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FORMULATION AND OPTIMIZATION OF CHLORTHALIDONE LOADED NANO-PARTICLES BY ANTISOLVENT PRECIPITATION USING BOX-BEHNKEN DESIGN

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Keywords:

Chlorthalidone, Nanoparticles, Box-Behnken factorial design, Freeze drying, Solubility

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ABSTRACT: Chlorthalidone is a long-acting diuretic recommended for treatment of oedema associated with congestive heart failure. It is oral active diuretic mainly acting on distal convoluted tubule of nephron. Chlorthalidone is poorly soluble in water at room temperature. Nanoparticles have great potential as a carrier and can improve the solubility of poorly water-soluble drugs like chlorthalidone. The aim of the present study was to formulate and optimize the chlorthalidone nanoparticles using Box-Behnken factorial design approach. Effect of three independent variables (concentration of polymer, amount of surfactant and ultrasonication frequency) on two dependent variables such as particle size and dissolution of the drug was studied. The nanoparticles of chlorthalidone were formulated by anti-solvent precipitation-ultrasonication-freeze drying technology to improve its solubility and dissolution. The samples were characterized using Horiba nanoparticles analyzer, Zeta potential analyzer, Differential Scanning Calorimetry (DSC), Fourier Transform Infrared Spectroscopy (FTIR), Powder X-ray Diffraction (PXRD), and Field Emission Scanning Electron Microscopy (FESEM). The average particle size of 342.5 nm with a Polydispersibility index 0.158 was confirmed by dynamic light scattering. Differential scanning calorimetry and powder X-ray diffraction revealed reduced crystallinity of chlorthalidone. Freeze-dried nanoparticles were observed as spherical shape under field emission scanning electron microscopy. The value of zeta potential was -15.5 mV. *In-vitro* dissolution study by dialysis bag investigated improvement of dissolution rate. The stability of the developed nanoparticle was confirmed by the accelerated stability study of developed nanoparticles. These results showed an increase in the saturation solubility and drug release of chlorthalidone due to particle size reduction and amorphous nature of the drug.

INTRODUCTION: Bioavailability, as well as dissolution of poorly water-soluble drugs, can be improved by the preparation of nanoparticles. Use of novel carriers such as micronization, modifications in excipients, liposomal drug delivery system, and solid dispersion, among others, have shown improved solubility [1].

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Novel carriers have been thoroughly investigated for improving drug solubility. The improvement of solubility was achieved by selecting a carrier system, a proper method of preparation, and optimal drug-carrier ratios.

Moreover, the combination of excipients with other materials can improve the functions of a dosage form [2]. The water solubility of drugs greatly influences pharmacokinetic and pharmacodynamic properties [3]. Biopharmaceutical Classification System (BCS Classification) has been a critical tool for the development of the formulation of various drugs [4]. Based on the solubility and intestinal permeability of the drugs, the BCS categorizes

them into one of four categories. Chlorthalidone is BCS class IV drug with low solubility and low permeability. Chlorthalidone has less than 0.1 mg/mL solubility at 37 ºC in an aqueous medium which makes it as a poor candidate for bioavailability. Solubility improvement by solid self micro-emulsifying drug delivery system of chlorthalidone has been reported by Dangre P. V *et al.;* Chlorthalidone is an oral active diuretic mainly acting on distal convoluted tubule of nephron. It shows it prolong action for 72 h. It is well known antihypertensive drug which can reduce the volume of extracellular fluid, the volume of plasma, output of cardiac muscle, glomerular filtration rate, and renal plasma flow. Chlorthalidone is rapidly absorbed on oral administration, and oral bioavailability of chlorthalidone is about 64% [5].

Several methods for improvement of the solubility of active moiety include nanosization by high shear mixing, uniform distribution and stabilization of drug by use of polymers or stabilizers, milling and antisolvent precipitation-ultrasonication [6, 7]. Out of these, antisolvent precipitation-ultra-sonication technology is simple, economical, rapid, and straight forward [8]. This is a promising technology to get nanosized particles, where higher super-saturation is achieved due to rapid precipitation of drug from water-miscible solvent to aqueous solvent. This phenomenon is helpful to improve the rate of supersaturation and nucleation and ultimately, it could reduce particle size [9, 10].

This study mainly concentrates on the formulation of nanoparticles for enhancement of solubility and dissolution rate of chlorthalidone with the aid of anti-solvent precipitation- ultrasonication-freeze drying technology. Further that, Box-Behnken factorial design approach was applied to find out effect of the independent variable on dependent variables. It was hypothesized that stable amorphous and nanosize particles of chlorthalidone formed due to nano-sization and freeze-drying technique would be capable of improving the solubility and bio-availability of chlorthalidone.

EXPERIMENTAL:

Materials: Chlorthalidone (Ipca Laboratory, Mumbai, India). Poloxamer-188 (Ipca Laboratory, Mumbai, India), Hydroxylpropyl methylcellulose (HPMC E_{50}), Sodium carboxymethylcellulose (NaCMC), Sodium dodecyl sulphate (SDS), Polyvinyl alcohol (PVA), Polyvinylpyrrolidone K-30 (PVK-30), and Polyvinylpyrrolidone K-90 (PVK-90) was procured from Aditya Chemicals (Pune, India). HPLC grade deionized water was used for the production of nanoparticles.

Formulation of Nanoparticles of Chlorthalidone: The nanoparticles were prepared by dissolving the exact quantities of HPMC E50, poloxamer, sodium CMC, PVA, PVK-30 and PVK-90 (0.5% w/v - 1.5% w/v) and SDS (0.02% w/v -0.08% w/v) in deionized water at room temperature (25 ºC ± 1 ºC) under constant stirring [10]. A filtered chlorthalidone solution in water-miscible solvent (methanol) was prepared. Then the drug solution was injected at a controlled speed of 0.2 ml/min in an aqueous system. The mixture was stirred by means of a mechanical stirrer (Emtek Instruments, Mumbai, India) at 5000 rpm for 10 min. Further, it was sonicated with a probe sonicator (Leelasonic 125-upp, Mumbai, India) at 5 mm spindle for 10-60 min. Finally, all the samples were centrifuged (Remi Instruments, Mumbai, India) for 15 min.

The resultant suspension was freezed (α LD plus 1-2, Germany) at -40 °C (time required 4 h). This study was continued by freeze-drying the suspension under controlled experimental parameters such as temperature (-48 ºC–0 ºC), time (24 h) and pressure (10 mbar). The freeze-dried nano-particles stored in a tightly closed container at room temperature until further use. The statistical analysis of developed formulation was performed by Box-Behnken factorial design approach.

Characterization of Freeze-Dried Nanoparticles: Analysis of Particle Size and Zeta potential: Particle size and particle size distribution of freeze-dried nanoparticles were determined by Horiba nanoparticles analyzer (Horiba SZ-100, ver. 1.90), which was working on the principle of Dynamic Light Scattering (DLS). The diluted samples were analyzed in triplicate at 25 °C ± 5 °C. Laser Doppler Anemometry (Horiba SZ-100, ver. 1.90) technology was employed for predicting the potential difference between nanosized particles. Zeta potential measurement was carried out in the electrophoretic cell with an applied potential of 3.3 V. Each sample was measured in triplicate.

Morphological Characterisation: A Field Emission Scanning Electron Microscopy (FESEM) technology (Nova NanoSEM 450, India) was used for further particle size and shape analysis of chlorthalidone and freeze-dried nanoparticles. Before analysis samples were coated with gold, and photograph was taken at different magnification power. All samples were observed with operating voltage 5.00 kV and images analyzed by software (FESEM xT microscope control).

Infrared Spectroscopy (FTIR): FTIR spectrum of chlorthalidone and freeze-dried nanoparticles were recorded by FTIR spectrophotometer (Jasco M-4100 type A filter, Japan) to study the interaction of chlorthalidone with other formulation ingredients. Samples for analysis were prepared by KBr press pellet technique where 3 mg of samples were dispersed in 9 mg of KBr and pressed by applying hydrostatic force 8 torr per square centimeter for 1 min.

