World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: https://wjpsonline.com/
Research Article

FORMULATION AND IN VITRO EVALUATIONOF DORAVIRINE SOLID DISPERSIONS BY USING HOT MELT EXTRUSION

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Received: 01-05-2025 / Revised Accepted: 10-05-2025 / Published: 22-05-2025

ABSTRACT:
The aim of the present work is to develop oral Nanosuspension of Macitentan by Emulsification solvent evaporation method using various Stabilizers & Surfactants such as Poloxamer-188, PVP K30, Pluronic® F-127, and SLS. Various formulation as well as process parameters were optimized in order to achieve desirable size and saturation solubility. Characterization of the prepared Nanosuspension was done with respect to particle size, zeta potential, saturation solubility, dissolution rate, morphology study (SEM), in-vitro dissolution study. Average particle size of Nano suspension of optimized formulations (NS12) was found to be 261.5 nm. From the in-vitro Diffusion studies we can say that formulation NS12 shows best drug release of 99.12±1.30%, within 30 minutes whereas all the other formulations didn't release the drug. The drug release from the Nanosuspension was explained by the using mathematical model equations such as zero order, first order, and equation methods. Based on the regression values it was concluded that the optimized formulation NS12 follows First order kinetics.
Keywords: Macitentan, Pluronic® F-127, SLS, FTIR, SEM and Nanosuspension.

INTRODUCT ION

Nanosuspension:

The effective formulation of medications depends on a number of factors, including solubility, stability at room temperature, and compatibility with solvent, excipient, and photostability. Currently, approximately 40% of newly created chemical entities resulting from drug development initiatives are lipophilic or poorly soluble in water substances.[1,2] Drugs with limited solubility and low bioavailability can be solved using a variety of formulation techniques. Conventional methods such as micronization, fatty solution application, penetration enhancer or cosolvent application, surfactant dispersion method, salt creation, precipitation, etc., have limited effectiveness in improving the solubility of poorly soluble pharmaceuticals. Other strategies include vesicular systems like liposomes, solids dispersion, emulsion and microemulsion techniques, and inclusion complexes with cyclodextrins. These strategies demonstrate promise as drug delivery systems, but their main drawback is that they are not universally applicable to all medications.[3] Nanoparticle engineering has been researched and reported for use in pharmaceuticals throughout the past few decades.[4] The challenges posed by the previously discussed methods can be resolved via nanotechnology. The study of science and engineering at the nanoscale, or 10–9 m, is known as nanotechnology. Techniques like Bottom-Up Technology and Top-Down Technology are used to transfer the drug microparticles/micronized drug powder to drug nanoparticles.[5] Submicron colloidal dispersions of medication particles that are nanosized and stabilized by surfactants are called nanosuspensions.[6] The weakly water-soluble medication is suspended in a dispersion with no matrix material in nanosuspensions.[7] These can be applied to improve the solubility of medications that have low solubility in lipid and water environments. Increased solubility causes the active ingredient to flood at a quicker pace, reaching the maximum plasma level more quickly. This method works well for compounds that are difficult for formulators to work with because they have poor permeability, poor solubility, or both. Because of the smaller particle size, poorly soluble medications can be administered intravenously without obstructing blood vessels. The suspensions can also be formed into a solid matrix by lyophilization. It also has the benefits of liquid formulations over other formulations in addition to these advantages.[8] The benefits, drawbacks, and pharmaceutical use of these various preparation techniques as a drug delivery mechanism are the primary topics of this review.

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How to Cite this Article: Kolluri Lakshmi Narasimha. FORMULATION AND IN VITRO EVALUATIONOF DORAVIRINE SOLID DISPERSIONS BY USING HOT MELT EXTRUSION. World J Pharm Sci 2025; 13(02): 57-66; https://doi.org/10.54037/WJPS.2022.100905

MATERIALS & METHODS USED: Macitentan API was procured from Gathi Lifesciences, and Poloxamer-188, PVP K30, Pluronic® F-127, Ethanol were procured from Lobachemie, Mumbai, SLS, Water were procured from Narmada chemicals.

Spectroscopic study:

Identification of pure drug:

Solubility studies:

Solubility of Macitentan was carried out in different solvents like- 0.1N HCL,7.4 pH buffer and 6.8 pH buffer, and also in organic solvents like ethanol, methanol. Solubility studies were performed by taking excess amount of drug in different beakers containing the solvents.The mixtures were shaken for 24hrs at regular intervals. The solutions were filtered by using whattmann's filter paper grade no.41. The filtered solutions were analyzed spectrophotometrically.

