International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 15, Issue 3, 2023

Original Article

FORMULATION DEVELOPMENT, OPTIMIZATION, AND EVALUATION OF LANSOPRAZOLE LOADED NANOSUSPENSION

LALITA S. NEMADE[1*], MORESHWAR P. PATIL[2]

[1,2]Department of Pharmaceutics, MET Institute of Pharmacy Adgaon, Nashik-422003, Maharashtra, India
*Email: lalitasnemade@gmail.com

Received: 13 Jan 2023, Revised and Accepted: 03 Apr 2023

ABSTRACT

Objective: To address issues with drug release, manufacturing, and drug product stability, the primary objective of this study was to optimize the solubility of poorly soluble lansoprazole medication by using an appropriate nanosuspension formulation.

Methods: The freeze-drying (nanopure) method was used to create lansoprazole nanosuspensions. The formulation and process variables affecting the properties of nanosuspensions (mannitol concentration, drug concentration, and PVP-K30 concentration) were optimized. Particle size, shape, zeta potential, drug content, stability study, and *in vitro* drug release study were performed on the nanosuspensions.

Results: Out of different batches of nanosuspension, batch F4 displayed the most optimizing parameters, i.e., drug content 97±1.2%, average particle size 125.5 nm, polydispersity index 0.361, and zeta potential-22.7 mV. In comparison to other formulations, the PVP-K30 polymer in F4 demonstrated an effective cumulative drug release of 120 min. In stability studies, optimized nanosuspension displayed favorable results and demonstrated significant stability for the said period of time.

Conclusion: The most promising drug release profile was shown by the optimized (F4) nanosuspension, which also demonstrated increased lansoprazole solubility. We have concluded from the current study that nanosuspension can serve as a better formulation for lansoprazole delivery.

Keywords: Lansoprazole, Nanopure method, Nanosuspension, Drug release, Solubility, Stability

DOI: https://dx.doi.org/10.22159/ijap.2023v15i3.47327. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

The effectiveness of a drug formulation depends on a number of factors, including the drug's solubility, stability at room temperature, compatibility with the solvent and excipient, and photostability. More than forty percent of the new chemical entities (NCEs) created thus far by drug development programs are lipophilic, or weakly water-soluble, molecules [1]. Insufficient medication solubility and bioavailability can be addressed by a wide variety of formulation strategies. The usual methods of improving the solubility of poorly soluble pharmaceuticals include micronization, fatty solution usage, penetration enhancer or cosolvent use, the surfactant dispersion method, salt creation, precipitation, etc. Vesicular systems, such as liposomes, solid dispersion, emulsion and microemulsion technologies, and inclusion complexes with cyclodextrins, also exhibit positive effects as drug delivery systems, but they have significant limitations [2]. Nanoparticle engineering has been invented and published for pharmaceutical applications throughout the past few decades [3].

Submicron colloidal dispersions of nanoparticle-sized medication particles that are stabilized by surfactants are called nanosuspensions. Nanosuspensions are dispersions in which only the weakly water-soluble medication is suspended. These can be used to make medications more soluble in both aqueous and lipid environments. An increase in solubility accelerates the flooding of the active ingredient, and the maximum plasma level is achieved sooner [4]. This method is helpful for compounds that present considerable difficulty to formulators due to their low solubility, poor permeability, or both. Because of the smaller particle size, even poorly soluble medications can be given intravenously without causing any blockage in the blood vessels. Lyophilization allows the suspensions to be transformed into a solid matrix. In addition to these benefits, it also offers the added convenience of liquid formulations [5–8].

The proton pump inhibitor (PPI) lansoprazole blocks the final step in the gastric acid release by parietal cells in the stomach [9]. When compared to other proton pump inhibitors, its 85% bioavailability after the first dose ensures that patients have rapid symptom alleviation. The bacterium Helicobacter pylori is inhibited by lansoprazole *in vitro*, demonstrating the drug's antibacterial action [9]. It has 85% bioavailability but showed less aqueous solubility and high permeability. Over the course of seventeen years of clinical experience around the world, lansoprazole has been shown to be an effective and well-tolerated treatment option for the management of acid-related disorders such as gastric and duodenal ulcers, gastroesophageal reflux disease, and the treatment or prevention of gastroduodenal lesions induced by NSAIDs. In terms of less water solubility and high bioavailability, lansoprazole is a BCS-II drug due to its biological half-life is 1.5 h. Another reason is, according to a market study, lansoprazole is available only as enteric coated pellets that are packed inside of capsules because it is acid labile. Although an enteric coating can delay the release of a drug, the procedure is time-consuming and expensive. The antiulcer drug lansoprazole is a classic case of a compound with poor water solubility. The drug lansoprazole is almost completely water-insoluble [10–13]. Therefore, in the present study, we tried to develop a nanosuspension containing lansoprazole to improve its solubility.

Nanopure technology based on freeze-drying is used to prepare the nanosuspension. The nanopure technique has several advantages, such as high drug entrapment efficiency for poorly soluble drugs, narrow particle size distribution, high batch-to-batch reproducibility, no homogenization required, simplicity, ease of scale-up, and low equipment cost [14, 15]. Therefore, we applied this method to prepare nanosuspension in the present study. The primary goal of this research is to improve the solubility and pharmacokinetic parameters of poorly soluble lansoprazole drugs using appropriate nanosuspension formulation in order to overcome drug release, manufacturing, and drug product stability problems.

MATERIALS AND METHODS

Material

Lansoprazole was obtained as a gift sample from Bioxera Pharma Pvt. Ltd. Mumbai, India. PVPK-30 (polyvinyl pyrrolidine) and PVA (polyvinyl alcohol) was purchased from Astron Chemical, Ahmedabad, India. HPLC-grade ethanol and dichloromethane was supplied by SD Fine Chemicals, Mumbai, India. All other materials of reagent grade

were purchased from SD Fine Chemicals, Mumbai, India and used as received. Double distilled water was used throughout the study.

Method

Calibration curve of lansoprazole

Approximately 50 mg of the drug was taken in a 50 ml of volumetric flask. A stock solution was prepared by adding methanol as a co-solvent and the volume was made with phosphate buffer pH 7.4. Stock solution ranged from 2-40µg/ml were prepared and absorbance's recorded at 281 nm using UV-spectrophotometer (Shimadzu 2450, Japan) [16].

Preparation of lansoprazole containing nanosuspension by nanopure method

The nanosuspension was formulated using PVP K30 as a stabilizer. Further, nanosuspensions were lyophilized to convert into solid nanocrystals using mannitol as a cryoprotectant. Freeze drying was accomplished by freezing solution (sucrose solution) (Lansoprazol+Mannitol+PVP-K30) in 2 ml 2R borosilicate glass vials submerged in liquid nitrogen, then drying with a Bio-base freeze dryer (NRI Technologies). To prevent melting of the frozen solutions, the shelf temperature was kept at-25 °C while the vials were loaded. The vials were equilibrated for 5 min before starting the primary drying cycle by lowering the chamber pressure to 65mTorr and the temperature to-25 °C for 24 h, followed by secondary drying at 20 °C. The solid nanocrystals was collected and stored in a dark, closed container [17, 18].

Formulation, development and optimization of nanosuspension

Different batches of lansoprazole contain nanosuspension were prepared based on the Box-Behnken design. Moisture content and % drug content were selected as responses. The statistical analysis of the factorial design formulations was performed using Design-Expert®software (Version 10, Stat-Ease Inc.). Seventeen batches were prepared as suggested by the software using box-behnken design with three levels, three factors and two responses. Different combinations used to prepare lansoprazole contain nanosuspension using box-behnken design is presented in table 1. ANOVA was used to establish the statistical validation of the polynomial equations generated by design expert® software [19–22].

Evaluation of nanosuspension

FTIR study

FTIR spectra (the Agilent Cary 630) of Mannitol, PVP-K30 and optimized nanosuspension (F4) were studied [nanosuspensions were lyophilized to convert into solid nanocrystals]. Above samples were mixed with KBr of IR grade in the ratio of 1:100 and compressed using a motorized pellet press at 10-12 tons of pressure. The pellets were then scanned using FTIR spectrophotometer (8400S Shimadzu, Japan) [23].