Differential Scanning Calorimetry (DSC): Thermal behavior of chlorthalidone, Physical mixture (PM-powder blend of drug and excipients) and freeze-dried nanoparticles were characterized by DSC (Shimadzu Co., Japan DSC-60). Three milligram of samples were placed in an aluminium pan and heated within temperature range 20 °C to 250 °C with heating rate 10 °C/min maintained in a nitrogen atmosphere.

PXRD Study: PXRD diffractogram of chlorthalidone and freeze-dried nanoparticles were recorded by X-ray diffractometer (Ultima-IV). Powder samples were placed in a glass sample holder and irradiated with the copper light source. The potential difference of 40 mV and current 40 mA was applied along with scan velocity 5°/min over a 2θ range 5°- 80°.

Determination of Saturation Solubility: Saturation solubility of chlorthalidone and freeze-dried nanoparticles was performed by flask shaking technique. Solubility variation in a different medium like deionized water and pH 6.8 phosphate buffer were evaluated. Excess samples were separately dispersed in 25 ml deionized water and pH 6.8 phosphate buffer in sealed vials. These vials were stirred on an orbital shaking thermostable incubator (Remi, Mumbai, India) overnight at 36 °C – 38 °C at 150 rotations per minute. Further, samples were centrifuged and separated by filtration. Analysis of filtered sample performed in triplicate on spectrophotometer at 230 nm. All data recorded as average with standard deviation (SD).

Drug Release Study: *In-vitro* dissolution study of freeze dried nanoparticles and chlorthalidone was performed by dialysis bag technique. Nanoparticles equivalent to 12.5 mg of chlorthalidone were transferred to dialysis bag and both ends of bag was closed. Then bags were tied to paddle of dissolution testing apparatus (Electrolab Dissolution Tester TDT-06P, India) containing deionized water and pH 6.8 phosphate buffer (900 ml) as dissolution medium. Study was performed at temperature 37 °C ± 0.5 °C with speed of rotation 75 rpm. Sampling was performed at particular time intervals and amount of drug released from nanoparticles estimated by UV visible spectrophotometer (Analytical Technologies 20108+, Germany). The collected data indicated as the mean ± SD from three different independent release experiments.

Stability Study: Accelerated stability study of freeze-dried samples were carried out in humidity control chamber (Lab Hosp, India) at 40 °C ± 2 °C / 75% ± 5% relative humidity wherein particle size, saturation solubility, dissolution rate, and retention of amorphous nature were analyzed over the period of six months.

RESULTS AND DISCUSSION: Traditional approach to pharmaceutical development is very time-consuming and costlier. Now a day, the statistical approach is widely used for development of nano-particles, where the relationship of independent variables with dependent variables is demonstrated in a systemic manner [11, 12].

Response surface methodology (RSM) is proven to be very significant for the optimization of pharmaceutical dosage form. In RSM, the Box-Behnken approach was used to measure linear and interactive relationships between independent and dependent variables to get optimized nanoparticulate formulation. As mentioned in experimental section, different polymers were tried to produce nanoparticles. In comparison to HPMC E50, poloxamer, sodium CMC, PVA, PVK-30, and

PVK-90; poloxamer produced stable nano-suspension. Therefore, further study was performed by poloxamer-188 as polymer. In the present invention Box-Behnken factorial design approach was used for the optimization of polymeric nanoparticles of chlorthalidone. Total 17 batches namely, CLNP1- CLNP17 were formulated. The concentration of polymer, amount of surfactant and ultrasonication frequency were chosen as independent variables. These three variables are designated as: A- Concentration of poloxamer, B- Concentration of surfactant, C- Ultrasonication frequency. These variables are set at three levels as high (+1), medium (0), and low (-1). Many researchers have been noted that influence of individual independent variables and combination of variables on dependent variables can be easily investigated by the use of three-dimensional surface response graph. Three dimensional, counter plot, interaction plot, perturbation graph, predicted verses actual graph, and cube graph were plotted.

Design Expert 11.1.2.0 response surface software was applied to the analyzed effect of independent variables on the response (dependent variables).

TABLE 1: INDEPENDENT VARIABLES USED IN BOX-BEHNKEN FACTORIAL DESIGN APPROACH OF CHLORTHALIDONE NANOPARTICLES

S. no.	Variable	Symbol	Unit	Levels		
				-1	0	+1
1	Concentration of polymer	A	mg	50	100	150
2	Concentration of surfactant	B	mg	20	40	60
3	Ultrasonication frequency	C	Hz	10	30	50

TABLE 2: DEPENDENT VARIABLES USED IN BOX-BEHNKEN FACTORIAL DESIGN APPROACH OF CHLORTHALIDONE NANOPARTICLES

S. no.	Variable	Symbol	Unit	Constraint
1	Particle size	R1	Nm	Minimum
2	Drug dissolution	R2	(%)	Maximum

TABLE 3: SUMMARY OF RESPONSE OF CHLORTHALIDONE NANOPARTICLES

Response	Analysis	Mini	Maxi	Mean	Std. Dev.	Ratio	Transform	Model
R1	Polynomial	282	349	329.97	24.82	1.24	None	Quadratic
R2	Polynomial	67.3	79.5	73.44	4.03	1.18	None	Linear

Effect of Independent Variables on Particle Size: Following polynomial regression equation shows the effect of independent variables on particle size.

$$R1 = +341.66 + 30.68A + 0.62B - 0.88C + 0.02AB + 1.55AC + 0.17BC - 23.00A^2 + 0.37B^2 - 2.20C^2$$

The three-dimensional graph (3D), counterplots (2D), and cube graph for a response (R1) are shown in **Fig. 1**.

TABLE 4: FIT STATISTICS OF RESPONSE R1 OF CHLORTHALIDONE NANOPARTICLES

Std. Dev.	1.60	**R^2**	0.9982
Mean	329.97	Adjusted R^2	0.9958
C.V. %	0.4848	Predicted R^2	0.9780
		Adeq Precision	54.6373

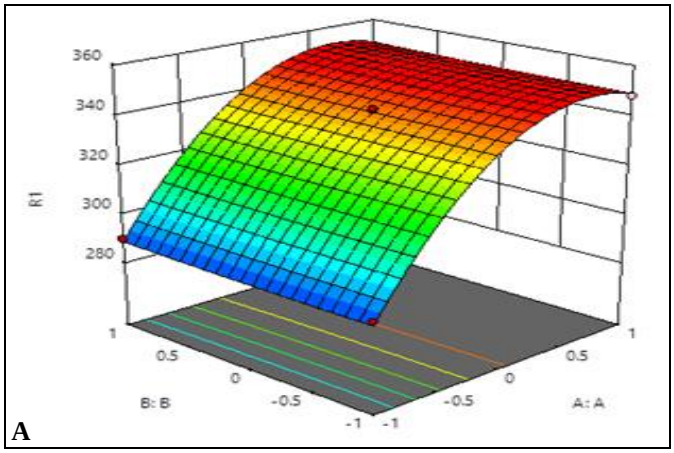
The influence of independent variables on response was predicted by the ANOVA test with a corresponding P-value. The suitable model for the analysis of main and interactive effects depends on the value of P. If the P-value is less than 0.0001 indicates a significant model, whereas the high value of P demonstrates non-significance.

The value of R^2 was found to be 0.9982. The suggested was a quadratic model with a corresponding P value less than 0.0001.

The obtained F value-427 was confirmed that the model is significant. In this invention, the positive coefficient of three factors, namely A, A2, C2 was found to be significant model terms while others were not. The non-significant lack of fit indicates that the model is good.

The difference between predicted R^2 (0.9780) and adjusted R^2 (0.9958) was less than 0.2 also confirmed that model is significant. Positive and negative values before coefficient are also indicator of synergistic or antagonistic effect on particle size.

The polymer concentration and surfactant concentration had a positive coefficient value, which signifies that particle size could be reduced as the concentration of surfactant and polymer reduced. The highest coefficient of concentration of polymer reflected that it had a pronounced effect on particle size.


