ORIGINAL ARTICLE

Formulation Characterization of Statistically Optimized Naproxen Nanocrystals with Quality by Design

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Abstract

Objective: The present objective of the study is to prepare nanoparticles of naproxen and then converting them into a suitable dosage form. Initially, the nanosuspension of naproxen is prepared using anti-solvent precipitation technique. The ideal and best formation obtained with the application of DOE. The best nanosuspension is freeze dried to get nanoparticles which is used for further study. In addition, the various process parameters which have a potential influence on the formulation are also studied. **Methods:** Solvent-anti-solvent precipitation method was employed as the preparation technique. A full factorial design (2^4) is used to design the experiment. Based on the previous studies and literature review, the parameters were selected. The experimental design is designed in such a way to assess various parameters such as critical process parameters, material attributes, and process parameters. Naproxen nanosuspensions were lyophilized with mannitol. The experimental data obtained were subjected to statistical analysis using various methods such as regression and ANOVA. Other evaluation procedures were followed to determine the various characteristics of the formulation and dissolution and pharmacokinetic studies are also performed. **Results:** The DOE and factorial design showed the point prediction as stirring time of 22.5 min and rate of injection as 0.4 ml/min, solvent-anti-solvent ratio of 1:15, and stabilizer drug concentration of 1:7.5. The nanoparticles were found to be intact in SEM/TEM analysis and there were no drug interactions between the formulation ingredients. The zeta potential was found to be 50.2 mv. The pharmacokinetic analysis of nanoparticles showed AUC of 836 ng/ml which is twice obtained with that of pure drug. Other parameters like bioavailability of the formed nanoparticles have improved significantly when compared with that of pure naproxen. **Conclusion:** Naproxen nanosuspensions have been successfully prepared and the various process parameters which have an effect on the characteristics are studied. The predicted formulation has been prepared and evaluated for various parameters and found pharmacokinetically better when compared to pure drug.

Key words: Bioavailability, DOE, Nanoprecipitation, Nanosuspension, Naproxen, SEM/TEM

INTRODUCTION

Solubility is a major problem faced by the formulation scientist in developing a formulation. Many technologies are being developed to improve the solubility and bioavailability. Among them, the nanotechnology offers a wide variety of applications. Nanosuspension is a process in which the size of the particles is reduced to nano range and thus the bioavailability is increased.[1-3] Nanosuspensions are generally considered as a dispersed nanoparticle. Nanosuspensions offer many advantages such as ease of preparation and formulation and can produce reproducible results. There are many techniques and procedures in preparation of nanosuspension.[4-7]

Bottom up approach is mostly used in the preparation of nanoparticles in which the active ingredient is solubilized in

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any particular solvent and they are crystallized or precipitated using any suitable anti-solvent.

The scientific approach of QBD and DOE has been utilized to understand the various parameters involved and interactions among them can be evaluated. It has various advantages over conventional trial and error method like decreased number of trials and estimation of interactions and statistical approach in getting the ideal formulation.[8,9]

Naproxen is a widely used NSAID (nonsteroidal anti-inflammatory drug) in various conditions of inflammation such as arthritis. It has its applications in the treatment of rheumatoid arthritis, spondylitis, gout, migraine, etc. Chemically, naproxen is a derivative of propionic acid a pale white and odorless substance. It is highly lipophilic and possesses a very low aqueous solubility.[10-12]

Our present study aims at the preparation of nanoparticles of naproxen and then converting them into a suitable dosage form. Initially, the nanosuspension of naproxen is prepared using anti-solvent precipitation technique. The ideal and best formation obtained with the application of DOE. The best nanosuspension is freeze dried to get nanoparticles which is used for further study. In addition, the various process parameters which have a potential influence on the formulation are also studied.

MATERIALS AND METHODS

Materials

Naproxen was obtained as a gift sample from Dr. Reddy's Laboratories, Hyderabad. All other ingredients such as acetone and Poloxamer 188 were procured from Sigma-Aldrich. All other ingredients were used of analytical grade or higher.

