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NANOCRYSTAL SUSPENSION OF POORLY WATER SOLUBLE ANTIBACTERIAL DRUG BY HIGH PRESSURE HOMOGENIZATION TECHNIQUE USING FULL FACTORIAL DESIGN

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ABSTRACT: Cefixime trihydrate belongs to third generation cephalosporin having poor water solubility. In the present study, nanocrystal suspensions of cefixime trihydrate were prepared with the objective of providing enhanced solubility and stability with reduced particle size and thus improving the bioavailability potential. Nanocrystal suspensions were prepared by high pressure homogenization technique and evaluated for particle size, polydispersity index, zeta potential, and drug release. 2^3 factorial design was used for the formulation designing purpose. Particles of average size 143.5nm having PDI of 0.269 were produced. Zeta potential was found to be -36.6mV and the formulation was found stable on the basis of results obtained from differential scanning calorimetry and scanning electron microscopy studies. The drug release and *ex-vivo* permeation studies revealed enhanced permeation of drug, as desired, indicating its potential for an attempt towards successful nanocrystal formulation.

INTRODUCTION: A major challenge in oral drug delivery is the low bioavailability of water insoluble therapeutics [1]. Two known mechanisms to increase the bioavailability of water insoluble drugs are to increase the surface area and to create stable amorphous forms [2 - 3]. A major hurdle that has prevented the commercialization of many promising poorly soluble drugs is dissolution rate limited bioavailability. Many approaches have been developed to improve solubility and to enhance the dissolution rate and oral bioavailability of poorly soluble drugs *e.g.* salt formation [4 - 6] solid dispersion [7 - 9] inclusion complex [10].

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Cefixime trihydrate, an orally active third generation cephalosporin antibiotic, is extensively used for treatment of urinary tract infection (UTI), upper and lower respiratory tract infection, acute otitis media, and Gonococcal urethritis [11].

On an oral administration, it gets slowly and incompletely absorbed from GIT. Antibacterial activity of cefixime exhibited by interfering with bacterial peptidoglycan synthesis after binding to the β-lactam binding proteins [11 - 12]. Cefixime fall in a low solubility and low permeability antibiotic drug. Bioavailability of drug is restricted due low solubility and due low solubility of cefixime, its bioavailability is only 30-40% if given orally and show maximum peak plasma concentration within 2-4 hours [13 - 14]. Here, it is necessary to realize that an ionic charge on cefixime creates difficulty to cross mucosal membrane of the intestine, resulting in poor bioavailability of 40 - 50% [15 - 16].

The poor water solubility of drugs such as CFX is a major hurdle for drug formulation. It results in poor bioavailability and therapeutic failure of the drug due to their inability to adequately reach their target sites [17 - 18]. Nanocrystal suspensions (NCS) are promising candidates that can be used to enhance the dissolution rates of such poorly soluble drugs [19-21]. In NCS, the drug is maintained in the required crystalline state with reduced particle size which results in an increase in surface area and creation of high energy surfaces due to the disruption of drug microcrystals to nanoscopic particles (NPs).

In addition, particles size reduction to the nanoscale decreases the diffusional distance on the surface of drug NPs, thus enhancing the concentration gradient. Moreover, NPs tend to have an increased adhesiveness to surfaces, cell membranes and intestinal wall, which contributes to increased drug absorption and reduced inter-individual variation with overall enhanced drug bioavailability [22]. Despite recent progresses and numerous researches on NCS technology, stability issues limit their applications in pharma industries [23 - 24].

Aggregation of the nanoscoic particles is the main stability issue which can occur during preparation process or storage and it is due to the Ostwald ripening phenomenon [25]. Among various ways to overcome NCS stability problem such as stabilizer addition [25 - 27], solidifying is one of the main solutions [23, 26, 28]. The solid state is preferred over the aqueous nanosuspensions, due to the fact that aggregation and other instability factors are significantly decreased. Therefore, nanosuspensions are commonly converted to the solid state which can be further formulated in to other dosage form [29 - 30].

