EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 7
Issue: 7
514-525
Year: 2020

FORMULATION DEVELOPMENT FOR SOLUBILITY ENHANCEMENT AND IN VITRO EVALUATION OF TERIFLUNOMIDE NANOSUSPENSIONS

P. Mounika*, T. Malyadri, P. Sreenivasa Prasanna and K. Thejomoorthy

Department of Pharmaceutics, M.L. College of Pharmacy, S. Konda-523101.

***Corresponding Author: P. Mounika**
Department of Pharmaceutics, M.L. College of Pharmacy, S. Konda-523101.

Article Received on 03/05/2020 **Article Revised on 23/05/2020** **Article Accepted on 13/06/2020**

ABSTRACT
Teriflunomide is used to treat multiple sclerosis-MS. It is not a cure for MS but is thought to work by decreasing certain immune system cells (lymphocytes) which can attack the nerves in your brain and spinal cord. This helps decrease the number of flare-ups (relapses) and may help slow down physical problems caused by MS. Teriflunomide is a BCS class II drug having low solubility and high permeability, so to increase its solubility Teriflunomide is formulated as a Nanosuspension. Nanosuspension is a novel technique used to increase the solubility of the drug prepared by Solvent evaporation method. 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 solvent evaporation method by using SLS, Polaxomer, PVP K30, PVA and acetone as organic solvent. The Nanosuspension were evaluated for parameters such as Entrapment efficiency, scanning electron microscopy, particle size analysis, zeta potential, in-vitro release, drug excipient interactions (FTIR). The entrapment efficacy of the formulated Nanosuspension was found to be in the range of 79.52%- 92.16% respectively. Zeta potential value for the optimized formulation (F3) was found to be within the acceptable limits. Average particle size of nanosuspension of optimized formulations (F3) was found to be 489.7nm. From the invitro studies we can say that formulation F3 shows best drug release of 99 % within 45 minutes where as all the other formulations takes about 60-180 minutes to release the drug. Based on the regression values it was concluded that the optimized formulation F3 follows Zero order kinetics.

KEYWORDS: Teriflunomide, Nanosuspension, SLS, Polaxomer, PVP K30, PVA.

INTRODUCTION
Nanotechnology opens up new vistas of research in the development of novel drug delivery systems. "Nano" word comes from the Greek word 'nanos" which means dwarf.[9] Nano means it is the factor of 10^{-9} or one billionth. Nanosuspension is submicron colloidal dispersion of drug particles. A pharmaceutical nanosuspension is defined as very finely colloid, biphasic, dispersed solid drug particles in an aqueous vehicle, size below 1 µm stabilized by surfactants and polymers prepared by suitable methods for drug delivery applications. Nanosuspension has revealed their potential to solve the problem associated with the delivery of poorly water soluble and poorly water and lipid soluble drugs. It enhances the absorption and bioavailability and help to reduce the dose of conventional oral dosage forms.[10]

For a long duration of time micronization of poorly soluble drugs by colloid mills or jet mills was preferred. The overall particle size distribution ranges from 0.1µm to approximately 25µm, only negligible amount being below 1µm in the nanometer range.

Depending upon the production technique applied changes in the crystalline structure of the drug particle may occur. An increase amount of amorphous drug fraction could induce higher saturation solubility. Nanosuspension not only solves the problem of poor solubility and poor bioavailability but also alters the pharmacokinetics of the drug and improves the drug safety and efficacy.

Teriflunomide is an enamide obtained by formal condensation of the carboxy group of (2Z)-2-cyano-3-hydroxybut-2-enoic acid with the anilino group of 4-(trifluoromethyl)aniline. Used for the treatment of relapsing forms of multiple sclerosis and rheumatoid arthritis. It has a role as an EC 1.3.98.1 [dihydroorotate oxidase (fumarate)] inhibitor, a tyrosine kinase inhibitor, a hepatotoxic agent, a drug metabolite and a non-steroidal anti-inflammatory drug. It is a nitrile, an enol, an aromatic amide, an enamide, a member of (trifluoromethyl)benzenes and a secondary carboxamide.

The chemical name of teriflunomide is (Z)-2-Cyano-3-hydroxy-but-2-enoic acid-(4- trifluoromethylphenyl) amide with molecular formula C12H9F3N2O2 and relative molecular mass 270.2 g/mol. Teriflunomide appears as a white to almost white, odourless, non-hygroscopic powder. It is a biopharmaceutical classification system (BCS) Class 2 compound, which is practically insoluble in water; sparingly soluble in acetone; and slightly soluble in ethanol, acetonitrile and methylene chloride.

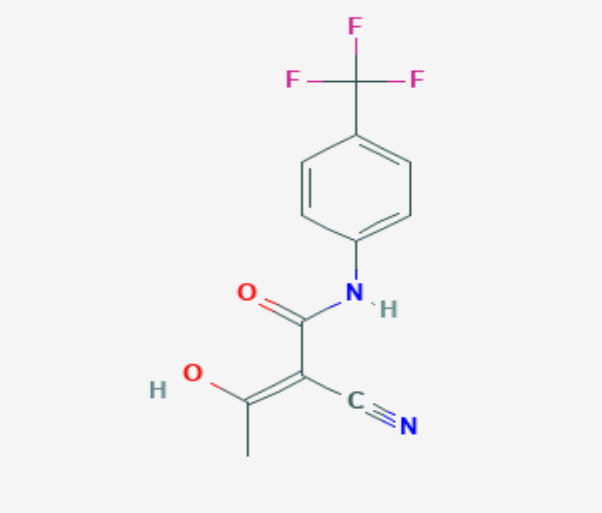

Figure 1: Chemical structure of the teriflunomide.

EXPERIMENTAL WORK

MATERIALS AND METHODS

Teriflunomide sample was collected form Biocon, Bangalore, Sodium lauryl sulphate Polaxomer 407, PVA, PVP K30, Acetone reagent LR grade Rankem, Mumbai, Dissolution test apparatus from LAB INDIA DS 8000, UV- Visible spectrophotometer from T60 UV Spectrophotometer, Particle size analyzer from Malvern zetasizer ZS, Hot air oven from MC Dalal and Co., Chennai, Digital balance from Shimadzu, (BL-220H), IR spectroscopy from 1615 series.

Pre-formulation studies

Prior to the development of dosage form, it is essential that certain fundamental physical and chemical properties of the drug molecule alone and when combined with excipients are determined. This first learning phase is known as pre-formulation. The overall objective of the pre-formulation is to generate information useful to the formulator in developing stable and bioavailable dosage forms which can be mass produced. The goals of pre-formulation studies are:

- To evaluate the drug substance analytically and determine its necessary characteristics
- To establish its compatibility with different excipients.

