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Research Article

Preparation And Evaluation Of Edoxaban Loaded Solid Lipid Nanoparticles Using Hot Homogenization Technique For Oral Delivery

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ABSTRACT
Solid lipid nanoparticles (SLN) are drug carriers in the submicron size range (50–500 nm) made of biocompatible and biodegradable lipids solid at room and body temperature. The main aim of the present study is to improve the solubility and bioavailability of the anticoagulant drug, Edoxaban by hot homogenization technique. Edoxaban is a member of the novel oral anticoagulants (NOACs) class of drugs and is a rapidly acting, oral, selective factor Xa inhibitor. The lipid selected was glycerol monostearate (GMS) on the basis of entrapment efficiency and particle size of SLNs along with surfactant as Tween 80. UV spectroscopy was performed for the identification of Edoxaban, melting point is carried out by capillary method and FTIR spectra illustrated functional groups of the drug. The optimized formulation shows controlled drug release ascompared to the release of pure drugs. Thus, the SLNs are a novel approach for improving the oral bioavailability of EDX.

Keywords: nanotechnology, solid lipid nanoparticles, lipid drug conjugates(LDC), anticoagulants,edoxaban.

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INTRODUCTION

Nanomaterials and nanotechnology play pivotal roles in emerging science and technology and are poised to have a broad and fundamental impact on the global economy. [1][2] To overcome the limitation of low loading capacity LDC was introduced. An insoluble drug-lipid conjugate bulk is first prepared either by salt formation (e.g. with a fatty acid) or by covalent linking (e.g. to ester or ethers). The obtained LDC is then processed with an aqueous surfactant solution (suchasTweens)toananoparticleformulationusi nghigh-pressurehomogenization (HPH). [3] Colloidal particles ranging in size between 10 and 1000 nm are known as SLNs. They are manufactured from synthetic/natural polymers and are ideally suited to optimize drug delivery and reduce toxicity[4,5]. The successful implementation of nanoparticles for drug delivery depends on their ability to penetrate through several anatomical barriers, sustained release of their contents, and their stability in the nano-meter size[5]. SLNs are colloidal carriers developed in the last decade as an
alternative system to existing traditional carriers (emulsions, liposomes, and polymeric nanoparticles). They are a new generation of submicron-sized lipid emulsions where the liquid lipid

(oil) has been substituted by a solid lipid. SLN offers unique properties such as small size, large surface area, high drug loading,and the interaction of phases the
interfaces, and are attractive for their potential to improve the performance of pharmaceuticals, neutraceuticals,and othermaterials[6,7].

Advantages:
Lipid nanoparticles have many advantages in comparison to other particulate systems such as:

- The ease of large-scale production[8],
- The biocompatible and biodegradable nature of the materials[9], low toxicity potential[10],
- The possibility of controlled and modified drug release[11], drug solubility enhancement and
- The possibility of both hydrophilic and lipophilic drug incorporation.

Lipid nanoparticles are different from micro-emulsions, which are clear thermodynamically stable dispersion of oil and water that are stabilized by surfactants and co-surfactants [12,13].

Objectives:
The main objective of this present research work is/are:

- To develop an improved drug delivery system for anti-coagulation drugs using the SLN approach.
- To achieve sustained and controlled drug delivery with reduced frequency of drug administration to have better management of cardiovascular events.

MATERIAL AND METHODS
EDOXABAN was purchased from CVR Life Science Pvt.Ltd., Hyderabad. Glycerol monostearate, GlycerylMonooleate(GMO), Disodiumhydrogenphosphate, Potassium dihydrogenphosphate, tween 80, n-octanol, methanol,etc procured from Meerut Institute of Engineering and Technology, Meerut India.

PREFORMULATION STUDIES
Solubility of EDX in different solvents
The amount of solute that dissolves in a unit volume of solvent to form a saturated solution under specific conditions of temperature and pressure is known as Solubility. The solubility of EDX is to be determined in different organic solvents like methanol (MeOH),water, and phosphate buffer pH 6.8 by using the vial method.[14,15] In this, an excess amount of drug was taken in 10ml glass vials containing a 2ml solvent system and shaken manually till saturated followed by some drug being added in excess. The vial containing the saturated solution of the drug was kept in a mechanical shaker for 24hrs at 37ºC. After 24hrs, the vial containing the drug solvent mixture was removed and centrifuged at 10000rpm for 20 min. to separate undissolved solids.The supernatant was withdrawn,diluted appropriately,and analyzed using a double-beam UV spectrophotometer at 290nm. Based on absorbance data, the concentration has been observed from a standard plot.Then, the concentration was multiplied by the dilution factor.The same procedure was applied in all thesolvent systems separately.

Partition Coefficient
The drug's partition coefficient was determined using n-octanol/water at room temperature. 10ml of n octanol and 10ml of water were taken and 10mg of the drug was added to this solution. when the drug was completely dissolved, the solutions were transferred into the separating funnel and the funnel was shaken clockwise horizontally for 15 minutes then the funnel was allowed to stand overnight so that the two phases were separated properly.[16] the drug content in both phases was analyzed by UV spectrometer

Partition coefficient(PC)=$C_f C_o/C_a$
C_f is the concentration of the total drug taken.
C_a is the concentration of the drug in aqueous phase.
C_o is the concentration of drug in n-octanol

Drug Excipients Compatibility
Drug excipients compatibility studies are an important parameter of pre-formulation studies.Compatibility of EDX with selected lipids was determined by visual interactions (changes due to physical instability like color, conversion of physical state and odor,etc) and physiochemical interaction.

Physical compatibility
The physical compatibility testing was carried out by the drug alone and by the drug with theexcipients. Samples were kept at accelerated conditions i.e. 4ºC and at 25ºC/60%RH for threeweeks. Drug and excipients were mixed till a saturated solution is obtained and divided into six equal parts, 3were sealed in vials and kept under different given temperature and relative humidity conditions[17]. The samples were checked for changes in color, texture, and physicalappearance.

Physiochemical compatibility(FTIR)
The physiochemical compatibility between drug and excipients was studied using Fouriertransform infrared spectroscopy using an FTIR spectrophotometer (Agilent Technologies, Cary630).TheFTIRspectrawererecordedforthe drug,physicalmixture(drug,lipids,andsurfactants), and drug-loaded formulations. The sample was placed on the diamond crystal knobadjusted so that it can touch the sample and scanned in between 4000-650cm^{-1} with theresolutionwas 4cm^{-1}.

