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FORMULATION, OPTIMIZATION AND CHARACTERIZATION OF NANOCRYSTALS OF AN ANTIBIOTIC

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Abstract: Nitrofurantoin is a broad-spectrum bactericidal antibiotic that affects both Gram-negative and Gram-positive bacteria. Nitrofurantoin exhibits bacteriostatic or bactericidal effects by inhibiting the synthesis of DNA, RNA, protein and cell wall synthesis. Nanocrystals of NFT were prepared by Cold High Pressure Homogenization Technique. NFT was dispersed in aqueous surfactant solution containing Poloxamer 188, PVPK 30 and HPMC E3 under continuous stirring. Poloxamer 188 was used as a surfactant for the preparation of the NCs Formulation NC9B3 have mean particle size 231 ± 9nm with Polydispersity index 0.09 ± 0.02 which indicates very narrow particle size distribution. % Entrapment efficiency was 98.3± 0.7 Slow drug release profile indicates the homogeneous dispersion of NFT in lipid matrix. NCs have crystalline nature with rough surfaces which has been confirmed using SEM analysis. XRPD spectra show the reduction in crystalline behaviour of the drug and the lipid after formation of the NCs. There was no significant change in the mean particle size and Polydispersity index after 6 month storage at 25°C/60% RH

Keywords: Nitrofurantoin (NFT), Nanocrystals (NCs)

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INTRODUCTION

Nitrofurantoin is a drug of choice for UTIs. Sustained release drug delivery system offer advantages of attenuation of adverse effects, fewer fluctuations in plasma drug concentration, improved patient compliance, reduction in dosing frequency, etc (Nalneesh Bhatt *et al.,* 2013).

The total therapeutic effectiveness of nanoformulation depends not only on the action of drug itself, but also on other factors related to the delivery system. The bioavailability of the drug and its absorption from the gastrointestinal tract can be greatly improved by particle size reduction. (Chattopadhyay P *et al.,* 2001)

In the proposed research work, we have to formulate, optimize and evaluate the nanoformulations of antibiotic Drug (Nitrofurantoin) by improving the solubility and bioavailability. The research work shall emphasize on the design, development and characterization of the nanoformulations of Nitrofurantoin (NFT) through the novel approach

The short biological half-life (0.3 to 1 hour) and dosing frequency more than one per day make Nitrofurantoin an ideal candidate for sustained release (Ayush *et al.,* 2016) Hence, there is a need of a Nitrofurantoin nanoformulations for bioavailability enhancement.

Nanocrystals (NCs) have been recognized as an effective formulation and drug delivery because of its advantages like higher bioavailability, better stability, high compatibility, low toxicity, ease of process scale up and large scale production. Several methods had been utilized for the formulation of NCs like High pressure homogenization, Solvent evaporation. High pressure homogenization and Sonication are used widely as efficient and promising technique for NCs.

In the present research, High pressure homogenization was utilized followed by ultra-sonication for the preparation of NCs. Prepared NCs were characterized mean particle size, entrapment efficiency, assay as well as *in-vitro* release of the formulation. Also, SEM and XRPD evaluation were performed on the Initial and Stability samples of the optimized formulation batch

MATERIALS:

Nitrofurantoin was kindly supplied by Hetero Labs Limited, Andhra Pradesh, India as a gift sample. Glyceryl Monostearate and Glyceryl Behenate (Compritol 888 ATO) were obtained as a gift sample from Gattefosse, Mumbai, India. Poloxamer 188, PVPK 30, HPMC E3 obtained from Sun pharma, Vadodoara. All remaining reagents and chemicals were of analytical grade. Purified water used for all experiments was MilliQ Plus, Millipore.

METHODS:

Analytical Method Development using HPLC-UV Technique:

The HPLC system used consists of LC-10AD/20AD pumps coupled with a Ultra-violet (UV) detector. The conditions on which these instruments run are as below.

Manufacturing Model: Perkin Elmer 200 series

Pump: Series 200 Binary pump

Detector: Variable single wavelength UV/VIS

Sampling Method: Auto sampler

Mobile Phase: Dissolve 6.8g. of monobasic potassium phosphate in 500ml water. add about 30ml 1.0N NaOH sufficiently to adjust pH 7.0 and dilute with water to 1000ml.

HPLC Column: L1

Wavelength: 254 nm

Injection Volume: 10 µl

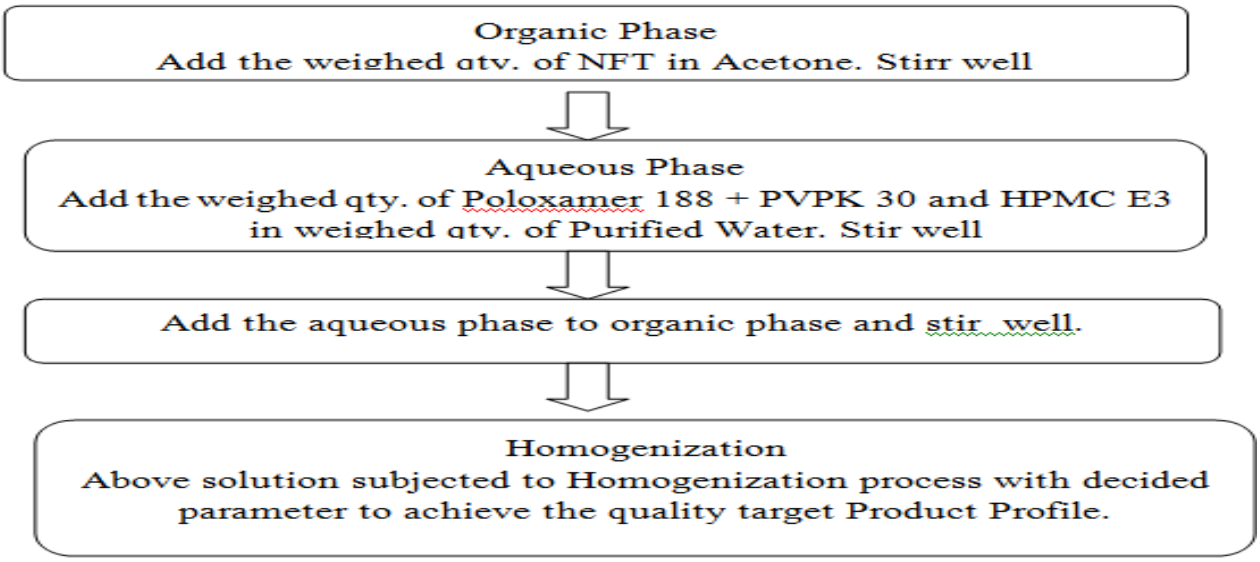
The NFT stock solutions were prepared by dissolving appropriate quantities of NFT in Dimethyl formamide + 50 ml Internal standard solution. (Internal standard : 1mg/ml acetanilide in water) mobile phase, sonicated for 30 minutes for complete dissolution and make up to the mark in the volumetric flask with mobile phase to yield a final concentration 1000ppm. Further 1 ml of above solution was diluted to 10 ml with mobile phase. (100 ppm).

