Dissolution Rate Enhancement of Dolutegravir Sodium Through Nanosuspension Technology Using 3^2 Factorial Design

Paul Bastyav Rodriques[1,2] , Prajapati Bhupendra Gopalbhai[3*]

[1]Faculty of Pharmacy, Ganpat University, Mahesana-384012, Gujarat, India.
[2]Department of Pharmaceutics, Krishna School of Pharmacy and Research, Drs Kiran & Pallavi Patel Global University (KPGU), Varnama, Vadodara-391243 Gujarat, India.
[3]Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Mahesana-384012 Gujarat, India.

ABSTRACT

Dolutegravir Sodium is a potent antiretroviral medication used in the treatment of HIV. It is a BCS Class II compound and is practically insoluble in the normal gastric pH range. This study aimed to discover and optimize the operational and formulation factors affecting the drug characteristics. 3^2 factorial design was used for the study. PVP K30, Polyvinyl Alcohol, Polxammer 407, and Polxammer 188 were used in the preparation of nanosuspension. The formulation variables tested were the polymer concentration, whereas the manufacturing variable was homogenization speed. Particle size and saturation solubility were the two responses evaluated in this study. The results of the analysis of variance indicate that the polymer concentration and homogenization speed significantly affect the saturation solubility and particle size of the nanosuspension. The contour plot and resulting polynomial formula help to estimate the values of the chosen independent factors to develop the best nanosuspension formulations with the required characteristics.

Key Words: *Nanosupension, Antiviral drug, Dolutegravir sodium, Factorial design, Solubility enhancement*

eIJPPR 2023; 13(6):1-7

HOW TO CITE THIS ARTICLE: Rodriques PB, Gopalbhai PB. Dissolution Rate Enhancement of Dolutegravir Sodium Through Nanosuspension Technology Using 3^2 Factorial Design. Int J Pharm Phytopharmacol Res. 2023;13(6):1-7. https://doi.org/10.51847/1wNkc9fDrX

INTRODUCTION

The bioavailability of the drug is primarily affected by its ability to dissolve and release from a formulation. Since roughly half of the active pharmaceutical ingredients being found through the novel approaches are either insoluble or poorly soluble in water, resolving solubility issues is a significant challenge for the pharmaceutical industry with the creation of new pharmaceutical products [1]. The inherent solubility and particle size of a drug determine its rate of dissolution. Research involving weakly water-soluble drugs has indicated that reducing the particle size to a sub-micron size can result in a greater dissolution rate and increased bioavailability [2, 3].

The development and reporting of nanoparticle engineering for medicinal purposes has occurred throughout the past ten years [4]. Sub-micron colloidal dispersions of solid medication particles in a liquid phase are known as nanosuspensions. High-speed homogenization, pearl milling, and Precipitation are the current nanosuspension engineering techniques employed in aqueous or nonaqueous environments [5]. Additionally, the amorphous proportion in the particles can be increased, or even entirely amorphous particles can be created by formulating nanosuspensions [6, 7].

Dolutegravir sodium (DTGS) is a novel integrase strand transfer inhibitor active against human immunodeficiency viruses (HIV). DTGS blocks HIV viral DNA integration into host DNA, which is a necessary stage in viral reproduction. DTGS is quickly absorbed after oral administration. It can be taken once daily without the requirement for a boosting dose, as the half-life of the drug is around 11–12 hours in HIV-positive adults. DTGS

Corresponding author: Prajapati Bhupendra Gopalbhai
Address: Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Mahesana-384012 Gujarat, India.
E-mail: ✉ bhpuendra.prajapati@guni.ac.in
Received: 12 August 2023; **Revised:** 01 November 2023; **Accepted:** 06 November 2023

belongs to BCS II with lesser aqueous solubility, minimal protein binding, and high permeability [8, 9].

Thus, the purpose of our research was to determine if it would be feasible to prepare DTG nanosuspension to accomplish fast dissolution. Fast onset of action in the bloodstream is particularly advantageous when using DTG for the treatment of HIV.