Methods

Preparation of nanosuspension

Solvent-anti-solvent precipitation method was employed as the preparation technique. Briefly, the API (10 mg) was dissolved in acetone (2 ml) which acts as a solvent and it is added slowly at a predetermined rate (0.2 ml/min or 0.6 ml/min) into an anti-solvent (water + Poloxamer 188 (stabilizer)) under stirring (Remi) at determined time (15 min or 30 min) and speed (2000 RPM).[13]

Lyophilization

Naproxen nanosuspensions were lyophilized with mannitol. Nanosuspensions changed into distributed into vials and frozen at −80° C for 4 h. Then, it was transferred to a freeze-dryer and dried at a pressure of 0.098 Mbar for 24 h at −80 ± 0.5° C. The lyophilized naproxen nanosuspensions were found to be redispersible.

Experimental design

A full factorial design (2^4) (Design Expert) is used to design the experiment. Based on the previous studies and literature review, the parameters were selected. The experimental design is designed in such a way to assess various parameters such as critical process parameters, material attributes, and process parameters. Each factor is used at two levels based on the preliminary experiments. The parameters along with the identified levels are given in Table 1.

The responses selected are particle size and PDI which play a key role in determining the stability of the product.

Statistical analysis

The experimental data obtained were subjected to statistical analysis using various methods such as regression and ANOVA. The polynomial expression obtained through the software is noted.

Evaluation of the nanoparticles

SEM/TEM

The optimized, stabilized nanosuspension was used further for the evaluation parameters. The SEM/TEM analysis

Table 1: Parameters along with levels

Type	Name of the parameter	Low level	High level
Critical process parameter	Rate of injection	0.2 ml/min	0.6 ml/min
	Stirring time	15 min	30 min
Formulation parameter	Solvent: anti-solvent ratio	1:20	1:10
	Stabilizer: drug	1:10	1:5

Table 2: Characteristic peaks observed in ATR spectrum

Type of peak	Characteristic value	Pure	Formulation
-OH stretching	3166	Yes	Yes
-CH_3 stretching	3002	Yes	Yes
-C=O stretching	1727	Yes	yes
-C-O stretching	1252	Yes	Yes

Table 3: Responses and critical process attributes of naproxen nanosuspension

A: Stirring time (min)	B: Rate of injection (ml/min)	C: Solvent: anti-solvent	D: Stabilizer: drug	Particle size (nm)*	PDI*
30	0.6	1:10	1:10	350.1±15.2	0.25±0.02
15	0.6	1:20	1:10	360.4±21.6	0.26±0.01
15	0.6	1:10	1:10	322.1±7.2	0.22±0.02
15	0.2	1:20	1:5	296.3±13.5	0.18±0.04
30	0.6	1:10	1:5	183.6±11.6	0.09±0.02
30	0.2	1:20	1:10	210.2±11.9	0.1±0.01
15	0.6	1:20	1:5	339.2±7.5	0.24±0.02
15	0.2	1:20	1:10	308.9±16.5	0.2±0.06
30	0.6	1:20	1:5	333.7±13.9	0.23±0.04
15	0.6	1:10	1:5	119.6±12.3	0.05±0.01
30	0.2	1:10	1:10	236.2±21.3	0.12±0.02
15	0.2	1:10	1:10	322.2±12.4	0.21±0.06
15	0.2	1:10	1:5	132.8±12.8	0.07±0.02
30	0.6	1:20	1:10	306.8±21.8	0.19±0.02
30	0.2	1:20	1:5	273.4±13.2	0.16±0.06
30	0.2	1:10	1:5	129.1±6.8	0.06±0.01

*Mean of 3 (n=3)

Table 4: ANOVA table for response particle size

Source	Sum of squares	df	Mean square	F-value	*P*-value	Significant
Model	60502.75	4	15125.69	3.70	0.0382	
A – Stirring time	1980.25	1	1980.25	0.4845	0.5008	
B – Rate of injection	10302.25	1	10302.25	2.52	0.1407	
C – Solvent: anti-solvent	24964.00	1	24964.00	6.11	0.0310	
D – Stabilizer: drug	23256.25	1	23256.25	5.69	0.0362	
Residual	44961.00	11	4087.36			
Cor total	1.055E+05	15				

Table 5: ANOVA table for response PDI

Source	Sum of squares	df	Mean square	F-value	*P*-value	Significant
Model	0.0437	4	0.0109	3.46	0.0461	
A – Stirring time	0.0033	1	0.0033	1.05	0.3281	
B – Rate of injection	0.0116	1	0.0116	3.66	0.0821	
C – Solvent: anti-solvent	0.0150	1	0.0150	4.75	0.0518	
D – Stabilizer: drug	0.0138	1	0.0138	4.37	0.0605	
Residual	0.0347	11	0.0032			
Cor total	0.0784	15				

Table 6: Point prediction for optimum formulation

Stirring time	Rate of injection	Solvent: anti-solvent	Stabilizer: drug
22.5 min	0.4 ml/min	1:15	1:7.5

(JEOL, JSM-6390, Tokyo, Japan) was generally used to identify the shape and surface morphology of the particles. Freshly prepared nanosuspension is suitably diluted and it was poured on carbon coated paper, dried, and observed under microscope with suitable magnification.

