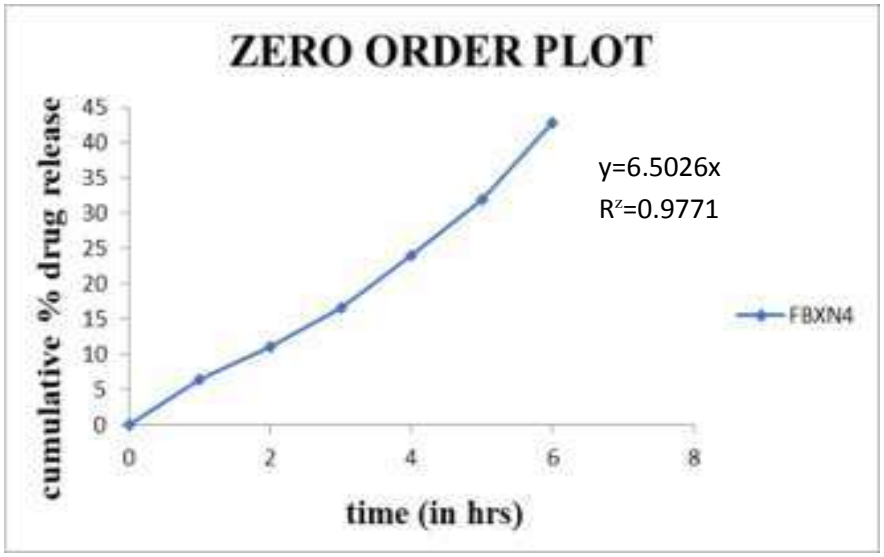


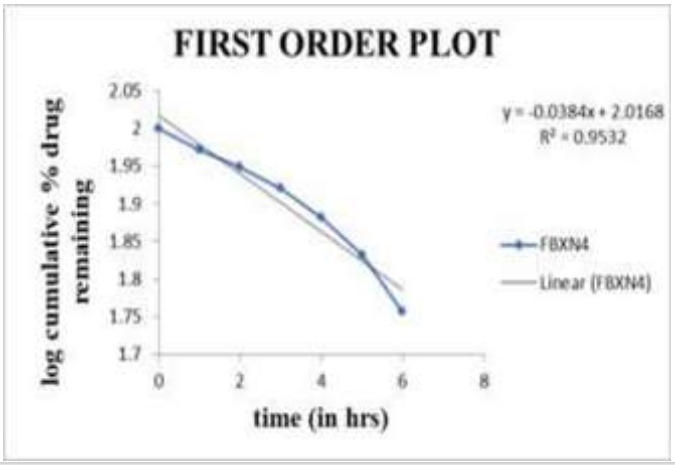
Fig : Zeta potential of TPXN4 formulation

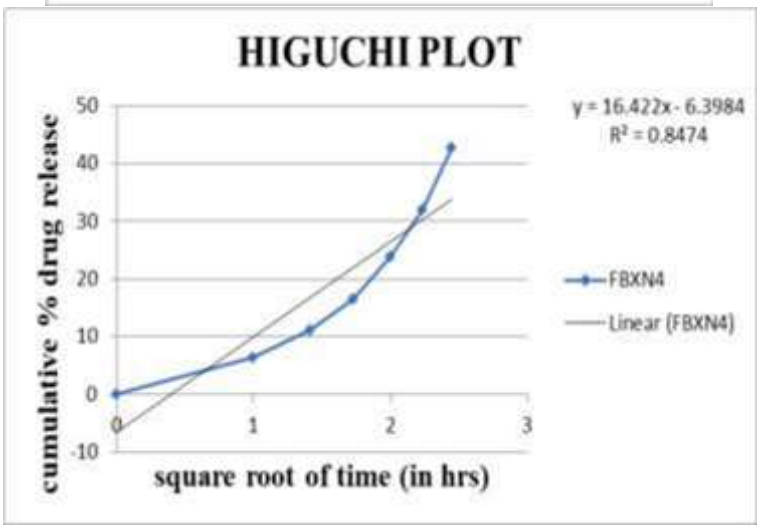
- Zeta potential value is -29.1mv which shows that the formulation is stable having good stability.

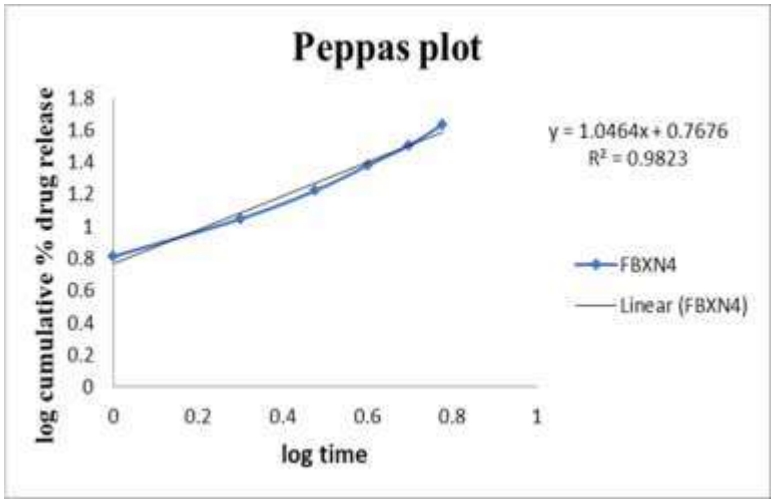
❖ **Drug release kinetics of Nano emulsion**

Different kinetic models were applied on the formulation TPXN4 and the results are


ZERO ORDER PLOT
cumulative % drug release
time (in hrs)
y=6.5026x
R²=0.9771
FBXN4


FIRST ORDER PLOT
log cumulative % drug remaining
time (in hrs)
y = -0.0384x + 2.0168
R² = 0.9532
FBXN4
Linear (FBXN4)


HIGUCHI PLOT
cumulative % drug release
square root of time (in hrs)
y = 16.422x - 6.3984
R² = 0.8474
FBXN4
Linear (FBXN4)



kinetic representation of prepared Nano emulsion

From the kinetic models, the drug release from prepared formulation TPXN4 was observed to follow zero order and the mechanism of drug release is non-fickian diffusion.

CONCLUSION

The present work concluded that Topiroxostat Nano emulsion formulation for solubility enhancement was successfully prepared by high-speed homogenization method. Now a day, Nano emulsion as carrier systems is more acceptable in drug delivery system. Isopropyl myristate (Oil), tween 80, span 80(surfactant) was successfully used as a suitable carrier system for incorporating Topiroxostat. Isopropyl myristate, span 80 are well-suited with the tween 80 and helps in solubilising the drug in the formulation of Nano emulsion. The % drug content was found to be 100%. The globule size of finalized formulation were in the range of 358.5 nm with good stability as confirmed by zeta potential values.

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