DSC study

Thermogram for Mannitol, PVP-K30 and optimized nanosuspension (F4) [nanosuspensions were lyophilized to convert into solid nanocrystals] were studied using DSC (Mettler DSC 1 star system, Mettler-Toledo, Switzerland). The drug was sealed in a perforated aluminum pan and heated at a constant rate of 10 °C/min over the temperature ranges of 30-350 °C at 20 ml/min nitrogen purging [24].

XRD study

The powder X-ray diffraction (PXRD) was recorded on Rigaku Analytical XRD, India. The PXRD pattern of optimized nanosuspension (F4) [nanosuspensions were lyophilized to convert into solid nanocrystals] was conducted using X-ray diffractometer with Cu as a target at a voltage of 40 kV. Samples were analyzed in 2θ angle range of 10–50° at a scanning rate of 3°/2U/min [25].

Scanning electron microscopy and transmission electron microscopy

SEM was recorded on Jeol, Tokyo, Japan model. TEM was recorded on JEM-1400 model. SEM and TEM was used to plan the surface geomorphology of the freeze-dried nanosuspension [26].

Particle size, polydispersity index measurement and zeta potential of freeze-dried nanosuspension

The particle size and distribution were determined using the Zetasizer Nano ZS and a technique known as dynamic light scattering. The zeta potential of a particle is the overall charge that the particle acquires in a particular medium [27].

Drug content in lyophilized nanosuspension

0.5 ml of each preparation was dispersed in 10 ml of isotonic (NaCl) solution and stored overnight, and 10 mg of drug was liquefied in 10 ml of isotonic (NaCl) solution and stored overnight. All preparations, including the drug, were filtered dilutions made at a concentration of 1µg/ml. These dilutions were estimated their content uniformity by reason a UV spectrophotometer at the wavelength 272 nm [28].

Viscosity determination

The nanosuspension viscosity was carried out on the Brookfield viscometer with the spindle no. 60 at 100 rpm [29].

Saturation solubility studies

Saturation solubility tests were performed on both the pure drug and various batches of the lyophilized lansoprazole nanosuspension. 10 mg of the pure drug compound and 10 mg of lyophilized lansoprazole nanosuspension powder were weighed and measured separately before being added to a 25 ml stoppard conical flask containing 10 ml distilled water. With the same methodology were used for phosphate buffers (1.2, 6.8 and 7.4). The flasks were sealed and placed in a rotary shaker at room temperature for 24 h before being equilibrated for two days. A UV spectrophotometer set to 272 nm was used for analysis [30].

***In vitro* release study**

The *in vitro* drug release was studied using USP Type II dissolution equipment, 900 ml of buffer pH 7.4, and a rotating speed of 50 rpm. Temperature was kept constant at 37±0.5 °C during sampling, and aliquot samples (5 ml) were collected and replenished with the same amount of fresh medium every 10-120 min. The aliquot sample (5 ml) was filtered using a 0.45m restricted membrane filter paper supporter, and the filtrate was properly diluted through the fresh medium and was predictable using a UV-Vis spectrophotometer (model UV-2600plus) at wavelength 272 nm [31, 32].

Stability study of nanosuspension

The nanosuspension stability investigations were conducted for 30 d under the following storage conditions. The intermediate storage condition for stability studies have been changed from 30 °C±2 °C to 65%±5% RH. For each condition, the optimized batch F4 Nanosuspension was used; the particle size, drug content, and *in vitro* dissolution are the most important specifications for activity and physical stability [33, 34].

RESULTS AND DISCUSSION

UV graph of lansoprazole

The lambda max of Lansoprazole was found to be 272 nm as shown in fig. 1.

Formulation, development and optimization of nanosuspension

Because it promotes effective freeze-drying and the better appearance of the products that have undergone freeze-drying, mannitol is utilized as a bulking agent and cryoprotectants. Biphasic dose forms consist of suspensions, although no perfect suspension without stability problems has yet been developed. Due to cake formation and sedimentation, any suspension formulation has stability problems. PVP-K30 has its unique position in the pharmaceutical industry, which uses a wide variety of suspending agents.

PVP-K30 is utilized for stabilizing and suspending purposes. The available literature lists numerous studies that scientists claim demonstrate the impact of PVP on suspension stability. Huertas *et al.*, where they investigated the impact of PVP adsorption on the stability of the silica suspension. Researchers looked at the impacts of silica concentration, polymer addition, and water dilution of the

suspension. The stability of the systems under investigation as a function of time was examined using the turbidimetry approach. It was demonstrated that the systems containing PVP (both before and after dilution) are gradually stable, whereas the suspension without polymer is characterized by the smallest stability.

Generally speaking, the bioavailability decreases with decreasing solubility. This is affected by a number of other elements as well. Considered a crucial factor in formulation creation is solubility. Researchers are constantly looking for novel approaches to raise solubility and thereby increase bioavailability. PVP has been used to increase the solubility of pharmaceuticals using the solid dispersions method [35]. In a study, Mahapatra *et al.* found that PVP was superior to hydroxypropyl β-cyclodextrin and β-cyclodextrin for improving the solubility of valsartan [35].

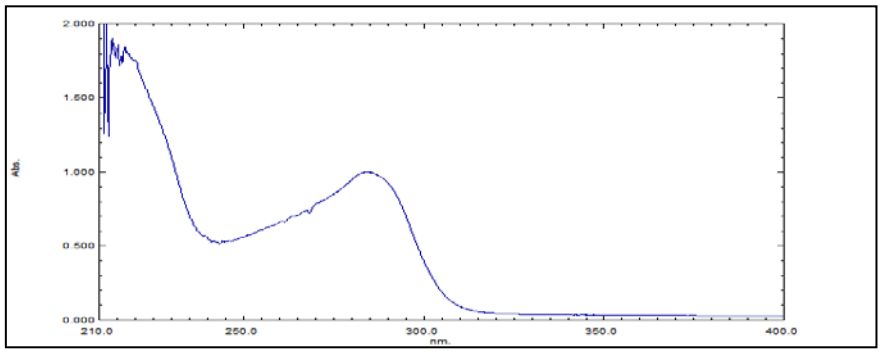


Fig. 1: UV spectrum of lansoprazole

Table 1: Formulation batches of nanosuspension

FC	Drug (mg)	Factor 1 A: Mannitol (mg)	Factor 2 B: PVP (mg)	Factor 3 C: Secondary drying temp (°C)	Response 1 % Drug content	Response 2 % Moisture content (w/w)
F1	20	12.5	5	20	61	2.8
F2	20	15	7.5	25	68	3.2
F3	20	12.5	7.5	22.5	70	3.2
F4	20	15	5	22.5	63	2.9
F5	20	12.5	7.5	22.5	65	3.1
F6	20	10	10	22.5	77	4.2
F7	20	10	7.5	25	72	3.5
F8	20	12.5	10	25	75	3.9
F9	20	12.5	7.5	22.5	69	3.3
F10	20	10	5	22.5	65	3
F11	20	12.5	7.5	22.5	70	3.3
F12	20	12.5	5	25	69	3.1
F13	20	12.5	7.5	22.5	68	3.2
F14	20	10	7.5	20	71	3.4
F15	20	12.5	10	20	78	4.5
F16	20	15	10	22.5	79	4.1
F17	20	15	7.5	20	73	3.7

Effect of concentration of mannitol and PVP on different batches

Because it promotes efficient freeze-drying, mannitol is utilized as a bulking agent and cryoprotectant. Mannitol at its highest concentration (15 mg) produces good freeze-dried products. For various batches, the PVP-K30 concentration was 5, 7.5, and 10 mg. Due to PVP-effectiveness K30's as a suspending agent and its ability to dissolve at low concentrations, less moisture is present, as demonstrated in table 1. High mannitol and low PVP-K30 concentrations exhibit the best drug and moisture contents, respectively.

Optimization data analysis and model-validation

The three factors with lower, middle and upper design points in coded and un-coded values are shown in table 2. As indicated in table 7, F4 exhibits promising outcomes when compared to the other 17 batches in terms of particle size, polydispersity index (PDI), and viscosity. The greater the surface area and the greater the solubility or bioavailability, the smaller the particle size. F4 was therefore chosen as the optimal batch. The lowest moisture content among all formulation batches was determined to be 2.9% w/w at high mannitol (15 mg) and low PVP-K30 (5 mg) values.