Physical Compatibility Study:

Compatibility studies were carried out for appropriate selection of excipients. Studies were carried out by mixing the drug with various excipients in required proportion in glass vials. Vials were closed with rubber stopper and kept at three conditions, namely 40ºC/75 % RH; 25ºC/60 % RH; and Photo stability for 1 month. Physical observations of the blend were done during the study at regular intervals. Compatibility of NFT with selected excipients was confirmed by FTIR

FORMULATION DEVELOPMENT OF NCS

Figure 1: Manufacturing flow chart



OPTIMIZATION OF THE COMPOSITION AND PROCESS:

Composition for the Preparation of the Nanocrystals

On the basis of literature survey and drug excipients compatibility we have formulate batch NC1 which is a placebo and batch NC2 is with drug by keeping ratio of organic phase, surfactant and polymer and stirring speed as well as homogenization pressure same to two cycles to check the feasibility

Table 1: Composition and Process Parameter for Preliminary Development Batches

Sr. No	Batch No	NC1	NC2
	Ingredients	**Quantity in gm**	
1	NFT	NA	1.2
2	Acetone	60.0	60.0
3	Poloxamer 188 +PVPK 30 (1:5)	1.2	1.2
4	HPMC E3	1.2	1.2
5	Purified Water@	Q.S	Q.S
Sr. no	**Parameter**	**NC1**	**NC2**

1	Stirring using Overhead stirrer	3000 RPM for 5 min	3000 RPM for 5 min
2	Homogenization Pressure (Bar)	1500	1500
3	Homogenization Cycle	2	2

High pressure homogenizations are used widely as efficient and promising technique for SLNs preparation. Optimized and robust process of High Speed Homogenization may help to achieve the desired LPV loaded SLNs.

Formulation Optimization by Formulation Variables

In present study formulation were optimized by considering the formulation variables to achieve the defined quality target product parameter. Composition and process parameter are mentioned as per following table.

Table 2: Formulation Optimization by Formulation Variables

Batch No.		**NC2**	**NC3**	**NC4**
Sr. No	**Ingredients**		**Qty in gm**	
1	NFT	1.2	1.2	1.2
2	Acetone	1.2g of API in60g of organic solvent		
3	Poloxamer 188 + PVPK 30 (1:5)	1.2	0.6	0.6
4	HPMC E3	1.2	0.6	0.3
5	Purified Water		Q.S	
Sr. No	**Parameters**	**NC2**	**NC3**	**NC4**
1	Stirring using Overhead Stirrer	3000 RPM for5 min	3000RPM for5 min	3000 RPM for 5 min
2	Homogenization Pressure (Bar)	1500	1500	1500
3	Homogenization Cycle	2	2	2

Formulation Optimization by Process Variables

In present study, process was optimized by considering the process variables to achieve the defined quality target product parameter. Composition was same as mentioned in Batch No.NC3 and Process parameter are change as per following table in batch no. NC5 to NC9 to achieve desired mean particle size, PDI, zeta potential and assay.

Table 3: Formulation Optimization by Process Variables

Sr.No	Parameters	NC5	NC6	NC7	NC8	NC9
1	Stirring using Overhead Stirrer	3000 RPM for 5 min	3000 RPM for 5 min	3000 RPM for 5 min	3000 RPM for 5 min	3000 RPM for 5 min
2	Homogenization Pressure (Bar)	500	750	1000	1250	1500
3	Homogenization Cycle	2	2	2	2	1

Composition and process parameter of Batch No. NC9 was taken forward to optimize the % Pearlitol 25C content as a cryoprotectant. Formulation and process parameters are as mentioned in below table.

Composition and Process Parameter for Pearlitol 25C Optimization Batches

Table 4: Composition and Process Parameter for Pearlitol 25C Optimization Batches

Sr. NO	Freeze Drying Parameter	NC9A	NC9B	NC9C
		Pearlitol 25C level 50% of the API	Pearlitol 25C level 100% of the API	Pearlitol 25C level 150% of the API
1	Temperature (Condenser)	-70°C		
2	Vacuum (mTorr)	100		
3	Cycle time (Hr)	24		

Pearlitol 25C levels were kept at 50, 100 and 150% of the API for the optimization purpose and batch no. were assigned as mentioned in above table.

Optimization of Freeze Drying Process

The final NC solution of Batch No. NC9B was divided into Batch No. NC9B1, NC9B2 and NC9B3 equally. The vials were first kept on the condenser surface.