Spectroscopic study[11-16]

Identification of pure drug

Melting Point

The temperature at which the first particle of the substance completely melts is regarded as melting point of the substance. The temperature at which the first particle starts to melt and last particle completely melts is regarded as the range of melting point.

Solubility studies

Solubility of Teriflunomide was carried out in different solvents –like water, Acetone, ethanol, and Acetone. Saturated solutions were prepared by adding excess drug to the vehicles and shaking on the shaker for 48 hr. at 25°C under constant vibration. Filtered samples (1ml) were determined spectrophotometrically at 282nm.

Drug-Excipient Interactions Studies: There is always possibility of drug- excipient interaction in any formulation due to their intimate contact. The technique employed in this study is IR spectroscopy.

IR spectroscopy is one of the most powerful analytical technique, which offers possibility of chemical identification. The IR spectra was obtained by KBr pellet method. (Perkin-Elmer series 1615 FTIR Spectrometer).

PREPARATION OF CALIBRATION CURVE OF TERIFLUNOMIDE

Procedure for standard curve in pH 6.8

10 mg of Teriflunomide was dissolved in 10 ml of pH 6.8 by slight shaking (1000 µg/ml). 1 ml of this solution was taken and made up to 10 ml with pH 6.8, which gives 100 µg/ml concentration (stock solution). From the stock solution, concentrations of 5, 10, 15, 20, 25 & 30µg/ml in pH 6.8 were prepared. The absorbance of diluted solutions was measured at 282nm and a standard plot was drawn using the data obtained.

Method of Preparation of Nanosuspension[13]

Solvent evaporation method

Nanosuspension was prepared by the solvent evaporation technique. Teriflunomide was dissolved in Acetone at room temperature (organic phase). This was poured into water containing different stabilizers of PVP K30, PVA, and poloxamer having SLS as surfactant 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 Nanosuspension at room temperature for 1 hour followed by sonication for 1 hour.

Table 1: Formulation table.

Ingredient(mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Teriflunomide	70	70	70	70	70	70	70	70	70
PVA(%)	0.5	1	1.5	--	--	--	--	--	--
PVP K30(%)	--	--	--	0.5	1	1.5	--	--	--
Polaxomer 407(%)	--	--	--	--	--	--	0.5	1	1.5
SLS (%)	1	1	1	1	1	1	1	1	1
Acetone (ml)	5	5	5	5	5	5	5	5	5
Water (ml)	50	50	50	50	50	50	50	50	50

Evaluation parameters of Nanosuspension Teriflunomide[14-26]

The Nanosuspension was evaluated for various parameters:-

1. Entrapment efficiency
2. Scanning electron microscopy
3. Particles size analysis
4. Zeta potential
5. In-vitro drug release studies

Entrapment efficacy: The freshly prepared 5ml of nanosuspension was centrifuged at 20,000 rpm for 20 min at 5°C temperature using cool ultracentrifuge. The amount of un incorporated drug was measured by taking the absorbance of the appropriately diluted 5 ml of supernatant solution at 282 nm using UV spectrophotometer against blank/control nanosuspensions. DEE was calculated by subtracting the amount of free drug in the supernatant from the initial amount of drug taken.

The entrapment efficiency (EE %) could be achieved by the following equation

%Entrapment efficiency= Drug content *100/Drug added in each formulation

Scanning electron microscopy: The morphological features of Teriflunomide nanosuspension are observed by scanning electron microscopy at different magnifications.

Particle size and shape

Average particle size and shape of the formulated nanosuspensions was determined by using Malvern Zetasizer ZS using water as dispersions medium. The sample was scanned 100 times for determination of particle size.

Zeta potential

There are three ways by which a solid particle (colloid) dispersed in a liquid media can acquire a surface charge. First, by the adsorption of ions present in the solution. Second, by the ionization of functional groups on the particle's surface. Third, due to the difference in dielectric constant between the particle and the medium. Attention should be paid to the formation of electric double layer at the solid-liquid interface. The zeta Potential is defined as the difference in potential between the surface of the tightly bound layer (shear plane) and the electro-neutral region of the solution. The potential gradually decreases as the distance from the surface increases.

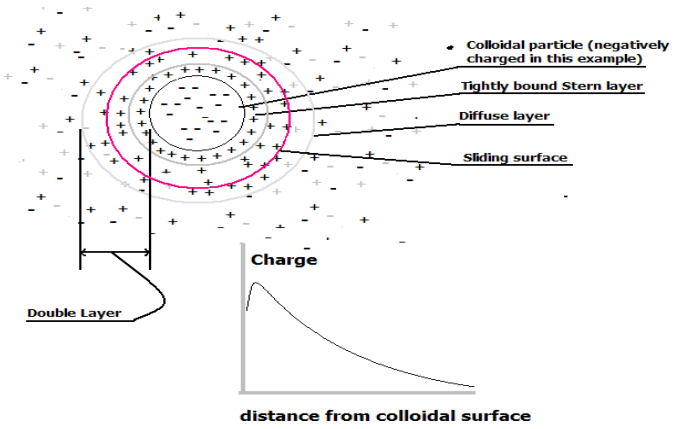

Figure 2: Schematic of the formation of electric double layer.

As the concentration of electrolyte increases in the medium, the zeta potential falls off rapidly due to the screening effect of the counter ions (Figure). The zeta potential cannot be measured directly; however, it can be calculated using theoretical models and from experimentally determined electrophoretic mobility data. The theory is based on electrophoresis and can be expressed as:

$$\mu = \zeta\varepsilon/\eta$$

Where (μ) is the electrophoretic mobility, (ε) is the electric permittivity of the liquid, (η)
Is the viscosity and (ζ) us the zeta potential.

Table 2: Zeta potential for colloids in water and their stability.

Zeta Potential [mV]	Stability behaviour of the colloid
0 to ±5	Rapid coagulationor flocculation
from ±10 to ±30	Incipient instability
from ±30 to ±40	Moderate stability
from ±40 to ±60	Good stability
more than ±61	Excellent stability

***In vitro* drug release study**[24]

In vitro dissolution study was performed by USP dissolution apparatus-type II using 900 ml of 6.8pH buffer as a dissolution medium maintained at 37 ± 0.5°C and stirring speed (100 RPM). The freshly prepared 5ml of nanosuspensions of drug: stabilizer ratios were added to the dissolution medium, five-milliliter samples were withdrawn at specific intervals of time, then filtered through a 0.45 µm filter paper and analyzed for their drug concentrations by measuring at 292 nm wavelength.

The results of in vitro release profiles obtained for the NDDS formulations were fitted into

Four models of data treatment as follows:

1. Cumulative percent drug released versus time (zero order kinetic model).
2. Log cumulative percent drug remaining versus time (first- order kinetic model).