R1
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340
320
300
280
B: B
A: A
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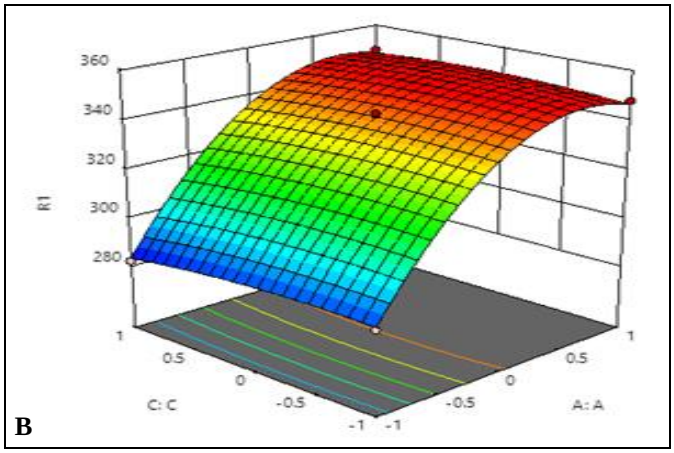
0.5
0
-0.5
-1

A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280
C: C
A: A
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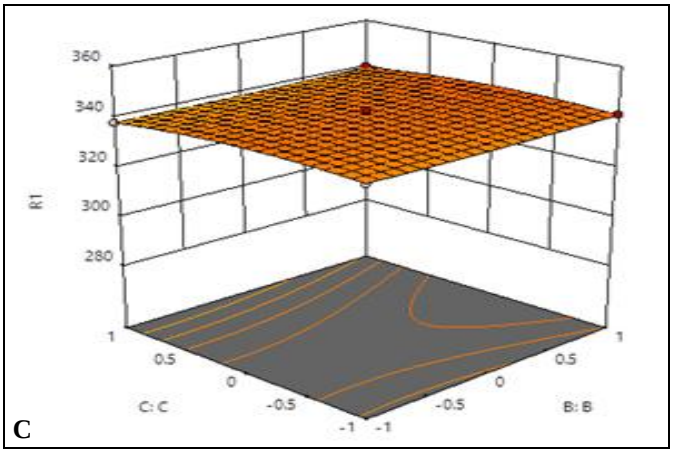
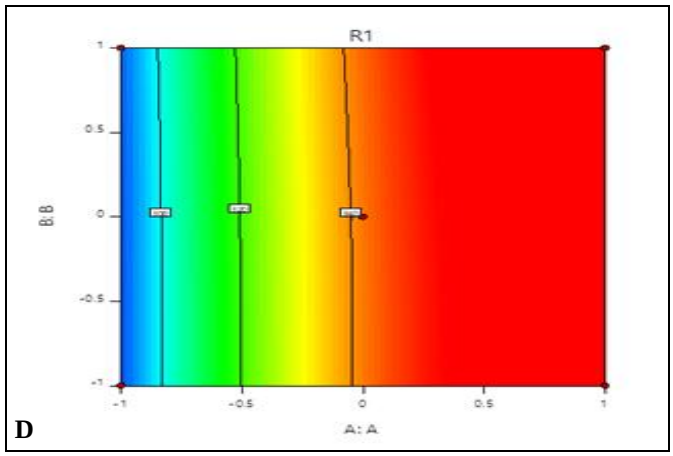
0
-0.5
-1

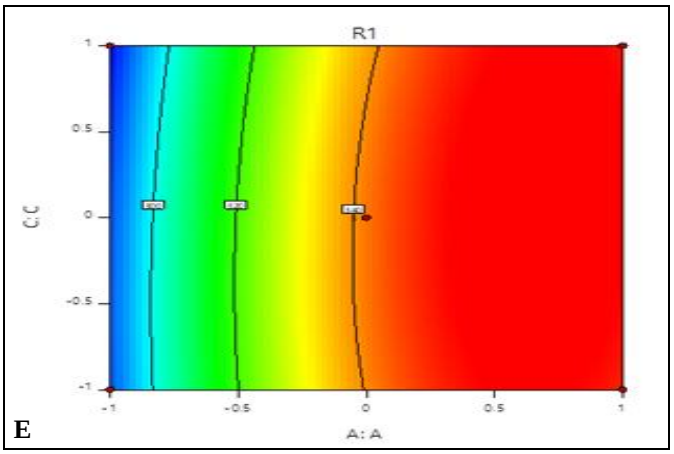
B


R1
360
340
320
300
280
C: C
B: B
1
0.5
0
-0.5
-1

C


R1
B: B
A: A
1
0.5
0
-0.5
-1

D


R1
C: C
A: A
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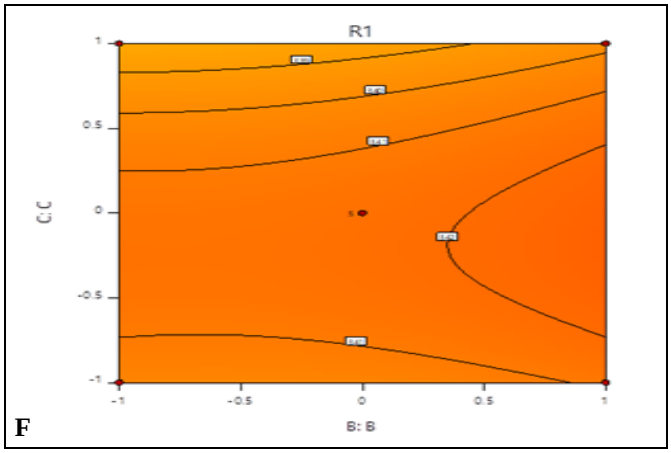
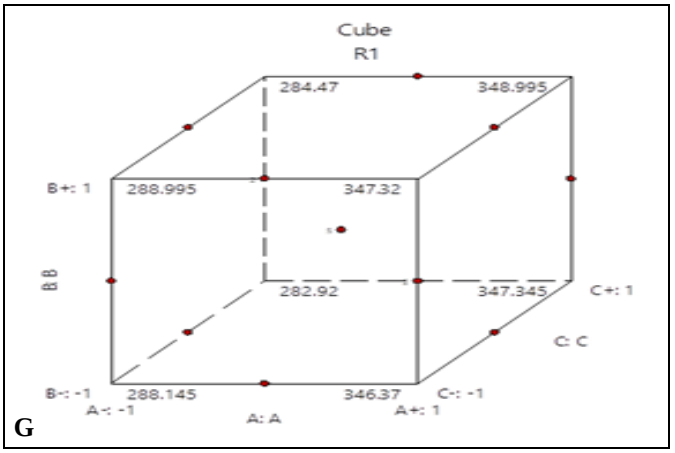
0.5
0
-0.5
-1

E


R1
C: C
B: B
1
0.5
0
-0.5
-1

F


Cube
R1
284.47
348.995
B+: 1
288.995
347.32
B: B
282.92
347.345
C+: 1
C: C
B-: -1
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346.37
C-: -1
A-: -1
A: A
A+: 1