MATERIALS AND METHODS

Materials

DTGS was obtained as a gift sample from Emcure Pharmaceutical Pvt. Ltd., Ahmedabad, India. PVP K30, Polyvinyl Alcohol, Polxammer 407, and Polxammer 188 were obtained as a gift sample from BASF, Mumbai. All other ingredients were of analytical grade.

Preparation method of nanosuspension

The Nanosuspension was prepared by solvent-antisolvent method. The weighed quantity of poloxamer 188 is mixed with water, and the weighed quantity of the drug is mixed in methanol in separate beakers with the help of a magnetic stirrer. The drug solution is injected slowly into the polymeric solution with a syringe. The mixer is subjected to high-speed homogenization at 10,000 rpm for 30 minutes for pulverization. The 6 g of dissolved mannitol in the nanosuspension was rapidly frozen in liquid nitrogen before being lyophilized for 24 hours at ambient temperature [10].

Design of experiment

To assess the preparation and processing characteristics of nanosuspensions, preliminary screening tests for different factors were conducted, like concentration of polymer, solvent for nanosuspension, homogenization time, and homogenization speed **(Table 1)**. A factorial design was employed to determine the impact of composition and manufacturing variables on the physical characteristics of nanosuspension, considering the quantity and degree of factors. It was found that the concentration of the polymer and the homogenization speed were essential for developing a nanosuspension in the nano range with the necessary stability. Each of these variables was controlled at three different levels: +1, 0, and −1. The type of polymer, quantity of solvent, and concentration of drug were kept the same for all the experiments. The investigation was carried out using Design Expert 13 software. A total of 9 experiments were designed by the software. To improve the accuracy of the model, trials were conducted in an arbitrary sequence. For every trial trial, a batch size of 25 g was maintained [11, 12].

Table 1. Factor level and responses for 3^2 factorial design investigation

	Batch	Level of poloxamer 188 (mg)	Level of shearing speed (RPM)	Particle size (nm)	Saturation solubility (µg/ml)
1	NS1	0.5% (-1)	5000 rpm (-1)	384.0 ± 5.2	175.83 ± 1.15
2	NS2	0.5% (-1)	7500 rpm (0)	325.0 ± 4.0	183.24 ± 1.09
3	NS3	0.5% (-1)	10000 rpm (1)	356.0 ± 4.7	195.36 ± 1.32
4	NS4	1 % (0)	5000 rpm (-1)	467.0 ± 3.2	145.75± 0.53
5	NS5	1 % (0)	7500 rpm (0)	324.0 ± 11.0	140.78 ± 1.21
6	NS6	1 % (0)	10000 rpm (1)	375.8 ± 3.2	136.12 ± 1.43
7	NS7	1.5% (1)	5000 rpm (-1)	348.9 ± 7.6	194.90 ± 1.28
8	NS8	1.5% (1)	7500 rpm (0)	230.6 ± 8.2	231.92 ± 1.34
9	NS9	1.5% (1)	10000 rpm (1)	189.0 ± 4.0	288.24 ± 1.32

Characterization of nanosuspension

Particle size and zeta potential

Malvern Zetasizer ZS200 was used to determine the particle size and zeta potential of the nanosuspension. A minimum of three measurements were taken for each sample. The response surfaces have been determined using the mean value [13].

Saturation solubility

To test the saturation solubility of the developed nanosuspension, it was placed in a container and stirred for 48 hours using a mechanical shaker. The nanosuspension was subsequently placed in a 4 ml centrifuge tube and spun at 5,000 rpm for 20 mins. The resulting solution was filtered and assessed using a UV-visible spectrophotometer after appropriate dilution with dissolving fluid that served as a blank. Every sample underwent analysis in triplicate [14, 15].

Fourier transform infrared (FTIR)

A Bruker FTIR Alpha II Spectrophotometer was used to perform FTIR spectrum analysis, and the spectra were obtained in the 4000–400 cm^{-1} wavelength band [16].