Table 7: Drug release kinetics of naproxen nanosuspension

Formulation	Zero order		First order	
	Equation	R^2	Equation	R^2
NP (naproxen pure)	y=0.5131×+1.4607	0.9844	y = –0.0027×+1.9966	0.9925
NNS (naproxen nanosuspension)	y=1.2351×+39.018	0.5998	y = –0.027×+1.8585	0.9481

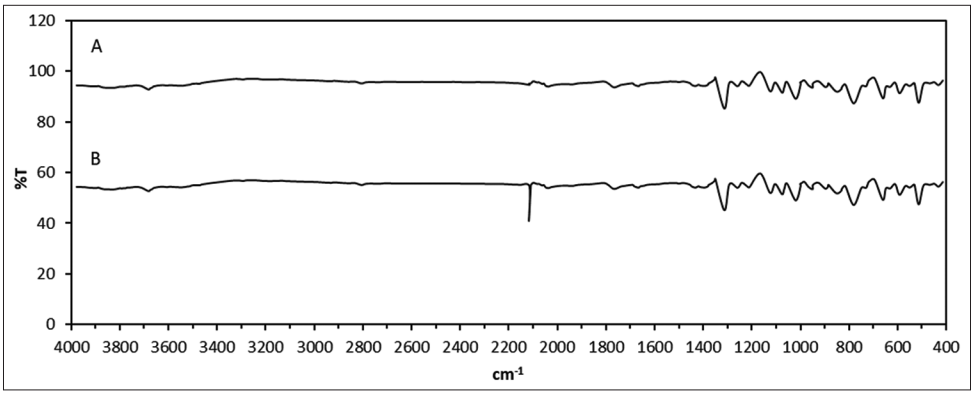

Figure 1: ATR spectrum. *A indicates pure naproxen and B indicated final formulation

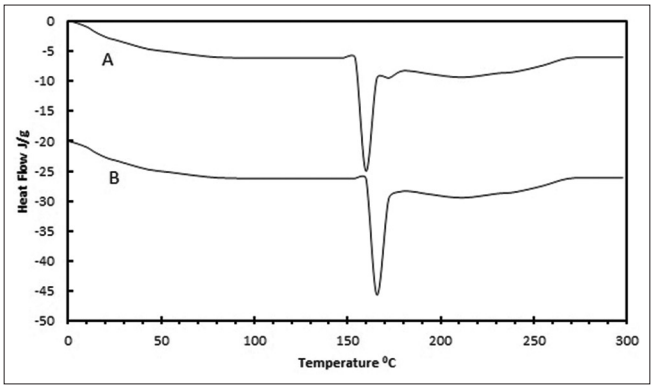

Figure 2: DSC thermogram. *A indicates pure naproxen and B indicated final formulation

Particle Size, Zeta Potential, and PDI

Zeta potential indicates the potential present at the double layer and it plays a major role in determining the stability of the nanosuspension. The measurement of zeta potential is done by sing Malvern Zetasizer (Zetasizer Nano ZS 90, Malvern Ltd., UK) by loading the nanosuspension into capillary cells.

ATR

The ATR (attenuated total reflectance) studies are performed to identify any interaction between the drug and other formulation ingredients. The pure drug and the final formulation ATR were recorded using Bruker instrument and analyzed for any interactions.

DSC

DSC is a measurement of the thermal behavior of the suspension. The freeze-dried suspension is loaded on to a standard aluminum pan and the temperature is increased at intervals of 10°C/min and the values are recorded. Q200 (TA instruments) is used to determine the sample thermal behavior.

Dissolution studies

Naproxen nanosuspension dissolution study was performed eight station dissolution test apparatus (Electrolab TDT08L) employing a USP II (Paddle) type apparatus at speed of 50 RPM and temperature of 37 ± 1° C. The dissolution media (900 ml) used was 0.1 N HCL (pH 1.2). The drug release from the nanosuspension is measured using the validated method, the dissolution data are analyzed for various release kinetics.