MATERIALS AND METHODS: Cefixime trihydrate was purchased from Parabolic Drug Ltd., Mumbai, India. PVP K-30, SLS, Tween 80 were purchased from Moylchem, Mumbai, India. Dialysis membrane and polyvinyl alcohol (PVA), was procured from Hi-media Labs New Delhi, India. All analytical grade chemicals and reagents were used in the present study.

Selection of Stabilizer: NCS were prepared using different stabilizers alone and in combination and were evaluated with respect to the particle size, PDI using Malvern Zeta sizer Nano-ZS90, UK. Selection of the stabilizer was made based on minimum particle size and PDI.

Preparation of Cefixime Trihydrate NCS: NCS were prepared by adding the cefixime trihydrate in water containing stabilizer which was added for the stabilization of the resultant suspension. This suspension of insoluble drug was first homogenized by using ultraturrex T-25 (IKA, Germany) at 10,000 rpm for 15 minutes then passed through high pressure homogenizer (HPH) at predetermined optimized pressure and cycles (temperature maintained below 10 °C during ultraturrex and HPH). On the basis of literature survey and trail batches various critical process variables and formulation variables were optimized by using 3^2 factorial designs with Design Expert 10.0.6 software (Stat-Ease, Inc., USA). The pressure, number of cycles in HPH and stabilizer concentration was selected as undependable variables and response on particle size, PDI and drug release were investigated.

Particle Size, Polydispersity Index, and Zeta Potential: The mean particle size analysis (Z-average) and polydispersity index (PDI) were determined by dynamic light scattering principle (Zetasizer Nano ZS90, Malvern Instruments UK). The samples were diluted as per user manual and results obtained for size and PDI were recorded. The test was conducted at an angle of 173º backscatter (NBS default) with measurement position set at 4.65mm. Measurements for zeta potentials were carried out at 25µ using double distilled water as a dispersant in a clear disposable zeta cell.

Differential Scanning Calorimetry (DSC): A differential scanning calorimeter (Mettler Toledo DSC 1) with STAR^{e}SW 10 software was used for recording and processing DSC thermograms of cefixime, PVP K-30 and optimized NCS. The accurately weighed sample (3 - 5mg) was placed in an aluminium pan. The experiment was carried out in nitrogen atmosphere at a scanning rate of 10µ/ min in the range of 40–340µ.

Fourier Transform Infrared Spectroscopy (FTIR): IR spectra of pure drug, physical mixture of drug and selected stabilizer and optimized formulation were recorded using FT-IR spectrophotometer (FTIR-8400S, Shimadzu, Japan) in the range of 4000 - 400cm^{-1} and compared for any significant change.

Scanning Electron Microscopy (SEM): The morphology of the cefixime trihydrate and optimized formulation were investigated by scanning electron microscopy (SEM, JOEL JSM-5600) at an accelerating voltage of 1.0 kV. Double coated carbon conductive tabs are mounted on SEM sample stubs.

Drug Release Study: *In-vitro* drug diffusion profile was obtained by dialysis-bag / dialysis-sac method [31]. Optimized NCS and raw drug suspension were filled in activated dialysis membrane bags (dialysis membrane 110 (LA 395), Hi-Media) and suspended in glass beakers containing methanolic phosphate buffer saline (PBS) (pH 6.8). Cefixime trihydrate has limited solubility in buffer but is soluble in methanol; hence methanol was added to PBS pH 6.8 to maintain the perfect sink conditions [32]. The beakers were placed on magnetic stirrers and stirred with magnetic beads and were covered with paraffin film to prevent any evaporative loss during the experimental run [33]. Aliquots were withdrawn from the receptor compartments at periodic time intervals for 24 hours and replaced with equivalent amounts of fresh diffusion medium. The aliquots were analysed spectrophotometrically at 288 nm. All the experiments were performed in triplicate.