Differential scanning calorimetry (DSC)

To investigate the thermal behavior of the sample (drug, stabilizer, physical mixture, and lyophilized nanosuspension), DSC was carried out using a DSC-60

ISSN (Online) 2249-6084 (Print) 2250-1029

(Shimadzu, Tokyo, Japan) calorimeter. The samples were heated in tightly sealed aluminum pans under nitrogen at a scanning rate of 5 °C/min from 50 °C to 400 °C. An unfilled aluminum pan served as a point of reference [17].

Powder X-ray diffraction (PXRD)

The crystalline state of the drug and the lyophilized nanosuspension were investigated by PXRD analysis. Diffraction patterns were assessed with a Miniflex II X-ray Diffractometer (Rigaku Co. Tokyo, Japan) [18].

In-vitro drug release study

The USP 24 paddle apparatus was used to conduct an *in-vitro* dissolution investigation. The medium was carefully poured into the dissolution jar to reduce the frothing of the media throughout the entire study. Dissolution was carried out at 37 ºC with a paddle speed. A nanosuspension corresponding to one dosage of the drug was introduced to the jar. 5 ml samples were taken at precise time intervals and promptly filtered through a filter paper and spectrophotometrically analyzed. Five milliliters of new medium were then added to the dissolving vessel. The trials were carried out in triplicate, and the average data were stated [6].

Scanning electron microscopy (SEM)

SEM was used to examine the surface properties of lyophilized nanosuspension formulation [19].

Accelerated stability study

The optimized formulation was stored for six months in a stability chamber at 40 ± 2 °C and 75 ± 5% relative humidity, following ICH recommendations for accelerated stability tests. Amber-colored vials of USP type I were filled with the optimized formulation. Sealed with aluminum caps and closed with rubber plugs. Following the time of stability, the samples were removed. Studies on dissolution were conducted. The in vitro drug release was assessed [20].

RESULTS AND DISCUSSION

Statistical analysis

After plugging the results of the regression coefficients into the formula, a comprehensive model was created. Design Expert software was used to carry out regression analysis.

The fitted equation for mean particle size (nm)

$$Y1=230-44.54X_1-21.00X_2 \quad 37.50X_1^2+23.19X_2^2+71.94X_1X_2 \qquad (1)$$

The fitted equation for saturation solubility (µg/ml)

$$Y2=231+29.86X1+6.87X2+33.25X12-5.31X22-40.81X1X2 \qquad (2)$$

The polynomial equations 1 and 2 demonstrate the empirical impact of the independent factors (X1 and X2) and their relationships with both responses. Every independent factor was shown to have a substantial impact on the response. The negative sign of the coefficient demonstrated that increasing the magnitude of the independent factor reduces the magnitude of the response and vice versa. The extent to which the independent factor affects the response is shown by the coefficient's absolute figure; the greater the figure, the greater the magnitude.

The contour plots **(Figure 1)** for the mean particle size (nm) and saturation solubility for a predetermined range of values are provided in **(Figure 2)**. It was discovered that the contour plot was multi-dimensional. Thus, there was a nonlinear connection between the independent factors and the particle size and saturation solubility as well [21].

FTIR

The infrared spectrum of DTGS nanosuspension revealed a little variation in the distinctive spectra of DTGS. In the spectrum DTGS nanosuspension, peaks are a little different and seem a bit wide **(Figure 1)**. Similar results were found in previous research [22].

DSC

The coarse drug particles showed a unique melting process with a sharp peaking at 340 °C and starting at 327 °C. The Lyophylized DTGS nanosuspension demonstrated a broad peak at 317 °C. Such a broad peak is an indication of the amorphous nature of the lyophilized nanosuspension. **(Figure 1)** [23].

Particle size and zeta potential

The particle size and zeta potential of the optimized formulation were found to be 144 nm and -26.9 mV, respectively **(Figure 2)** [24].