In vivo pharmacokinetic studies

The optimized formulation was selected for the determination of various pharmacokinetic parameters and bioavailability studies. The institutional animal ethical committee (Proposal Number: 03/GNIP/CPCSEA/IAEC/2020) approved all the protocols and the procedures were followed according to the established guidelines. Male Wistar albino mice were selected for the study (procured from Sri Vyas Labs, Hyderabad) and they are acclimatized for 7 days before the experiment. All the animals had free access to food and water. The mice were fasted overnight before the administration of the nanosuspension (p.o 8 mg/kg BW). The mice divided into two groups and control group had access to pure water. The blood was collected from retro-orbital plexus, centrifuged at 2500 RPM, and analyzed for the drug content according to the developed and validated method.[14-16]

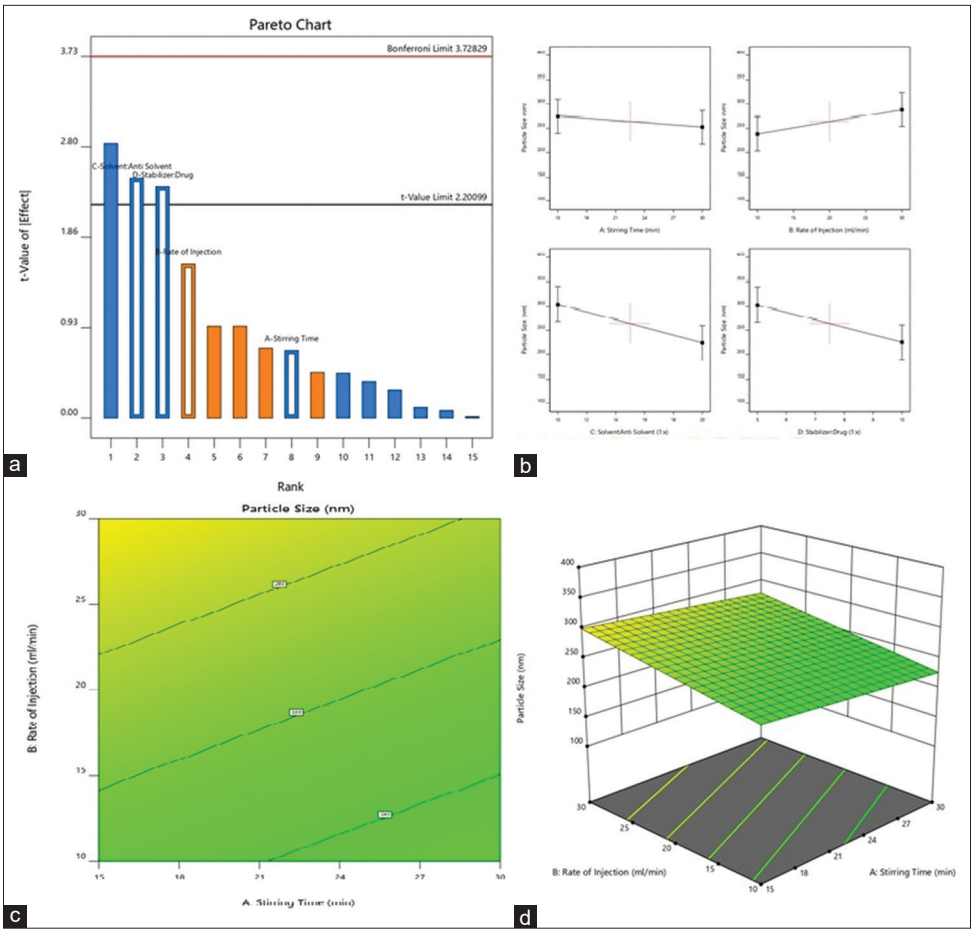

Figure 3: (a) Pareto chart, (b) main effects, (c) contour plot, and (d) 3D surface response plot of response particle size

RESULTS AND DISCUSSION

ATR

The ATR spectrum of the pure naproxen along with the final formulation is shown in Figure 1, which shows that there is no sign of major interactions between the drug and the other ingredients. The characteristic peaks of naproxen showed are given in Table 2.

DSC

The DSC of the pure drug is showed in Figure 2 and peak at 160°C while the peak of the combined formulation showed slightest change in peak at 168°C which shows no interaction between the drug and the ingredients. The retention of the peak showed the crystalline nature of the drug.