G

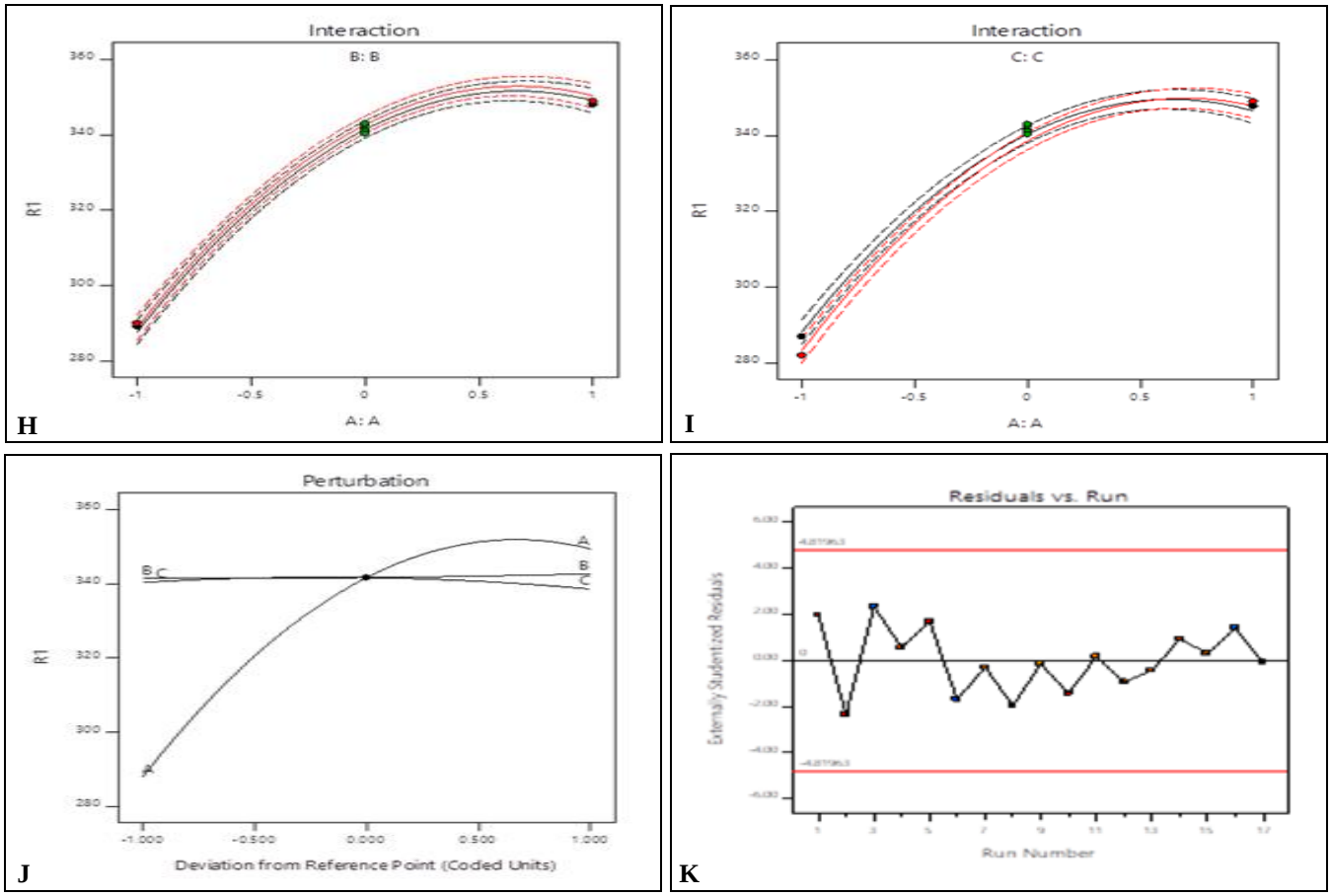


FIG. 1: RESPONSE SURFACE MODEL GRAPHS (3D), COUNTER PLOTS (2D), CUBE GRAPHS, INTERACTION GRAPHS SHOWS EFFECT OF VARIABLES (A; THE CONCENTRATION OF POLYMER, B; THE CONCENTRATION OF SURFACTANT, C; ULTRASONICATION FREQUENCY) ON THE RESPONSE PARTICLE SIZE (R1)

Effect of Independent Variables on Drug Dissolution (%): Following equation represent the correlation of various variables with drug dissolution:

$$R^2 = + 73.44118 - 0.337500\ A + 5.62500\ B - 0.012500C$$

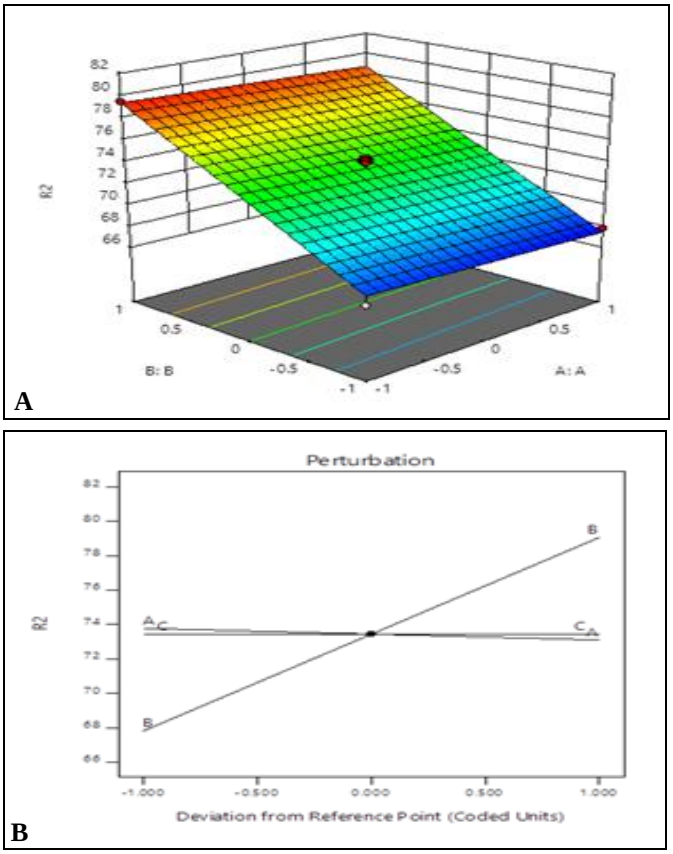
TABLE 5: FIT STATISTICS OF RESPONSE- DRUG DISSOLUTION (R^2) OF CHLORTHALIDONE NANO-PARTICLES

Std. Dev.	0.6716	**R²**	0.9774
Mean	73.44	Adjusted R²	0.9722
C.V. %	0.9145	Predicted R²	0.9623
		Adeq Precision	36.6049

The positive coefficient of factor B was indicated that rate of dissolution increase with an increased concentration of surfactant. R² value was 0.9774 indicated that the linear model was significant.

The non-significant value of lack of fit (1.38) also concluded that linear model was significant.

The three-dimensional graph for response R^2 is shown in **Fig. 2**.



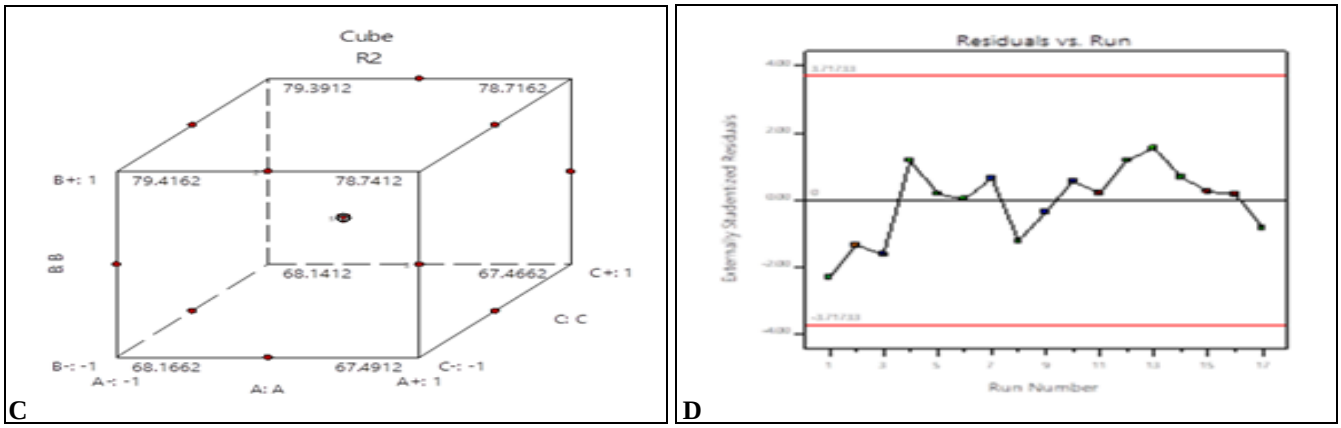


FIG. 2: RESPONSE SURFACE MODEL GRAPHS (3D), COUNTER PLOTS (2D), CUBE GRAPHS, INTERACTION GRAPHS SHOWS EFFECT OF VARIABLES (A; THE CONCENTRATION OF POLYMER, B; THE CONCENTRATION OF SURFACTANT, C; ULTRASONICATION FREQUENCY) ON THE RESPONSE DRUG DISSOLUTION (R^2)

Validation of Optimization Model: The level of factors for the optimum formulation was suggested by the desirability function in the software **Fig. 3.** The optimized formulation of nanoparticles showed 0.1 % w/V of polymer, 40 mg surfactant, and 30 Hz ultrasonication frequency was necessary. The low % error in the prepared formulation suggests good fitting of the model.