Table 3: Drug release kinetics.

Kinetic Model	Relation	Systems Following the Model
First order	$\ln Q_t = \ln Q_o + K_t$ release is proportional to amount of drug remaining	Water-soluble drugs in porous matrix
Zero order	$f_t = K_o t$ (independent of drug concentration)	Transdermal systems Osmotic systems

Where, ft = fraction of dose released at time‘t’
KH, Ko, and Ks = release rate constants characteristic to respective models
Qo = the drug amount remaining to be released at zero hour
Qt = the drug amount remaining to be released at time‘t’
Wo = initial amount of drug present in the matrix
Wt = amount of drug released at time‘t’

1. Zero Order Kinetics: A zero-order release would be predicted by the following equation.

$$A_t = A_0 - K_0 t$$

Where:
A_t = Drug release at time ‘t’
A_0 = Initial drug concentration.
K_0 = Zero-order rate constant (hr^{-1}).
When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys zero-order release kinetics, with a slope equal to K_0.

2. First Order Kinetics: A first-order release would be predicted by the following equation

$$\text{Log } C = \text{Log } C_0 - \frac{Kt}{2.303}$$

Where:
C = Amount of drug remained at time‘t’
C_0 = Initial amount of drug
K = First-order rate constant (hr^{-1}).

When the data is plotted as log cumulative percent drug remaining versus time yields a straight line, indicating that the release follows First-order kinetics. The constant ‘K’ can be obtained by multiplying 2.303 with slope values.

Mechanism of Drug Release: To find out the drug release mechanism due to swelling (upon hydration) along with gradual erosion of the matrix, first 60% drug release data can be fitted in Krosmeyers–Peppas model which is often used to describe the drug release behaviour from polymeric systems when the mechanism is not well-known or when more than one type of release phenomena is involved.

$$\text{Log } (M_t / M_\infty) = \text{Log } K_{KP} + n \text{ Log } t$$

Where,
M_t = is the amount of drug release at time t,
M_∞ = is the amount of drug release after infinite time,
K_{KP} = is a release rate constant incorporating structural and geometrical characteristics of Tablet
n = is the release exponent indicative of the mechanism of drug release.

RESULTS AND DISCUSSION

Determination of melting point

The melting point of Teriflunomide was found to be in range of 230°C which was determined by capillary method and complies with IP standards.

Solubility studies

Saturation solubility was carried out at 25^0C using 0.1N HCL, 6.8 phosphate buffer, and other solvents.

The solubility studies in various buffers we can say that pH 6.8 phosphate buffer has more solubility when compared to other buffer solutions. So pH 6.8 buffer is used as dissolution medium, based upon the solubility studies on organic solvents Acetone has more solubility than others so Acetone was used in the nanosuspension formulation.

Table 4: Solubility data.

Media	Solubility(mg/ml)
0.1N HCL	0.361
Ethanol	0.705
Methanol	0.756
Acetone	0.956
0.1N HCl	0.236
pH 4.5 acetate buffer	0.365
pH 6.8 phosphate buffer	0.568

Determination of absorption maximum (λmax)

Determination of Teriflunomide λ-max was done in pH 6.8 buffer medium for accurate quantitative assessment of drug dissolution rate.

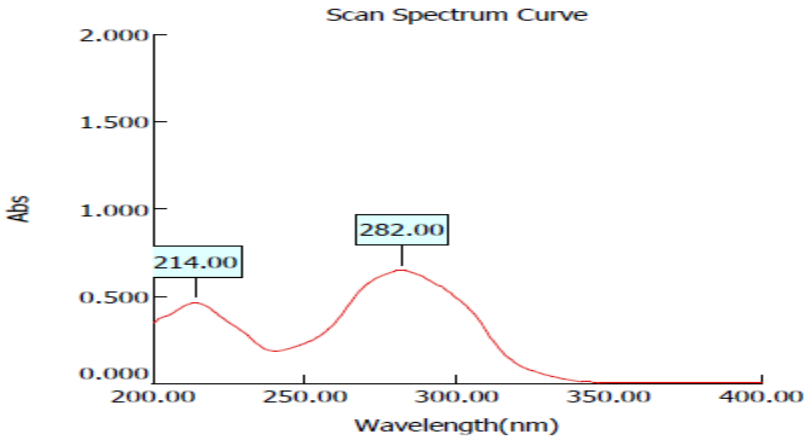

Figure 3: Uv Spectrum of Teriflunomide.

Linearity

The linearity was found to be in the range of 2-12 µg/ml in acetone, pH 6.8 buffer. The regression value was closer to 1 indicating the method obeyed Beer-lamberts' law.

Table 5: Standard graph of Teriflunomide in pH 6.8 (λmax 282nm).

Concentration (µg/ml)	Absorbance
0	0
2	0.128
4	0.254
6	0.410
8	0.552
10	0.671
12	0.812

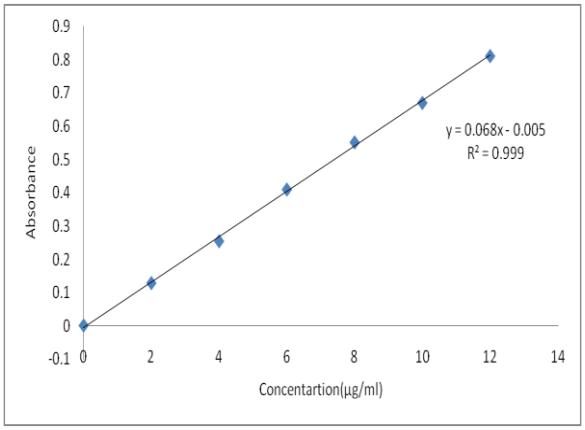

Figure 4: Standard calibration curve of Teriflunomide in pH 6.8.

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. Form the drug excipient compatibility studies we observe that there are no interactions between the pure drug

(Teriflunomide) and optimized formulation (Teriflunomide+ excipients) which indicates there are no physical changes.