Table 2: ANOVA for quadratic model (Response 1: drug content)

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	391.52	9	43.50	9.84	0.0032	significant
A-Mannitol	0.5000	1	0.5000	0.1131	0.7465	
B-PVP	325.13	1	325.13	73.53	<0.0001	
C-Secondary drying temp	0.1250	1	0.1250	0.0283	0.8712	
AB	4.00	1	4.00	0.9047	0.3732	
AC	9.00	1	9.00	2.04	0.1967	
BC	30.25	1	30.25	6.84	0.0346	
A^2	8.55	1	8.55	1.93	0.2070	
B^2	5.81	1	5.81	1.31	0.2892	
C^2	5.81	1	5.81	1.31	0.2892	

Table 3: ANOVA for quadratic model (Response 2: moisture content)

Source	Sum of squares	df	Mean square	p-value		
Model	3.72	9	0.4137	81.58	<0.0001	significant
A-Mannitol	0.0050	1	0.0050	0.9859	0.3538	
B-PVP	3.00	1	3.00	591.80	<0.0001	
C-Secondary drying temp	0.0612	1	0.0612	12.08	0.0103	
AB	0.0000	1	0.0000	0.0000	1.0000	
AC	0.0900	1	0.0900	17.75	0.0040	
BC	0.2025	1	0.2025	39.93	0.0004	
A^2	0.0442	1	0.0442	8.72	0.0213	
B^2	0.2179	1	0.2179	42.97	0.0003	
C^2	0.0684	1	0.0684	13.50	0.0079	

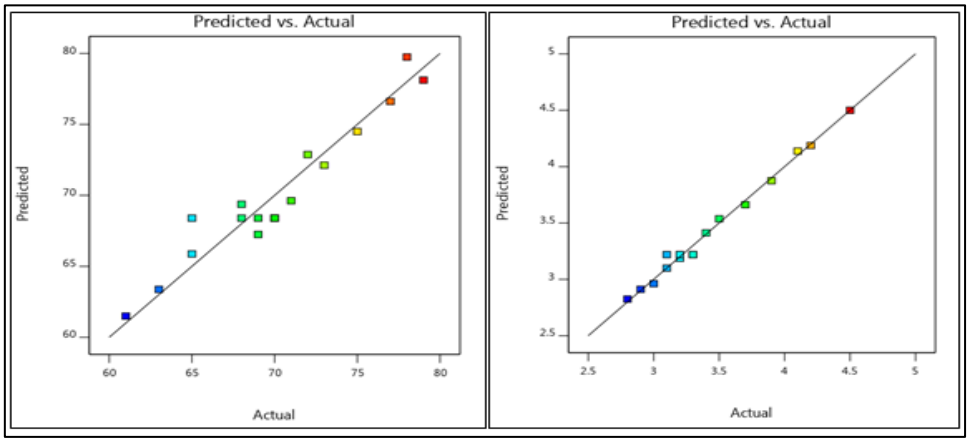


Fig. 2: (a) Predictive Vs actual response for response-1, (b) Predictive Vs actual response for response-2

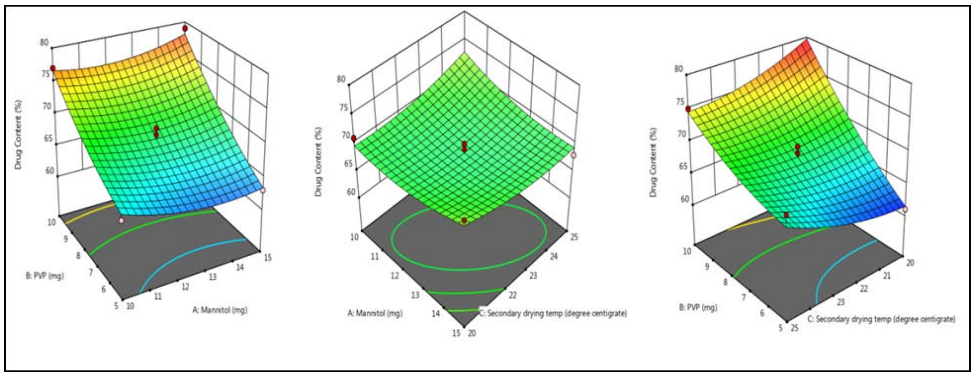


Fig. 3: (a) 3D response surface for response 1(Drug content) plotted between A Vs B; (b) 3D response surface plotted between A Vs C; (c) 3D response surface plotted between B Vs C

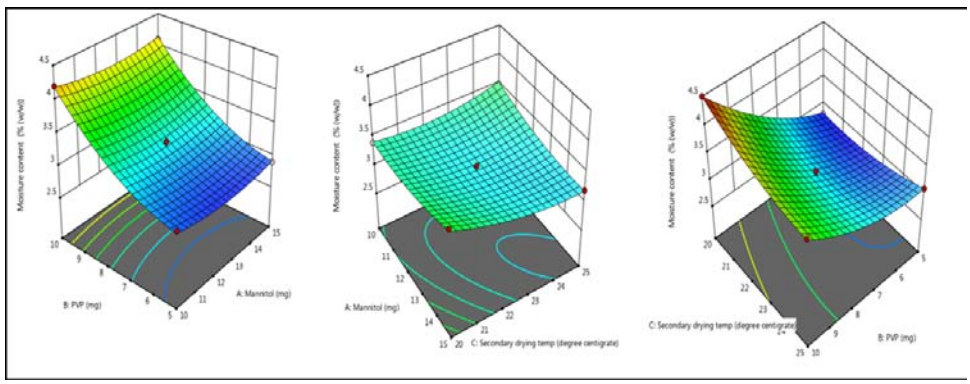


Fig. 4: (a) 3D response surface for response 2 (Moisture content) plotted between A Vs B; (b) 3D response surface plotted between A Vs C; (c) 3D response surface plotted between B Vs C

It was observed and inferred from fig. 3 and 4 that the concentration of mannitol and PVP-K30 is directly inversely related to the drug and moisture contents. i.e., the medication content and moisture content will increase with concentration. However, it was not discovered in a case of optimized batch, which is another reason to choose the F4 as the optimized batch.

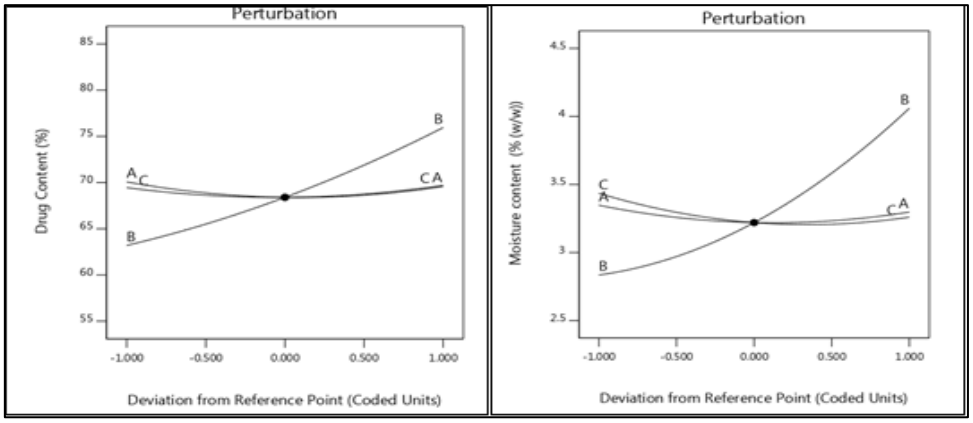


Fig. 5: Perturbation plot showing the deviation from the reference point (a) Drug content (b) Moisture content