TABLE 6: RESULTS OF EXPERIMENTS FOR CONFIRMING OPTIMIZATION CAPABILITY OF CHLORTHALIDONE NANOPARTICLES

Batch	Response	Predicted value	Actual Value	Error	StdDev
CLNP4	Particle Size	341.66	342.5	0.24	1.59975
	Drug dissolution	73.4412	74.2	1.03	0.671604

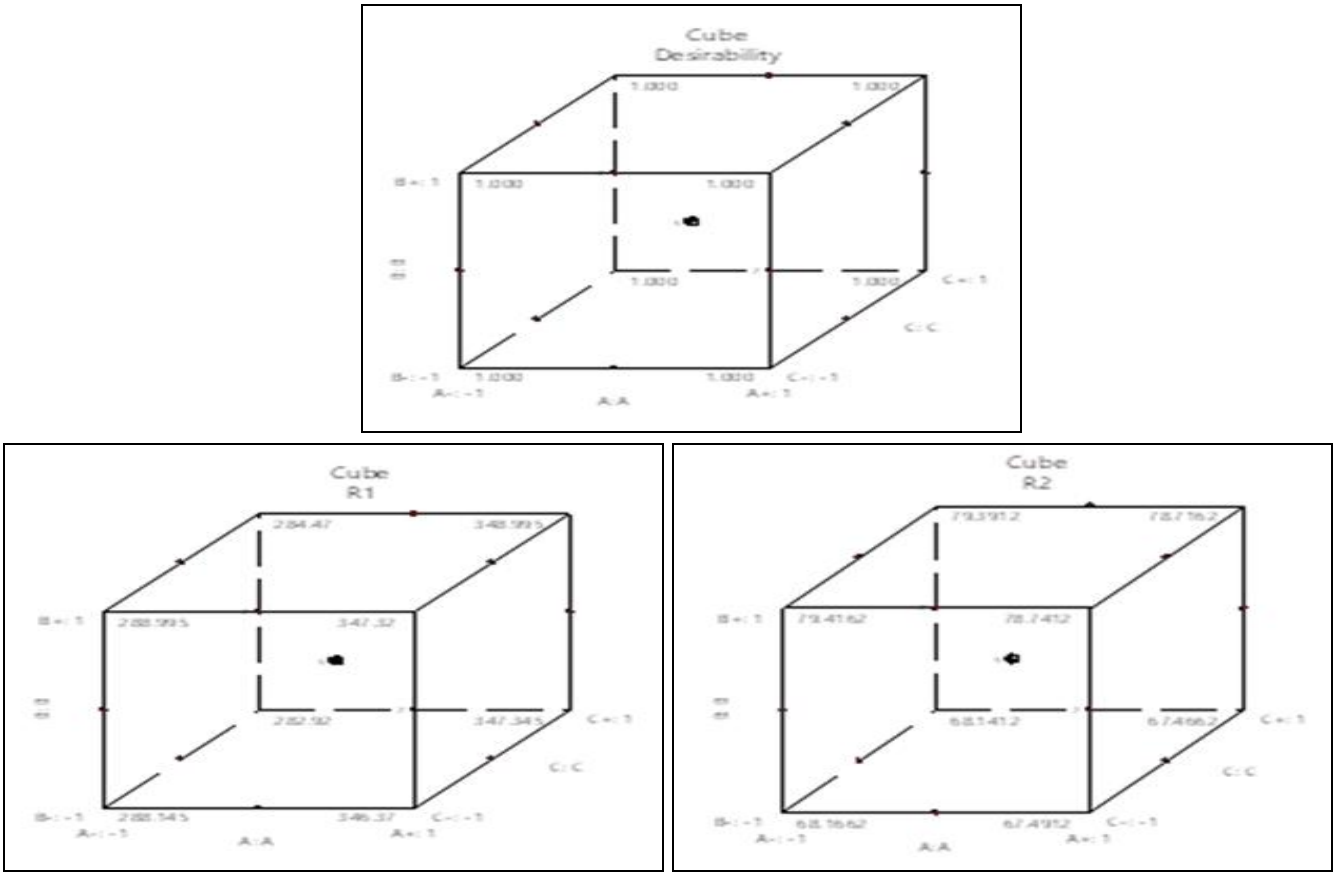


FIG. 3: DESIRABILITY PLOTS OF RESPONSE: R1 AND R2

Analysis of Particle Size: In this study, the particle size of lyophilized nanoparticles of chlorthalidone was reduced to nanometre size (342.5 nm) with a Poly dispersibility index 0.158 **Fig. 4**. The value of the Zeta potential of nanoparticles was found to be -15.5; indicated nanoparticles were stable **Fig. 5**.

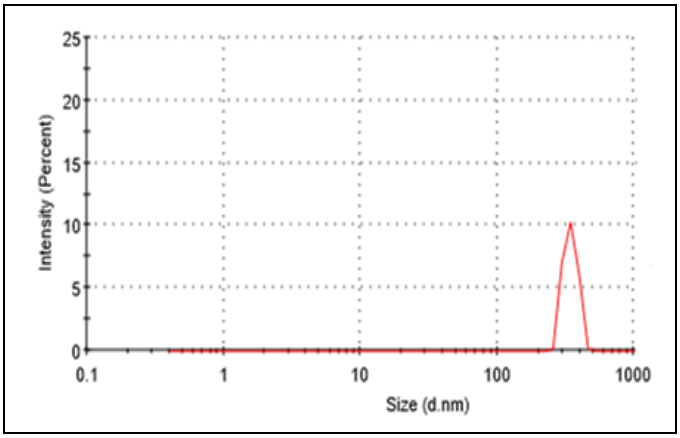


FIG. 4: PARTICLE SIZE ANALYSIS OF LYOPHILISED NANOPARTICLES OF CHLORTHALIDONE

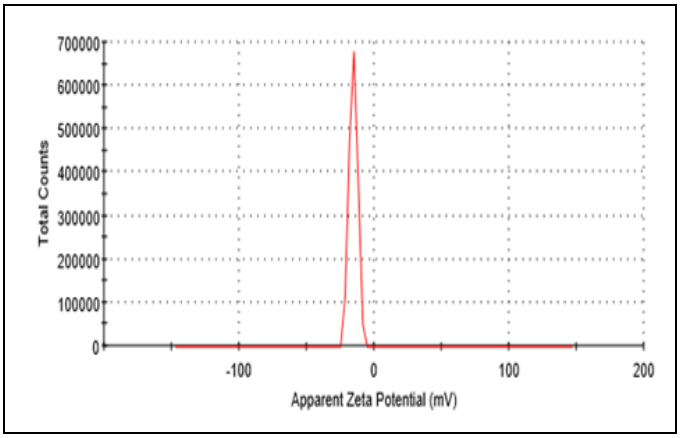


FIG. 5: ZETA POTENTIAL OF LYOPHILISED NANOPARTICLES OF CHLORTHALIDONE

Morphology Characterization: In FESEM analysis of freeze-dried nanoparticles of chlorthalidone, agglomerated nanoparticles were observed at lower magnification, but when magnification power increased to 1,00,000, it was found that uniform and spherical shape nano-particles were entrapped in the polymer structure. FESEM image of lyophilized nanoparticles of chlorthalidone taken after six months also indicated that the morphology of the nanoparticles was preserved **Fig. 6**.