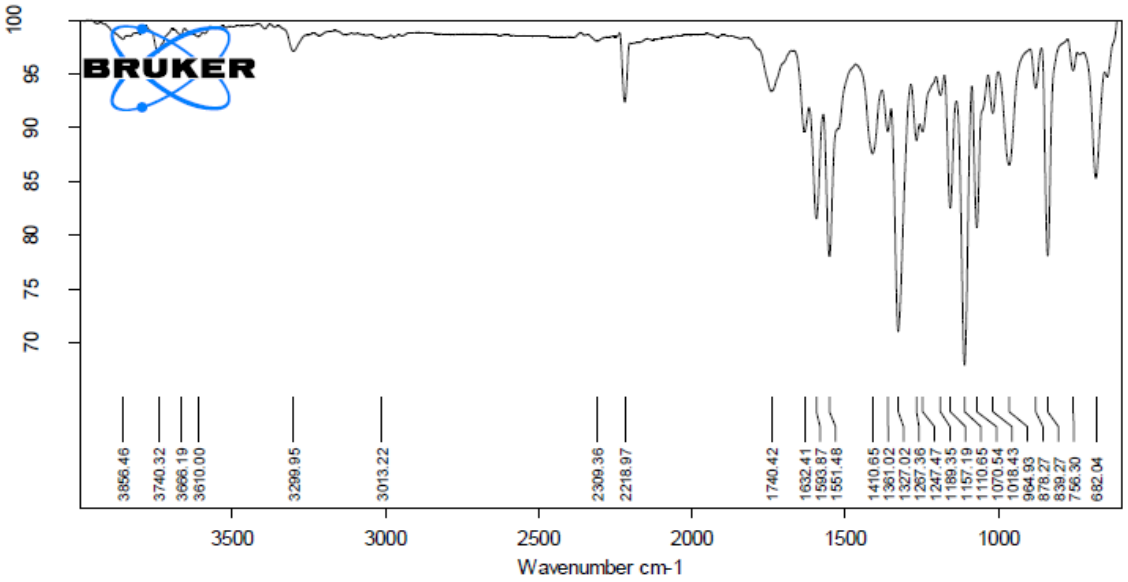

Figure 5: IR spectrum of Teriflunomide.

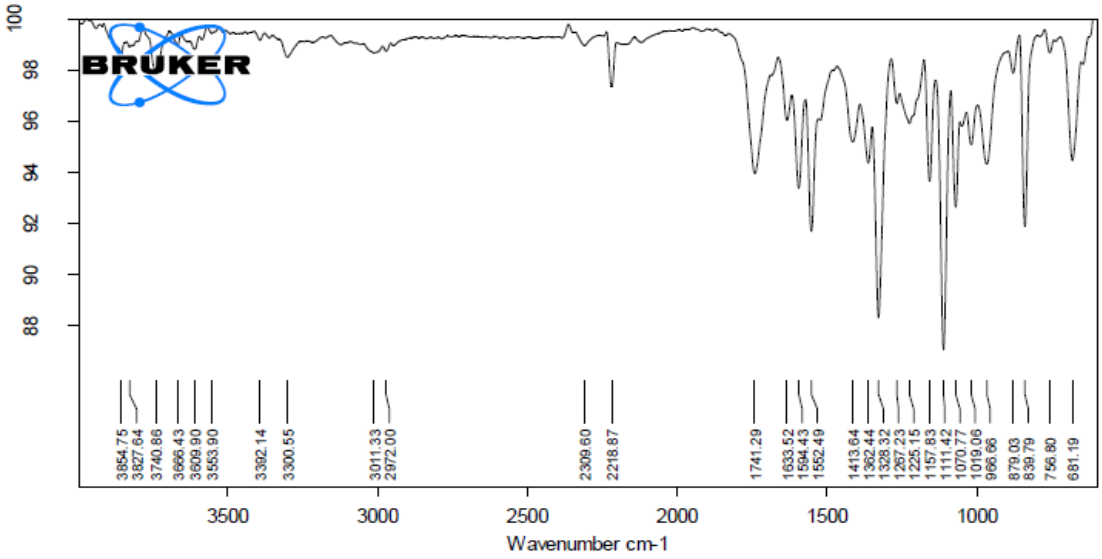

Figure 6: IR spectrum of Teriflunomide Optimised Formulation.

Table 6: Entrapment efficiency of formulated Nanosuspensions.

The entrapment efficacy of formulation F1-F9 was found to be 79.52%- 92.16%.

Formulation code	Mean % entrapment efficiency
F1	79.52±0.52
F2	86.16±0.26
F3	92.16±0.03
F4	80.16±0.20
F5	87.20±0.41
F6	90.06±0.85
F7	80.42±0.42
F8	83.36±0.51
F9	87.02±0.26

SCANNING ELECTRON MICROSCOPY

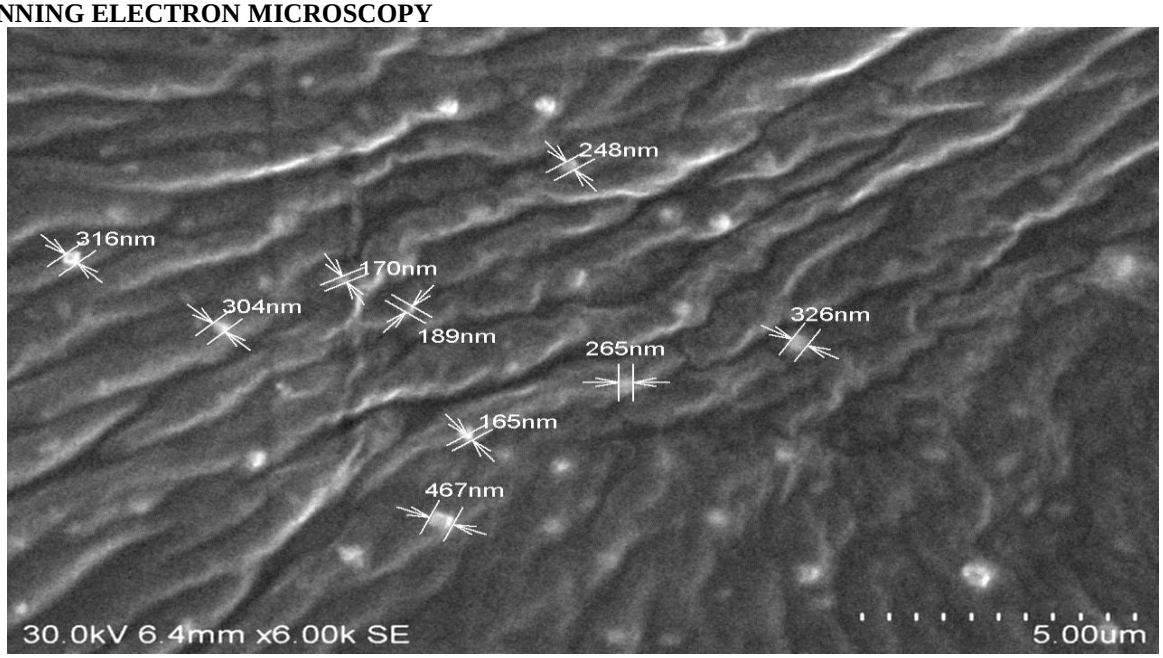

Figure 7: SEM image of Teriflunomide Nanosuspension.