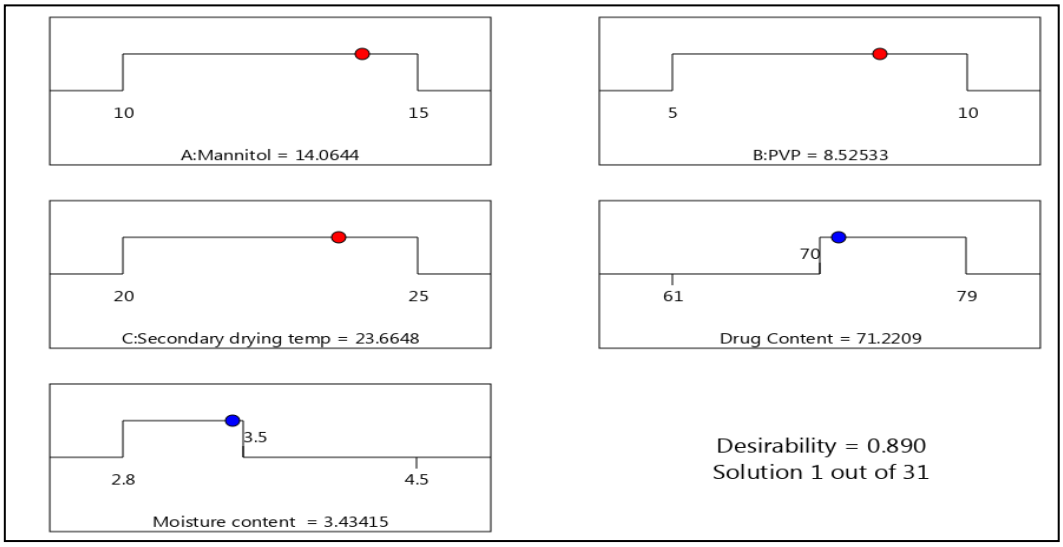


Fig. 6: Numerical method of finding the value for optimized formulation

Table 4: Coefficients of all variable included in Box-Behnken study

	Intercept	A	B	C	AB	AC	BC	A^2	B^2	C^2
Drug content	68.4	-0.25	6.375	0.125	1	-1.5	-2.75	1.425	1.175	1.175
p-values		0.7465	<0.0001	0.8712	0.3732	0.1967	0.0346	0.2070	0.2892	0.2892
Moisture content	3.22	-0.025	0.6125	-0.0875	1.40	-0.15	-0.225	0.1025	0.2275	0.1275
p-values		0.3538	<0.0001	0.0103	1.0000	0.0040	0.0004	0.0213	0.0003	0.0079

Applying uncoded values of factor levels, the least square regression method was performed using statistical software for the estimation of coefficients in polynomial function.

Drug content= 68.4-0.25A+6.37B+0.125 C+1.0 AB-1.5 AC-2.75 BC+1.425 A^2+1.175 B^2+0.175 C^2 ..... (1)

Moisture content=3.22-0.025A+0.612B-0.087 C+1.4 AB-0.15 AC-0.22 BC+0.102 A^2+0.22 B^2+0.1275 C^2..... (2)

Evaluation of nanosuspension

FTIR study

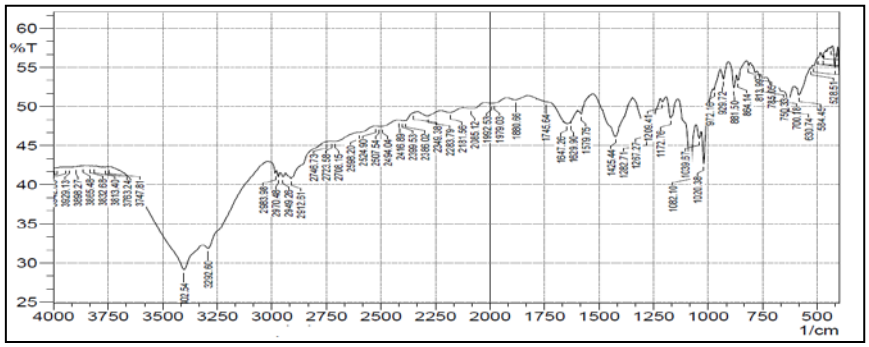
FTIR spectra ascertained characteristic peaks of lansoprazole. A characteristic broad spectrum at 3402.54 cm^{-1} appeared which indicates N-H stretching. Presence of pyridine ring exhibited at 1647 whereas 1425.44 observed for C-F stretching. The peak observed at 1267.27 cm^{-1} observed for C=C bending. The IR spectrum of Mannitol was characterized by a strong peak at 3400.62 cm^{-1} due to O-H stretching, a peak at 2970 cm^{-1} due to C-H stretching, and peaks at 1082 cm^{-1} and 1020 cm^{-1} due to C-O stretching. Meanwhile, characteristic peak at 3423 cm^{-1}, 1641 cm^{-1} and 1078.24 cm^{-1} appeared for PVP. The optimized nanosuspension subjected to FTIR analysis and evaluated for peak and intensity. A characteristic broad spectrum at 3423.76 cm^{-1} appeared, which indicates N-H stretching. Presence of pyridine ring exhibited a characteristic peak at 1641.48 cm^{-1}. The FTIR spectrum in fig. 7 indicated that the drug is well compatible with excipients (PVP and mannitol).

Table 5: Optimized concentration level of freeze-dried product

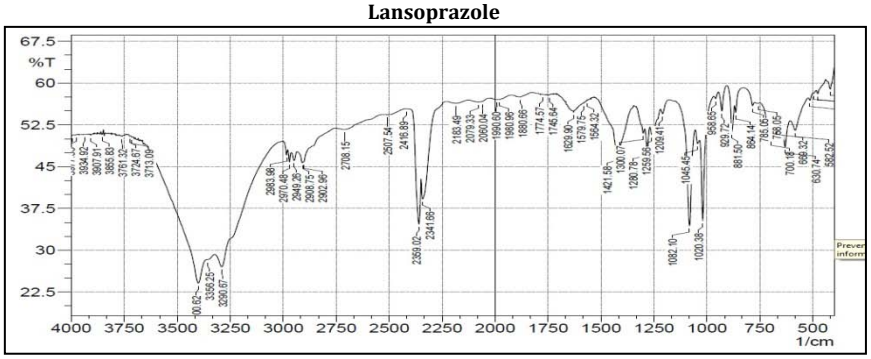
Factor	Name	Level	Low level	High level	SD	Coding
A	Mannitol	14.06	10.00	15.00	0.0000	Actual
B	PVP	8.53	5.00	10.00	0.0000	Actual
C	Secondary drying temp	23.66	20.00	25.00	0.0000	Actual

Table 6: Measured responses observed of optimized formula

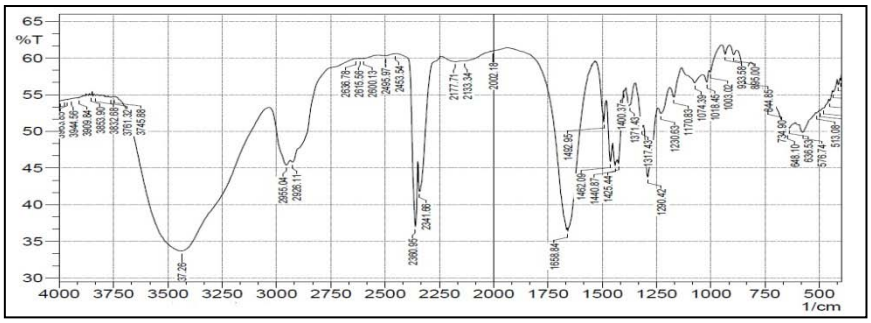
Response	Predicted mean	Predicted median	Observed	Std. Dev.	SE mean	95% CI low for mean	95% CI high for mean	95% TI low for 99% Pop	95% TI high for 99% Pop
Drug content	71.2209	71.2209	73.27	2.10272	1.02631	68.7941	73.6478	59.5427	82.8992
Moisture content	3.43415	3.43415	3.18	0.071214	0.0347585	3.35196	3.51634	3.03863	3.82966