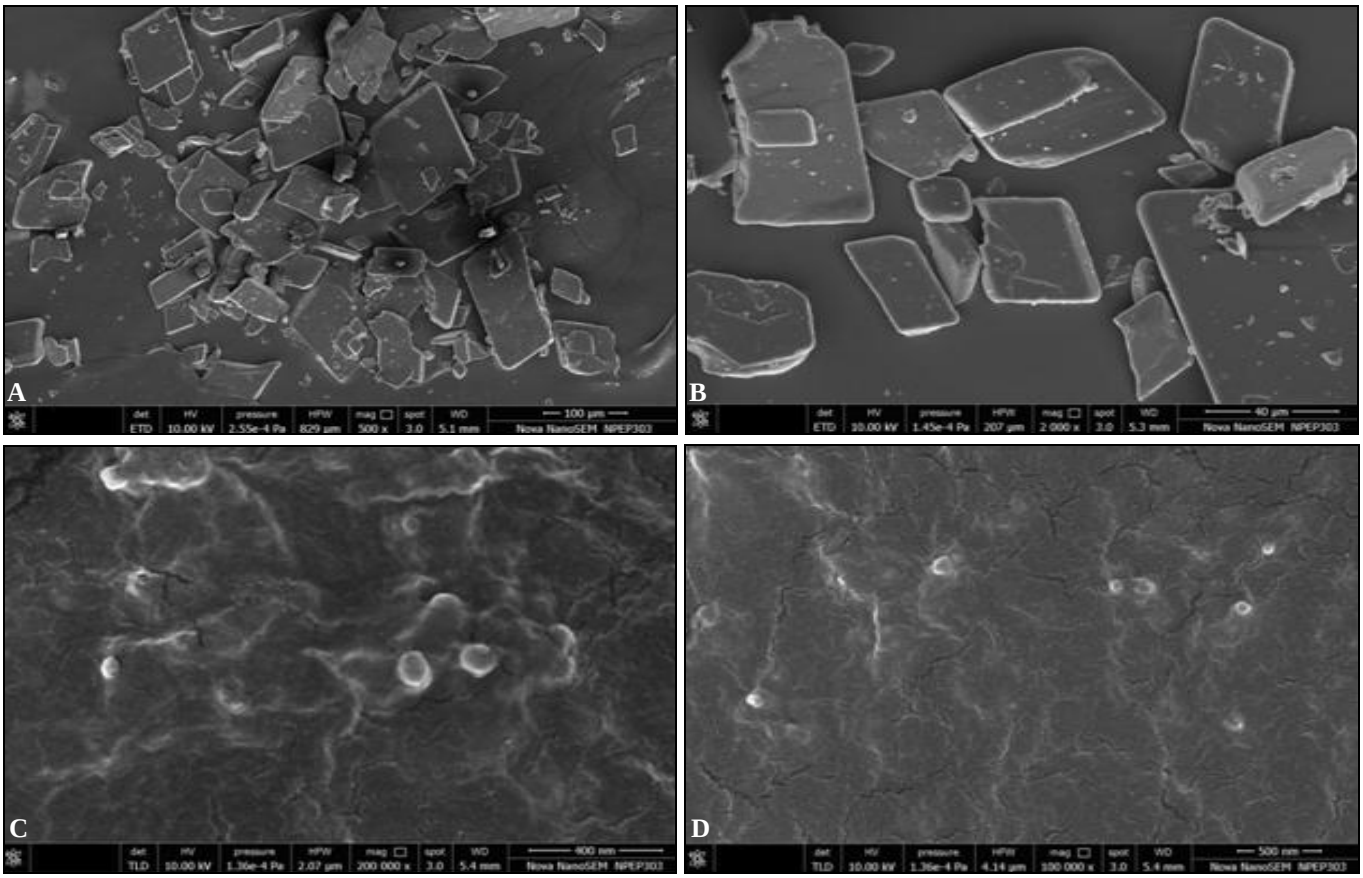


FIG. 6: A. FESEM OF CHLORTHALIDONE, B. FESEM OF PHYSICAL MIXTURE, C. FESEM OF LYOPHILISED NANOPARTICLES OF CHLORTHALIDONE AND D. AFTER THREE MONTHS STORAGE OF CHLORTHALIDONE NANOPARTICLES

FTIR Analysis: FTIR spectroscopic analysis **Fig. 7** was carried out to evaluate possible interaction between chlorthalidone and polymer molecules. IR spectral patterns of pure chlorthalidone, physical mixture and lyophilized samples of chlorthalidone were interpreted, and it was concluded that IR spectral pattern remained the same after nanosization of chlorthalidone. This finding also confirmed that selected excipients and chlorthalidone were compatible with each other.