Zeta Potential: The measurement itself is a particle electrophoresis, the particle velocity is determined via the doppler shift of the laser light scattered by the moving particles. The field strength applied was 20 V/cm. The electrophoretic mobility was converted to the zeta potential in mV using the Helmholtz-Smoluchowski equation. At standard measuring conditions (room temperature of 25°C, water) this equation can be simplified to the multiplication of the measured electrophoretic mobility (µm/cm per V/cm) by a factor of 12.8, yielding the ZP in mV.

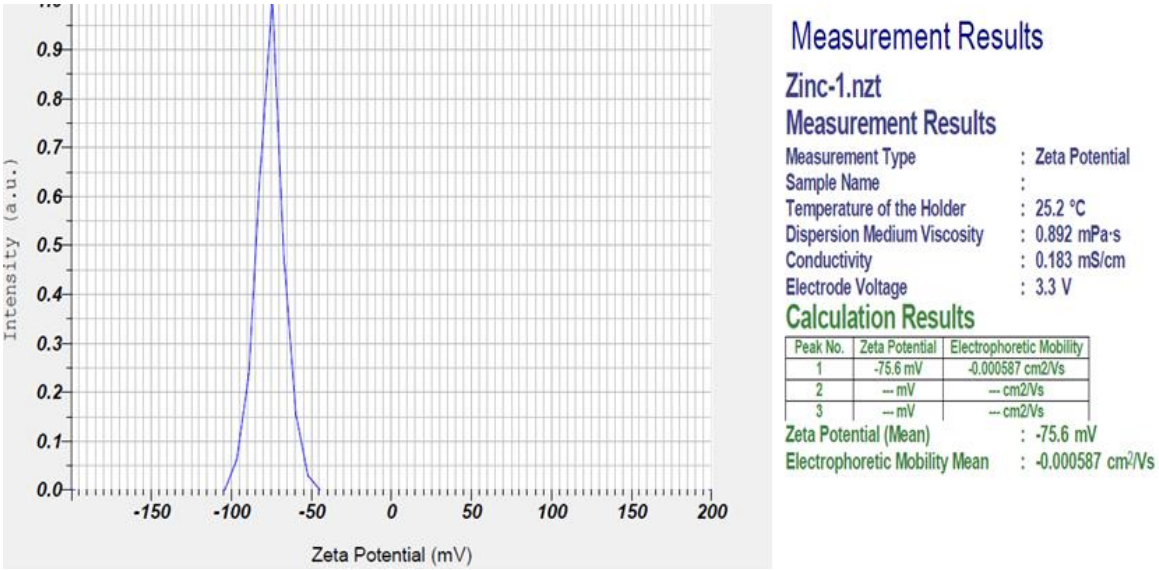

Figure 8: Zeta potential curve for formulation F3.

Particle size analysis: Average particle size of nanosuspension of optimized formulations (F3) was found to be having maximum particles at a range of 489.7 nm.

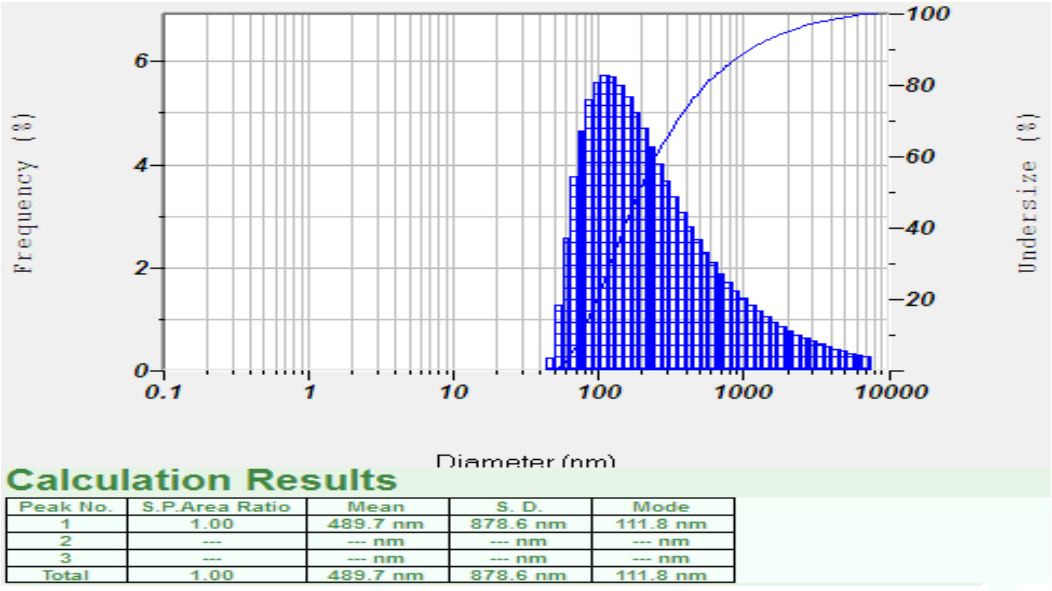

Figure 9: Particle size analysis curve for formulation F3.

In vitro Dissolution studies

Table 7: *In-vitro* drug release data of formulation F1 to F9.

Time (Min)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
5	11.02	17.02	34.05	9.32	26.45	35.41	23.02	30.05	39.74
10	18.04	26.45	47.61	15.06	34.85	40.85	37.74	42.74	51.85
20	24.42	34.85	61.41	29.02	49.96	52.89	46.85	59.85	64.62
30	30.86	49.96	79.56	42.95	56.06	65.06	58.62	73.62	76.16
45	44.85	56.06	99.31	58.86	65.74	76.31	69.02	85.05	88.96
60	58.96	65.74		66.87	74.56	87.48	77.31	91.96	98.03
90	69.36	74.56		80.85	88.23	98.95	85.15	99.31	
120	85.14	98.23		89.61	98.74		97.62		
160	98.52			92.23					
180				99.02					