Effect of process and parameters

The effects of the various parameters and the responses are shown in Table 2, the parameters along with the responses are shown in Table 3.

The ANOVA table for the particle size was showed in the Tables 4-6 showing P <0.05 indicating that the model is significant and the responses can be predicted.

The Pareto chart shown in Figures 3 and 4 showed that the selected process variables and material variables showed a response over the mean particle size of the naproxen nanosuspension. The main effects of all the parameters are shown in figure which showed that the material variables have more effect than the process variables. Among all the parameters, the anti-solvent: solvent ratio has most influence on the particle size. An interaction also reported that as the anti-solvent ration increases the particle size decreases and the opposite effect was observed with stabilizer concentration. Among all the parameters, the stabilizer: drug concentration has most influence on the particle size. An interaction also reported that as the anti-solvent ratio increases the PDI decreases and the opposite effect was observed with stabilizer concentration. Among all the combinations, the point predicted formulation obtained was selected for further evaluation. The final selected formula was shown in the table.

Evaluation of nanosuspension

SEM/TEM

The SEM and TEM images are shown in Figures 5 and 6; the images showed the spherical nature of the particles and showed no sign of aggregation and the surface morphology is also clear.

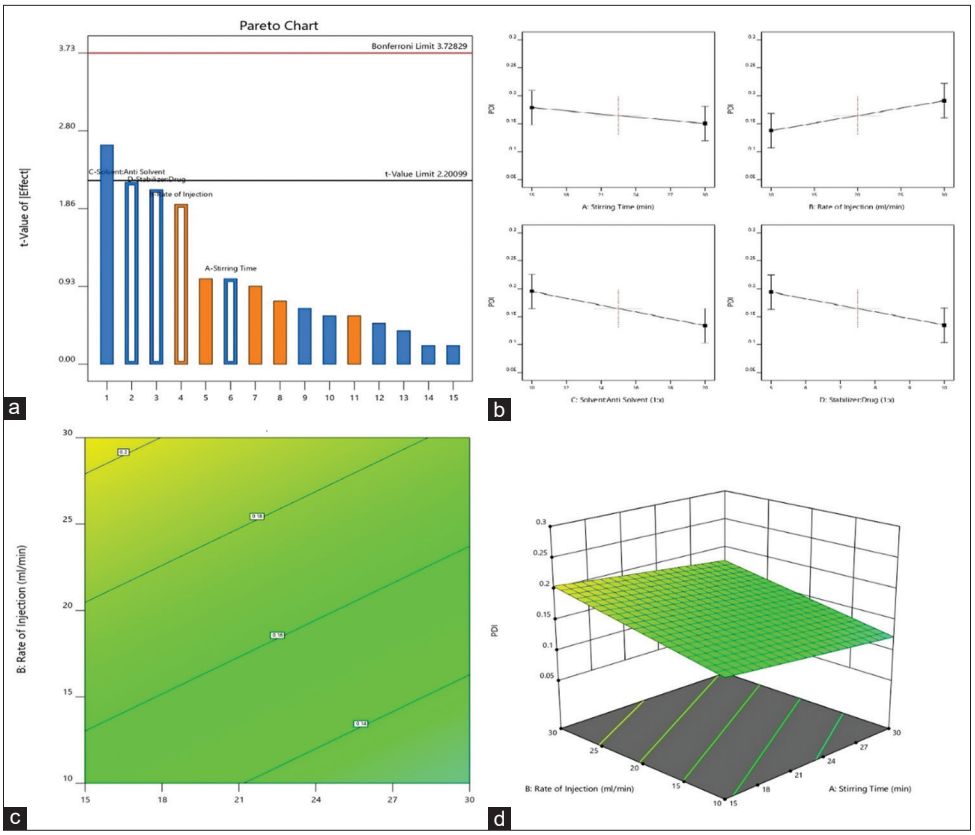

Figure 4: (a) Pareto chart, (b) Main effects, (c) Contour plot, and (d) 3D surface response plot of response PDI