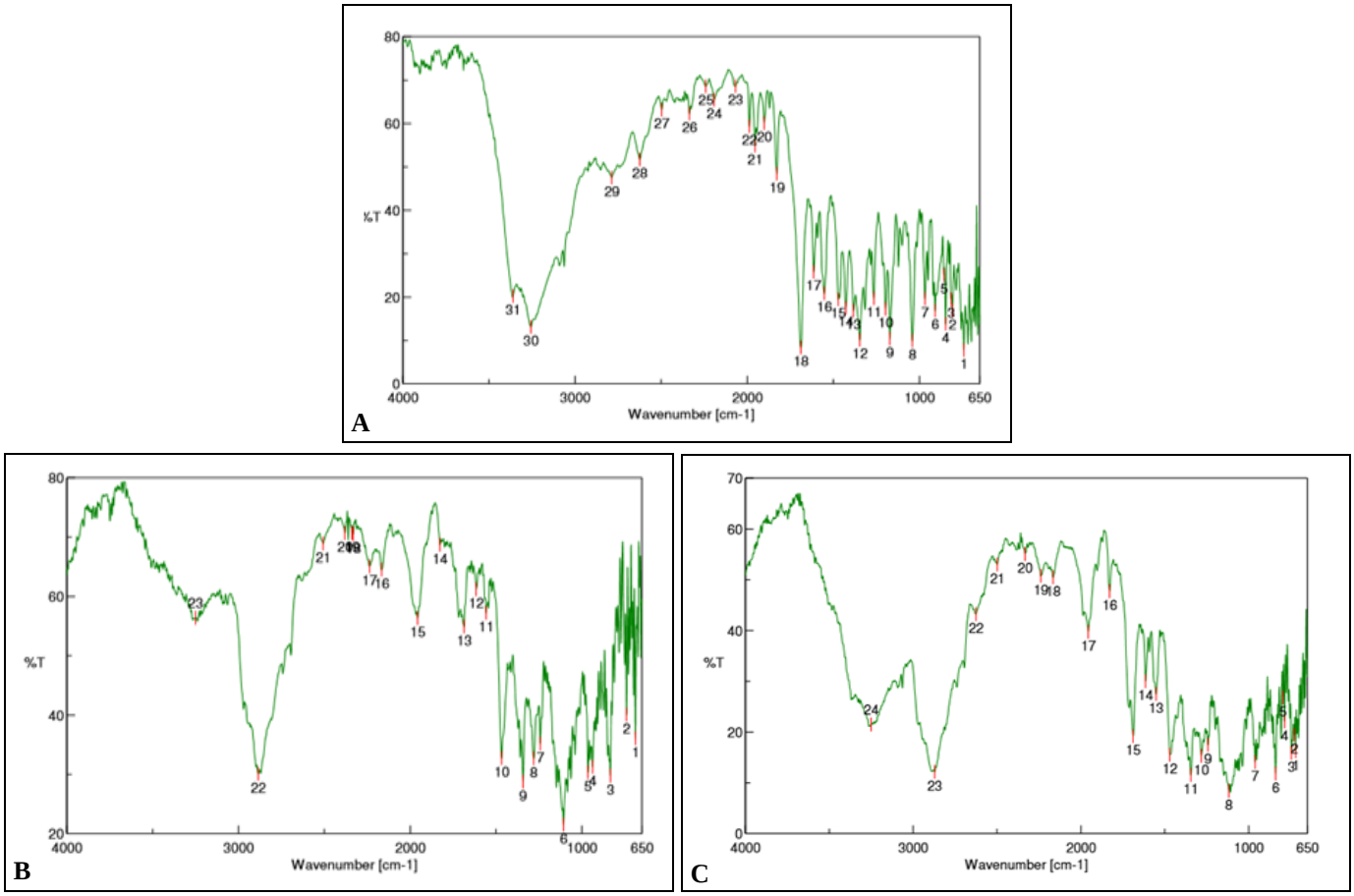


FIG. 7: FTIR SPECTRA (A) CHLORTHALIDONE (B) PM (C) LYOPHILISED NANOPARTICLES OF CHLORTHALIDONE

TABLE 7: CHARACTERISTIC PEAKS IN FTIR SPECTRUM OF CHLORTHALIDONE

S. no.	Functional groups	Chlorthalidone	Physical Mixture	Freeze-dried nanoparticle of Chlorthalidone
1	C-H $_{(S)}$	3256.22	3250.43	3250.43
2	C-C $_{(S)}$	1689.22	1689.34	1686.44
3	C=C	1615.09	1615.09	1616.06
4	C=C	1552.42	1551.45	1557.24
5	C=C	1472.38	1470.46	1467.56

DSC: Energy change in chlorthalidone, PM and lyophilized nanoparticles were carried out by purging inert nitrogen gas. The DSC analysis of chlorthalidone shown a sharp endothermic peak at 226.06 °C correspondings to melting of a crystalline form of chlorthalidone while, DSC of PM indicated a peak at 227 °C. DSC thermogram of freeze-dried nanoparticles of chlorthalidone characterized by absence of a sharp peak at 226.06 °C, clearly revealed that drug was converted to an amorphous form in polymeric nanoparticles **Fig. 8**.

PXRD Study: PXRD data **Fig. 9** of the chlorthalidone exhibited intense peaks at 2θ 7.3°, 14.70°, which are particular peaks for the crystalline drug.

The diffractogram of the nanoparticles depicted a featureless diffraction PXRD pattern, indicating nanosize drug particles were surrounded by added additives as well crystallinity of the drug was reduced. This amorphous form of the drug could improve the aqueous solubility of chlorthalidone.

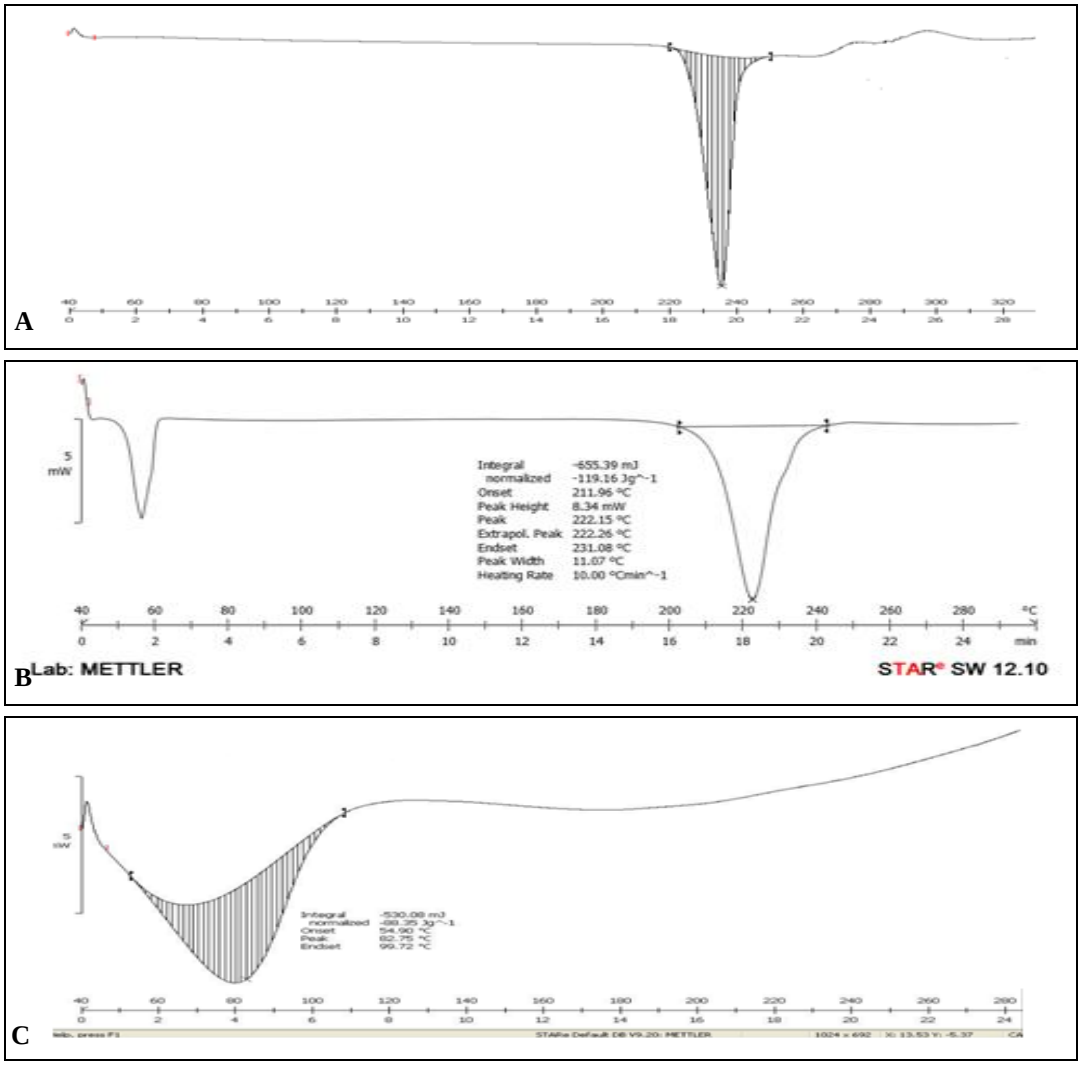


FIG. 8: DSC STUDY (A) CHLORTHALIDONE (B) PM (C) LYOPHILISED NANOPARTICLES OF CHLORTHALIDONE

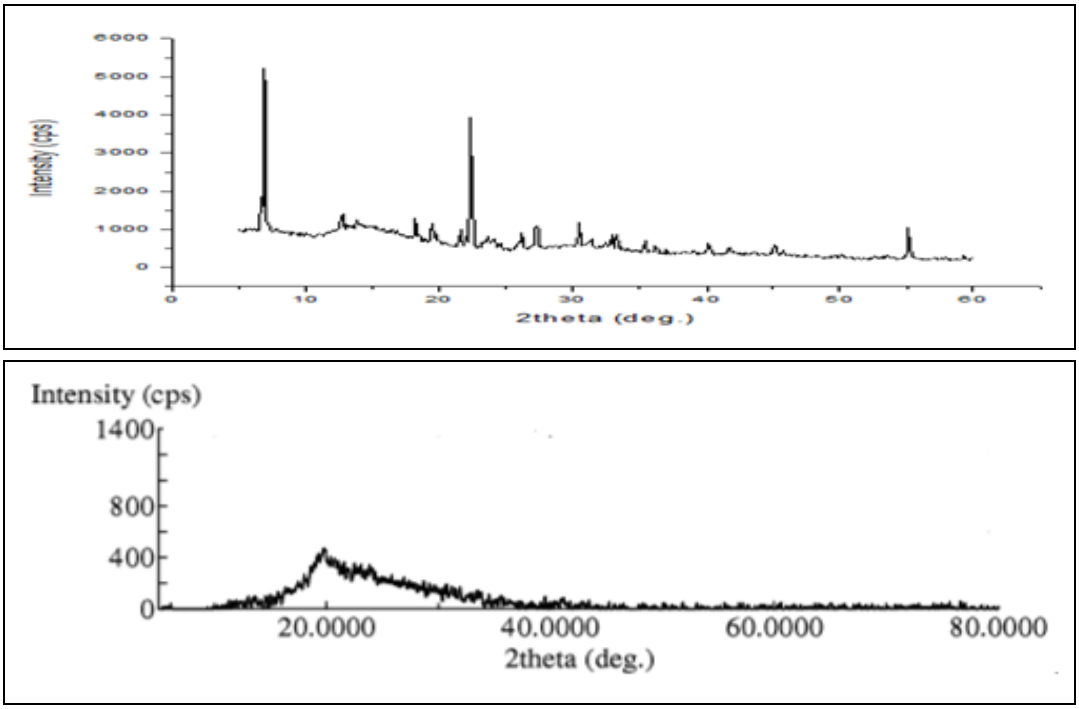


FIG. 9: PXRD OF LYOPHLISED NANOPARTICLES OF CHLORTHALIDONE

Saturated Solubility: The saturation solubility of lyophilized nanoparticles of chlorthalidone was greater than that of raw chlorthalidone **Table 8**. The mechanisms for solubility enhancement were nanosization of drug molecule which in turn increased surface area, the formation of a porous and amorphous structure with a high vapour pressure of nanoparticles as revealed in PXRD and DSC data, electrostatic interaction between drug-stabilizer [13].