ANALYTICAL METHODOLOGY
Intrinsic stability
To study the Intrinsic stability of EDX inthe release medium, a known concentration of a drugsolution(10µg/ml) in the release medium was prepared and divided into three parts. Eachpart was kept at a different temperature i.e. refrigeration(4ºC), and room temperature(25ºC), thestudy was done for 3 days. The UV spectrum was taken initially and after

24hrs -48hrs andobserved for any change in λ_{max}or any other significant change in absorbance to ascertain the intrinsic stability studies of the solution

Determination of λmax Using UV spectrophotometer

In order to ascertain the wavelength of maximum absorption (λ max) of EDOXABAN(EDX),10µg/mlEDXsolution in phosphate buffer pH6.8 was scanned between200–400nm against phosphate buffer pH 6.8 as blank. The spectrophotometric identification was carried out using UV Visible double beam spectrophotometer (V-630)with1cm matched quartz cells.

Preliminary Trials for Selection of Excipients and Technique

Selectionof Formulation Technique

Hot homogenization followed by ultrasonication and double emulsion techniques were triedfor the formulation of nanoparticles, the technique was selected based on entrapmentefficiency.

Ultra sonication technique

EDX loaded SLNs were prepared by hot homogenization followed by the ultrasonication method with slight modifications.[18]EDX and lipid were heated at 80±5° C in a hot water bath.To the lipid and drug mixture,a solution of surfactant was added which was also heated at the same temperature as of lipid a nd drug. Then the mixture was homogenized at UltraturaxT25 and then immediately sonicated withthesonicator.After sonication,the emulsion was suddenly cooled to 4°C in an ice bath. To the freshly preparedformulation,sucrose(cryoprotectant) was dissolved and then the mixture was subjected to freeze dryingusinglyophilization for 72hrs.

- **Screening of Excipients**

Selection of Lipid

Different lipids were tried: Glyceryl Monostearate (GMS) and Glyceryl monooleate (GMO)asthesearecommonlyusedlipidspreparedbyhotho mogenizationfollowedbyultrasonication. The selection of lipids was based on the entrapment efficiency and particle size ofSLNs.

selection of Surfactants

Different surfactants were tried: Tween 80 and Span 20 as these are commonly use dsurfactants

- **Selection of homogenization time and speed and sonication time**

After the selection of desiredexcipientsdifferentbatchesof nanoparticleswerepreparedatdifferenthomogenizationsp eeds at constant sonication time.Followingtheaboveprocedurenanoparticleswerepre paredatconstanthomogenizationspeedbut at different sonication times.Afterthe selectionofdesiredhomogenizationandsonicationtime,di fferenthomogenizationspeedsweretried to selectthe optimized parameter.The homogenization speed and time exhibit a significant effect on theparticle size, PDI, andZeta Potential. Not only homogenization but sonication time also have a great impact on theparticlesize, PDI,and Zeta Potential ofthe formulation.

Preparation of SLNs by Hothomogenization method followed by ultra sonication[18]

EDX-loaded SLNs were prepared by hot homogenization followed by the ultrasonicationmethod with slight modifications.[18]EDX and lipid were heated at 80±5° C in a hot water bath.To the lipid and drug mixture, a solution of surfactant was added which was also heated at the same temperature as of lipidand drug.ThenthemixturewashomogenizedatUltraturaxT25a ndthenimmediatelysonicatedwiththesonicator.Aftersoni cation,theemulsion was suddenly cooled to 4°C in an ice bath. To the freshly preparedformulation,sucrose(cryoprotectant) was dissolved and then the mixture was subjected to freeze dryingusinglyophilization for 72hrs.

Lyophilization of SLNs

Lyophilization is the most common method for manufacturing pharma products that havetobedriedthoroughlytoensurestability[19].Itisa processthatrequires a n inputofenergy for a certain period ranging from days to even weeks, which depends on whetherthe cycle is optimized or not. The stability of the drug during the process and storage andduration of the cycle are two major considerations for the optimization of the freeze-drying process.Theprocess oflyophilization consistsofthreestages

Freezing

The main function of freeze drying is to separate the solvent from the solute, minimize thethermaldegradationintheproduct,and preventthe productfrom foamingwhena vacuumisapplied[20].Itis the stage where most ofwater is removed from the drug and excipients, and the interfaces betweenice and drug phases form. The formulation must be frozen below its triple point(temp. atwhich solid, liquid, and gas exist at the same time). This process induces many stresses. A coolingrate of about 1ºC/min yields moderate supercooling with large ice crystals which producesslowfreezing(annealing).

Primary drying

In this stage, pressure is reduced inside the chamber, and heat is applied to initiate the processof sublimation of ice crystals formed during the freezing stage. As the sublimation processproceeds, frozen mass changes into a cake-type structure. As there is a loss of latent heat duringthe process, heat must be applied to the product throughout primary drying. Primary drying isa slow process too much heat during the process alters the structure and causesthe removal of 95%ofthe water from theproduct.

Secondary drying

This is the last stage of lyophilization in which water that did not get freezed, is removed bythe process of desorption from the solute phase. The main objective is to reduce the unboundwater to a level that is optimal for the stability of the final product. The temp. in secondary drying ismuch higher than primary drying so the desorption of water may occur at a practical rate.Thisprocess is also knownas"Isothermal Desorption".Aftercompletionofthe lyophilizationprocess,the vacuumisbrokenwithinertgasandtheproductissealed.

Characterization of formulation

Entrapment efficiency

Entrapment efficiency is defined as the amount of drug entrapped in the nanoparticles. Theentrapment efficiency of SLN was determined by the centrifugation method. A volume of 1ml of eachEDX–SLN was centrifugedat10000 rpm for45min toseparate thelipidandaqueous phases.1mlofsupernatantwasthendilutedwithphosphatebuffer6.8andanalyzedusingUVspectroscopyat 290 nm. Thepercentageentrapment efficacywascalculated asfollows:

% Entrapment efficiency= (Weight initial drug– Weight free drug)/ Weight initial drug*100

Where,

$Weight_{initialdrug}$istheweightofEDX used

The weight-free drug was the weight of the unencapsulated drug in the formulation

$Weight_{lipid}$was the weightof lipid usedin theformulation

ParticlesizeandPolydispersityIndex(PDI)[21,22,23]

Analysis of the particle size was performed by using the dynamic light scattering technique. Themean particle size and the polydispersity index (PDI) were measured for all preparations byusing a Particle Size Analyzer by MalvernZetasizer NanoZS90. Particle size measurementswere performed on diluted lipid nanoparticles dispersed in Milli Q water at 25^0 C. SLNs(200µL) weredispersed in 10ml ofMilli-Q water.