Determination of Melting Point:

Melting point of Macitentan was determined by capillary method. Fine powder of Macitentan was filled in glass capillary tube (previously sealed at one end). The capillary tube was tied to thermo meter and the thermometer was placed in the Thais tube and this tube was placed on fire. The powder at what temperature it melted was noticed.

Determination of absorption maximum (λmax):

The wavelength at which maximum absorption of radiation takes place is called as λmax. This λmax is characteristic or unique for every substance and useful in identifying the substance. For accurate analytical work, it is important to determine the absorption maxima of the substance under study. Most drugs absorb radiation in ultraviolet region, as they are aromatic or contain double bonds.

Accurately weighed 10mg Macitentan separately was dissolved in 10 ml of dichloromethane in a clean 10ml volumetric flask. The volume was made up to 10ml with the same which will give stock solution-I with concentration 1000µg/ml. From the stock solution-I, 1ml was pipette out in 10ml volumetric flask. The volume was made up to 10ml using 0.1N HCl to obtain stock solution-II with a concentration 100µg/ml. From stock solution-II, 1ml was pipette out in 10ml volumetric flask. The volume was made up to 10ml using 0.1N HCl to get a concentration of 10µg/ml. This solution was then scanned at 200-400nm in UV-Visible double beam spectrophotometer to attain the absorption maximum (λ-max).

Construction of calibration curve using 0.1N HCl Buffer:

Accurately weighed 10mg Macitentan was dissolved in 0.1N HCl taken in a clean 10ml volumetric flask. The volume was made up to 10ml with 0.1N HCl buffer which gives a concentration of 1000µg/ml. From this standard solution, 1ml was pipette out in 10ml volumetric flask and volume was made up to 10ml using 0.1N HCl buffer to obtain a concentration of 100µg/ml. From the above stock solution, aliquots of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml each was transferred to a separate 10ml volumetric flask and solution was made up to 10ml using 0.1N HCL buffer to obtain a concentration of 1, 2, 3, 4, 5, & 6 µg/ml respectively. The absorbance of each solution was measured at 280 nm.

Drug excipient compatibility study:

The drug and excipient compatibility was observed using Fourier Transform – Infra Red spectroscopy (FT-IR). The FT-IR spectra obtained from Bruker FT-IR Germany (Alpha T) was utilized in determining any possible interaction between the pure drug and the excipients in the solid state. The potassium bromide pellets were prepared on KBr press by grounding the solid powder sample with 100 times the quantity of KBr in a mortar. The finely grounded powder was then introduced into a stainless steel die and was compressed between polished steel anvils at a pressure of about 8t/in2. The spectra were recorded over the wave number of 4000 to 400cm-1.

Preparation of Nanosuspensions:

Preparation of Macitentan Nanosuspension by Emulsification solvent evaporation method:

Nanosuspensions was prepared by the solvent evaporation technique. Azilsartan was dissolved in methanol at room temperature (organic phase). This was poured into water containing different stabilizers of PVP K25, pluronic F127 and SLS maintained at room temperature and subsequently stirred on magnetic stirrer which is stirred at rpm 800-1000 for 30 min to allow the volatile solvent to evaporate. Addition of organic solvents by means of a syringe positioned with the needle directly into stabilizer containing water. Organic solvents were left to evaporate off under a slow magnetic stirring of the Nanosuspensions at room temperature for 1 hour followed by sonication for 1 hour. Emulsification solvent evaporation was used to formulate the nanosuspension. At room temperature, Macitentan was dissolved in ethanol to form the organic phase this solution is then emulsified into water containing various stabilizers such as Poloxamer-188, PVP K30, Pluronic® F-127, and SLS while kept at room temperature. To add organic solvents, insert a syringe into a water-based stabilizer and mix on a magnetic stirrer at 1000 RPM for 1 hour at 40oC. After that, sonicate the sample for 15 minutes.70-75 Then the nanosuspension was filtered using a membrane filter.