Permeation Study: Optimized NCS formulation permeation study was conducted on wistar albino rats against drug solution as control for permeation across rat intestine. The rats kept on overnight fasting were sacrificed and their intestine were excised into 5cm long pieces from distal to the duodenojejunal flexure and washed thoroughly with saline solutions. Each piece of intestine was legated at one end with silk thread, filled with NCS (equivalent to 10mg of drug) and the other end was legated to form a balloon-shaped sac. The sac containing NCS were immersed in Krebs solution (100ml) contained beaker with stirring (200 rpm). Aliquots (2ml) were withdrawn from beaker at specified time interval, *i.e.* 15, 30, 45, 60, 90 and 120 min and same volume of fresh buffer was added to beaker in order to maintain sink condition.

RESULT AND DISCUSSION:

Selection of Stabilizer: For the selection of stabilizer, NCS were prepared with six different stabilizers and were evaluated for particle size and PDI. PVP K-30 was found to give minimum particle size and PDI. Stabilizers are used to provide short term and long-term storage stability for nanosuspension along with the stabilization of nanocrystals during particle production. Non-ionic stabilizers are reported to sterically stabilize the nanoparticles [34]. In general, bulky and non-ionic surfactants are reported to be less toxic than single-chain and ionic surfactants [35]. Hence, PVP K-30 was selected for further investigations for the formulation.

Optimization of Process and formulation Variables: Critical process and formulation variables which may have significant effect on the critical quality attributes were identified for each step involved in the formulation, that is, stabilizer concentration, pressure and number of cycles in HPH. Preliminary optimization of stirring time and RPM of high speed homogenizer (IKA, Germany) were carried out by conducting the experiments at three different RPM (5,000 to 15,000) for three-time durations (10 to 20 minutes) at room temperature.

From the results obtained (results not shown) in terms of particle size and PDI, it was observed that as the homogenization time and/or homogenization speed increased, the particle size decreased which may be attributed to increased force of deformation at higher speed leading to smaller particles. It was also observed that with the increase in homogenization time PDI also increased which may be due to formation of foam and more aggregation in the formulation. Best results were obtained by stirring at 10,000 RPM for 15 minutes. Hence further investigations were done with homogenization / stirring speed of 10000 RPM for 15 min. Critical process variables involved during the HPH were pressure, number of cycles and stabilizer's concentration.

These were optimized using 2^3 factorial designs with Design Expert 10.0.6 software (Stat-Ease, Inc., USA). Eight runs were carried out with pressure (400 to 800 bars) number of cycles (1 to 5) and stabilizer concentration (30 to 60% wt of drug) as independent variables and particle size dependent variables. Full factorial design used for optimization of process variables as shown in **Table 1**.

ANOVA was applied to determine the significance and the magnitude of the effects of the variables and their interactions showed in **Table 2**.

TABLE 1: FACTORIAL DESIGN WITH CODED VALUES OF FORMULATION VARIABLES (INDEPENDENT VARIABLE: CONCENTRATION OF STABILIZER, CYCLE AND PRESSURE IN HPH, DEPENDENT VARIABLE: PARTICLE SIZE)

Run	Stabilizers conc.	Cycle	Pressure	Response (PS in nm)
1	+1	-1	+1	291.6
2	+1	+1	+1	143.5
3	-1	-1	+1	309.3
4	-1	+1	-1	399.1
5	+1	+1	-1	376.3
6	-1	+1	+1	253.3
7	+1	-1	-1	403.7
8	-1	-1	-1	468.2

TABLE 2: ANOVA FOR RESPONSE SURFACE MODEL FOR RESPONSE (PARTICLE SIZE)

Term	Sum of squares	% contribution	P value
A- stabilizer	5.40	8.30	0.1372
B- Cycle	10.40	15.98	0.0618
C- Pressure	42.99	66.07	.0064
AB	0.76	1.16	-
AC	0.68	1.04	-
BC	2.43	3.34	-
ABC	2.41	3.71	-