Zetapotential

Zeta potential is highly useful for the assessment of the physical stability of colloidal dispersions.Zeta potential can be measured by the determination of the movement velocity of the particles inan electric field (electrophoresis measurements) by Malvern Zetasizer NanoZS90. Zeta limitsare ranged from - 200 mV to + 200 mV. In the present work, the EDX). Each sample wassuitably diluted with filtered distilled water (10 times) and placed in a small disposable zetacellandzetapotentialwasmeasuredintriplicatemanner 100.Zetapotentialstudywasperformedforoptimized SLN formulations.

In-vitrodrugrelease

*In-vitro*release studieswereperformed in phosphate buffer pH 6.8 using a dialysis bag (Hi-Media, Mumbai) of molecular weight 12,000 Da. The dialysis bag was prepared one day beforethe*in-vitro*studiesandthepreparationwasdescribedinsection.10 mlofSLNsloadedEDXwas filled in the dialysis bag and 150ml of phosphate buffer pH 6.8 was taken receptor media.The dialysis bag was dipped in the receptor media and stirred at 300rpm and the temperature wasmaintainedat37ºC.2mlofsampleswerewithdrawnatdifferenttimeintervals(0.25,0.5,0.75,1,2,3,4,6,8,10,12,18 and 24hrs). Fresh receptor media was replaced at eachintervaltomaintainsinkconditions.SampleswereanalyzedbyusingUV-VisibleSpectrophotometer[23].Theconcentrationofdrugreleasewascalculatedbyusingthestandardcurve. The experiments wereperformed in triplicate

RESULT AND DISCUSSION

MeltingPoint

Meltingpoint of EDX was found to be 260ºC± 0.5ºC. The reported melting point is 260º- 263ºC ±0.5ºC [18].

Fourier Transform Infrared Absorption Spectroscopy(FTIR) of EDX

FTIR was carried out for the identification of EDX and the resolution was recorded between400-4000 cm^{-1}as per IP procedure. The characteristic peaks are found to be similar to thespectrumofpuredrugs ofEDX[20,].

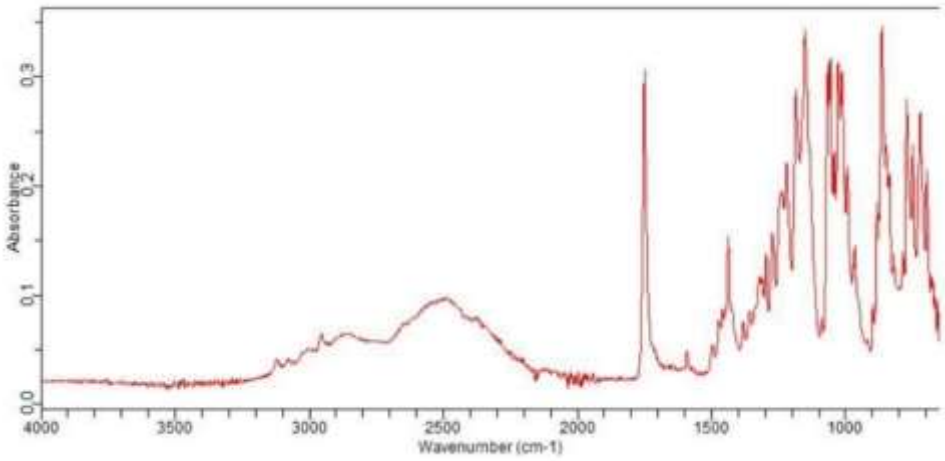
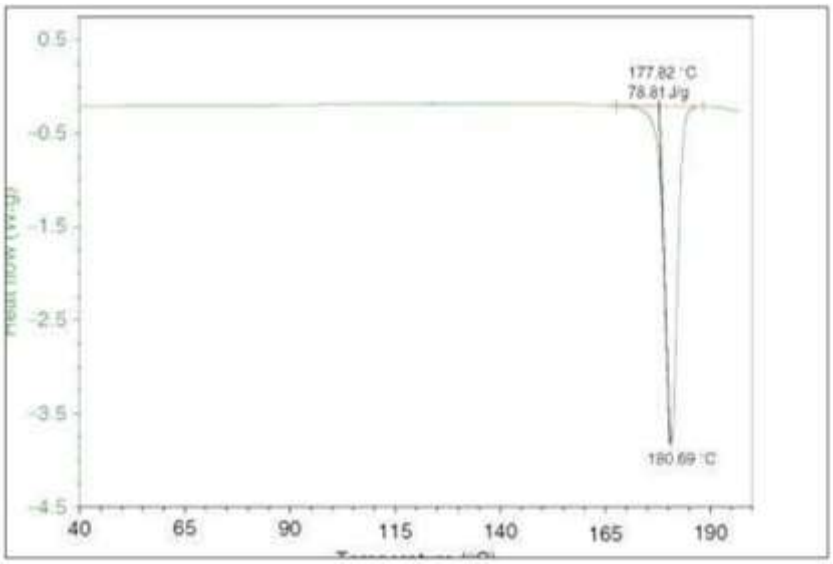


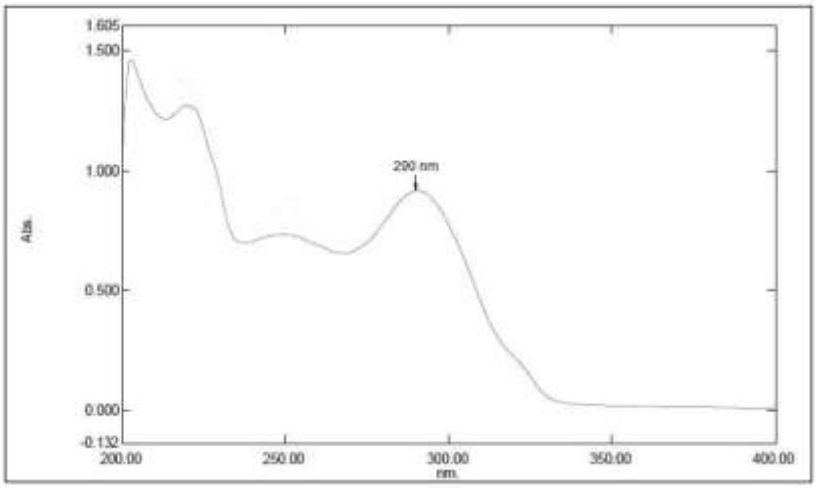
Fig. FTIR Spectrum of EDX Determination of Differential Scanning Calorimetry (DSC)

DSC of EDX wasconductedusinga *thermalanalyzer*.The peak obtained in DSC of EDX was found to be endothermic causing heatabsorptionduringthecycleandreachingthe peakof180.69ºC.