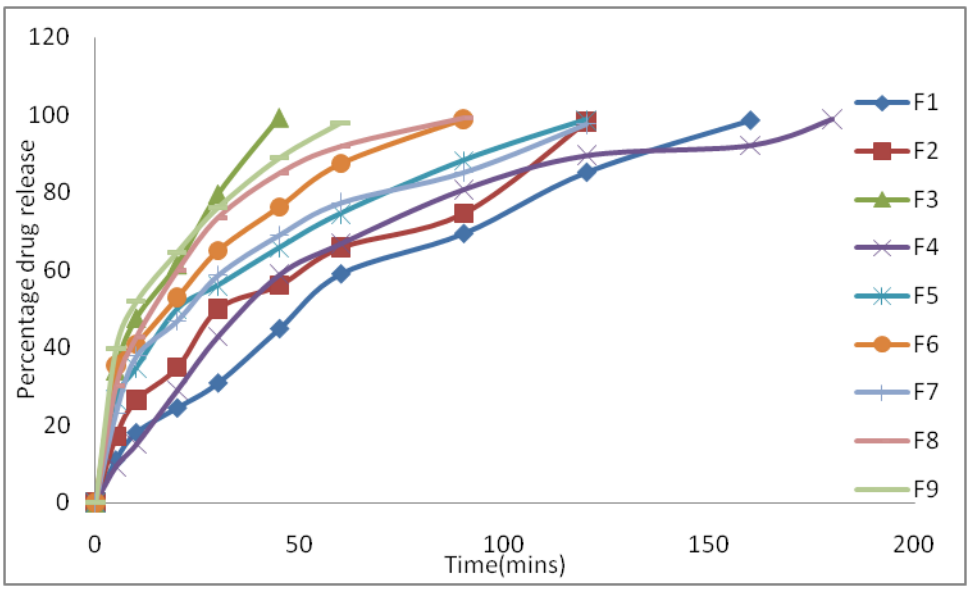

Figure 10: Dissolution parameters for the formulations F1-F9.

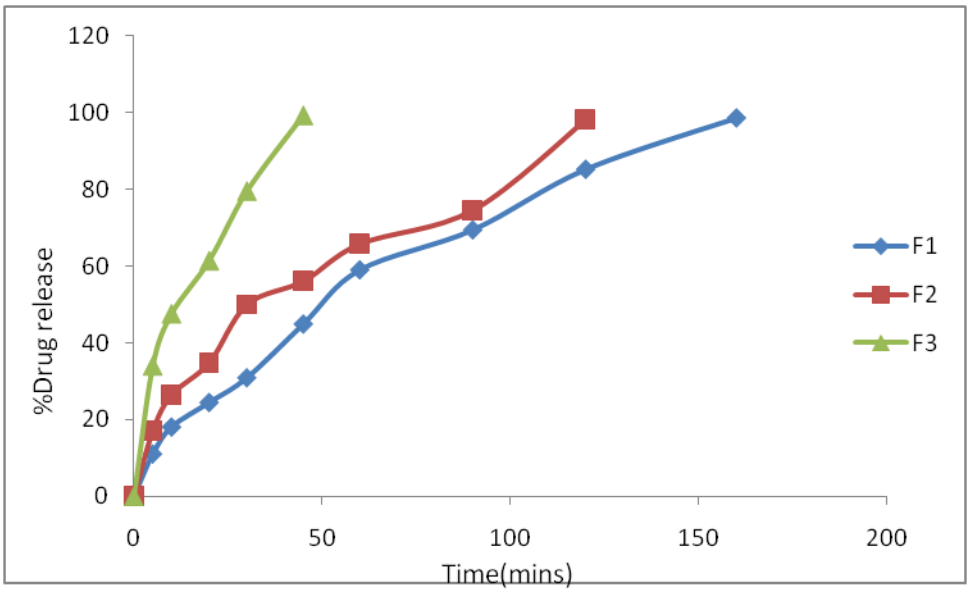

Figure 11: Dissolution parameters for the formulations F1-F3.

From the above invitro drug release studies of formulations F1-F3 containing PVA in different concentrations i.e, from 0.5-1.5% along with 1% surfactant concentration shows that F3 shows 99% drug release in 45 minutes when compared to other formulations.

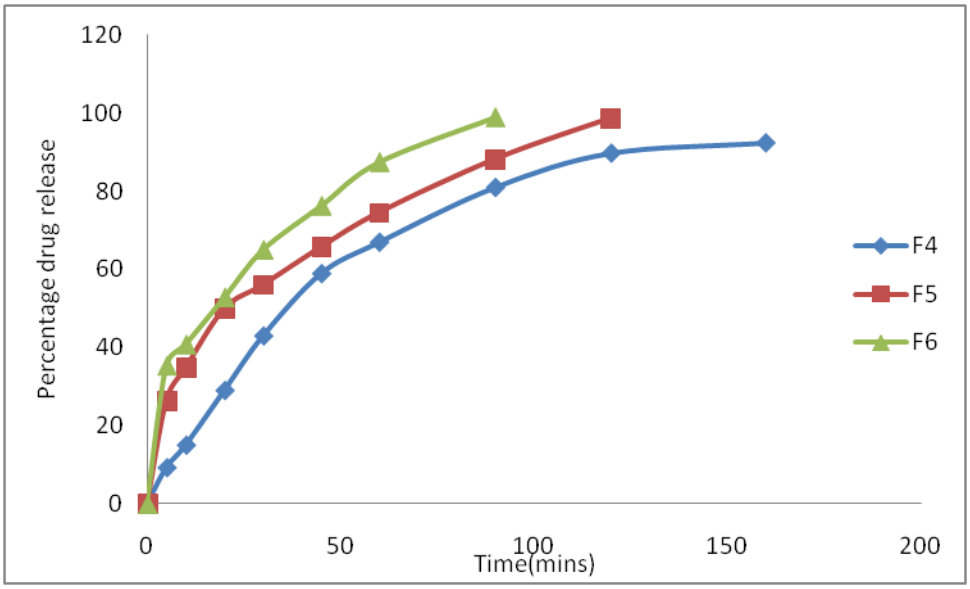

Figure 12: Dissolution parameters for the formulations F4-F6.

From the above invitro drug release studies of formulations F1-F3 containing PVP K30 in different concentrations i.e, from 0.5-1.5% along with 1% surfactant concentration shows that F6 shows 98% drug release in 90 minutes when compared to other formulations.

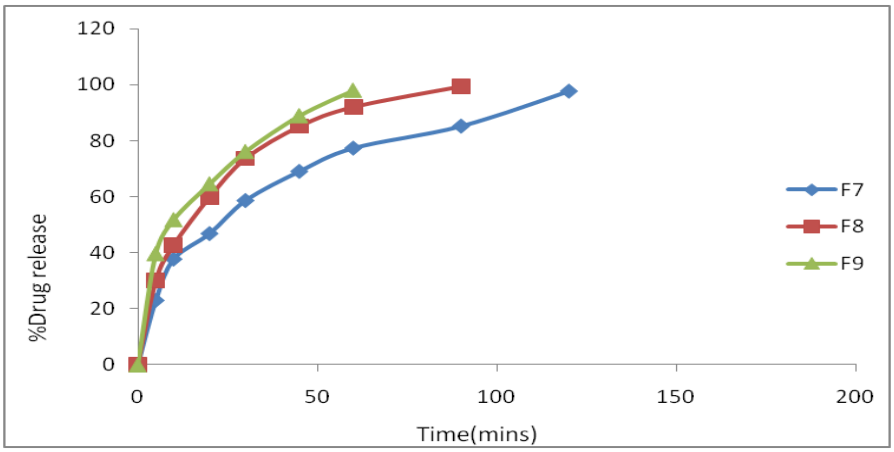

Figure 13: Dissolution parameters for the formulations F7-F9.