Full model equation for particle size in terms of coded factors was obtained as

Sqrt (Particle size) = +17.96 – 0.82∗A – 1.14∗B– 2.32∗C ------------- (1)

The individual effect of surfactant concentration (A) was found to be insignificant (p value = 0.1372), number of cycles (B) was found to be marginally significant (p value = 0.0618) and of pressure (C) was significant (p value = 0.0064). Pareto chart reveals that pressure has most significant effect on particle size compared to HPH cycle and stabilizer concentration as given in **Fig. 1**.

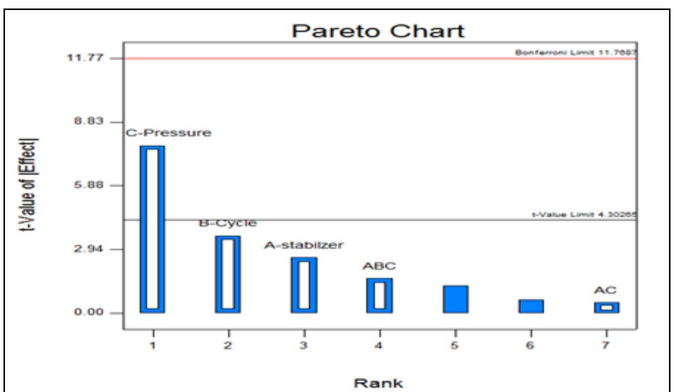

FIG. 1: RELATIVE SIZE OF DIFFERENT FACTORS ON RESPONSE

The regression model obtained was used to generate the contour and 3D surface plots as shown in **Fig. 2** for analysing interactions of the independent factors reveals as the pressure and/or numbers of cycles were increased, the particle size was found to be reduced.

Particle Size, Polydispersity Index (PDI), Zeta Potential: The average particle size, PDI and zeta potential of the optimized NCS formulation determined using Malvern Zeta sizer Nanoseries Nano-ZS, were found to be 143.5 nm, 0.269 and −36.6 mV respectively, as shown in **Fig. 3**. Most NCS formulations produced by high pressure homogenization (HPH) are characterized by an average particle size below 500nm, attainable particle size is especially dependent on the concentration of stabilizer, pressure and numbers of cycles in HPH. Low PDI value (<0.3) indicated the narrow distribution of size (mono-dispersity) and stability of the formulation was indicated by the zeta potential value (- 36.6mV). Zeta potential is an important physicochemical parameter that influences the stability of nanosuspensions. Extremely positive or negative zeta potential values cause larger repulsive forces, whereas repulsion between particles with similar electric charge prevents aggregation of the particles and thus ensures easy redispersion. A minimum zeta potential of ± 20mV is desirable for the stabilization achieved by steric stabilizers [26].