Identification by UV spectroscopy

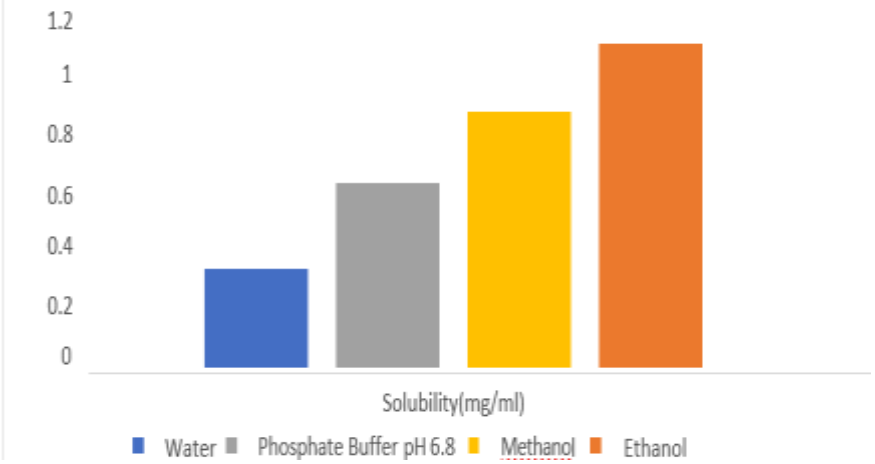
Thepeakmaximawereobserved at 290 nm as shown in Fig, the observed peak complies withthe reported peak maxima[20,]. The UV Spectrum of EDX in phosphate buffer pH 6.8 is showninFig.



- **Pre-formulationstudies**

SolubilityofEDXinDifferentSolvents

The solubility studies of EDX were carried out in various solvents**.** The EDX was found to be 0.64±0.34mg/ml soluble in phosphate buffer pH 6.8. The EDX wasfound tobe 1.12±0.21mg/ml soluble in ethanol i.e. freely soluble, 0.88±0.28 mg/ml solubleinmethanol,andlesssolubleinwateri.e.0.34±0.15 mg/ml.



Partition Coefficient

The experimentally observed value and theoretical value of the partition coefficient are tabulated

ORGANIC PHASE	**AQUEOUS PHASE**	**OBSERVED VALUE (Log P)**
N- Octanol	Water	1.72

- **Drug-Excipient Compatibility Studies**

Physio chemical Interaction

The physiochemical interaction is mainly observed by the chemical instability between thedrug and the selected excipients. These were examined by FTIR. The FTIR of pure drug andpure drug with excipients were recorded in between scanning range of 4000-400 cm^{-1} asshownin Figs. No changes were observed in the absorption peaks of the drug when loaded with thephysicalmixture of excipients.

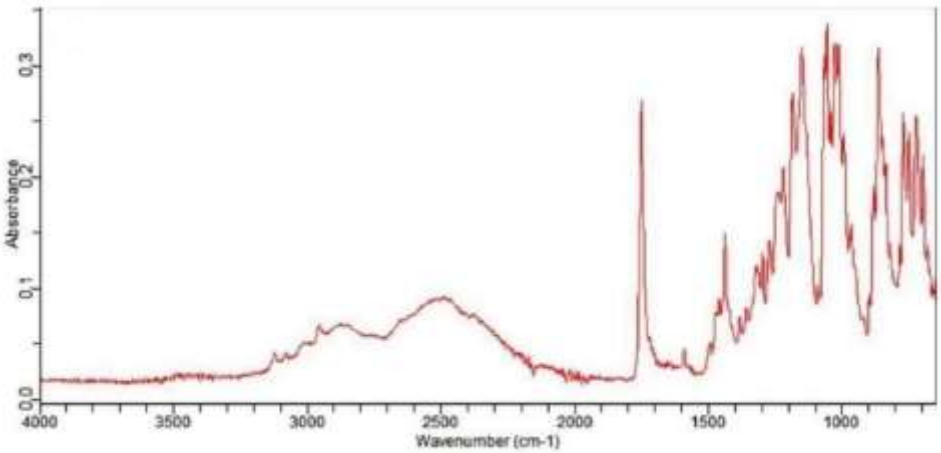


Fig 1. A physical mixtureofEDXandGMS

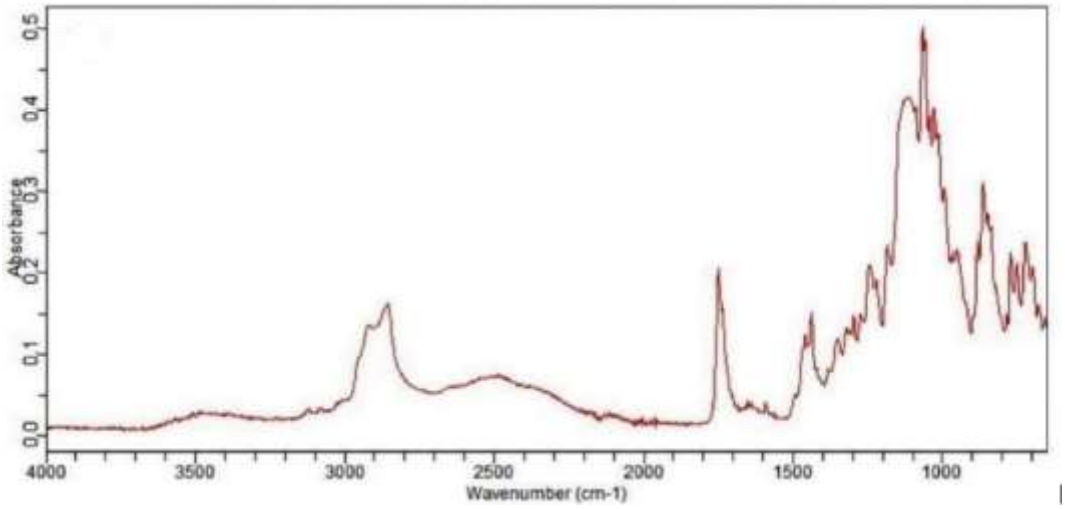


Fig.2 PhysicalmixtureofEDXandTween80

• **Analytical methodology Intrinsic stability**

The samples of known concentration of EDX in phosphate buffer pH 6.8 were stored atdifferentconditionsandanalyzedforanychangeinabsorbanceatspecifictimeintervalsfor2 daysasshown in Table.