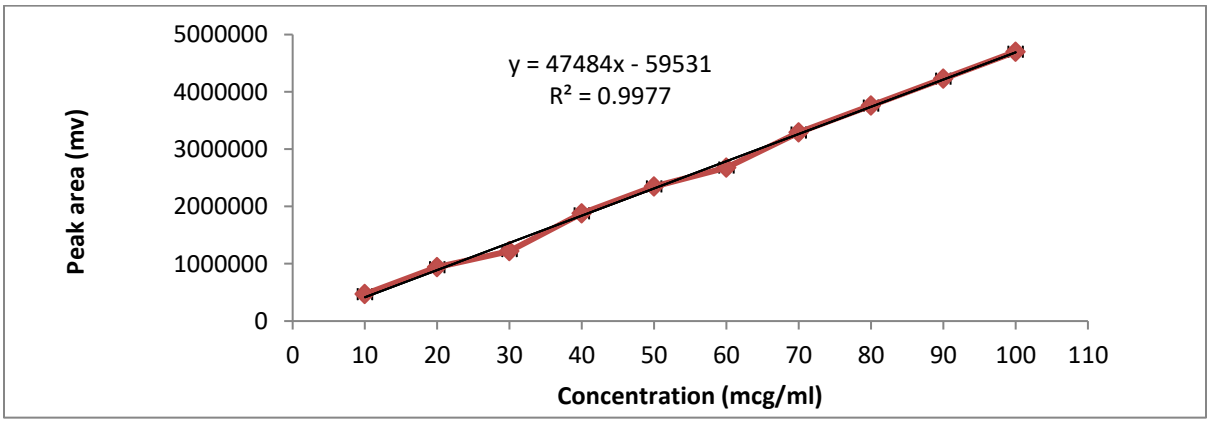
Table 5: Optimization of Freeze Drying Process

Sr. no	Freeze drying parameter	NC9B1	NC9B2	NC9B3
		Pearlitol 25C Level 100% of the API		
1	Temp	-70°C	-70°C	70°C
2	Vacuum (mTorr)	100	100	100
3	Cycle Time (Hr)	12	18	24

RESULTS AND DISCUSSIONS:

A suitable stability-indicating analytical method development is very critical. The standard curve was generated for the entire range from 0 to 100µg/ml. The results of standard curve preparation are shown in Figure 3

Figure 3: Linearity curve of Nitrofurantoin



Observation of Drug-Excipients Compatibility Studies

Compatibility study of drugs with excipients were done at Initial, after 2 week at 25°C/60%RH and 1Month 25°C/60%RH. Change in appearance and molecular structure were observed by FTIR

Table 6: Observation of Compatibility Study of Drug and Excipients

Run No.	Sample name	Initial	2 week 25°C/60%RH	1Month 25°C/60%RH
1	Nitrofurantoin (NFT)	Yellow color powder	Yellow color powder	Yellow color powder
2	NFT + Poloxamer 188	Yellow color powder	Yellow color powder	Yellow color powder
3	NFT + PVPK 30	Yellow color powder	Yellow color powder	Yellow color powder
4	NFT + Poloxamer 407	Yellow color powder	Blackish yellow color powder	Blackish yellow color powder
5	NFT + HPMC E3	Yellow color powder	Yellow powder	Yellow color powder
6	NFT + HPMC E5	Yellow color powder	Yellow color powder Slight Sticky	Yellow color powder Sticky behaviour

Figure 4: IR graph of Drug (Nitrofurantoin)and drug with excipiens overlay

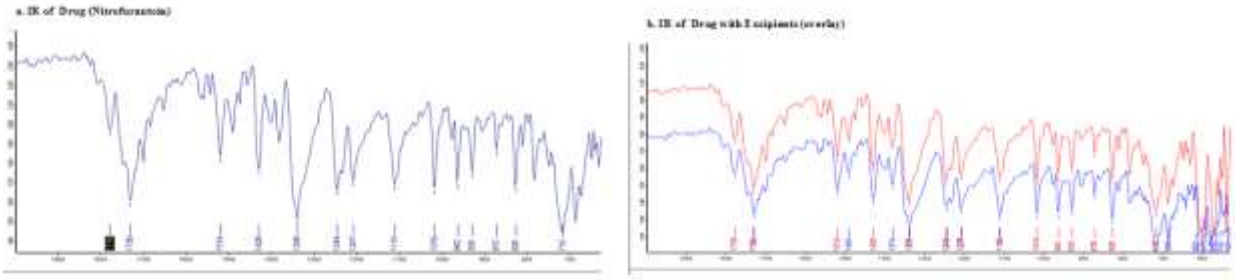
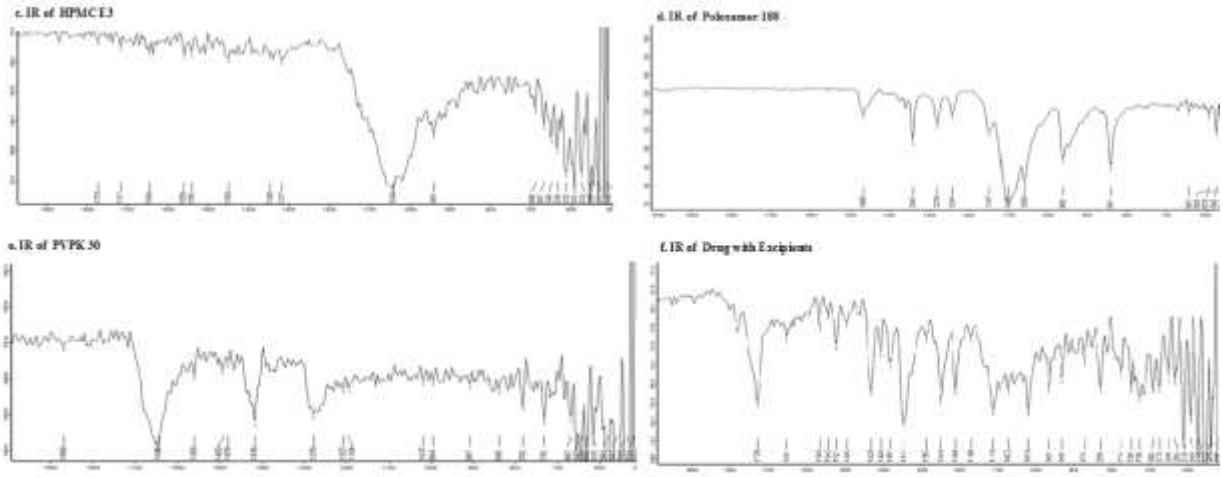


Figure 5: IR graph of excipients and drug with excipients



Evaluation and Characterization of NC

Evaluation of the Preliminary Development Batches

Preliminary batches were manufactured as per the Composition and Process Parameter mentioned in Table 1. Results are mentioned in below table.