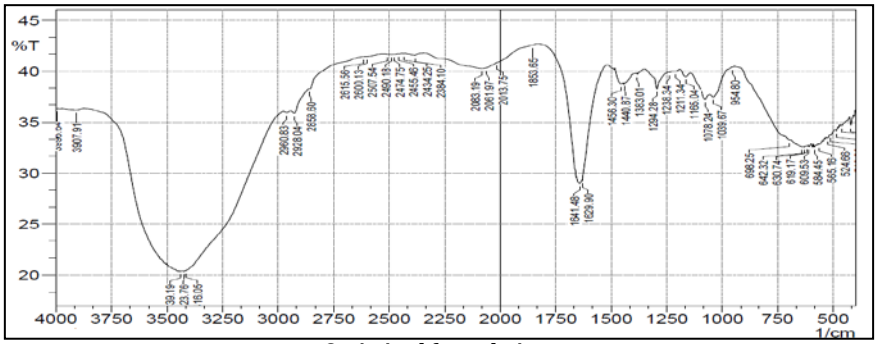
Lansoprazole



Mannitol



PVP K30



Optimized formulation

Fig. 7: The FTIR spectrum of drug and excipients to monitor compatibility

DSC study

Differential scanning calorimetry (DSC) is typically used to evaluate any potential interactions between a medicine and its excipients regarding the peak's emergence, disappearance, and shift toward either an endothermic or exothermic peak. At 186.89 °C, which corresponds to the melting point, a prominent endothermic characteristic peak for lansoprazole was found during the investigation. Additionally, it was noted that for mannitol and PVP, respectively, distinctive peaks formed at 171 °C and 169 °C coupled with a broad peak at 60.70 °C. While in nanosuspension, there is a noticeable reduction in peak intensity. At 81.06 °C, a wide broad peak could be seen, and at 175.01 °C, a little peak of poor strength could be seen. Peak intensity loss could be the result of poor storage conditions or a solvent system interaction. The DSC overlain curve of lansoprazole, mannitol, PVP K30, and optimized formulation (F4) is depicted in fig. 8.

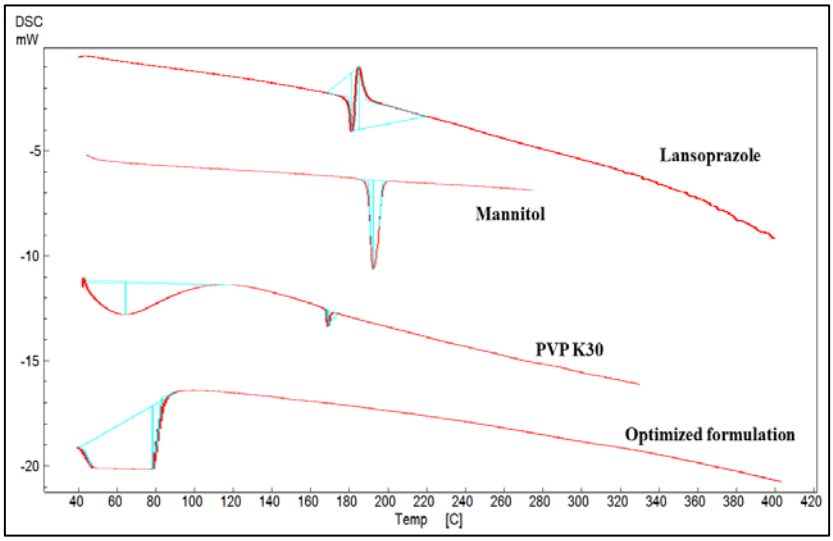


Fig. 8: DSC overlain curve of Lansoprazole, mannitol, PVP K30, and optimized formulation (F4)

XRD study

The diffractograms of lansoprazole and optimized nanosuspension shown in fig. 9. Change in the crystallinity of the drug can be determined by this technique. Pure drug was analyzed by the help of XRD 7000, Shimadzu. Pure lansoprazole showed the classical diffractogram of the crystalline substance. The drug provided a clear diffractogram with sharp and tall peaks. Lansoprazole showed definitive peaks at 16.8 °, 17.8, 24.5, 27.8, and 32 °25. It also noticed few other sharp peaks observed as seen in the optimized nanosuspension DSC curve. Whereas there is a significant loss of peak observed in lansoprazole nanosuspension. It could be stated that, the crystallinity of the pure drug lost as it got formulated in a freeze-dried nanosuspension form.

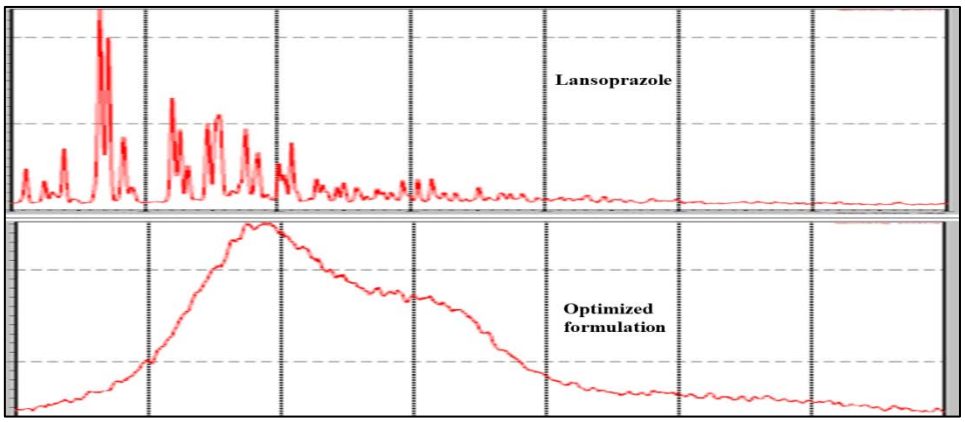


Fig. 9: XRD curves of lansoprazole and optimized formulation (F4)

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM)

The SEM and TEM were used to examine the shape and surface morphology of pure lansoprazole and freeze-dried optimized nanosuspension (F4) after preparation of suitable samples. For TEM pure drug and the optimized formulation was dissolved in a suitable solvent. Fig. 10 shows SEM and TEM images of optimized nanosuspension at 2000 magnification F4 revealing a 200 nm spherical shape of nanosuspension.

Particle size, polydispersity index, zeta potential, drug contents, and viscosity

The particle size of the nanosuspension is a fundamental factor because it decides the rate and extent of drug release as well as drug absorption. The smaller particle size offers a larger interfacial surface area for drug absorption and improves bioavailability. The calculation of the polydispersity index takes into account the particle mean size (125 nm), the refractive index of the solvent, the measurement angle and the variance of the distribution. Low

polydispersity index value might be associated with high homogeneity in the particle population, whereas high polydispersity index (PDI) values suggest a broad size distribution or even several populations. The polydispersity index (PDI) was 0.218 PDI to 0.396 PDI, and the zeta potential was-16.6 mV to-23.9 mV. It was observed that lansoprazole nanosuspensions displayed viscosity ranges from 44.6±1.6 cps to 88.7±1.8 cps. The drug contents of all the batches were determined and it was observed that it ranges from 70±2.8% to 97±1.5%. The optimized formulation (F4) showed maximum drug content of 97±1.5% out of all the trial batches. The particle size (nm), polydispersity index (PDI), zeta potential (ZP, mV), drug content (%±SD), and viscosity (cps) of all the batches are tabulated in table 7. The particulate distribution from 125.5 nm to 273.6 nm of optimized formulation (F4) is depicted in fig. 11.

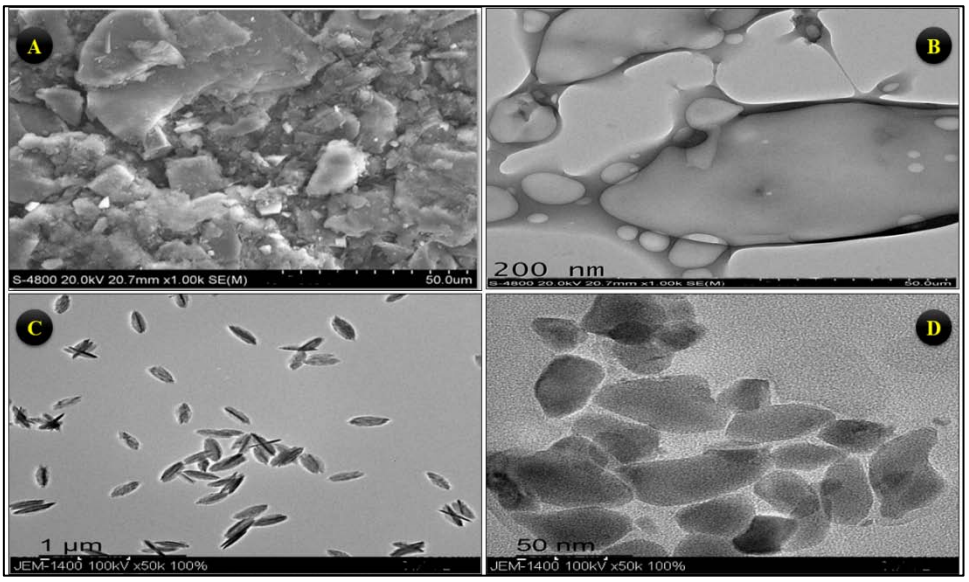


Fig. 10: a) SEM of lansoprazole (at ×1,00k); b) SEM of optimized formulation (F4, at ×1,00k); c) TEM of lansoprazole (at ×50k); d) TEM of optimized formulation (F4, at ×50k)