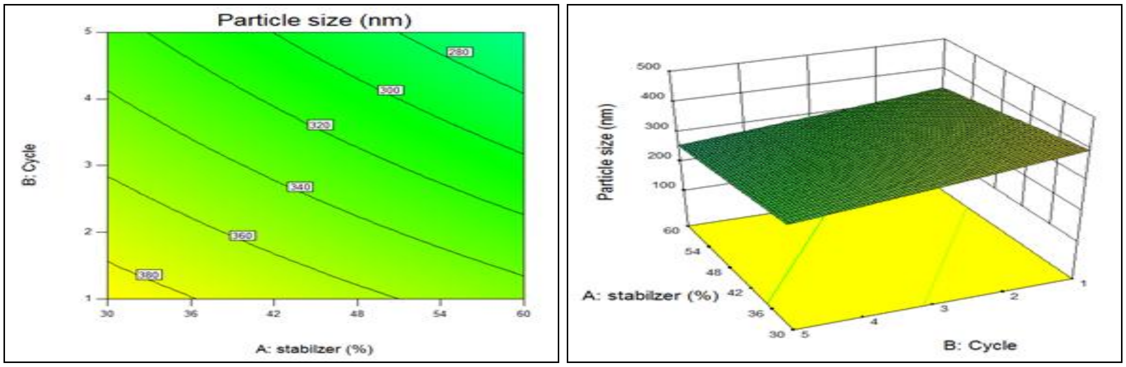

FIG. 2: CONTOUR AND 3D RESPONSE SURFACE PLOT SHOWING EFFECT OF PRESSURE AND CYCLE OF HPH ON PARTICLE SIZE

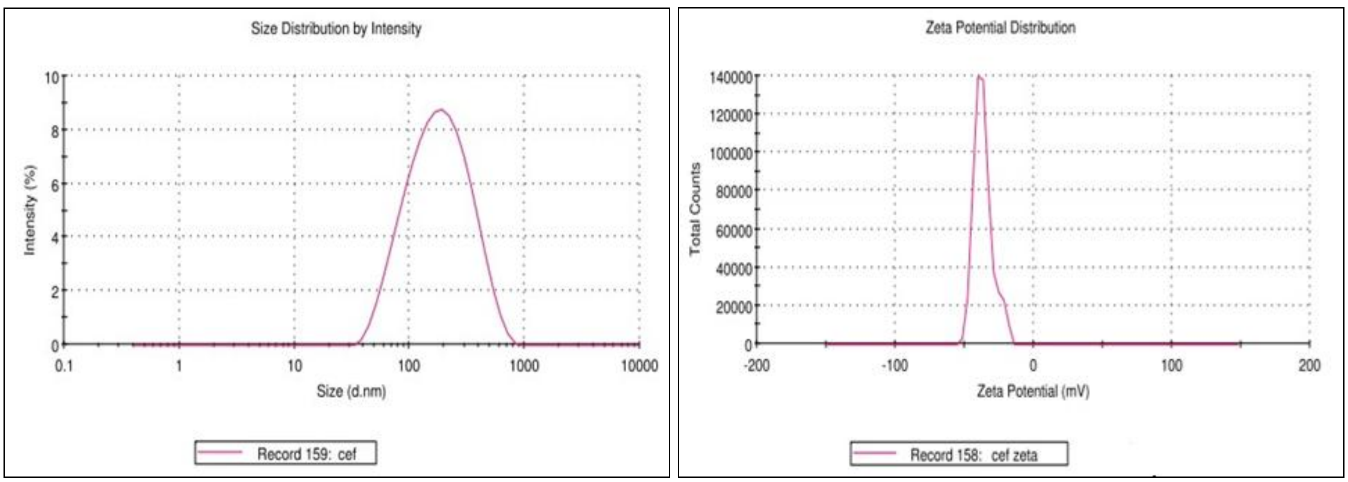

FIG. 3: PARTICLE SIZE AND ZETA POTENTIAL OF OPTIMIZED NCS FORMULATION

Differential Scanning Calorimetry (DSC): The DSC thermograms of cefixime trihydrate, PVP K-30 and optimized formulation have been represented in **Fig. 4**, which can provide valuable information about physiochemical state of nanoparticles. The influence of the heat flow on the thermal properties of stabilizer, drug, and optimized formulation are illustrated. For raw cefixime trihydrate, the endothermic peak around 100 - 110 °C represents the evaporation of water molecules from the crystal lattice and exothermic peaks at 215 °C and 250 °C could be related to the crystalline state transition and drug decomposition, respectively. In the thermogram of PVP K-30, a broad endothermic peak ranging from corresponding to the loss of water was observed and can be due to the hygroscopic nature of PVP polymers. In CFX nanoparticles thermogram, both endothermic peaks of cefixime and PVP-k 30 were shifted to 180 °C and the exothermic peak of CFX decomposition was still visible at 250 °C.