Table 7: Results of the Preliminary Development Batches

Sr. No		Evaluation	NC2
1		Mean Particle Size (nm)	413 ± 49
2		Polydispersity Index	0.39 ± 0.05
3		Zeta Potential (-mv)	-29.3 ± 0.13
4	A	Assay	99.1± 1.5

Evaluation of the Formulation Optimization Batches

Ideal NC have small particle size with narrow particle size distribution (Polydispersity Index <1 %). In present study composition and process parameter are as mentioned in Table 2 Different Ratio of Drug: Poloxamer 188+PVPK 30: HPMC E3 was optimized. Results are as mentioned below Table.

Table 8: Results of the Formulation Optimization Batches

Sr No.	Evaluation	NC2	NC3	NC4
1	Mean Particle Size (nm)	413 ± 49	198 ± 13	279 ± 38
2	Polydispersity Index	0.39 ± 0.05	0.12 ± 0.03	0.22 ± 0.02
3	Zeta Potential (-mv)	-29.3 ± 0.13	-38.1 ± 0.12	-22.3 ± 0.24
4	Assay	99.1 ± 1.5	100.0 ± 0.5	98.9 ± 1.1

Evaluation of the Process Optimization Batches

Optimized and robust process of NC may help to achieve the desired quality target product profile. Compositions are same as Batch No. NC3 and process parameter are as mentioned in the Table 3. Batches are evaluated as mentioned in below table.

Table 9: Results of the Process Optimization Batches

Sr. No	Evaluation	NC5	NC6	NC7	NC8	NC9
1	Mean Particle Size	829±109	745± 69	332 ± 41	213 ± 17	238 ± 12
2	Polydispersity Index	2.39 ± 0.29	2.11 ± 0.41	1.01 ± 0.33	0.14 ± 0.08	0.09 ± 0.06
3	Zeta Potential (-mv)	----	---	---	- 35.4 ± 0.31	-36.1 ± 0.19
4	Assay	---	---	---	101.1 ± 0.7	99.8 ± 0.3

Table 10: Results of the Pearlitol 25C Optimization Batches

Sr. No	Evaluation	NC9A	NC9B	NC9C
1	Mean Particle Size (nm)	--	223 ± 8	241 ± 11
2	Polydispersity Index	--	0.11 ± 0.03	0.12 ± 0.02

3	Zeta Potential (-mv)	--	-41.1 ± 0.22	-43.4 ± 0.18
4	Assay	--	99.1± 0.5	98.8± 0.6
5	Water Content	--	2.1± 0.2	1.8± 0.2
6	Residual Solvent (PPM)	--	97 ± 3	83 ± 5
7	Appearance	Loose Self-Supporting Cake	Acceptable Porous Cake	Acceptable Porous Cake

It has been observed that physical appearance of the Batch No. NC9A was not acceptable as loose self-supporting cake was observed which is due to ineffective concentration of Pearlitol 25C as a cryoprotectant or bulking agent. Hence Pearlitol 25C concentration needs to be increased further to get the desired target product profile. There is no significance difference between the target product parameters of Batch No. NC9B and NC9C. They all are within the acceptable range including the physical appearance and residual solvent content. Therefore Batch No. NC9B having the 100% Pearlitol 25C of the API has been taken forward for the optimization of the freeze drying process.

Evaluation of the Optimization Batches for Freeze Drying Process

Freeze drying is an essential process to enhance the stability of the formulation. Appearance and the Water content of the formulated cake are the key evaluation parameter. The final NC dispersion of Batch No. NC9B was divided into Batch No. NC9B1, NC9B2 and NC9B3 equally. These were then subjected to lyophilization process in a Virtis bench top lyophilizer with the parameters as shown in table 12. Results are as mentioned in the below table.

Table 11: Results of the Optimization Batches for Freeze Drying Process

Sr. No	Evaluation	NC9B1	NC9B2	NC9B3
1	Mean Particle Size (nm)	--	327 ± 13	231 ± 9
2	Polydispersity Index	--	0.21 ± 0.04	0.09 ± 0.02
3	Zeta Potential (-mv)	--	-29.4 ± 0.12	-38.8 ± .15
4	Assay	--	97.9 ± 0.3	98.3± 0.7