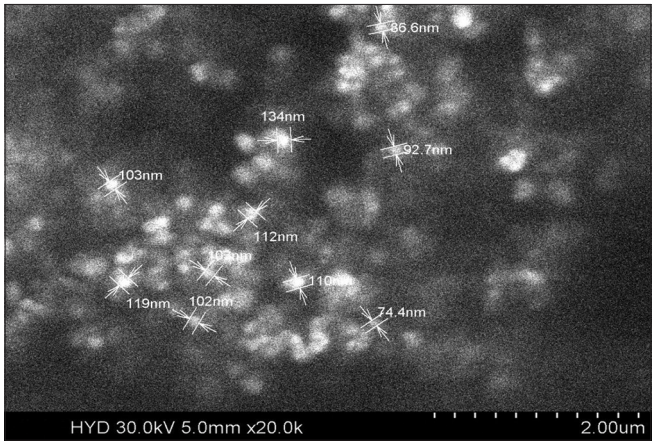

Figure 5: SEM image of naproxen nanosuspension

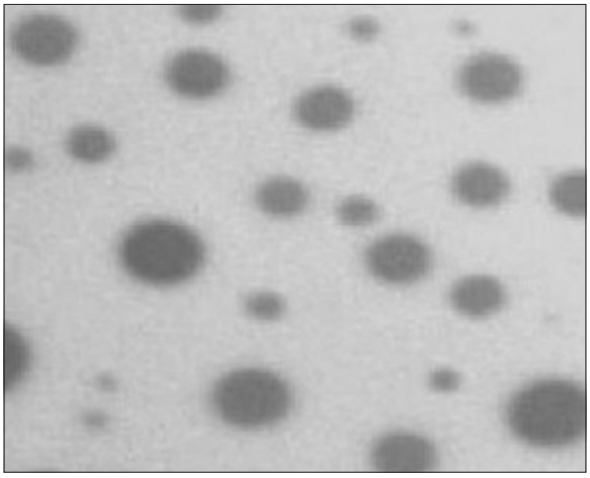

Figure 6: TEM image of naproxen nanosuspension

PXRD

The PXRD studies of the pure drug and the final formulation are shown in Figure 7. The spectra indicate the crystalline nature of the drug. The peaks at 19.100, 24.260, 26.100 are retained shows that there are no major interactions between the drug and excipients and crystallinity of the drug is retained.

Particle size

The particle size of the nanosuspension is determined by the Malvern Zetasizer and the values are noted in the table. All the values obtained were in acceptable size and it has been showed that the process parameters can be a potential influencer in determining the particle size and PDI.

Zeta potential

The zeta potential of the final selected formulation was found to be 50.2 which is generally considered as stable formulation as the zeta potential is sufficient enough to make stable nanosuspension. The zeta potential graph is shown in Figure 8.