Table.1 Composition of Nanosuspension of Macitentan

Ingredients	NS1	NS2	NS3	NS4	NS5	NS6	NS7	NS8	NS9	NS10	NS11	NS12
Macitentan(mg)	50	50	50	50	50	50	50	50	50	50	50	**50**
Poloxamer-188	50	100	150	200	--	--	--	--	--	--	--	--
PVP K30	--	--	--	--	50	100	150	200	--	--	--	--
Pluronic®F-127	--	--	--	--	--	--	--	--	50	100	150	**200**
SLS	10	10	10	10	10	10	10	10	10	10	10	**10**
Ethanol (ml)	5	5	5	5	5	5	5	5	5	5	5	**5**
Water(ml)	50	50	50	50	50	50	50	50	50	50	50	**50**
Stirring RPM	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**
Sonication time (mins)	**30**	**30**	**30**	**30**	**30**	**30**	**30**	**30**	**30**	**30**	**30**	**30**

Note:

- Total Drug content of Macitentan in nanosuspension is 50mg/5ml.
- Label claim is 10mg/1ml.

Evaluation parameters of Nanosuspensions:

The Nanosuspensions was evaluated for various parameters:-

- Entrapment efficiency
- Scanning electron microscopy
- Particles size and shape
- In-vitro drug release studies
- Drug release kinetics studies

Entrapment efficiency

The 50mg of the Macitentan weight equivalent Nanosuspensions was analysed by dissolving the sample in 10ml of ethanol. After the drug was dissolved 10ml of clear layer of dissolved drug is taken. There after the amount of drug in the water phase was detected by a UV-Spectrophotometric method at 280 nm (U.V Spectrophotometer). The concentration of the drug is determined with the help of calibration curve. The amount of drug inside the particles was calculated by subtracting the amount of drug in the aqueous phase from the total amount of the drug in the Nanosuspensions. The entrapment efficiency (%) of drug was calculated by the following equation.

$$\%\ \text{of Drug entrapment} = \frac{\text{Mass of drug in Nanosuspensions}}{\text{Mass of drug used in formulation}} * 100$$

Particle size measurement:

The particle size was determined using the particle size analyzer (Zeta sizer Nano series, UK). The formulations were diluted with an appropriate volume of 0.1N HCl. The measurements were carried out three times where the mean value was used.

Scanning electron microscopy:

The morphological features of prepared Nano suspensions are observed by scanning electron microscopy at different magnifications.

Diffusion study:

Diffusion Parameters

Medium : 0.1N HCl
Apparatus : Basket (USP-I)
RPM : 50
Temperature : 37º C±0.5
Time Points : 5,10,15,20, 30, 45 & 60 minutes

Procedure:

The dialysis membrane diffusion technique was used. Five millilitre of the nanosuspension was placed in the dialysis membrane (Mw cutoff 12,000–14,000 Hi-media), fixed in a Franz diffusion cell with the receptor volume of 20 ml. The entire system was kept at 37 °C with continuous magnetic stirring. Sample of 1 ml was withdrawn from the receptor compartment at predetermined time intervals and replaced by fresh medium. The amount of drug dissolved was determined using UV spectrophotometer.

Modelling of Diffusion Profile

In the present study, data of the in vitro release were fitted to different equations and kinetic models to explain the release kinetics of Macitentan from the nanosuspension. The kinetic models used were Zero order equation, First order, Higuchi release and Korsmeyer-Peppas models.

Kinetic Studies: Mathematical models:

Different release kinetic equations (zero-order, first-order, Higuchi's equation and Korsmeyer-peppas equation) were applied to interpret the release rate of the drug from matrix systems for the optimized formulation. The best fit with higher correlation (r2) was calculated.

Zero-order model:

Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equation

$$Qt = Q0 + K0t$$

Where Qt is the amount of drug dissolved in time t, Q0 is the initial amount of drug in the solution (most times, Q0 = 0) and K0 is the zero order release constant expressed in units of concentration/time. To study the release kinetics, data obtainedfrom in vitro drug release studies were plotted as cumulative amount of drug released versustime.

Application: It is used to describe the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems, as well as tablets with low soluble drugs in coated forms, osmotic systems, etc.

First Order Model:

The first order equation describes the release from systems where the dissolution rate is dependent upon the concentration of the dissolving species.

Release behavior generally follows the following first order equation:

$$Log\ C = Log\ C_o - kt/2.303$$

Higuchi model:

The first example of a mathematical model aimed to describe drug release from a system was proposed by Higuchi in 1961. Initially conceived for planar systems, it was then sustained to different geometrics and porous systems. This model is based on the hypothesis that initial drug concentration in the is much higher than drug solubility; drug diffusion takes place only in one dimension (edge effect must be negligible); drug particles are much smaller than system thickness; swelling and dissolution are negligible; drug diffusivity is constant; and Perfect sink conditions are always attained in the release environment.