Solvent	**Absorbance values of samples stored at different temperature**					
	4ºC			**25ºC**		
Phosphate buffer ph 6.8	**Initial**	**After 24 hrs**	**After 48 hrs**	**Initial**	**After 24 hrs**	**After 48 hrs**
	0.321	0.321	0.321	0.321	0.323	0.321

Determination ofλ_{max}

The λ_{max} of EDX. in phosphate buffer pH 6.8 was found to be 290 nm .

Preparation of Calibration Curve forEDX

The calibration curve of EDX. in phosphate buffer pH 6.8was preparedby usingUVspectroscopy. Different concentration solutions ranging from 2-20µg/ml of EDX in phosphate bufferpH6.8 The absorbance recorded for each concentration is shown in Table.

The calibration curve of EDX in phosphate buffer pH 6.8 showed a linear response across theconcentrationrangeof2-20µg/mlhavingacorrelationcoefficientof**R^2=0.9985 andy=0.0446x-0.0218.**

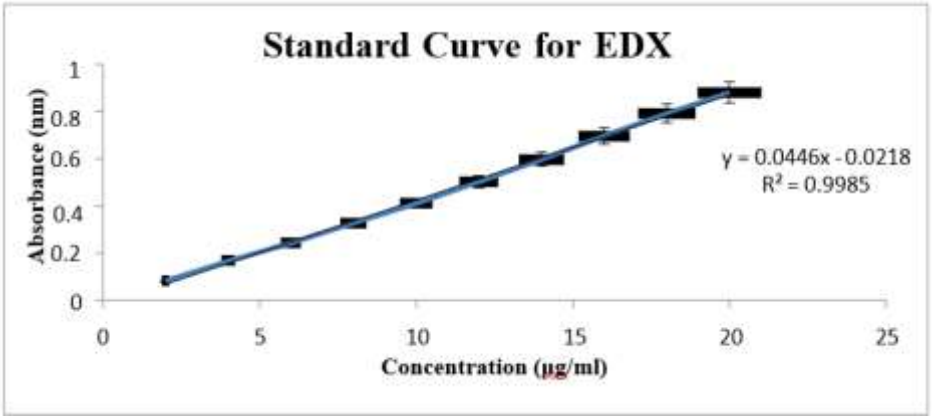


Fig CalibrationCurveofEDXat290nm

Concentration(µg/ml)	**Absorbance(nm)±S.D.**
2	0.081±0.02
4	0.166±0.05
6	0.243±0.04
8	0.326±0.03
10	0.410±0.06
12	0.501±0.05

14	0.596±0.08
16	0.695±0.04
18	0.791±0.07
20	0.880±0.03

Table.
StandardcalibrationcurveofEDXinphosphatebuffer pH6.8

- **PreparationofSLNsbyHotHomogenizationmethodfollowedbyUltrasonication LyophilizationofSLNs**

SLN is fabricated and lyophilized for further characterization. Lyophilization is a promisingwaytoincreasephysicalandchemicalstabilityoveran extendedperiodoftime.[18]

F1-F7 SLNsFormulations

Formulation Codes	Lipid (mg)	Surfactant (%w/v)	Homo. Time(min.)	Homo. Speed(rpm)	Sonication Time(min.)
F1	100	1.5	20	10000	10
F2	200	1.5	20	10000	10
F3	300	1.5	20	10000	10
F4	200	1	20	10000	10
F5	200	1.5	20	10000	10
F6	200	2	20	10000	10
F7	200	1.5	10	10000	10

- **Characterization of Formulation**

Fromthepreliminarystudies,variousfactorslikelipidrange(100-300mg),surfactantconcentration(1-2%w/v), homogenization time (20 min.), and sonication time (10min) werefixed.Afterpreliminarytrials,7formulationswerepreparedbyhot homogenizationfollowed by ultrasonication technique.

Entrapment Efficiency

The minimum and maximum value of E.E. obtained was 49.2±0.04 for formulation F6 and74.86±1.9 for formulation F3 respectively. As increasing the lipid concentration decreases theentrapment efficiency but increasing the surfactant concentration increases the entrapment efficiency Increasing lipid concentration increases the entrapment efficiency attributed to the increasinglipid core leads to the reduction of the crystallinity, and increases imperfections which leaves enough space to accommodate more drug molecules. The E.E .of all the formulations was summarized inTable 5

Particle Size and Zeta Potential

TheparticlesizeandzetapotentialofalltheformulationsF1-F7werepresentedinTable

Theminimumparticlesizewasfoundtobeofformulation F3188.9±3.1nmand the maximum for formulation F5 401.2 ±4.6nm. After incorporation of EDX into SLNs, makesthe size bigger, suggesting that the loaded drug is either adsorbed onto the particle surface or enters the lipid core resulting in in creasing the particle size on increasing the lipid concentration.

The minimum and maximum Zeta potential was found to be of formulation F7 +6.3±3.1 andF2-26.6±7.76 respectively. A high the value of Polydispersity Index (PDI) indicates a wide range of particle sizes. Less thePDI narrow will be the particle size and was found to be a homogeneous distribution of SLNs.Zeta potential is the surface charge responsible for the stabilization of SLNs.

***In-vitro* Drug Release**

Cumulative Drug Release (%) up to 24 hours for all formulations F1-F7 is summarized inTable5

High lipid content encapsulates the drug, thus reducing the drug partition in the outer phaseandconsequentlyitsreleaseinreceivermedia.With highlipidintheformulation,the thicknessof the lipidcoatingwillbehightherebyincreasingthelengthofdiffusionresulting ina decreaseindrugrelease.Assurfactantslowertheinterfacialtensionbetweentheproductandtheaqueousmedia,formorerapidandpossiblycompletepenetrationofthedrugrelease