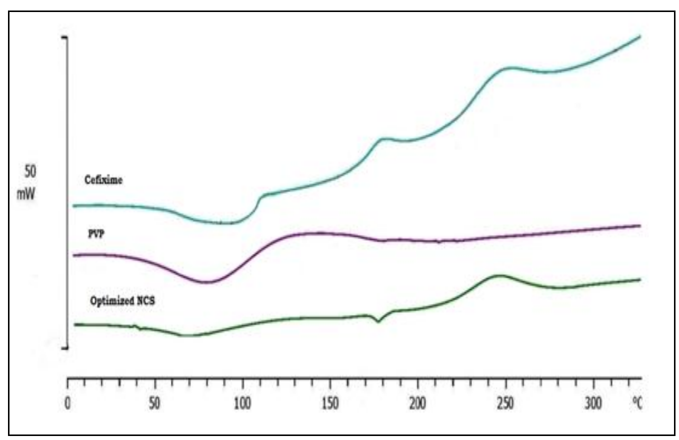

FIG. 4: DSC THERMOGRAM OF PURE DRUG, STABILIZER AND OPTIMIZED FORMULATION

Fourier Transform Infrared Spectroscopy (FTIR) Studies: FT-IR spectroscopy of cefixime trihydrate, physical mixture and optimized NCS have revealed important information regarding possible interactions between functional groups of added ingredients in formulation and are presented in **Fig. 5**.

The changes observed *i.e.* frequency shifts / disappearance, attenuation and broadening in characteristics peaks of optimized formulation were observed, which could be attributed to the loss of water from crystal lattice and slight crystalline to amorphous transition.