In a general way the Higuchi model is simply expressed by following equation

$$Q = K_H - t^{1/2}$$

Korsmeyer-Peppas model:

Korsmeyer et al.(1983) derived a simple relationshipwhich described drug release from a polymeric system equation. To find out the mechanism of drug release, first60% drug release data were fitted in Korsmeyer-Peppas model,

$$Mt / M\infty = Kt^n$$

RESULTS AND DISCUSSIONS

Determination of melting point:

The melting point of found to be in range of 134.8°C, which was determined by capillary method.

Saturation Solubility:

Saturation solubility was carried out at 25^0C using Methanol, Ethanol, 0.1N HCl, 6.8 phosphate buffer, and 7.4 pH buffer.

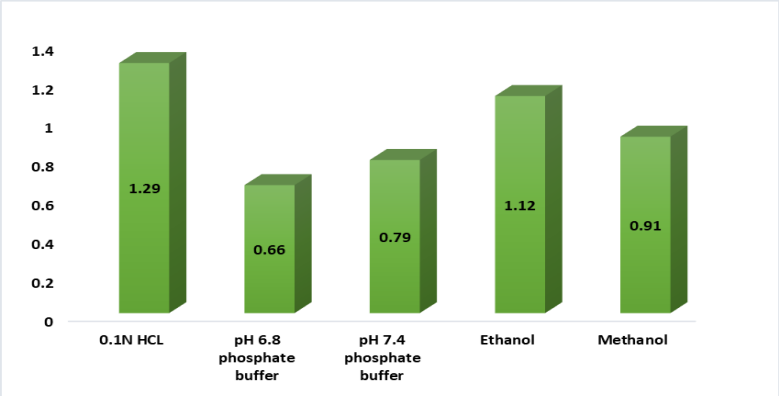


Figure.No:1 Solubility studies of Macitentan

Discussion: From the above conducted solubility studies in various buffers we can say that 0.1N HCl buffer has more solubility when compared to other buffer solutions. So 0.1N HCl buffer is used as Diffusion medium, based upon the solubility studies on organic solvents ethanol has more solubility than others so ethanol was used in the nanosuspension formulation.

Determination of absorption maximum (λmax):

Determination of Macitentan λ-max was dne in 0.1N HCl buffer medium for accurate quantitative assessment of drug Diffusion rate.

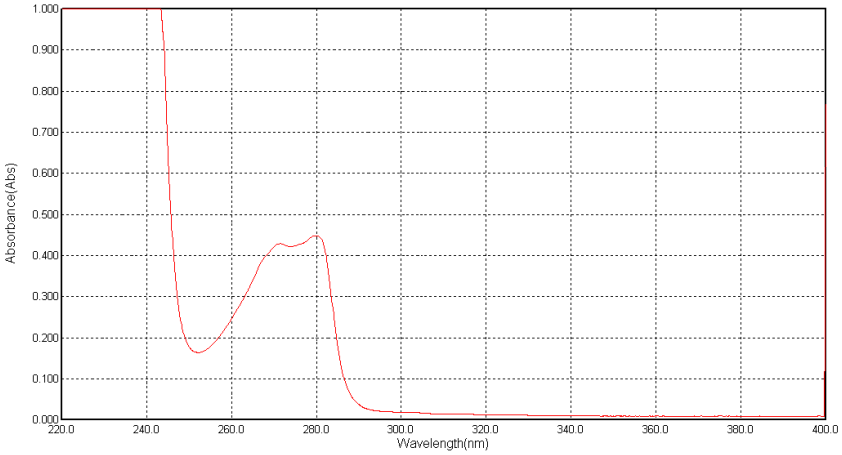


Figure.No.2 UV spectrum of Macitentan

Discussion: The λ-max of Macitentan of 100% solution i.e 4ppm (µg/ml) by using Single Beam Spectrophotometer (YIS-294) was found to be at 280 nm by using 0.1N HCL buffer.