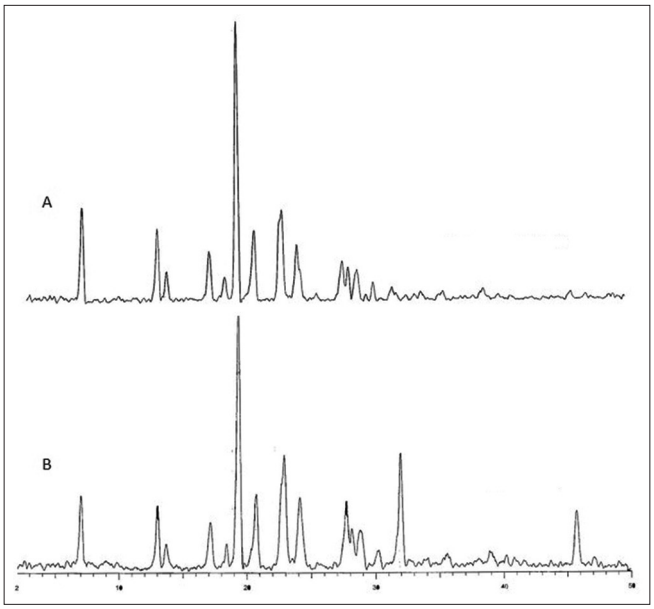

Figure 7: PXRD. *A indicates pure naproxen and B indicated final formulation

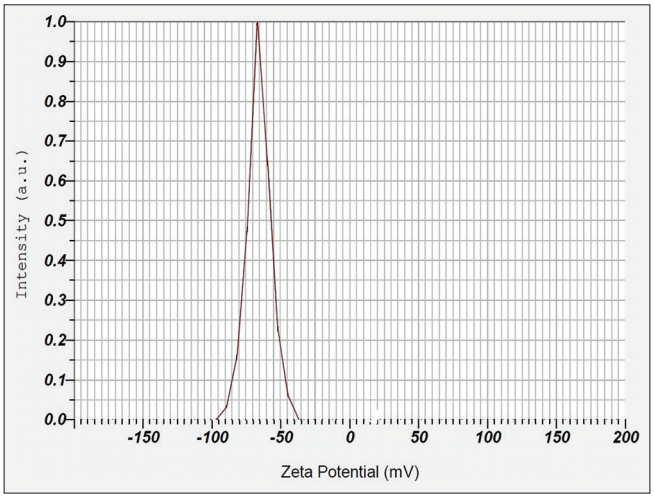

Figure 8: Zeta potential graph of naproxen nanosuspension

In vitro dissolution studies

The best selected naproxen nanosuspension was subjected to dissolution and the data were shown in tables and graphs, the pure naproxen does not show any significant dissolution whereas the naproxen nanosuspension showed a maximum release within 30 min. The release kinetics showed that it followed first-order mechanism and the release kinetics is shown in Figure 9 and Table 7. The naproxen nanosuspension follows first-order release kinetics.

Pharmacokinetic data

The pharmacokinetic data of the pure drug and the naproxen nanosuspension formulation are shown in Table 8 and Figure 10, it was evident that the naproxen nanosuspension formulation showed better pharmacokinetic parameters when compared to pure drug.

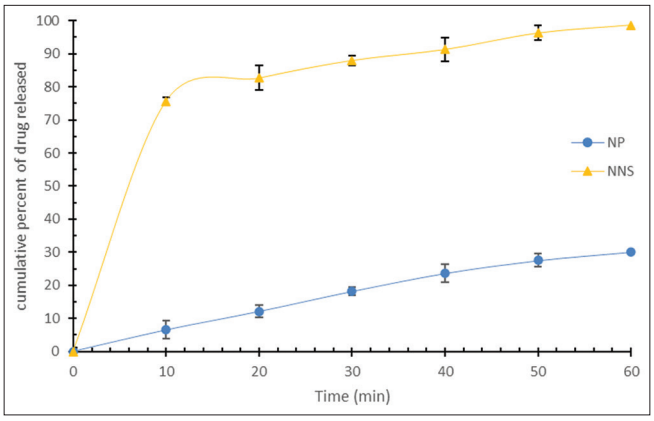

Figure 9: Drug release plot of naproxen nanosuspension. *Mean of 3 (*n*=3)

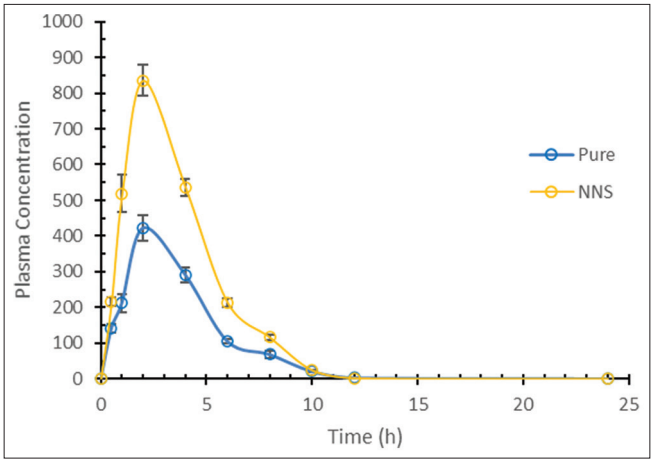

Figure 10: Pharmacokinetic drug profile of naproxen nanosuspension. *Mean of 3 (*n*=3)

Table 8: Pharmacokinetic profile of naproxen nanosuspension

Parameter	Unit	Pure	NNS
t1/2	h	0.9	0.68
Tmax	h	2	2
Cmax	ng/ml	421	836
AUC 0-t	ng/ml*h	1869.25	3587.25
AUC 0-∞	ng/ml*h	1873.09	3589.22
MRT	h	3.545	3.395

CONCLUSION

Naproxen owing its low solubility and irritability nanoparticulate approach is preferable as it overcomes the major disadvantages of the drug. The nanosuspension can be prepared conveniently and the various process and material attributes play a major role in determining the efficiency of

the delivery system, the material variable interactions are also taken into consideration while designing a suitable dosage form. The results obtained were in confirmation to already reported literature on naproxen nanosuspension.[10] The naproxen nanoparticles have been successfully evaluated for various pharmacokinetic parameters.

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