From the above invitro drug release studies of formulations F1-F3 containing Polaxomer 407 in different concentrations i.e, from 0.5-1.5% along with 1% surfactant concentration shows that F9 shows 98% drug release in 60 minutes when compared to other formulations.

From the above in vitro studies we can say that at F3 formulation containing PVA at 1.5% concentration and 1% SLS as surfactant shows immediate release whwn compared to polaxomer and PVP K30 so F3 formulation was considered as the best formulation and drug release kinetics were performed for F3 formulation.

ZERO ORDER RELEASE KINETICS

Drug release kinetics studies: Best formulation F9

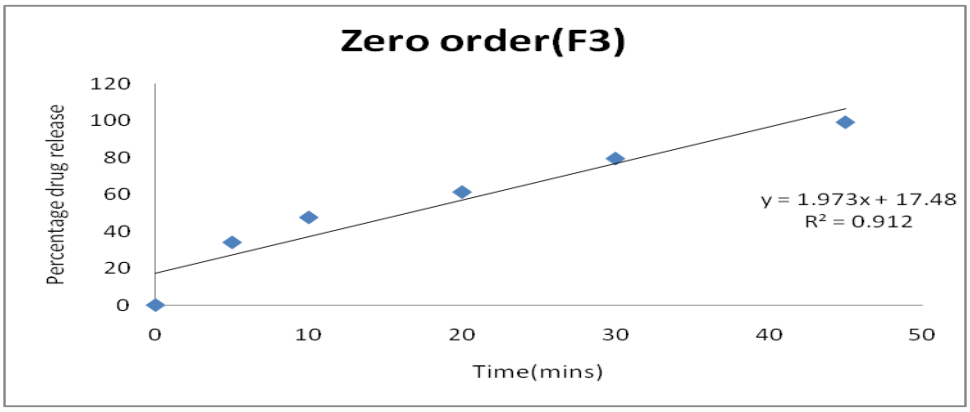

Figure 14: Zero order release profile of formulation F3.

FIRST ORDER RELEASE KINETICS

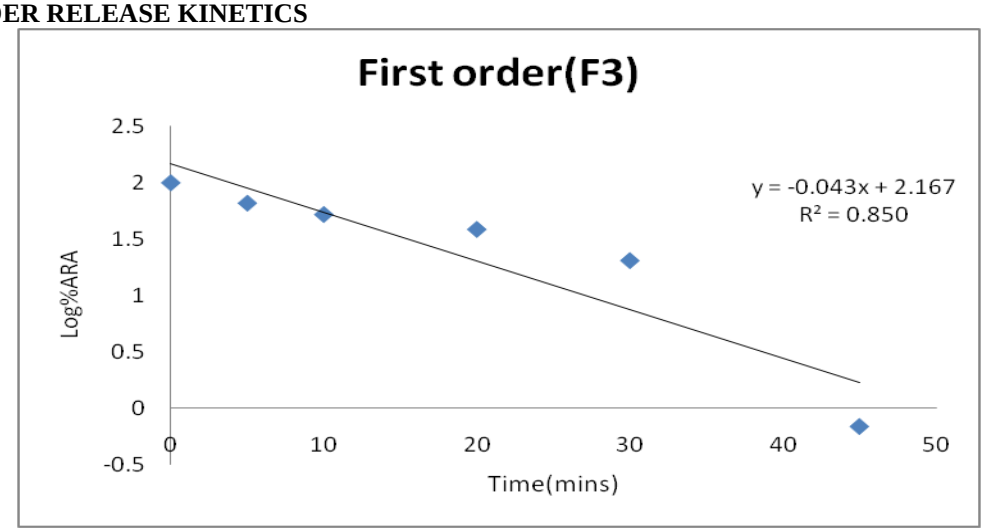

Figure 15: First order release profile of formulation F3.

Table 8: Kinetic data of the formulation F3.

ORDE OF KINETICS	ZERO ORDER	FIRST ORDER
REGRESSION	0.912	0.850

DISCUSSION

The in vitro dissolution data of all the designed formulations are shown and dissolution profiles depicted in figures. In vitro drug release data of all the Nanosuspension formulations of Teriflunomide was subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetics and according to equations of drug release. The results of linear regression analysis including regression coefficients from the above data it is evident that the optimized formulation (F3) follows zero-order release kinetics.

SUMMARY

Teriflunomide is used to treat multiple sclerosis-MS. It is not a cure for MS but is thought to work by decreasing certain immune system cells (lymphocytes) which can attack the nerves in your brain and spinal cord. This helps decrease the number of flare-ups (relapses) and may help slow down physical problems caused by MS. Teriflunomide is a BCS class II drug having low solubility and high permeability, so to increase its solubility Teriflunomide is formulated as a Nanosuspension. Nanosuspension is a novel technique used to increase the solubility of the drug prepared by 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 solvent evaporation method by using SLS, ,Polaxomer, PVP K30, PVA and acetone as organic solvent. In present investigation Nanosuspension is prepared by 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 solvent evaporation method by using combinations of SLS, Polaxomer, PVP-K30, PVA, and Acetone and quantity sufficient water). Estimation of Teriflunomide was carried out spectrophotometrically at 282nm. The Nanosuspension were evaluated for parameters such as Entrapment efficiency, scanning electron microscopy, particle size analysis, zeta potential, in-vitro release, drug excipient interactions (FTIR).

The melting point of Teriflunomide was found to be in range of 230°C which was determined by capillary method.

Saturation solubility was carried out at 25^0C using 0.1N HCL, 6.8 phosphate buffer, and organic solvents. Form the drug excipient compatibility studies we observe that there are no interactions between the pure drug (Teriflunomide) and optimized formulation (Teriflunomide+ excipients) which indicates there are no physical changes.

The entrapment efficacy of the formulated Nanosuspension was found to be in the range of 79.52%-92.16%respectively.

Zeta potential value for the optimized formulation (F3) was found to be within the acceptable limits. Average particle size of nanosuspension of optimized formulations (F3) was found to be 489.7nm.

From the invitro studies we can say that formulation F3 shows best drug release of 99 % within 45 minutes where as all the other formulations takes about 60-180 minutes to 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 F3 follows Zero order kinetics.