PXRD

The PXRD of DTGS exhibited intense peaks at 2Θ of 13.74, 15.19, 18.9, 17.4, 18.94, 19.1, and 21.68, which reveals the crystalline nature of DTGS. Such sharp peaks were not found in the diffractogram of lyophilized DTGS nanosuspension. Such results were also demonstrated in previous research **(Figure 2)** [25].

In-vitro drug release study

The in vitro drug release study has demonstrated 97.55% cumulative drug release from DTGS nanosuspension up to 45 minutes, while 32.98% cumulative drug release was observed from pure DTGS. Therefore, nanosuspension

significantly increased the rate of solubility of DTGS. **(Figure 2)** [26].

SEM

The surface morphology of DTGS, as assessed by SEM, was found to be prolonged, flat, and round, with particles ranging in size from 4-30 micrometers. Nevertheless, after being converted to lyophilized nanosuspension, particles that were deposited on the exterior of mannitol employed as a cryoprotectant got a smaller size (approximately 200 nm), perhaps due to hydrophobic contact [27].

Stability study

The % drug content and % CDR of optimized DTGS nanosuspension formulation were found to be 98.94 ± 0.23% and 97.63 ± 0.23%, respectively, after six months at 40 ± 2 °C/75% RH, which is quite similar to the results of the initial. The result of the stability study revealed that the formulation was stable after six months [28, 29].

Optimized formulation composition **(Table 2)**, its physicochemical parameters **(Table 3)**, *In vitro* release % CDR and release kinetic **(Table 4)**, and stability study data **(Table 5)** are compiled in **Table 2**.

Table 2. Optimized batch formulation

1	**Amount of DTGS**	**20 mg**
2	Amount of polaxamer 188	150 mg
3	Solvent-antisolvent Ratio	1:20
4	Shearing speed	10,000 rpm
5	Shearing time	30 mins
6	Amount of mannitol 1:1 (cryoprotectant)	170 mg

Table 3. Physicochemical parameters of optimized batch

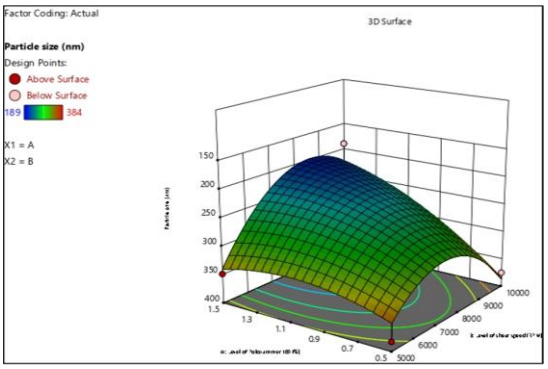
Sr. No.	Parameters	Results
1	Particle size	144.5 nm
2	Zeta potential	-26.9 mV
3	PDI	0.393
4	Drug content	98.87%
5	Saturation solubility	288.67 (µg/ml)
6	In vitro drug release	97.54 % uptil 45 mins

Table 4. *In vitro* release % CDR and release kinetic

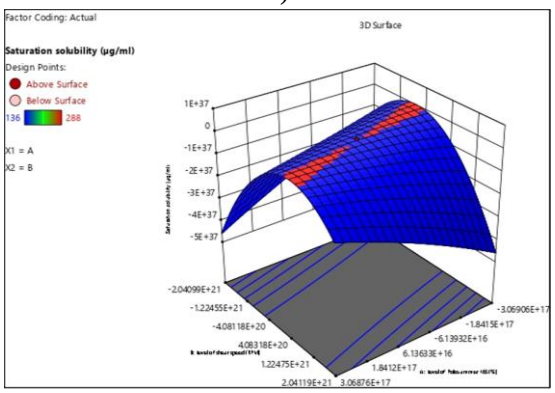
Formulations	% Cumulative drug release in 45 min	Release kinetics R^2
NS Optimized	97.55 ± 0.49	R^2 = 0.997 (Zero order)
Pure drug	32.98 ± 0.24	R^2 = 0.996 (Zero order)