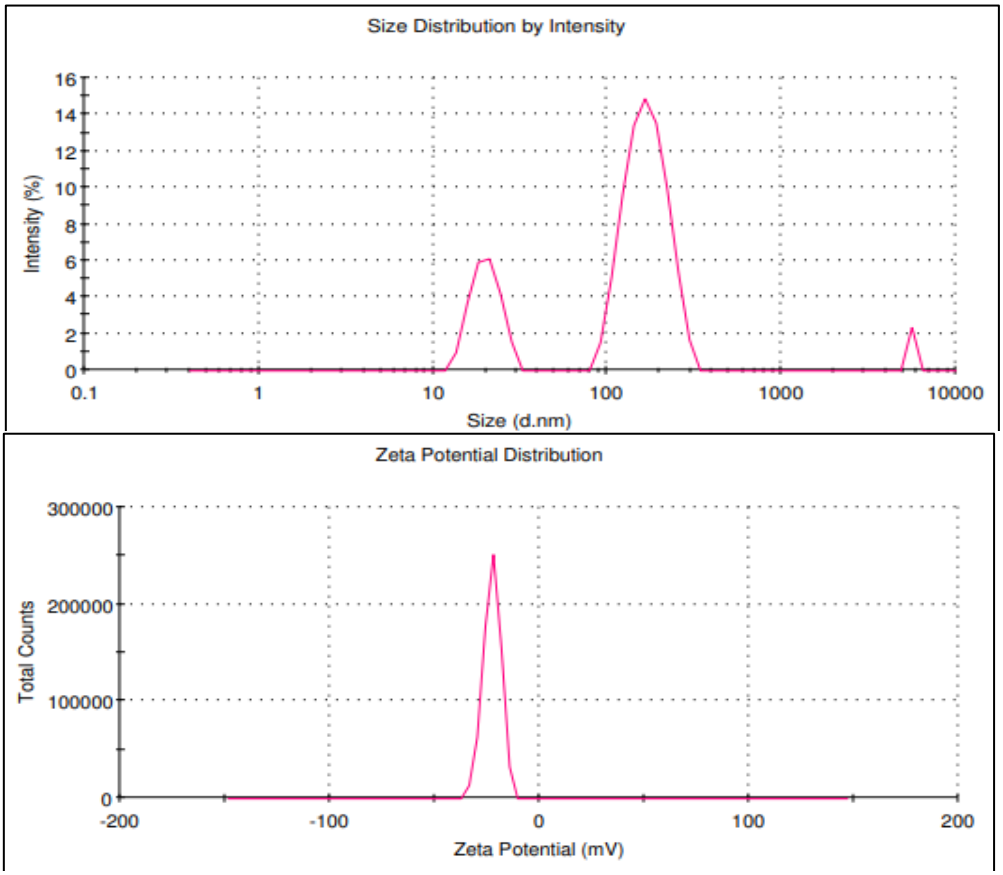


Fig. 11: The particle size distribution and zeta potential of optimized formulation (F4)

Table 7: The particle size (nm), polydispersity index (PDI), zeta potential (ZP, mV), drug content (%±SD), and viscosity (cps) of nanosuspension

Formulation code	Particle size (nm)	PDI	ZP (mV)	Drug content (%±SD)	Viscosity (cps)
F1	266.8	0.293	-16.6	94±1.3	45.1±2.1
F2	211.4	0.218	-21.9	92±2.6	47.3±1.8
F3	264.7	0.288	-21.4	90±2.8	54.6±1.6
F4	125.5	0.361	-22.7	97±1.5	88.7±1.8
F5	246.3	0.278	-23.2	86±1.7	64.9±2.3
F6	198.4	0.331	-21.1	83±1.9	67.2±2.1
F7	237.2	0.353	-19.8	91±2.0	65.8±1.9
F8	273.6	0.396	-23.9	83±2.1	66.3±3.0
F9	366.8	0.393	-26.6	74±1.3	55.1±2.1
F10	311.4	0.318	-11.9	72±2.6	67.3±1.8
F11	364.7	0.388	-29.4	70±2.8	44.6±1.6
F12	225.5	0.461	-24.7	77±1.5	48.7±1.8
F13	256.3	0.378	-21.2	76±1.7	54.9±2.3
F14	298.4	0.231	-23.1	80±1.9	63.2±2.1
F15	337.2	0.253	-18.8	81±2.0	64.8±1.9
F16	373.6	0.296	-26.9	73±2.1	61.3±3.0
F17	375.9	0.396	28.3	74±1.3	55.1±2.1

For drug content and viscosity; the experiments performed as n=3, the values are expressed as mean±SD

Saturation solubility

The saturation solubility of pure drug and the optimized formulation was determined and compared in different solvents. It was observed that optimized formulation displayed 4-fold more solubility of the drug in buffers at different pH. According to the Ostwald-Freundlich equation, this is due to a reduction in particle size when compared to a pure drug. The saturation solubility of pure drug and optimized formulation (F4) is depicted in table 8. Because they aid in keeping the pH level in a certain environment constant, phosphate buffers are frequently utilized. Since the properties closely resemble those of the human body, most scientists generally attempt to maintain a pH of 7.4 as frequently as possible. Phosphate buffers, however, have a variety of other applications as well. This is mostly due to the fact that the solutions are both isotonic and non-toxic (to the majority of cells).

Table 8: Saturation solubility of pure drug and F4

Medium	Pure drug concentration	F4 concentration
Distilled Water	2.50±0.13	3.50±0.19
Methanol	35±9.61	83±11.56
Buffer pH 1.2	135±29.61	653±21.56
Buffer pH 6.8	336±21.16	1240±39.41
Buffer pH 7.4	536±28.10	1840±30.50

The experiments performed as n=3, the values are expressed as mean±SD

***In vitro* drug release studies**

The *in vitro* drug release profile of pure drug and all the formulations are tabulated in table 9 and the graph is depicted in fig. 12. Out of all formulations, F4 showed 95.59% drug release up to 120 min, which was better than the other formulations. The release patterns of formulation nanosuspension were similar to pure drug suspension, differing only slightly in terms of burst and cumulative drug release. The release of free drug was found to be rapid, reaching 25% cumulative within a short period of time (120 min). In the case of nanosuspension, an initial burst release (23-64%) was observed for the first 30 min, after which drug release from nanosuspension increased steadily up to 120 min. A burst drug release was observed at first, which could be attributed to the smaller particle size attributed to the large surface area of the nanosuspension, or it could be due to diffusion of the drug from the outer shell of the nanosuspension. When nanosuspension was compared to pure drug solution, it was discovered that drug incorporation in nanosuspension results in controlled release. For about 120 min, nanosuspension demonstrated a significantly enhanced release pattern.