Table5.VariousEvaluationParametersof7FormulationsF1toF7

mulationCodes	'article size(nm)±S.D	PDI ±S.D	ZetaPotential(mV) ±S.D	EntrapmentEfficiency ±S.D	*In-vitro* drugRelease±S.D
F1	235.1±8.4	0.220±0.01	-18.4±1.34	56.2±0.03	53.161±0.4
F2	330.4±2.23	0.263±0.05	-26.6±7.76	60.32±0.90	57.65±0.62
F3	188.9±3.1	0.401±0.04	-26.4±0.86	74.86±1.9	72.74±0.31
F4	365.4±9.19	0.320±0.07	-13.3±1.40	69.1±0.13	67.31±0.16
F5	401.2±4.6	0.102±0.03	-22.3±0.13	67.86±1.9	64.74±0.31
F6	297.7±2.3	0.380±0.05	-19.6±3.15	49.2±0.04	58.02±0.86
F7	245.3±3.14	0.415±0.04	+6.3±3.1	59.1±0.04	67.12±0.34

Table5.*In-vitro*DrugRelease(%)ofF1-F7

CumulativeDrugReleaseofF1-F7 withSD(n=3)												
Time(hrs.)	**30 (min.)**	**45 (min)**	**1**	**2**	**3**	**4**	**6**	**8**	**10**	**12**	**18**	**24**
F1 (%)	2.12 ±0.25	4.93 ±0.42	6.20 ±0.85	10.73 ±0.32	13.53 ±0.26	19.56 ±0.70	25.70 ±0.92	31.20 ±0.25	36.02 ±0.67	41.11 ±0.22	48.19 ±0.54	53.16 ±0.4
F2 (%)	1.98± 0.46	5.92 ±0.60	13.07 ±0.42	17.59 ±0.31	23.86 ±0.53	27.90 ±0.45	34.13 ±0.37	39.12 ±0.65	42.72 ±0.29	45.12 ±0.16	49.68 ±0.45	57.65 ±0.62
F3 (%)	2.84 ±0.45	5.75 ±0.78	9.09 ±0.15	13.01 ±0.08	17.97 ±0.72	24.27 ±0.02	31.07 ±0.41	38.33 ±0.52	45.65 ±0.63	53.14 ±0.54	62.94 ±0.15	72.74 ±0.31
F4 (%)	3.07 ±0.26	7.02 ±0.76	20.62 ±0.29	23.02 ±0.21	27.25 ±0.92	33.60 ±0.41	37.12 ±0.85	42.23 ±0.61	46.98 ±0.27	51.02 ±0.75	59.33 ±0.43	67.31 ±0.16
F5 (%)	2.98 ±0.34	5.73 ±0.36	19.20 ±0.53	25.43 ±0.46	32.16 ±0.22	35.73 ±0.31	43.02 ±0.74	49.56 ±0.88	52.63 ±0.08	55.90 ±0.41	58.32 ±0.28	64.74 ±0.31
F6(%)	1.93 ±0.84	2.02 ±0.32	4.13 ±0.70	9.53 ±1.03	15.76 ±0.86	19.83 ±0.11	25.78 ±0.77	31.12 ±0.21	36.03 ±0.84	40.16 ±0.96	46.04 ±0.31	58.02 ±0.86
F7 (%)	3.09 ±0.54	7.12 ±0.63	20.83 ±0.32	22.07 ±0.82	25.50 ±0.52	28.63 ±0.84	32.30 ±0.44	36.40 ±0.14	39.04 ±0.87	42.21 ±0.28	48.18 ±0.77	60.55 ±0.19

- **Characterization of Optimised Formulation**

ParticleSizeandPDI

Optimized formulation was selected for size determination using a particle size analyzer. Onevaluationparticlesizeoftheformulationwasfoundtobe 188.1±3.1andthePDIoftheformulationwasfoundto be0.401±0.04

			Size (d.n...	% Intensity	Width (d.n...
Z-Average (d.nm):	188,9	Peak 1:	208,5	100,0	68,19
PdI:	0,102	Peak 2:	0,000	0,0	0,000
Intercept:	0,942	Peak 3:	0,000	0,0	0,000
Result quality	Good				

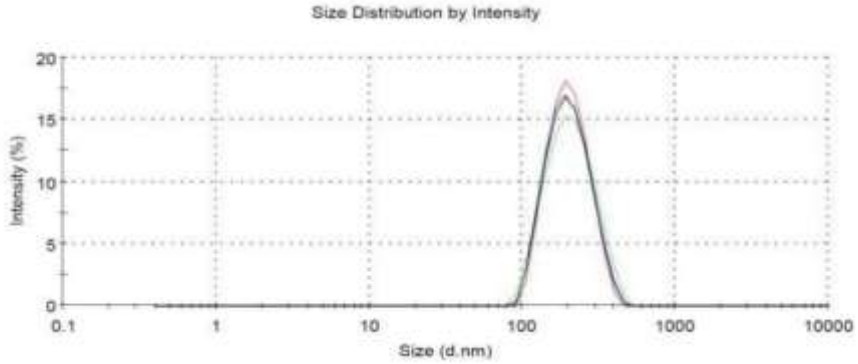


Fig. Particle Size and PDI

Zeta Potential

The Z.P. of the optimized formulation was found to be -22.3, indicating that the preparedformulationdoesnotsufferanyinstability

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-22.3	Peak 1:	-22.3	100.0	7.76
Zeta Deviation (mV):	7.76	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.116	Peak 3:	0.00	0.0	0.00
Result quality	Good				

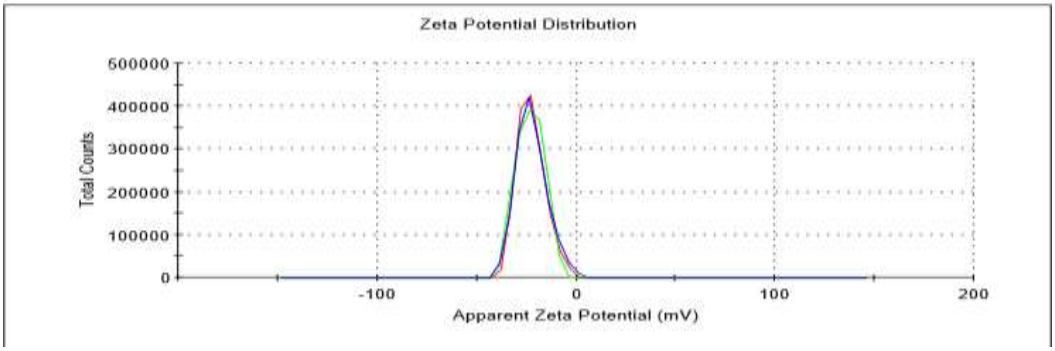


Fig. Zeta Potential

FTIR of Fopt

The FTIR Spectraof F_{opt} revealed that the drug was completely entrapped in SLNs.Therewas no individual peak of the drug and lipid observed in the spectra of F_{opt}