Table 5. Stability study of nanosuspension

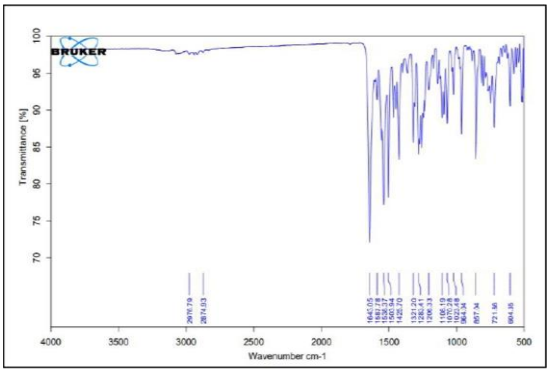
Formulation	% Drug content (40 ± 2 °C/75% RH)	% CDR (40 ± 2 °C/75% RH)
Optimized formulation	98.94 ± 0.23	97.63 ± 0.23



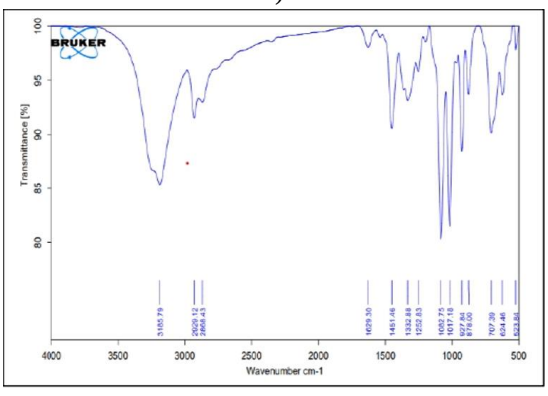
a)



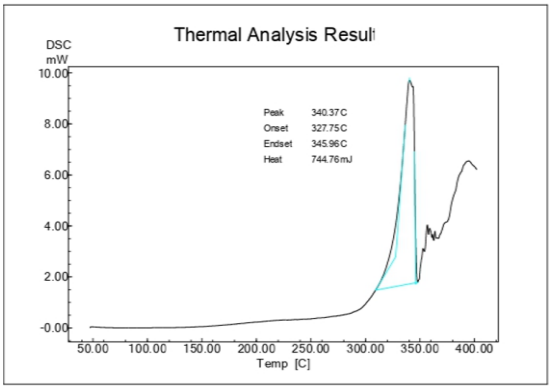
b)



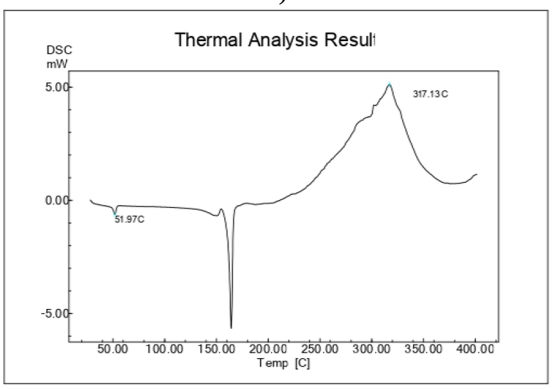
c)



d)

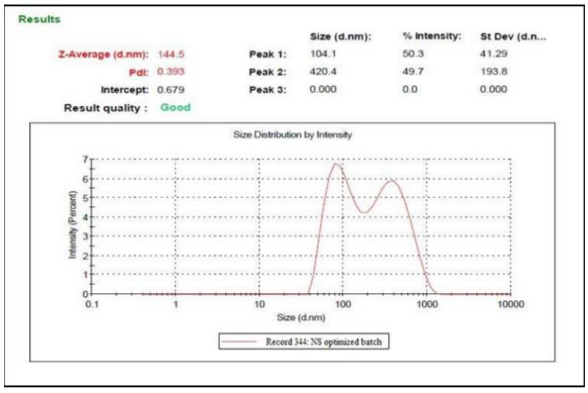


e)