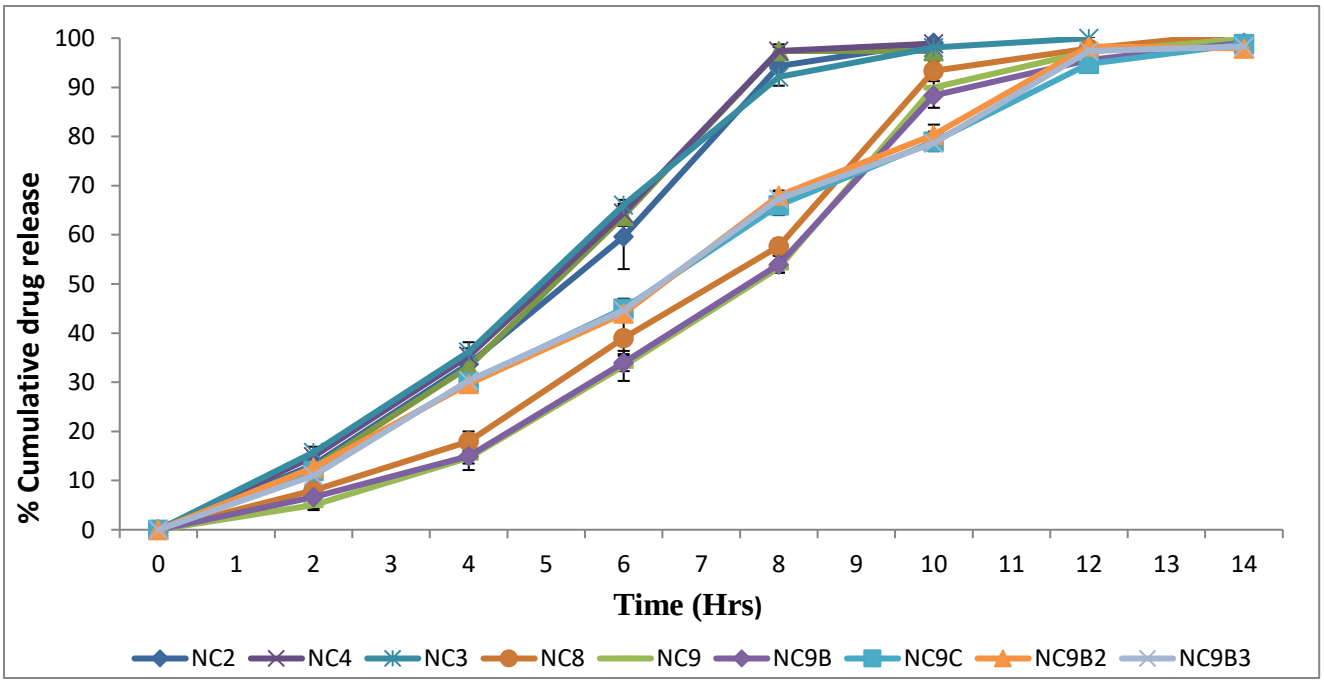
5	Water Content	--	4.3 ± 0.4	1.9 ± 0.3
7	Appearance	Loose self supporting cake	Acceptable Porous cake	Acceptable Porous cake

Batch No. NC9B1 shows the loose self supporting cake indicates the higher water content which is due to insufficient cycle time of 12 Hrs. Batch No. NC9B2 and NC9B3 seem to be satisfactory. There is no significance difference between Batch No. NC9B2 and NC9B3. By considering the lower value of residual solvent content Batch No. NC9B3 was taken further for complete analysis and to be loaded in stability study.

Evaluation of the Optimized Batch for drug release profile

All batches NC2, NC3, NC4, NC8, NC9, NC9B, NC9C, NC9B2, NC9B3 were packed in capsule. *In-vitro* drug release study was performed (figure 9) in USP-II apparatus with sinker using 900 ml of Phosphate buffer (pH 7.2) at 50 RPM for 12hrs..

Figure 6: Drug release profile of optimized batches



Stability study of Optimized batch

Table: 12 Stability Data of the Batch No. NC9B3 (Capsules Containing NC in HDPE Bottle)

Evaluation	25°C/60 RH Condition				
	Initial	1M	2M	3M	6M
Mean ParticleSize (nm)	231 ± 9	229 ± 5	241 ± 6	239 ± 7	251 ± 13
PolydispersityIndex	0.09 ± 0.02	0.11 ± 0.01	0.11 ± 0.02	0.09 ± 0.01	0.16 ± 0.01
Zeta Potential(mV)	-38.8 ± 0.15	-35.8 ± .08	-38.1 ±.07	-40.4 ± 0.12	-41.1 ± 0.14
% Assay	98.3 ± 0.7	97.3 ±0.9	98.2± 1.2	99.1 ± 2.3	97.8 ± 1.3

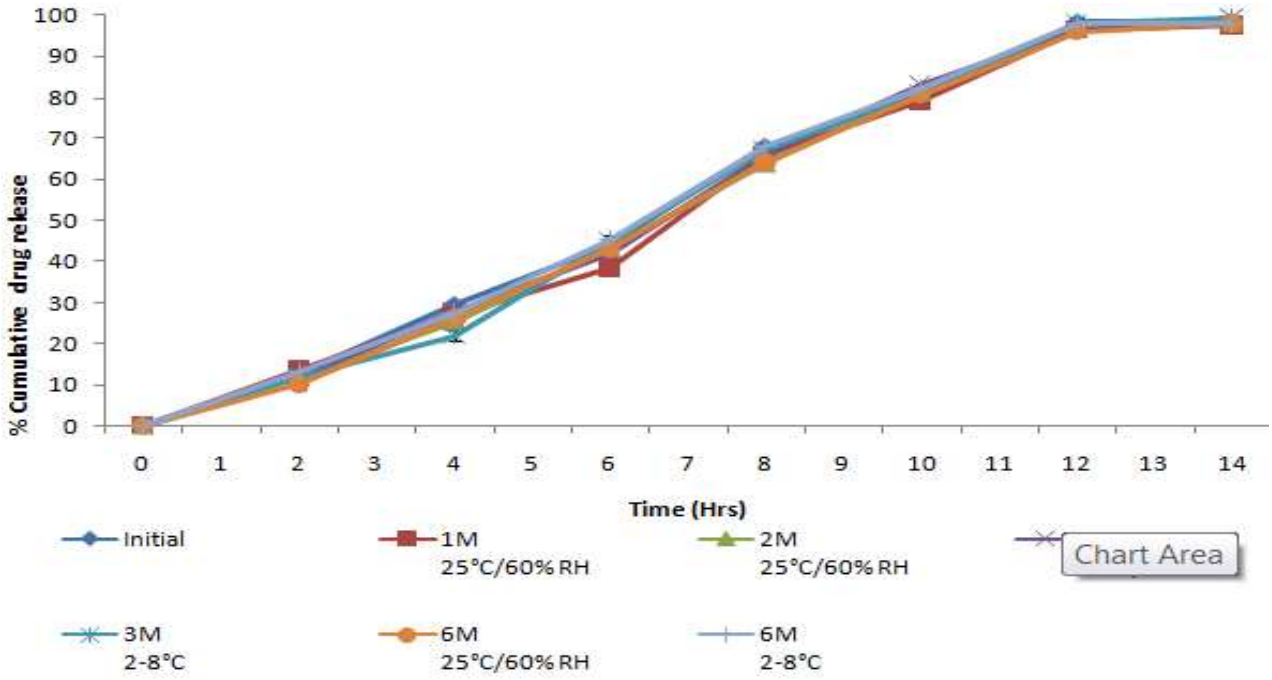
Inference: Batch No. NS9B3 is complies as per target specification at 2-8°C and 25°C/60% RH up to 6 months.