Table 9: *In vitro* drug release profile of Lansoprazole and batches of nanosuspension

Batches	Time (min)				
	15	30	60	90	120
F1	21.23	61.85	81.26	90.27	92.06
F2	20.25	62.65	82.71	90.2	93.36
F3	37.92	56.45	70.98	78.64	84.04
F4	23.62	64.93	79.55	83.8	95.59
F5	16.85	47.92	61.44	71.31	87.22
F6	19.72	58.03	76.14	81.26	91.34
F7	21.38	65.16	69.25	80.59	90.67
F8	17.93	64.87	72.04	79.86	84.73
F9	20.23	51.85	71.26	72.27	78.06
F10	18.25	52.65	62.71	70.2	83.36
F11	17.92	36.45	50.98	68.64	81.04
F12	22.62	54.93	69.55	73.8	85.59
F13	21.85	57.92	61.44	71.31	80.22
F14	19.72	48.03	56.14	71.26	81.34
F15	18.38	55.16	69.25	79.59	83.67
F16	17.93	54.87	62.04	79.86	84.73
F17	15.93	44.87	62.04	75.86	81.73
Pure drug	1.607	4.857	8.116	14.23	25.08

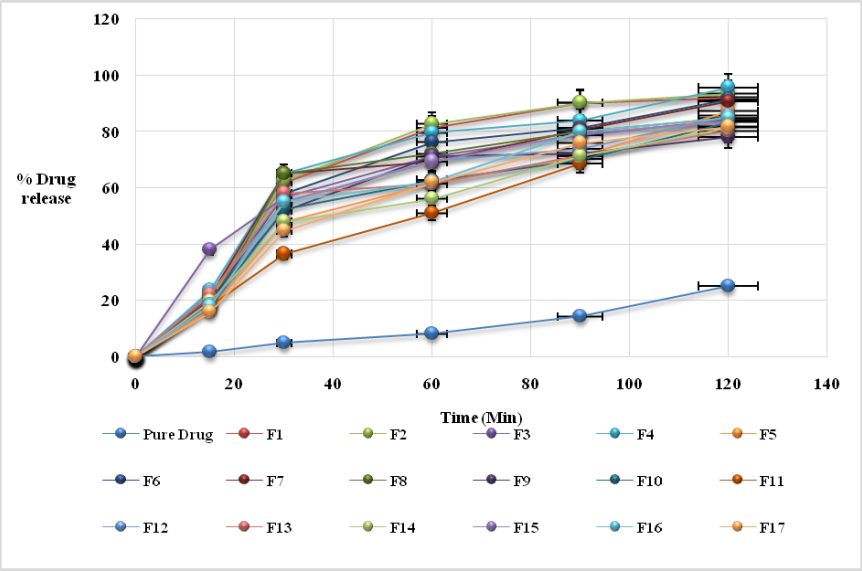


Fig. 12: *In vitro* drug release profile of pure drug and nanosuspension formulations

Stability studies

Stability studies were conducted for the optimized formulation (F4) in accordance with the ICH guidelines, as shown in table 10. The intermediate storage condition for stability studies has been changed from 30 °C±2 °C and 65%±5% RH. It focuses on the fact that there was no change in drug content, *in vitro* drug release, or particle size, but it was within acceptable limits.

Table 10: The stability studies of optimized formulation (F4)

Period	% Drug content	Particle size	% Drug release
8 d	97.40	124.1	95.59
14 d	96.97	123.9	95.13
21 d	97.21	125.3	94.96
30 d	97.88	125.6	95.49

The drug lansoprazole, also marketed under the name prevacid, lowers stomach acid. It is used to treat peptic ulcer disease, gastroesophageal reflux disease, and zollinger-ellison syndrome. It is categorized as a BCS class-II medication with limited solubility and high permeability and is now offered in tablet dose form. It is, therefore not very bioavailable. Any effort to increase the solubility of lansoprazole may boost the drug's bioavailability [13]. Taking this into consideration, the current study aimed to develop a lansoprazole-containing nanosuspension and evaluate the formulation for its physicochemical properties as well as *in vitro* release studies.

Amongst various pharmaceutical approaches, the nanoparticle approach is important today because it influences the physicochemical and biological properties of the drug. Lansoprazole was attempted to formulate in a nanosuspension in the current study based on this factor. For the preparation of nanosuspension, various methods have been reported. The nanopure method was chosen for the current study because it is simple to follow at the laboratory scale and can ensure small particle formulation, among other benefits. Many factors influence the particle size of nanosuspension, with the influence of nanosuspension carriers being particularly important.

A suspension is homogenized in a water-free medium using the Nanopure method. It is "deep-freeze" homogenization, which involves homogenizing drug suspensions in the non-aqueous medium at 0 °C or lower. Because water, oils, and fatty acids have extremely high boiling points and low vapor pressures, a static pressure drop is insufficient to initiate cavitation in nanopure technology [35]. According to the findings of this study, these significant variables also play an important role in controlling the mean particle size and zeta potential in nanosuspension, and it may be possible to prepare nanosuspension with a mean particle size less than 200 nm by optimizing these variables.

The large surface area and high total surface energy generated by nanosuspension are thermodynamically unfavorable, resulting in a tendency to agglomerate to minimize surface energy. Agglomeration can result in a variety of problems, such as crystal growth, rapid settling, and creaming [8]. PVP-K30 was used as a suspending agent in this study to address these issues. These agents are frequently thought to be beneficial in the formation of nanosuspension. The type and quantity of agents also influenced the physical stability of the nanosuspension. As a result, different agent concentrations were used to control the stability of various formulations. According to the results, batch F4 is more stable than the other batches.

Crystallization theory can explain particle size formation. This theory includes steps such as particle nucleation, molecular growth, and agglomeration or aggregation. The driving force in this process is supersaturation, which is useful in determining the nucleation rate and diffusion-controlled growth rate. The faster crystal growth, the greater the supersaturation [36]. After various variables were optimized, submicron-sized particles with an average diameter of 100-250 nm were successfully produced.

When compared to pure lansoprazole, which has a mean particle size of 125.5 nm, the results showed that lansoprazole nanosuspension had a significantly smaller particle size. Following the nanopure process, the release rate of selected batches was significantly improved. When compared to the control, the release rate of lansoprazole nanosuspensions was more than four-fold. The increased release rate could be attributed to the increased surface area of the dissolvable nanosized drug particles as well as the reduced diffusion layer thickness.

A stable nanosuspension should have a zeta potential greater than 20mV, because higher zeta potential values produce repulsive force between the particles in the nanosuspension, preventing particle aggregation and thus stabilizing the nanosuspension. According to the findings of this study, the zeta potential of formulation F4 was discovered to be-22.7 mV, ensuring the stability of formulation F4. PVP-K30 polymer in F4 demonstrated an effective cumulative drug waiver of 120 min when compared to other formulations.

CONCLUSION

Very low bioavailability is a key issue with poorly soluble medicines. The situation is much more complex for medications like lansoprazole which is poorly soluble in both aqueous and nonaqueous environments, corresponding to BCS class II as categorized by the biopharmaceutical classification system. Formulation as nanosuspension is an appealing and promising solution to tackle these challenges. Dispersion of the pure, poorly water-soluble medication, without any matrix ingredient, is what makes up a nanosuspension. Preparation of nanosuspension is easy and applies to all medicines which are water-insoluble. A nanosuspension not only solves the problems of poor solubility and bioavailability but also affects the pharmacokinetics of medicine and so increases drug safety and efficacy. In the present study, we have used nanopure method to formulate lansoprazole containing nanosuspension. By adjusting the operation parameters, the particle size of lansoprazole can be obtained in the nano-size ranges. The drug content was 97.2±1.2 percent, the average particle size was 125.5 nm, the polydispersity index was 0.361, and the zeta potential was-22.7 mV. The optimized nanosuspension displayed most promising drug release profile and showed improved solubility of lansoprazole. From the present study we have concluded that nanosuspension can serves as better formulation for delivery of lansoprazole.

ACKNOWLEDGMENT

The authors are thankful to Trustees and Dr. Sanjay Kshirsagar sir Principal, BKC MET Institute of Pharmacy, Nashik for providing all the necessary facilities and support to conduct the research work.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

All the authors confirm that there is no conflict of interest.