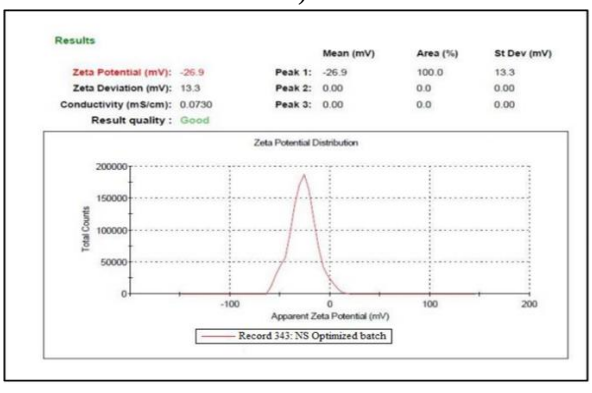


f)

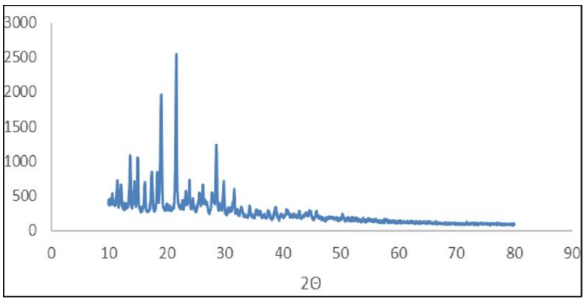
Figure 1. a) Counter plot of particle size, b) Counter plot of saturation solubility, c) FTIR of DTGS, d) FTIR of DTGS nanosuspension, e) DSC thermograph of DTGS, and f) DSC of DTGS nanosuspension



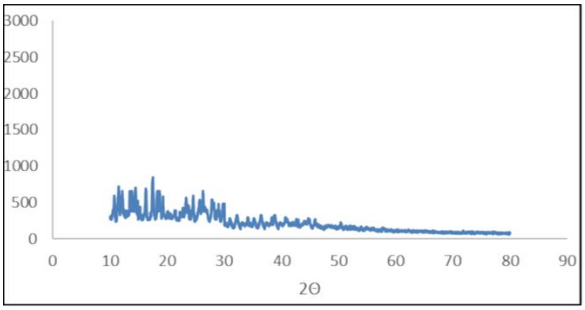
a)



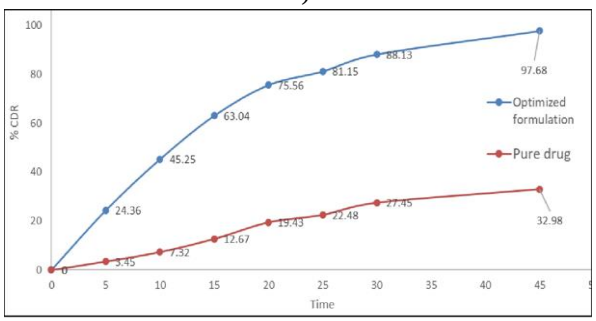
b)



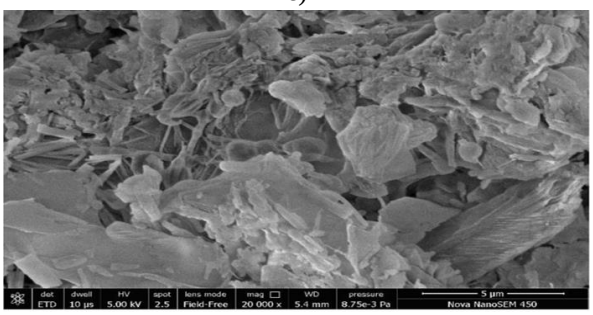
c)



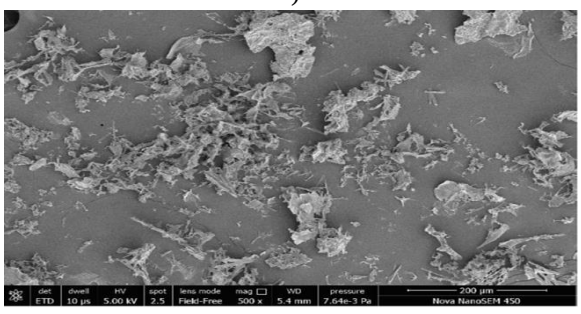
d)



e)



f)



g)