NC9B3 was chosen for the stability study.Selected formulae were packed in HDPEsealed glass bottles and stored at ambient room temperaturein desiccators over anhydrous CaCl2 and at 2-8°C in refrigerator. 25±2°C/60% relativehumidity (RH) or 40±2°C/75% relative humidity for6 months. The chosen formulae were evaluated for Mean ParticleSize (nm), Polydispersity Index, Zeta Potential, % assay, degradation product, related substances, microbial limit and zone of inhibition.

Characterization of Nanocrystal

***In-Vitro* Characterization of the Optimized Batch (Batch No. NC9B3)**

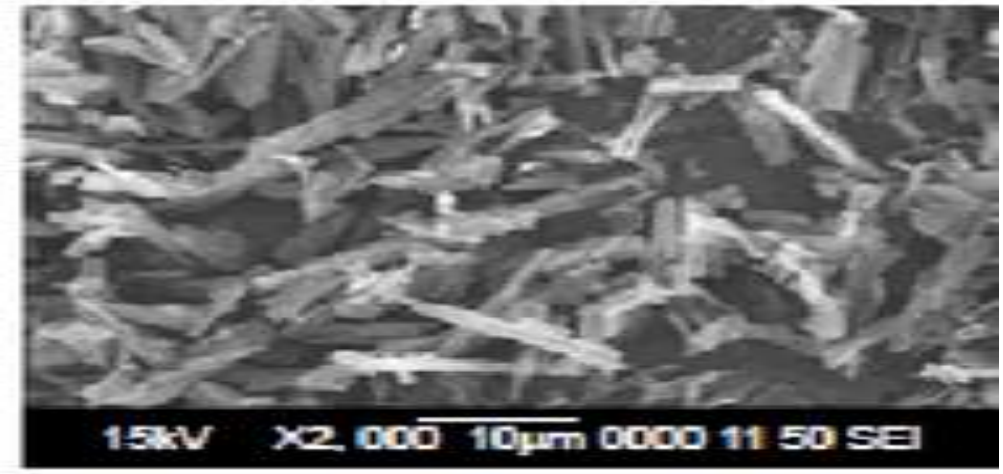
Figure 7: *InVitro* Characterizaion of stability batch Batch No. NC9B3