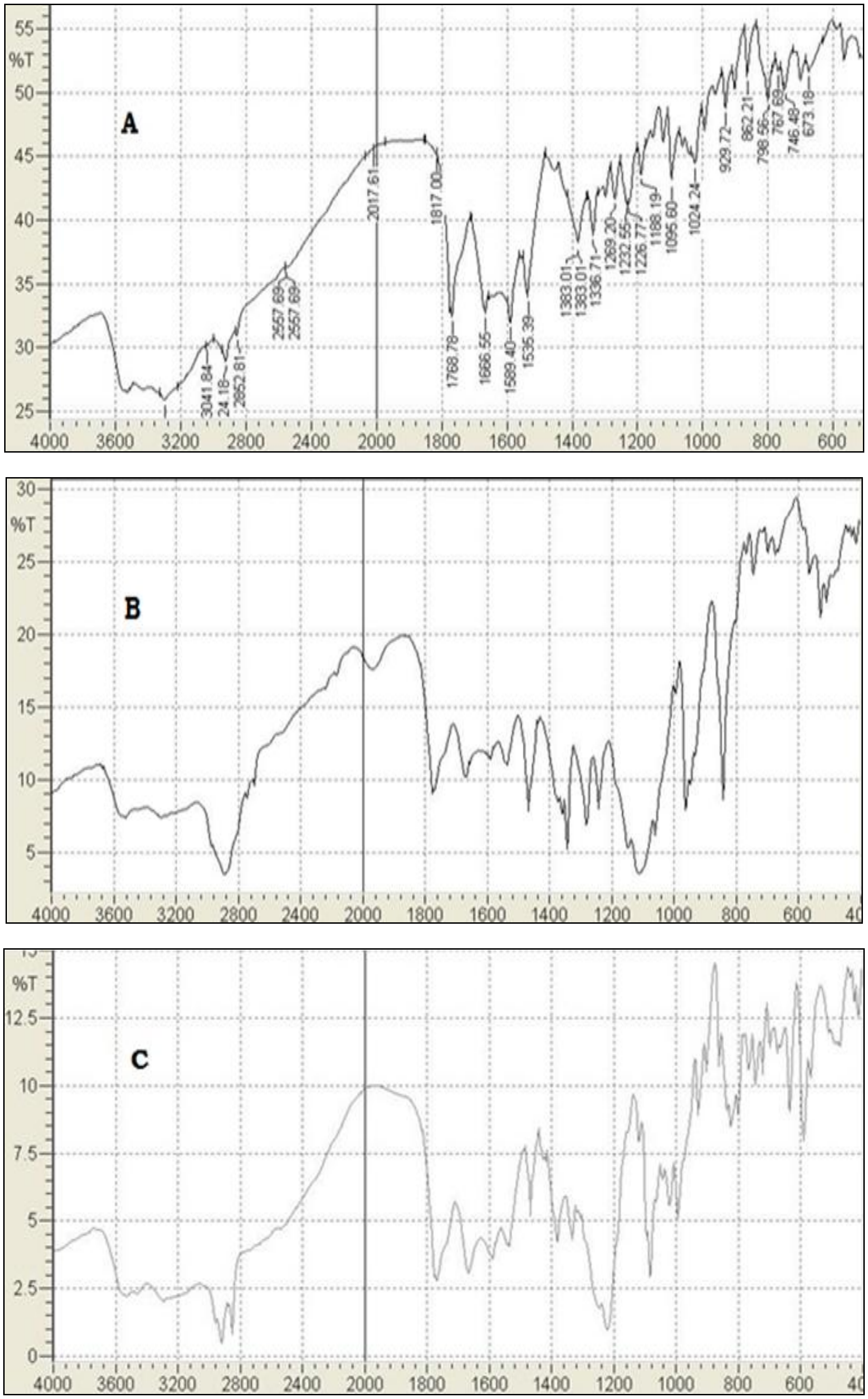

FIG. 5: FTIR OF CEFIXIME TRIHYDRATE (A), PHYSICAL MIXTURE (B) AND OPTIMIZED FORMULATION (C)

Scanning Electron Microscopy (SEM): SEM images of the raw drug and optimized NCS (freeze dried) formulations are shown in **Fig. 6**. It can be observed that raw cefixime trihydrate was of crystalline nature with rough surfaces and optimized formulation was relatively spherical shaped. It indicates that stabilizer may have affected the morphology of optimized formulation.

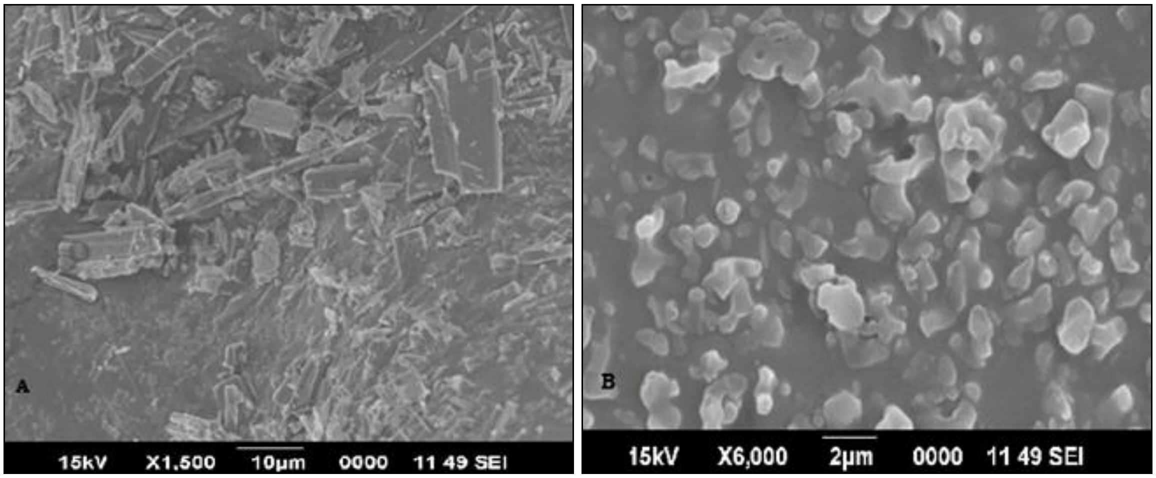

FIG. 6: SEM IMAGES OF PURE CEFIXIME TRIHYDRATE (A) AND OPTIMIZED FORMULATION (B)