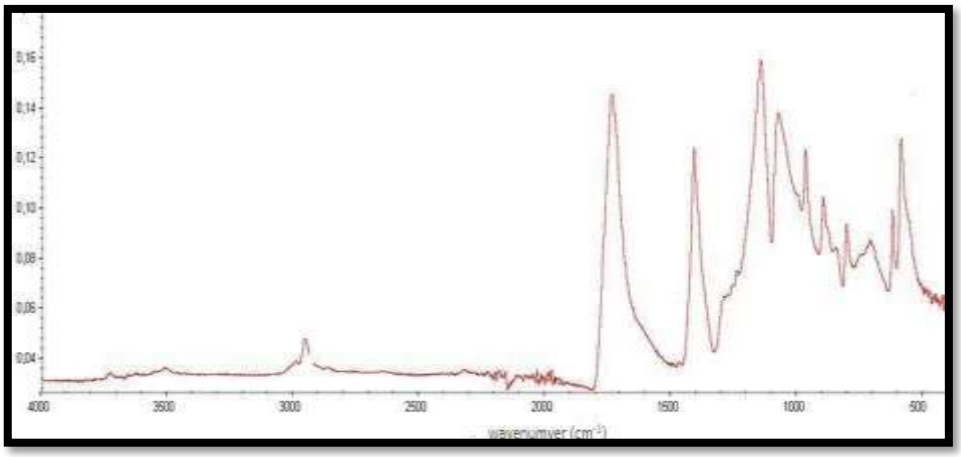


Fig . FTIR of Optimized Formulation Differential Scanning Calorimetry(DSC)

EDX showed as harp end othermic peak at 180.96ºC.The absence of the characteristic peak in the EDX-loaded SLN sconfirms that EDX was successfully encapsulated.

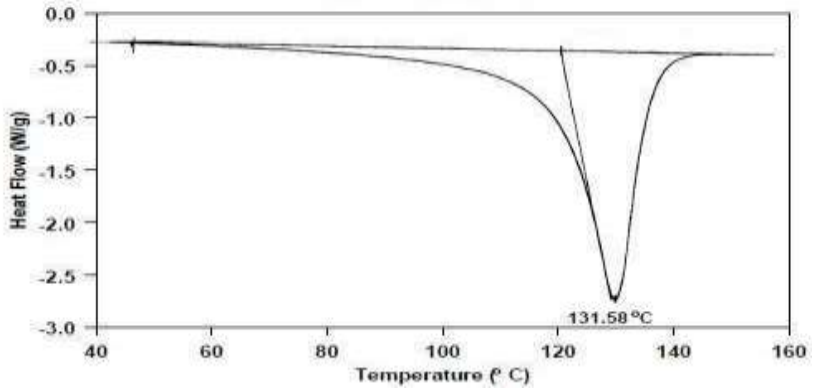


Fig. DSC of SLNs

Scanning Electron Microscopy (SEM)

SEM images of lyophilized EDX-loaded SLNs are shown in Fig. SLNs were roughly spherical withsmoothsurfaces.Aggregationwasalsoseeninsomeim ages and mayoccurduringLyophilization process. Small particles were seen due to the contribution of surfactants thatwereadsorbedontothedrugparticlesurfaceinhibitin gparticlegrowth.

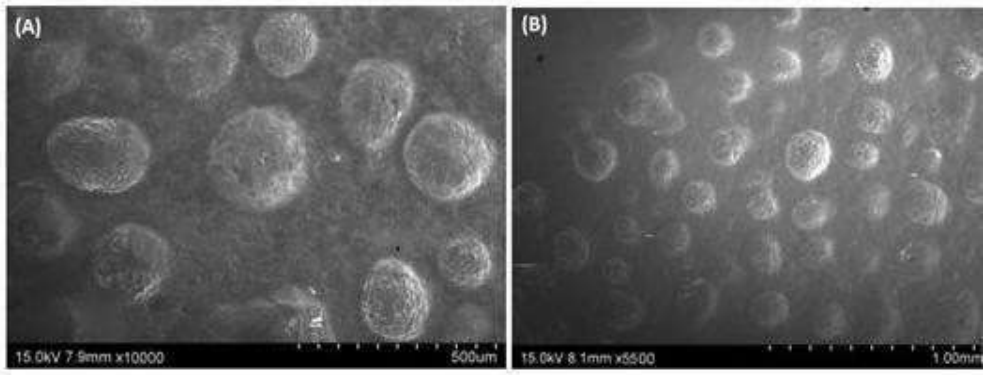


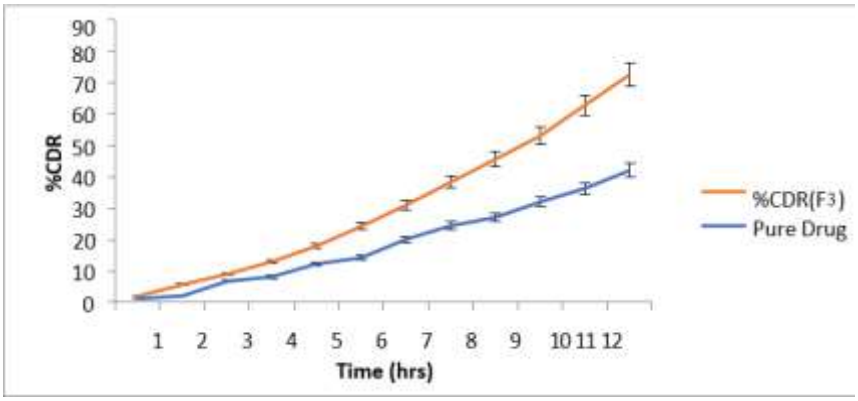
Fig.ScanningElectronMicroscopyat10000Magnificationand5500Magnification

In-vitro Drug Release

Table. *In-vitro* Drug Release ofF $_{opt}$vs Drug Release of Pure Drug

TIME(Hrs.)	%DrugReleaseof PureDrug	%DrugReleaseof Fopt
0.5	1.7±0.21	2.84±0.45
0.75	2.14±0.25	5.75±0.78
1	6.81±0.23	9.09±0.15
2	8.11±0.66	13.01±0.08
3	12.13±0.47	17.97±0.72
4	14.33±0.22	24.27±0.02
6	18.08±0.69	31.07±0.41
8	22.44±0.51	38.33±0.52
10	25.13±0.30	45.65±0.63
12	29.21±0.92	53.14±0.54

18	32.33±0.41	62.94±0.15
24	36.14±0.29	72.74±0.44



***In-vitro* ofF $_{opt}$ Formulation**

The cumulative % drug release of optimized SLNs was determined for 24 hrs. The release behavior of EDX-loaded SLNs is characterized byan initial burst during the first 4h, followed by slowand sustained release. This prolonged-releasebehaviorwasdesirablebecause thatmakesit possibletobypassgastricandintestinaldegradation of the encapsulated drug. The cumulative % drug release from EDX-loaded SLNsand pure drugs was72.74% and 36.14% after 24 hrs. respectively. While % CDR after 6 hrswasfoundtobe31.07%ofdrug-loadedSLNsandaround18.08%ofpuredrugsin6hours.The high load of EDX resulted in its improper encapsulation, thus more initial burst effectfollowed by slow release.