Calibration curve of pure Drug:

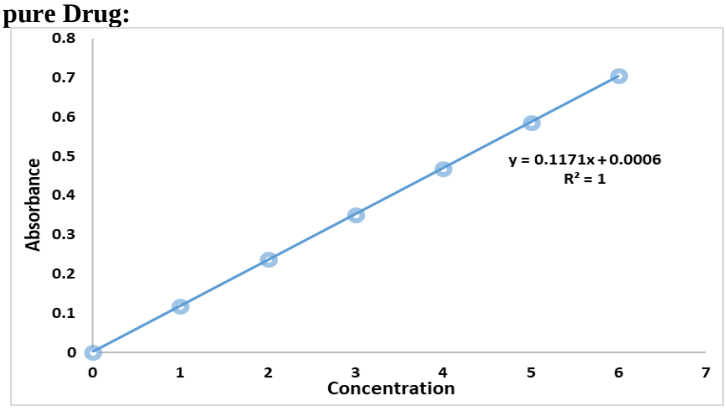


Figure.No .3 Standard calibrationcurve of Macitentan in 0.1N HCl buffer

Discussion:

The linearity was found to be in the range of 1-6 µg/ml in 0.1N HCl buffer. Regression analysis was selected because it minimizes the deviation and correct the variance heterogeneity. The regression line was defined by its slope (m) and its intercept (C) for normal regression analysis was found as 0.1171 and 0.0006, with regression coefficient of 1 respectively. The regression value was closer to 1 indicating the method obeyed Beer-lamberts' law.

Drug excipient compatibility: Drug and excipient compatibility was confirmed by comparing spectra of FT-IR analysis of pure drug with that of various excipients used in the formulation.

Pure Drug :

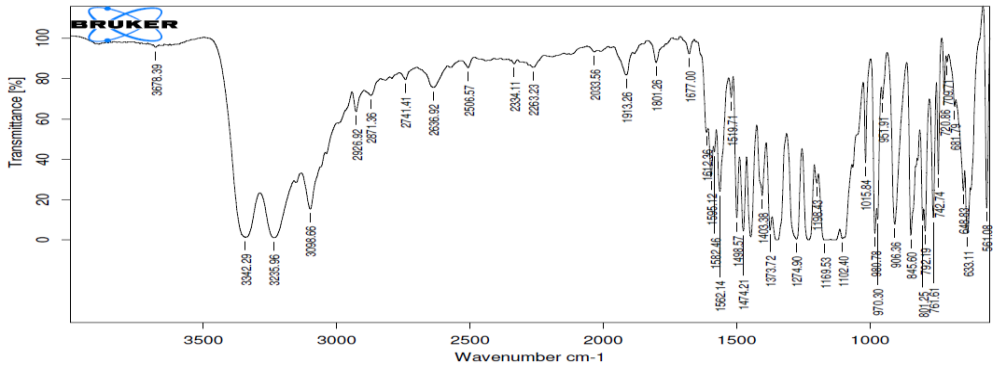


Figure.No .4 IR spectrum of Macitentan

Optimized Formulation

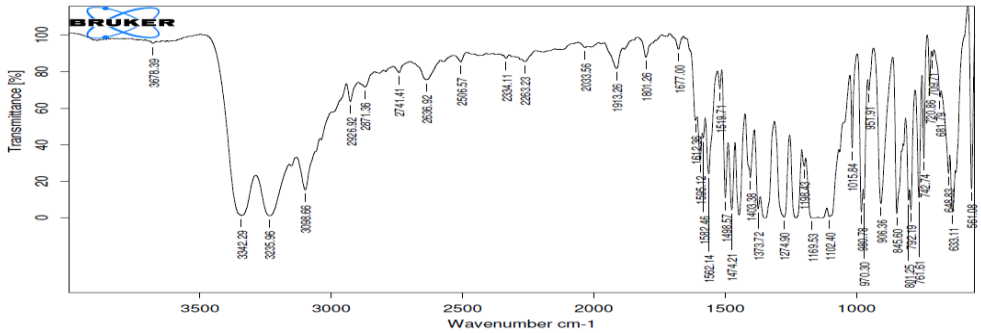


Figure.No .5 IR spectrum of Optimized formulation

Discussion: Form the drug excipient compatibility studies we observe that there are no interactions between the pure drug (Macitentan) and optimized formulation (Macitentan + excipients) which indicates there are no physical changes.