CONCLUSION

oral Nanosuspension of Teriflunomide by precipitation method using various polymers such as SLS, Polaxomer, PVP-K30, PVA, and Acetone. The entrapment efficacy of the formulated Nanosuspension was found to be in the range of 78.02%- 97.02% respectively. As the polymer concentration increases, the drug release rate decreases, whereas Nanosuspension strength increases. Optimized formulations of Nanosuspension displayed zero order release kinetics and drug release. IR spectroscopic studies indicated that there are no drug-excepient interactions. When compared to other all the formulations F9 is the best formulation which showed 99.31% of drug released respectively with in 25 min and follows Zero order release kinetics. Hence from the study it was concluded that the solubility of Febuxostat drug was successfully enhanced by using Nanosuspension prepared by solvent evaporation method using polaxomer(15mg), as a carrier and PVA as a stabilizer.

REFERENCES

1. Rao GCS, Kumar MS, Mathivanam N, Rao MEB. Advances in Nanoparticulate Drug Delivery Systems. Indian Drugs, 2004; 41(7): 389-395.
2. Velmula M, Pavuluri P, Rajashekar S, Dr. Rao VUM. Nanosuspension Technology for Poorly Soluble Drugs - A Review. World Journal of

Pharmacy and Pharmaceutical Sciences, 2015; 4(7): 01612-1625.
3. Kamble kk, mahadik kr, preparation & characterization of olmesartan medoxomil nanosuspensions prepared by emulsion diffusion technique ijprs v-3, i-3, 2014.
4. Neha Tyagi et.al., Formulation And Evaluation Of Zidovudine Nanosuspensions Using A Novel Bio Polymer From The Seeds Of Buchanania Lanzan, Journal of Drug Delivery & Therapeutics, 2013; 3(4): 85-88.
5. Natarajan r, prabhu c and rajendran nn, formulation development and evaluation of tadalafil oral jelly comparative with marketed product, ijrpc, 2014; 4(2): 479-483.
6. Mayur H. Patil, Dr Surajj Sarode, Dr. P. V. Jain, Prof. B. S. Sathe, B. V. Jain, Dr. G.P. Vadnere, design and development of orodispersible tablets of tadalafil using novel superdisintegrants, World Journal of Pharmacy and Pharmaceutical Sciences, 3(4): 1367-1377.
7. V. Ravi Kumar*, M. Sevukarajan, Jayasri Vulava, A.G. Pavankumar, Y. Deepthi, M. Manjunath, A. Anand, improvement of dissolution characteristics and bioavailability of tadalafil by solid dispersion technique using water-soluble polymers, International Journal of Advanced Pharmaceutics Vol 2 | Issue 2 | 2012 | 56-63.
8. Majid Saeedi1,2*, Mohammad Reza Rafati3 Katayoun Morteza-Semnani4, Atieh Yazdani Rostam5, Hamid Reza Kelidari, Evaluation of effect of tween 80 on characteristics of tadalafil 0.1% suspension, Pharm Biomed Res., 2015; 1(2): 35.
9. Bhanu P Sahu. et.al., Optimization of felodipine nanosuspensions using Full Factorial Design, International Journal of Pharm Tech Research, April-June 2013; 5(2): 553-561.
10. Sara Salatin,1,2 Jaleh Barar,1,3 Mohammad Barzegar-Jalali,3 Khosro Adibkia, 3,4 Farhad Kiafar, 3,5 and Mitra Jelvehgari3, Development of a nanoprecipitation method for the entrapment of a very water soluble drug into Eudragit RL nanoparticles, Res Pharm Sci., 2017 Feb; 12(1): 1–14.
11. Prasanth Kumar Acholu. et.al., Enhancement Of Water Solubility And Dissolution Rate Of Felodipine Using Modified B-Cyclodextrins, Journal of Global Trends in Pharmaceutical Sciences, October-December 2013; 4(4): 1291-1299.
12. Chaudhari Bharat. et.al., Preparation And Evaluation Of Nanosuspension Of Poorly Soluble Drug Albendazole, Journal of Drug Discovery and Therapeutics, 2013; 1(1): 37-42.
13. Bhanu P. Sahu.et.al., Nanoprecipitation With Sonication For Enhancement Of Oral Bioavailability Of Furosemide, Acta Poloniae Pharmaceutica n Drug Research, 2014; 71(1): 129n137.
14. R. Suresh Kumar. et.al., Development and Characterization of Oral Disintegrating Tablet Containing Nanosuspension of Lurasidone Hydrochloride antipsychotic Drug, Asian Journal of Pharmaceutics • Apr-Jun 2017 • 11 (2) | 102.
15. Sara Salatine. et.al., Development of a nanoprecipitation method for the entrapment of a very water soluble drug into Eudragit RL nanoparticles, Research in Pharmaceutical Sciences, February 2017; 12(1): 1-14.
16. CongLuo. et.al., Felodipine nanosuspension: a faster *in vitro* dissolution rate and higher oral absorption efficiency, Journal of Drug Delivery Science and Technology, 2014; 24(2): 173-177.
17. Suryakanta Nayak. et.al. Formulation Design And In Vitro Characterization Of Felodipine Nano-Suspension, IJPSR, 2015; 6(6).
18. Mori D. et.al., Formulation, evaluation and optimization of the felodipine nanosuspension to be used for direct compression to tablet for in vitro dissolution enhancement. Pak J Pharm Sci., 2016 Nov; 29(6): 1927-1936.
19. Vezin W.R., Khan K.A. and Pang H.M., Journal of Pharmacy and Pharmacology, 1983; 35: 555-558.
20. Aulton ME: Pharmaceutics; The Science of Dosage Form Design. Churchill Livingstone, London, Second Edition, 2002.
21. Aulton ME: Pharmaceutics; The Science of Dosage Form Design. Churchill Livingstone, London, Second Edition, 2002.
22. Oth M, Franze M *et al*. The bilayer floating capsule: a stomach directed drug delivery system for misoprostol. *Pharm Res*, 1992; 9(8): 298-302.
23. Gergogiannins YS *et al*. Floating and swelling characteristic of various excipients used in controlled release technology. *Drug Dev Ind Pharm*, 1993; 19(6): 1061-1081.
24. Costa P., Sousa L. J. M., Modelling and comparison of dissolution profiles, European Journal Pharmaceutical Sciences, 2001; 13:2: 123–133.
25. Vijay Agarwal1,2* and Meenakshi Bajpai3, Preparation and Optimization of Esomeprazole Nanosuspension using Evaporative Precipitation–Ultrasonication, Tropical Journal of Pharmaceutical Research, April 2014; 13(4): 497-503.
26. Dash S, Murthy PN, Nath L and Chowdhury P: Kinetic modeling on drug release from controlled drug delivery systems. Acta Poloniae Pharmaceutica - Drug Research, 2010; 67: 217-223.