REFERENCES

1. Sharma P, Denny WA, Garg S. Effect of wet milling process on the solid state of indomethacin and simvastatin. Int J Pharm. 2009 Oct 1;380(1-2):40-8. doi: 10.1016/j.ijpharm.2009.06.029, PMID 19576976.
2. Aher SS, Malsane ST, Saudagar RB. Nanosuspension: an overview. Int J Curr Pharm Sci. 2017;9(3):19-23. doi: 10.22159/ijcpr.2017.v9i3.19584.
3. Verma S, Lan Y, Gokhale R, Burgess DJ. Quality by design approach to understand the process of nanosuspension preparation. Int J Pharm. 2009;377(1-2):185-98. doi: 10.1016/j.ijpharm.2009.05.006, PMID 19446617.
4. VDN S, SVN P. Nanosuspensions: a promising drug delivery systems. Int J Pharm Sci Nanotechnol. 2009;2(4):679-84.
5. Patravale VB, Date AA, Kulkarni RM. Nanosuspensions: a promising drug delivery strategy. J Pharm Pharmacol. 2004;56(7):827-40. doi: 10.1211/0022357023691, PMID 15233860.
6. Rabinow BE. Nanosuspensions in drug delivery. Nat Rev Drug Discov. 2004;3(9):785-96. doi: 10.1038/nrd1494, PMID 15340388.
7. Wang Y, Zheng Y, Zhang L, Wang Q, Zhang D. Stability of nanosuspensions in drug delivery. J Control Release. 2013;172(3):1126-41. doi: 10.1016/j.jconrel.2013.08.006, PMID 23954372.
8. Wang L, Du J, Zhou Y, Wang Y. Safety of nanosuspensions in drug delivery. Nanomedicine. 2017;13(2):455-69. doi: 10.1016/j.nano.2016.08.007, PMID 27558350.
9. Barradell LB, Faulds D, McTavish D. Lansoprazole a review of its pharmacodynamic and pharmacokinetic properties and its therapeutic efficacy in acid-related disorders. Drugs. 1992;44(2):225-50. doi: 10.2165/00003495-199244020-00007, PMID 1382017.
10. Fort FL, Miyajima H, Ando T, Suzuki T, Yamamoto M, Hamashima T. Mechanism for species-specific induction of leydig cell tumors in rats by lansoprazole. Fundam Appl Toxicol. 1995;26(2):191-202. doi: 10.1006/faat.1995.1090, PMID 7589908.
11. Hishinuma T, Suzuki K, Yamaguchi H, Yamagishi H, Koike T, Ohara S. Simple quantification of lansoprazole and rabeprazole concentrations in human serum by liquid chromatography/tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2008;870(1):38-45. doi: 10.1016/j.jchromb.2008.05.034, PMID 18555753.
12. Kasture SV, Gondkar SB, Darekar AB, Priyobrata D, Bhambar KV. Enhancement of dissolution rate of lansoprazole using liquisolid tablet technique. Int J Pharm Res. 2011;3(2):27-31.
13. Landes BD, Petite JP, Flouvat B. Clinical pharmacokinetics of lansoprazole. Clin Pharmacokinet. 1995;28(6):458-70. doi: 10.2165/00003088-199528060-00004, PMID 7656504.
14. Keck CM, Muller RH. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenisation. Eur J Pharm Biopharm. 2006;62(1):3-16. doi: 10.1016/j.ejpb.2005.05.009, PMID 16129588.
15. Nida DL, Rahman MS, Carlson KD, Richards Kortum R, Follen M. Fluorescent nanocrystals for use in early cervical cancer detection. Gynecol Oncol. 2005;99(3) Suppl 1:S89-94. doi: 10.1016/j.ygyno.2005.07.050, PMID 16139342.
16. Özaltín N. Determination of lansoprazole in pharmaceutical dosage forms by two different spectroscopic methods. J Pharm Biomed Anal. 1999;20(3):599-606. doi: 10.1016/s0731-7085(99)00061-8, PMID 10701978.
17. Geetha G, Poojitha U, Khan KAA. Various techniques for preparation of nanosuspension-a review. Int J Pharm Res Rev. 2014;3(9):30-7.
18. Vedaga SB, Gondkar SB, Saudagar RB. Nanosuspension: an emerging trend to improve the solubility of poorly water-soluble drugs. J Drug Deliv Ther. 2019;9(3):549-53.
19. Vittal GV, Deveswaran R, Bharath S, Basavaraj B, Madhavan V. Formulation and characterization of ketoprofen liquisolid compacts by box-behnken design. Int J Pharm Investig. 2012;2(3):150-6. doi: 10.4103/2230-973X.104398, PMID 23373006.
20. Ferreira SLC, Bruns RE, Ferreira HS, Matos GD, David JM, Brandao GC. Box-behnken design: an alternative for the optimization of analytical methods. Anal Chim Acta. 2007;597(2):179-86. doi: 10.1016/j.aca.2007.07.011, PMID 17683728.
21. Robinson TJ. Box-behnken designs. Wiley STATS Ref Stat Ref Online; 2014.
22. Zhang TF, Yang JF, Lin DKJ. Small box-behnken design. Stat Probab Lett. 2011;81(8):1027-33. doi: 10.1016/j.spl.2011.02.024.
23. Almeida MO, Barros DAS, Araujo SC, Faria SHDM, Maltarollo VG, Honorio KM. Study on molecular structure, spectroscopic properties (FTIR and UV–vis), NBO, QTAIM, HOMO-LUMO energies and docking studies of 5-fluorouracil, a substance used to treat cancer. Spectrochim Acta A Mol Biomol Spectrosc. 2017;184:169-76. doi: 10.1016/j.saa.2017.04.070, PMID 28494379.
24. Menczel JD, Judovits L, Prime RB, Bair HE, Reading M, Swier S. Differential scanning calorimetry (DSC). Therm Anal Polym Fundam Appl. 2008:7-239.
25. Epp J. X-ray diffraction (XRD) techniques for materials characterization materials characterization using nondestructive evaluation (NDE). Methods. 2016:81-124.
26. Erdman N, Bell DC, Reichelt R. Scanning electron microscopy. In: Springer handbooks; 2019. p. 229-318. doi: 10.1007/978-3-030-00069-1_5.

27. Hsieh YL, Taylor LS. Salt stability-effect of particle size, relative humidity, temperature and composition on salt to free base conversion. Pharm Res. 2015;32(2):549-61. doi: 10.1007/s11095-014-1484-2, PMID 25145335.
28. Siddiqui N, Rauf A, Latif A, Mahmood Z. Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal unani drug gul-e-zoofa (Nepeta bracteata Benth). J Taibah Univ Med Sci. 2017;12(4):360-3. doi: 10.1016/j.jtumed.2016.11.006, PMID 31435264.
29. Mischenko SV, Mordasov MM, Savenkov AP, Sychev VA. Analysis of the influence of sizes of a vessel with a liquid on the readings of Brookfield viscometer. Meas Tech. 2020;63(4):288-94. doi: 10.1007/s11018-020-01784-2.
30. Frank KJ, Rosenblatt KM, Westedt U, Holig P, Rosenberg J, Magerlein M. Amorphous solid dispersion enhances permeation of poorly soluble ABT-102: true supersaturation vs. apparent solubility enhancement. Int J Pharm. 2012;437(1-2):288-93. doi: 10.1016/j.ijpharm.2012.08.014, PMID 22951865.
31. Batheja P, Sheihet L, Kohn J, Singer AJ, Michniak Kohn B. Topical drug delivery by a polymeric nanosphere gel: formulation optimization and *in vitro* and *in vivo* skin distribution studies. J Control Release. 2011;149(2):159-67. doi: 10.1016/j.jconrel.2010.10.005, PMID 20950659.
32. Galey WR, Lonsdale HK, Nacht S. The *in vitro* permeability of skin and buccal mucosa to selected drugs and tritiated water. J Invest Dermatol. 1976;67(6):713-7. doi: 10.1111/1523-1747.ep12598596, PMID 1033956.
33. Dasari V, Shaikh A, Sisodiya D, Bhargava T, Dangi R, Nagwe S. Stability study of mucoadhesive microsphere containing nateglinide by using biodegradable polymer chitosan. J Pharm Res Int. 2021:866-72. doi: 10.9734/jpri/2021/v33i47A33086.
34. Tiwari V, Kumar BMN, Sharma V, Gautam DNS. Accelerated stability study and quantitative estimation of piperine in draksharishta. Int J Res Pharm Sci. 2017;8(2):209-15.
35. Awasthi R, Manchanda S, Das P, Velu V, Malipeddi H, Pabreja K. Poly(vinylpyrrolidone). Inengineering of biomaterials for drug delivery systems. Woodhead Publishing; 2018. p. 255-72.
36. Moschwitzer J, Muller RH. Drug nanocrystals-the universal formulation approach for poorly soluble drugs. In: Nanoparticulate drug delivery systems. CRC Press; 2007. p. 71-88. doi: 10.1201/9781420008449.ch5.