Drug Release Profile: *In-vitro* drug diffusion profile of the optimized NCS formulation was obtained by dialysis-bag/dialysis-sac method and was compared with that of plain drug suspension (PDS). The results obtained are as shown in **Fig. 7A**. The release of drug from the optimized formulation was found to be more consistent in comparison to the release from plain drug suspension. 89.79 ± 0.115% drug was released from optimized formulation compared to 62.29 ± 0.173% drug release from plain drug solution used as control in 12 hrs. The small size of nanoparticles and the presence of stabilizer in the developed optimized formulation may have improved the solubilisation, dissolution and resulted in enhanced drug release.

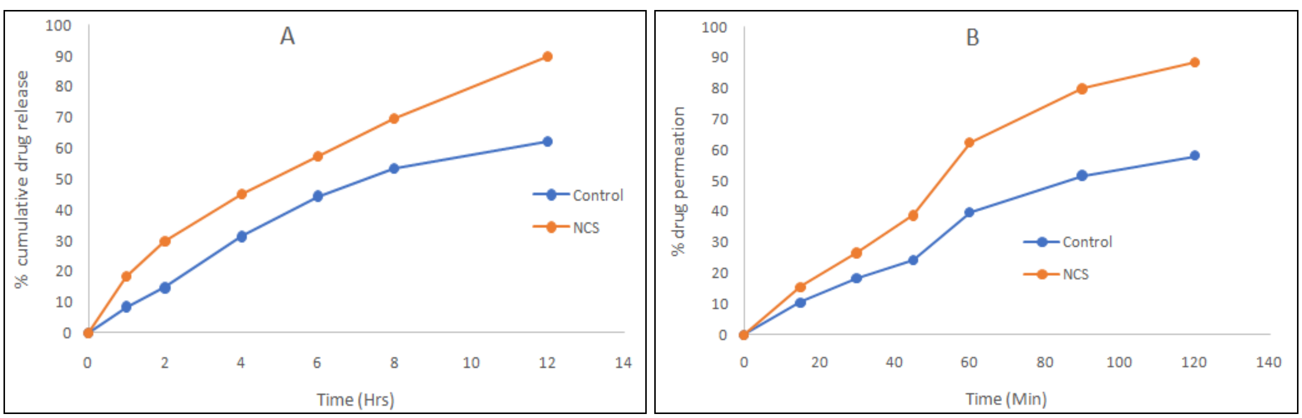

FIG. 7: DRUG RELEASE AND *EX- VIVO* PERMEATION PROFILE OF OPTIMIZED FORMULATION Vs PLAIN DRUG SOLUTION IN A AND B RESPECTIVELY

Permeation Study: The permeation study revealed that 88.38 ± 0.134% drug within 2 h was permeated from optimized formulation compared to 57.91 ± 0.165% of control (pure drug solution) as showing in **Fig. 7B**, indicating increased permeation rate of drug from intestinal barrier. This effect is attributed to improved physiochemical properties of optimized formulation compared to free drug.

CONCLUSION: With the help of results obtained from present investigations, it may be concluded that nanocrystal suspension of a poorly aqueous soluble drug cefixime trihydrate were successfully formulated and can be optimized using the factorial design approach of design of experiments (DoE) by high pressure homogenization technique. In present study cefixime trihydrate NCS was prepared with a mean size of 143.5nm (PDI = 0.269) without any additive or organic solvents. Increased saturation solubility can lead to dose reduction with probability of elimination high dose related side effects. Higher solubility and dissolution can lead to increased drug release and permeation performance reflected by better results in drug release and permeation compared to control formulation. Thus, it may be concluded that the developed formulation has better potential for increased solubility and bioavailability.

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CONFLICT OF INTEREST: The authors declare that they have no competing interests.

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