TABLE 8: SATURATED SOLUBILITY STUDY OF CHLORTHALIDONE AND LYOPHILISED NANO-PARTICLES OF CHLORTHALIDONE

S. no.	Medium	Solubility of Pure drug (µg/ml) Mean± SD*	Solubility of Lyophilized sample (µg/ml) Mean± SD*
1	Water	11.856 ± 0.150	27.816 ± 0.168
2	Phosphate buffer pH 6.8	11.723 ± 0.151	29.633 ± 0.276

Drug Content: The % drug content of optimized batch of chlorthalidone was found to be 95.65±2.1.

Drug Release Study: A drug release study of chlorthalidone and developed lyophilized nano-particles of chlorthalidone was performed in deionized water and phosphate buffer pH 6.8 in 60 min at 75 rpm. **Fig. 10** revealed the dissolution profile of the pure drug and lyophilized nano-particles. The highest cumulative dissolution rate was achieved within 45 min. This phenomenon might be occurred due to the large surface area of nanosized particles comes in contact of the solubilizing medium, which make the surface more hydrophilic and converts in aqueous solution form.

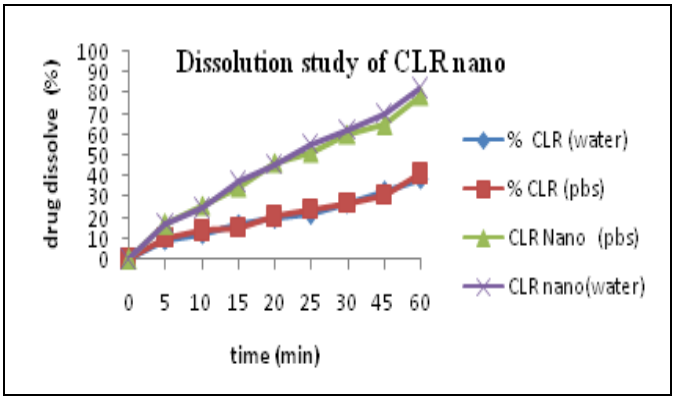


FIG. 10: DISSOLUTION STUDY OF CHLOR-THALIDONE AND LYOPHILISED NANOPARTICLES OF CHLORTHALIDONE

Stability Study: Results of the stability study of Chlorthalidone nanoparticles shown that it was remained stable at 40 °C / 75% RH for a period of 3 months. While stability study, the particle size of optimized nanoparticles was analyzed by DLS and remained stable for three months, which revealed that the absence of physicochemical degradation of nanoparticles and confirmed the formulation's stability. The solid-state stability of lyophilized nanoparticles was characterized by DSC, PXRD, FTIR, and FESEM analysis.

CONCLUSION: Amorphous freeze-dried nanoparticles of chlorthalidone were formulated by the anti-solvent precipitation-ultrasonication technique. The box-Behnken factorial design approach was used to study the simultaneous effect of formulation variables for optimizing the formulation. Comparing the actual and predicted responses indicated that surface response methodology is suitable for optimization of chlorthalidone nano-particles. The particle size of freeze-dried nanoparticles of chlorthalidone was 342.5 nm, and the Poly dispersibility index was 0.158. The DSC thermo-gram indicated the disappearance of a sharp endothermic peak at 226.06 °C, which proved that chlorthalidone was converted to an amorphous form.

Also, an intense peak of chlorthalidone was not observed in PXRD study of chlorthalidone confirmed that the drug was converted to amorphous form. The solubility and dissolution profile of chlorthalidone was improved due to the conversion of the drug to nanosize. FTIR study has indicated drug, and selected excipients are compatible. The stability studies of developed nanoparticles confirmed developed formulation remains stable at 25 °C / 60% RH and 40 °C / 75% RH for a period of 3 months.

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REFERENCES:

1. Tran P, Pyo YC, Kim DH, Lee SE, Kim JK and Park JS: Overview of the manufacturing methods of solid dispersion technology for improving the solubility of poorly water-soluble drugs and application to anticancer drugs. Pharmaceutics 2019; 11(3): 132.

2. Zhang X, Xing H, Zhao Y and Ma Z: Pharmaceutical dispersion techniques for dissolution and bioavailability enhancement of poorly water-soluble drugs. Pharmaceutics 2018; 10(3): 74.
3. Khames A: Investigation of the effect of solubility increase at the main absorption site on bioavailability of BCS class II drug (risperidone) using liquisolid technique. Drug Deliv 2017; 24(1): 328-38.
4. Mahmoud A, Lassaad B and Amine BM: Biopharmaceutics Classification System (BCS) based biowaiver studies of lenalidomide capsules (25 mg) – an alternative to *in-vivo* bioequivalence studies for generic oncology drug products. Journal of Bioequivalent Availability 2019; 11: 386.
5. Dangre PV, Gilhotra RM and Dhole SN: Formulation and development of solid self micro-emulsifying drug delivery system (S-SMEDDS) containing chlorthalidone for improvement of dissolution. Journal of Pharmaceutical Investigation 2016; 46: 633-44.
6. Ahire E, Thakkar S, Darshanwad M and Misra M: Parenteral nanosuspensions: a brief review from solubility enhancement to more novel and specific applications. Acta Pharmaceutica Sinica B 2018; 8(5): 733-55.
7. Wu W, Zu Y, Wang L, Wang L, Wang H, Li Y, Wu M, Zhao X and Fu Y: Preparation, characterization and antitumor activity evaluation of apigenin nanoparticles by the liquid antisolvent precipitation technique. Drug Deliv 2017; 24(1): 1713-20.
8. Wu W, Wang L, Wang L, Zu Y, Wang S, Liu P and Zhao X: Preparation of honokiol nanoparticles by liquid antisolvent precipitation technique, characterization, pharmacokinetics, and evaluation of inhibitory effect on HepG2 cells. Int J Nanomedicine 2018; 17(13): 5469-83.
9. Agrawal S and Paterson A: Secondary nucleation: mechanisms and models, Chemical Engineering Communications 2015; 202(5).
10. Zhang X, Chen H, Qian F and Cheng Y: Preparation of itraconazole nanoparticles by anti-solvent precipitation method using a cascaded microfluidic device and an ultrasonic spray drier. Chemical Engineering Journal 2018, 334(15): 2264-72.
11. Gidwani B and Vyas A: Preparation, characterization, and optimization of altretamine-loaded solid lipid nanoparticles using Box-Behnken design and response surface methodology. Artificial Cells, Nanomedicine and Biotechnology 2016, 44(2): 571-80.
12. Shaikh MV, Kala M and Nivsarkar M: Formulation and optimization of doxorubicin-loaded polymeric nanoparticles using Box-Behnken design: *ex-vivo* stability and *in-vitro* activity. Eur J Pharm Sci 2017; 30(100): 262-72.
13. Jog R and Burgess DJ: Pharmaceutical Amorphous Nanoparticles. J Pharm Sci 2017; 106: 39-65.

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