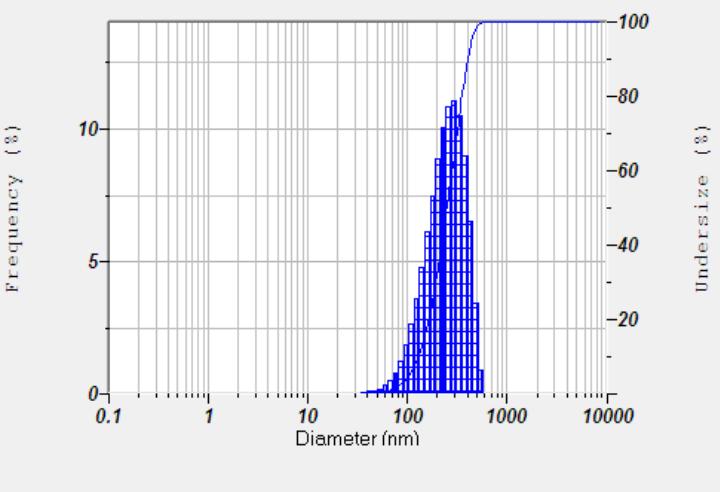
Entrapment efficacy:-The entrapment efficacy of the formulated Nanosuspension was found to be in the range of 87.84±1.24%-98.74±1.76% respectively.

Table.No.2 Entrapment efficiency of formulated Nanosuspensions

Formulation code	Mean % entrapment efficiency
NS1	59.84±1.46%
NS2	63.28±1.12%
NS3	65.61±1.07%
NS4	68.42±1.78%
NS5	62.10±1.36%
NS6	65.38±1.12%
NS7	69.40±1.54%
NS8	72.18±1.93%
NS9	65.37±1.20%
NS10	68.49±1.34%
NS11	73.32±1.10%
NS12	76.10±1.57%

Discussion: The entrapment efficiacy of formulation NS1 was found to be 59.84±1.46%, formulation NS2 was found to be 63.28±1.12%, formulation NS3 was found to be 65.61±1.07%, formulation NS4 was found to be 68.42±1.78%, formulation NS5 was found to be 62.10±1.36%, formulation NS6 was found to be 65.38±1.12%formulation NS7 was found to be 69.40±1.54%, formulation NS8 was found to be 72.18±1.93%, formulation NS9 was found to be 65.37±1.20%, formulation NS10 was found to be 68.49±1.34%, formulation NS11 was found to be 73.32±1.10%, formulation NS12 was found to be 76.10±1.57%.

Particle size analysis:



Measurement Results

Measurement Type	: Particle Size
Sample Name	:
Scattering Angle	: 90
Temperature of the Holder	: 25.0 °C
Dispersion Medium Viscosity	: 0.895 mPa·s
Transmission Intensity before Meas.	: 33
Distribution Form	: Standard
Distribution Form(Dispersity)	: Monodisperse
Representation of Result	: Scattering Light
Count Rate	: 143 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	261.5 nm	104.8 nm	296.1 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	261.5 nm	104.8 nm	296.1 nm

Figure No.6 Particle Size Analysis of Optimized Formulation

Discussion: Average particle size of nanosuspension of optimized formulations (NS12) was found to be having maximum particles at a range of 261.5 nm.

Scanning Electron Microscopy:

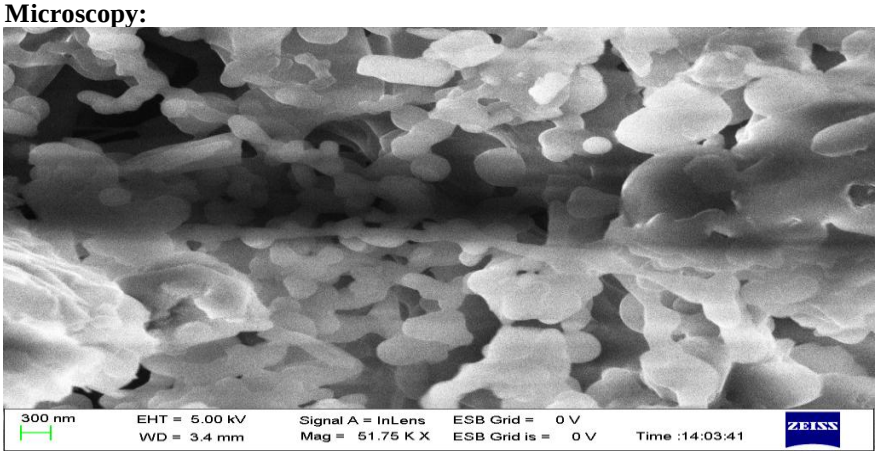


Figure No.7 Scanning Electron Microscopy of Optimized Formulation

Discussion: The surface structure of optimized formulation was observed by scanning electron microscopy at different magnifications. In this SEM, the nanosuspension particles are appeared within slightly spherical in shape and particle size was reduced up to 300 nm.