Characterization of NC using SEM and XRPD

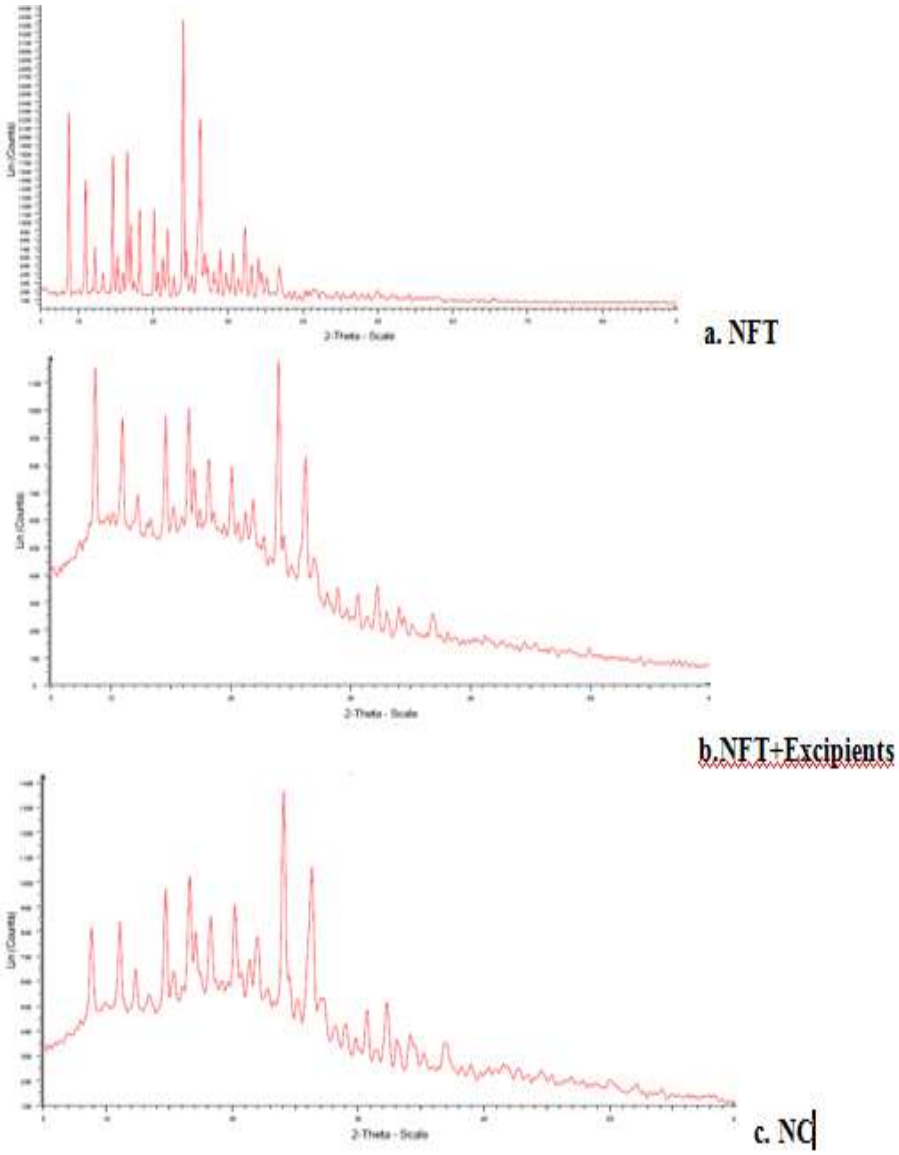
a. Characterization of NC using SEM

Figure 8: SEM Evaluation of the NC



b. XRPD Spectra of Optimized batch

Figure 9: XRD spectra



CONCLUSION:

In the present investigation, NCs of NFT were successfully manufactured using Cold High Pressure homogenization technique. The effect of the formulation composition and process parameter on the NCs were evaluated and optimized. Optimized formulation shows desired quality target product profile. Bioavailability increment indicates higher GI uptake of Nitrofurantoin nanocrystals in comparison to nitrofurantoin solution. Stability data shows that there is no significant difference in the optimized formulation after 6 months at 2-8°C and 25°C/60% RH conditions. These storage conditions of NCs were found appropriate for drug delivery. The study opens the chances of manufacturing by competitive cost at commercial level.

REFERENCES

1. Abdelwahed W., Degobert G., Fessi H. A pilot study of freeze drying of poly(epsilon-caprolactone) nanocapsules stabilized by poly(vinyl alcohol): formulation and process optimization. Int. J. Pharm. 2006; 309, 178-188.
2. Ambrus R., Kocbek P., Kristl J., Sibanc R. Investigation of preparation parameters to improve the dissolution of poorly water-soluble Meloxicam. Int. J. of Pharm. 2009; 381, 153–159.
3. Ayush G., Anshu S., Indrajeet S. Preparation and evaluation of sustained release film: Coated tablets of nitrofurantoin. Int. J. of Res. in Pharmacy and Pharm. Sci. 2016; 1(1), 47-53
4. Mahesh R, Umang K, Dhaval D.Formulation, optimization and characterization of candesartan cilexetil nanosuspension for in vitro dissolution enhancement. African J. of Pharmacy and Pharmacology.2015; 9(2), 102-113
5. Varaporn B. Junyaprasert A, Boontida M. Nanocrystals for enhancement of oral bioavailability of poorly water-soluble drugs. Asian J. of pharm. Sci. 2015; 10, 13-23.
6. Zhang D, Gao L, Chen M, Preparation and characterization of an oridonin Nanocrystal for solubility and dissolution velocity enhancement, Drug Dev. Ind. Pharm. 2007; 33, 1332–1339.
7. Abdul H, Gopinath M. Formulation and evaluation of Paliperidone Nanocrystals. BioMedRx 2013; 1(5),422-438
8. Rajveer B, Prakash H. Nanocrystal suspension of poorly water soluble antibacterial drug by high pressure homogenization technique using full factorial design. Int. J. of Pha.Sci. and Res. 2018; 9(1), 114-122.
9. Auweter H., Andre V., Horn D., Luddecke E. The function of gelatin in controlled precipitation processes of nanosize particles. J. Dispersion Sci. Tech. 1998; 19, 163–184.
10. Auweter H., Bohn H., Heger R., Horn D., Siegel B., Siemensmeyer K. Precipitated water-insoluble colorants in colloid disperse form, 2002; US Patent 6,494,924.
11. Chattopadhyay P., Gupta P. Production of Griseofulvin nanoparticles using supercritical CO_2antisolvent with enhanced mass transfer. Int J Pharm 2001; 228: 19-31.