Figure 2. a) Particle size, b) Zeta potential graph of nanosuspension, c) PXRD of DTGS, d) PXRD of Lyophilized nanosuspension of DTGS, e) in vitro drug release in phosphate buffer 6.8, f) SEM of DTGS, g) SEM of Lyophilized nanosuspension of DTGS

CONCLUSION

The DTGS nanosuspensions were effectively developed through the high-speed homogenization approach. Homogenization factors have a significant impact on nanoparticle particle size. By optimizing the homogenization speed and concentration of surfactant, nanosuspensions with mean diameters less than 200 nm and with low PDI can be developed. There was a substantial increase in the dissolution rate of DTGS. This innovative delivery method shows promise as a substitute conventional formulation for DTGS.

Acknowledgments: The authors are grateful to Emcure Pharmaceuticals Ltd. Ahmedabad, Gujarat, India, and BASF Pvt Ltd for providing gratis samples. The authors would also like to thank Ganpat University, Shri S K College of Pharmaceutical Education & Research, Kherva, for providing facilities to conduct this research.

Conflict of interest: None

Financial support: None

Ethics statement: None

REFERENCES

[1] Varia U, Prajapati B, Katariya H. Formulation and development of bosentan loaded once a daily tablet for pulmonary artery hypertension using lipid matrices by 3 (2) full factorial design. Int J Pharm Sci Res. 2018;9(11):4729-40.

[2] Alaa Abdulelah A, Nawal Ayash R. Bioavailability study of Posaconazole in rats after oral Poloxamer P188 Nano-micelles and oral Posaconazole pure drug. J Adv Pharm Educ Res. 2023;13(2):140-3.

[3] Bhattacharya S, Rodriques P, Prajapati B. Introductory chapter: advanced drug delivery systems. InAdvanced Drug Delivery Systems 2023 Feb 15. IntechOpen.

[4] Khunt D, Prajapati BG, Prajapti M, Misra M, Salave S, Patel JK, et al. Drug delivery by Micro, Nanoemulsions in tuberculosis. InTubercular Drug Delivery Systems: Advances in Treatment of Infectious Diseases 2023 Mar 15 (pp. 173-188). Cham: Springer International Publishing.

[5] Patel RJ, Pandey P, Patel AA, Prajapati BG, Alexander A, Pandya V, et al. Ordered mesoporous silica nanocarriers: An innovative paradigm and a promising therapeutic efficient carrier for delivery of drugs. J Drug Deliv Sci Technol. 2023:104306.

[6] Prajapati BG, Paliwal H, Patel M. Fabrication and evaluation of polymeric nanoparticles of acitretin for the solubility enhancement. Res J Pharm Technol. 2023;16(6):2655-60.

[7] Kingsley JD, Dou H, Morehead J, Rabinow B, Gendelman HE, Destache CJ. Nanotechnology: a focus on nanoparticles as a drug delivery system. J Neuroimmune Pharmacol. 2006;1(3):340-50.

[8] Paul Bastyav R, Bhupendra Gopalbhai P. Formulation and evaluation of Dolutegravir Sodium Nanoemulsion for the treatment of HIV. Pharmacophore. 2022;13(6):1-8.

[9] Kandel CE, Walmsley SL. Dolutegravir–a review of the pharmacology, efficacy, and safety in the treatment of HIV. Drug Des Devel Ther. 2015;9:3547-55.

[10] Dattatraya Manohar S, Sonal Sanjay J, Prashant Laxman P, Sahebrao Sampat B, Sunil V. Formulation, evaluation, and optimization of Glimepiride Nanosuspension by using antisolvent evaporation technique. Pharmacophore. 2022;13(4):49-58.