Release kinetics/models ofF$_{opt}$

The *in-vitro* drug release data of F_{opt} was applied to various kinetics models to predict thedrug release mechanism and kinetics models zero order, first order, Higuchi, and Koresmeyer -Peppas models as shown in Fig.

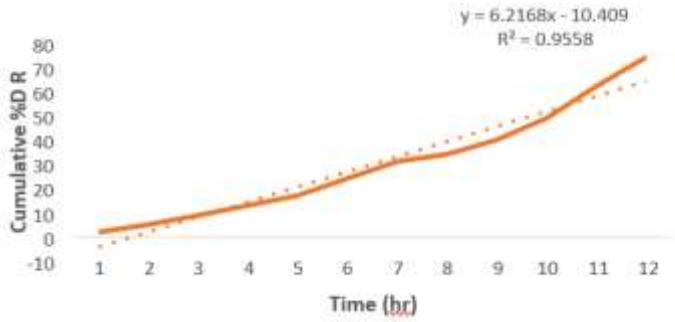


Fig.ZeroOrderModel

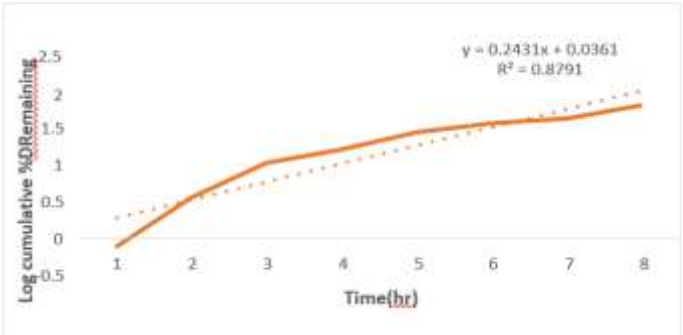


Fig first oder model

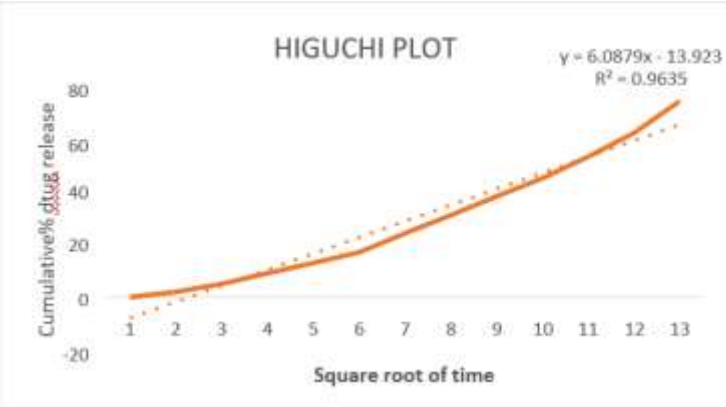


Fig. Higuchi Release Model

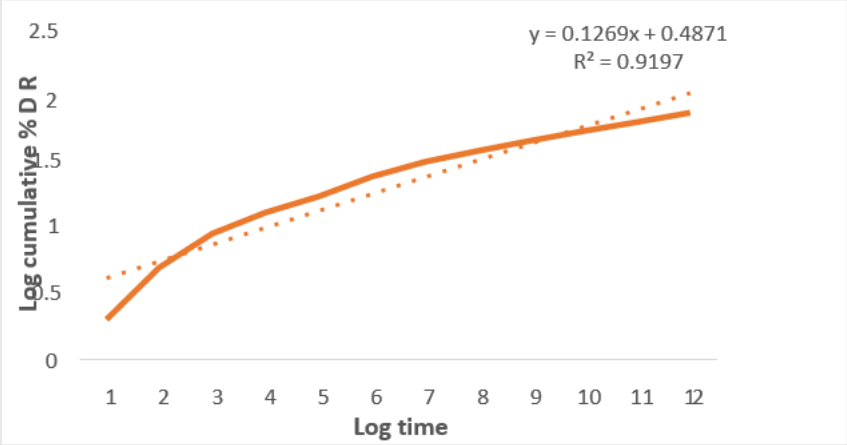


Fig.Koresmeyer-Peppasmodel

The release profile ofallSLNsbest fit into the Higuchimodel thatdescribes the diffusionofthe drug from homogeneous and granular matrix systems. The drug release from a matrix systemis said to follow Higuchi's release kinetics and allows follows the highest linearity (r^2) of 0.9638.

CONCLUSION

In the present study, EDX loaded SLNs were prepared by hot homogenization followed byultrasonication technique. The pre-formulation studies of drug were performed and Results were helped in selecting the suitable solvent for the preparation of formulation. The excipients ranges were selected on the basis of results of preliminary trials. From the FTIRspectraitwasobservedthatsimilarcharacteristicspeaksappearforthedruganditsformulations.Hence itmay concluded thatthere wasnochemicalinteraction between thedrug and excipients used.7 formulations were prepared and characterized by entrapmentefficiency, particle size and *in-vitro* drug release study. The optimized formulation shouldhave maximum entrapment efficiency, minimum particle size and sustained drug release, F3wasfoundtobeoptimizedformulationhaving74.86±1.9 %entrapmentefficiency,231.1±3.1nm particle size, -22.3±0.13mV zeta potential and 72.74±0.31 *in-vitro* drug release.The surface morphology of optimized formulation was examined using SEM. DSC was alsocarried out to determine the melting point of pure drug and formulation. *In-vitro* were carriedout for optimized formulation, the optimized formulation shows controlled drug release ascompared to the release of pure drug. Results shows that drug release follows higuchi modelattributingtothecontrolleddrugrelease.ThustheSLNsisanovelapproachforimprovingtheoral bioavailabilityof EDX.

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