12. Gao L., Zhang D., Chen M. Drug nanocrystals for the formulation of poorly soluble drugs and its application as a potential drug delivery system. J. Nanopart. Res. 2008; 10, 845–862.
13. Kayser O., Grau J., Muller H. Nanocrystals of poorly soluble drugs reproducibility of small-scale production. Int. J. Pharm. 2000; 196, 155-159.
14. Hanafy A., Spahn-Langguth H., Vergnault G., Grenier P. Pharmacokinetic evaluation of oral Fenofibrate Nanocrystals and SLN in comparison to conventional suspensions of micronized drug. Advanced Drug Delivery Reviews. 2007; 59, 419–426.
15. Hany M., Peter Y., Nicholas B. Preparation of hydrocortisone Nanocrystal through a bottom-up nanoprecipitation technique using microfluidic reactors. Int. J.of Pha. 2009; 375, 107–113.
16. Hetal P., Bindesh P., Sneha T. Development and characterization of Nanocrystals of Olmesartan Medoxomil for bioavailability enhancement. J. Pharm. Bio. Sci. 2011; 3, 426-434.
17. Jacobs C., Kayser O., Muller R. Production and characterization of mucoadhesive Nanocrystals for the formulation of Bupravaquone. Int. J. of Phar. 2001; 214, 3–7.
18. Joshi M., Patravale V. Nanostructured lipid carrier (NLC) based gel of celecoxib. Int. J. Pharm. 2008; 346, 124-132.
19. Kakran M., Sahoo N., Li L., Judeh Z., Wang Y., Chong K., Loh L. Fabrication of drug nanoparticles by evaporative precipitation of Nanocrystal. Int. J. of Pha. 2010; 383, 285–292.
20. Kayser O., Olbrich C., Yardley V., Kiderlen L., Croft S. Formulation of Amphotericin B as Nanocrystal for oral administration. Int. J. of Pha. 2003; 254, 73–75.
21. Muller H. Keck M., Drug nanocrystals of poorly soluble drugs produced by high pressure homoginisation. Eur. J. Pharm. Biopharm. 2006; 62, 3-16.
22. Nalneesh B, Shammi Goyal. Sustained-Release Matrix Tablets of Nitrofurantoin: Formulation and Evaluation, Int. J.of Chem.Tech. Research. 2013; 1, 491-501.
23. Maria J, Munoz D. Role of Old Antibiotics in the Era of Antibiotic Resistance. Highlighted Nitrofurantoin for the Treatment of Lower Urinary Tract Infections. J. of antibiotics. 2014; 3 (1), 39-48
24. Flexner J, Hartmann R. Megaloblastic anemia associated with anticonvulsant drugs. Am. J. Med. 2010; 3(28), 386–396
25. Ayush G, Anshu S, Indrajeet S, Preparation and evaluation of sustained release film: Coated tablets of nitrofurantoin, Int. J. of Res. in Pha. Sci. 2016; 1, 47-53.
26. Gleckman R, Alvarez S, Drug therapy reviews: nitrofurantoin, Am J Hosp Pharm. 1979; 36(3), 342-51
27. Mavromanolakis E, Maraki S, Samonis G, Tselentis Y, Cranidis A.Effect of norfloxacin, trimethoprim-sulfamethoxazole and nitrofurantoin on fecal flora of women with recurrent urinary tract infections. J. of Chemother. 1997; 9(3), 203-7
28. Zili Z, Sfar S, Fessi H. (2005) Preparation and characterization of polycaprolactone nanoparticles containing griseofulvin. Int. J. Pharm. 294: 261– 267

29. Trotta M, Gallarate M, Carlotti ME, Morel S.Preparation of griseofulvin nanoparticles from water-dilutable microemulsions. Int. J. Pharm. 2003;Mar 26, 254(2), 235-42
30. Dodiya S, Chavhan S, Korde A, Sawant K, Solid lipid nanoparticles and Nanocrystal of adefovir dipivoxil for bioavailability improvement: formulation, characterization, pharmacokinetic and biodistribution studies, Drug Dev. Ind. Pharm. 2013; 39, 733–743.
31. Wongmekiat A, Yuichi T, Keiko S,Co-grinding with Cyclodextrin as a Nanoparticle Preparation Method of a Poorly Water Soluble Drug, J. of Inclusion Phenomena and Macrocyclic Chemistry. 2002; 50: 67–71.
32. Biman B , Prakash R, Nath L, Nanocrystal Technology And Drug Delivery, World J, of Pharm. Res. 2014; 3(2), 2940-2971
33. Muller, Sucker H, Hydrosole E, Alternative fór die parenterale Anwendung von schwer was serloslichen Wirkstoffen. In: RH., GE. Hildebrand, editors. Pharmazeutische Technologie; Modern Arzneiformen. 1998; 2 nd ed. 142-150
34. Muller R, Peters K, Becker R. Nanocrystal for IV administration of poorly soluble drugs-Stability during sterilization and long term storage. Proc Int Symp Control Rel. Bioact Mater. 1995; 22, 574-5.
35. Chowdary K, Madhavi B., Novel drug delivery technologies for insoluble drugs. Ind. Drugs 2005; 42, 557-563.
36. Andrej D., Julijana K., Sasa B., Odon P. Advantages of celecoxib Nanocrystal formulation and transformation into tablets. Int. J. of Pharm. 2009; 376, 204–212
37. Dodiya S., Chavhan S., Sawant K., Korde A. Solid lipid nanoparticles and Nanocrystal formulation of Saquinavir: Preparation, characterization, pharmacokinetics and biodistribution studies. Journal of microencapsulation. 2011; 28, 515-527.
38. Hanafy A., Spahn-Langguth H., Vergnault G., Grenier P. Pharmacokinetic evaluation of oral Fenofibrate Nanocrystals and SLN in comparison to conventional suspensions of micronized drug. Advanced Drug Delivery Reviews. 2007; 59, 419–426.
39. Hany M., Peter Y., Nicholas B. Preparation of hydrocortisone Nanocrystal through a bottom-up nanoprecipitation technique using microfluidic reactors. Int. J.of Pharm. 2009; 375, 107–113.
40. Hetal P., Bindesh P., Sneha T. Development and characterization of Nanocrystals of Olmesartan Medoxomil for bioavailability enhancement. J Pharm Bioallied Sci. 2011; 3, 426-434.
41. Jacobs C., Kayser O., Muller R. Production and characterization of mucoadhesive Nanocrystals for the formulation of Bupravaquone. Int. J. of Pha. 2001; 214, 3–7.
42. Wei L., YonggangY. Preparation and in vitro/in vivo evaluation of Revaprazan hydrochloride Nanocrystal. Int. J. of Pharm. 2011; 408, 157–162
43. Gao Y, Qian S, Zhang J, Physicochemical and pharmacokinetic characterization of a spray-dried cefpodoxime proxetil Nanocrystal, Chem. Pharm. Bull. 2008; 58, 912–917.

44. Rabinow B, Nanocrystals in drug delivery, Nat. Rev. Drug Discov. 2007; 3, 785–796.
45. Patravale V, Date A, Kulkarni R, Nanocrystals: a promising drug delivery strategy, J. Pharm. Pharmacol. 2004; 56, 827–840.
46. Zhang D, Gao L Chen M, Preparation and characterization of an oridonin Nanocrystal for solubility and dissolution velocity enhancement, Drug Dev. Ind. Pharm. 2007; 33, 1332–1339.