[11] Prajapati BG, Jivani M, Paliwal H. Formulation and optimization of topical nanoemulsion based gel of mometasone furoate using 32 full factorial design. Indian Drugs. 2021;58(6).

[12] Akshay P, Bhupendra Gopalbhai P. Response surface methodology for an improved Nanoemulsion of Ivacaftor & its optimisation for solubility and stability. Pharmacophore. 2023;14(5):1-8.

[13] Ammanage A, Rodriques P, Kempwade A, Hiremath R. Formulation and evaluation of buccal films of piroxicam co-crystals. Future J Pharm Sci. 2020;6(1):16.

[14] Ameerah Abdulelah R, Iman Sabah J. Factors influencing the dissolution behavior of meloxicam dispersions. J Adv Pharm Educ Res. 2022;12(3):9-14.

[15] Shekhawat P, Pokharkar V. Risk assessment and QbD based optimization of an Eprosartan mesylate nanosuspension: in-vitro characterization, PAMPA and in-vivo assessment. Int J Pharm. 2019;567:118415.

[16] Rodriques PB, Prajapati BG. Design and development of dolutegravir sodium co-crystal loaded orodispersible tablet with improved dissolution behavior. Indian Drugs. 2023;60(3):30-9.

[17] Na YG, Pham TMA, Byeon JJ, Kim MK, Han MG, Baek JS, et al. Development and evaluation of TPGS/PVA-based nanosuspension for enhancing dissolution and oral bioavailability of ticagrelor. Int J Pharm. 2020;581:119287.

[18] Priyal P, Ashok M, NileeThakor. Enhancement of solubility and dissolution of Clopidogrel bisulfate by solid dispersion in combination with surface adsorbent. Pharmacophore. 2020;11(5):1-13.

[19] Macwan M, Prajapati B. Development, optimization and characterization of ocular nanoemulsion of an

antifungal agent using design of experiments. Res J Pharm Technol. 2022;15(5):2273-8.

[20] Prajapati BG, Patel DV. Formulation and optimization of domperidone fast dissolving tablet by wet granulation techniques using factorial design. Int J PharmTech Res. 2010;2(1):292-9.

[21] Paliwal H, Parihar A, Prajapati BG. Current state-of-the-art and new trends in self-assembled nanocarriers as drug delivery systems. Front Nanotechnol. 2022;4:836674.

[22] Prajapati BG, Prajapati B, Khunt D. Formulation and evaluation of self-nanoemulsifying drug delivery system for improved oral delivery of exemestane hydrochloride. IP Int J Compr Adv Pharmacol. 2023;8(1):42-8.

[23] Patel VM, Prajapat BG, Patel JK, Patel MM. Physicochemical characterization and evaluation of buccal adhesive patches containing propranolol hydrochloride. Curr Drug Deliv. 2006;3(3):325-31.

[24] Prajapati BG, Patel DV. Comparative study of the efficiency of different super disintegrants for fast dissolve tablet of domperidone. J Pharm Res. 2010;3(1):151-5.

[25] Bhattacharya S. Formulation design and development of anti-EGFR-BSA-CYP-SLNs In Situ gel for nasal administration. Asian J Pharm. 2016;10(04).

[26] Bhattacharya S. Preparation and evaluation of celecoxib polysaccharide based matrix tablets using nanoparticular approach. Asian J Pharm. 2017;11(02):S374-81.

[27] Prajapati B, Modasiya MK. Studies on solubility of meloxicam by solid dispersion method. J Pharm Res. 2012;5(4):2290-2.

[28] Kapoor DU, Garg R, Gaur M, Prajapati BG, Agrawal G, Bhattacharya S, et al. Polymeric nanoparticles approach and identification and characterization of novel biomarkers for colon cancer. Results Chem. 2023;6:101167.

[29] Shah S, Patel AA, Pandya V, Trivedi N, Patel SG, Prajapati BG, et al. Breaking barriers: intranasal delivery of brexpiprazole-nanostructured lipid carriers targets the brain for effective schizophrenia treatment. J Drug Deliv Sci Technol. 2023;90:105160.