Diffusion results:

Table.3 In vitro Dissolution studies

Time												

(min)	NS1	NS2	NS3	NS4	NS5	NS6	NS7	NS8	NS9	NS10	NS11	NS12
0	0	0	0	0	0	0	0	0	0	0	0	0
5	21.17 ±1.17	37.49 ±1.71	44.12 ±1.75	49.07 ±1.26	24.65 ±1.45	28.53 ±1.74	40.15 ±1.25	49.64 ±1.29	37.07 ±1.54	40.42 ±1.02	46.75 ±1.34	59.12 ±1.46
10	44.16 ±1.75	46.18 ±1.62	58.48 ±1.45	57.94 ±1.46	35.63 ±1.51	39.18 ±1.62	55.42 ±1.34	66.53 ±1.46	48.94 ±1.61	58.42 ±1.39	63.20 ±1.14	65.32 ±1.19
15	57.75 ±1.23	55.49 ±1.48	67.49 ±1.22	70.19 ±1.27	50.86 ±1.36	48.86 ±1.54	67.24 ±1.51	78.61 ±1.24	59.19 ±1.75	66.12 ±1.52	71.45 ±1.34	75.57 ±1.20
20	64.94 ±1.08	69.89 ±1.36	79.52 ±1.20	79.65 ±1.65	67.16 ±1.74	63.63 ±1.20	79.29 ±1.10	90.49 ±1.74	71.65 ±1.45	80.34 ±1.25	78.25 ±1.15	87.46 ±1.15
30	76.85 ±1.75	75.49 ±1.46	85.34 ±1.96	89.45 ±1.74	76.08 ±1.52	79.35 ±1.61	87.25 ±1.37	98.57 ±1.02	80.45 ±1.69	87.42 ±1.95	89.42 ±1.34	99.12 ±1.30
45	85.18 ±1.69	89.04 ±1.09	98.27 ±1.12	98.76 ±1.52	87.65 ±1.52	89.58 ±1.45	98.45 ±1.12		88.36 ±1.54	98.45 ±1.10	98.14 ±1.02	
60	98.29 ±1.24	98.68 ±1.26			98.48 ±1.54	98.81 ±1.12			98.85 ±1.10			

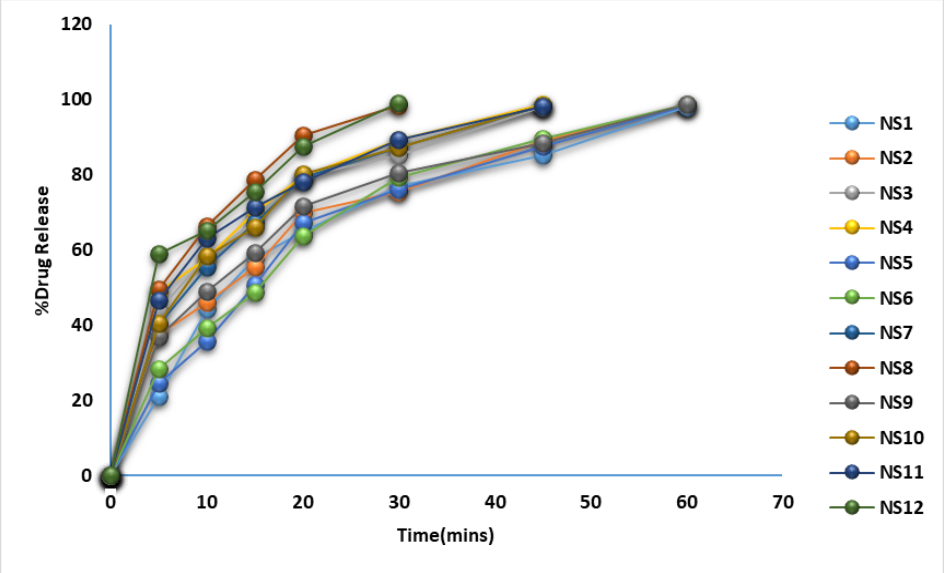


Figure. No.8 Diffusion parameters for the formulations NS1-NS12

Discussion: From the above invitro studies we can say that increase in the polymer concentration of polymers decrease in the Diffusion time of all the formulations.

From the above invitro studies we can say that at low polymer concentrations the drugs release time was increased. So NS12 is considered as optimized formulation as it shows drugs release with in 30mins.

Among all the four stabilizers we have used NS12 containing Pluronic® F-127 releases maximum drugs release at the end of 30 mins.

Increase in the stabilizer concentration of Pluronic® F-127 shows 99.12±1.30% of drugs release, so the formulations prepared by using Pluronic® F-127 releases more drugs release at the end of 30mins than the other stabilizers.

Drugs release kinetics studies: Best formulation NS12

ZERO ORDER RELEASE KINETICS:

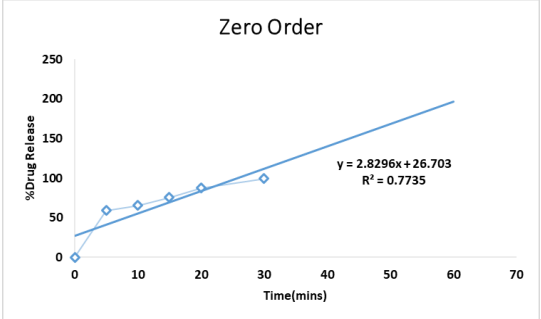


Figure.No.9 Zero order release profile of formulation NS12

FIRST ORDER RELEASE KINETICS:

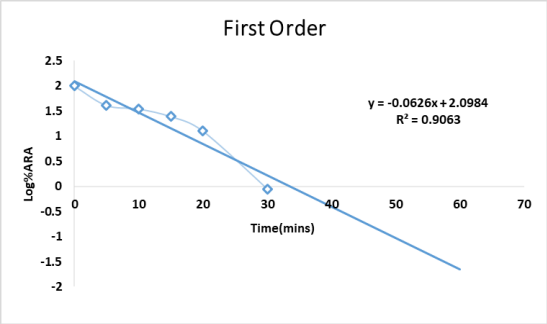


Figure.No .10 First order release profile of formulation NS12

Table.No.3 Kinetic data of the formulation NS12

ORDER OF KINETICS	ZERO ORDER	FIRST ORDER
REGRESSION	0.773	0.906

Discussion:

The drugs release from the Nanosuspension was explained by using mathematical model equations such as zero order, first order, and equation methods. Based on the regression values it was concluded that the optimized formulation NS12 follows first order kinetics.

CONCLUSION:

In present investigation Nanosuspensions of was prepared by Emulsification solvent evaporation method. The Nano suspensions are novel promising target and controlled released dosage form which is gaining importance because of ease of manufacturing and diversified applications. The present trend of pharmaceutical research lies in the usage of biodegradable polymer because of its availability and low toxicity. Nanosuspension containing drug was prepared by emulsification solvent evaporation method by using combinations of Poloxamer-188, PVP K30, Pluronic® F-127 , SLS, Ethanol and quantity sufficient water). Estimation of Macitentan was carried out spectrophotometrically at 301nm.The Nanosuspension were evaluated for parameters such as drug content uniformity, scanning electron microscopy, particle size analysis, in-vitro release, drug excipient interactions (FTIR). The stability data was also subjected to statistical analysis. The melting point of Macitentan was found to be in range of 75°C which was determined by capillary method. Saturation solubility was carried out at 250C using 0.1N HCl, 6.8 phosphate buffer, 7.4 pH buffer, methanol & ethanol. From the drug excipient compatibility studies we observe that there are no interactions between the pure drug (Macitentan) and optimized Formulation (Macitentan+ excipients) which indicates there are no physical changes. The entrapment efficacy of Formulation NS1 to NS12 was found in between 59.84±1.46%-76.10±1.57% Average particle size of nanosuspension of optimized Formulations (NS12) was Found to be 261.5 nm. From the in vitro Diffusion studies we can say that Formulation NS12 shows best drug release of 99.12±1.30% within 30 minutes whereas all the other Formulations didn't release the drug. The drug release from the Nanosuspension was explained by the using mathematical model equations such as zero order, first order, and equation methods. Based on the regression values it was concluded that the optimized Formulation NS12 follows first order kinetics.

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