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Original Article

FORMULATION DEVELOPMENT AND CHARACTERIZATION OF LYOPHILIZED FEBUXOSTAT NANOSUSPENSION

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ABSTRACT

Objective: The study aims to prepare and evaluate febuxostat nanosuspension to improve oral bioavailability.

Methods: Febuxostat nanosuspension was prepared by the solvent-antisolvent method, followed by a lyophilization technique using PVP K-30 as a stabilizer and sodium lauryl sulfate as a surfactant. Drug content, differential scanning calorimetry, powder x-ray diffraction, Fourier transform infrared spectroscopy, and *in vitro* dissolution studies were used to characterize the nanosuspension.

Results: The results of the characterization studies indicated the formation of nanosuspension. The lyophilized FXT NS particle size is 2170.2 nm, the PDI value is 0.63, the negative zeta potential is 1.6 mV, and the drug content is 19.02%. Functional characterization studies demonstrated that the particle size reduced due to the interaction between the stabilizer and surfactant.

Conclusion: It can be concluded that the prepared febuxostat nanosuspension enhances the aqueous solubility of FXT and improves its oral bioavailability.

Keywords: Febuxostat, Nanosuspension, PVP K-30, Sodium lauryl sulfate, Lyophilization

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INTRODUCTION

Febuxostat (FXT) (IUPAC name: 2-(3-cyano-4-isobutoxyphenyl)-4-methyl-1,3-thiazole-5-carboxylic acid), a xanthine dehydrogenase inhibitor, is used in the management of hyperuricemia [1-3]. FXT exhibits a short half-life, rapid metabolism, and elimination [4]. It exhibits poor aqueous solubility (~12.9) [5], and thus, it is classified as a Biopharmaceutical Classification System (BCS) class II compound with low solubility and high permeability. Furthermore, FXT has a poor and variable absorption profile from the GI tract, resulting in low oral bioavailability (49%) [6]. Considering FXT's significant pharmacological activity and its existing drawbacks, there is a strong need for unique formulations that can improve the solubility and permeability of FXT.

Different formulation strategies have been reported so far to improve the dissolution rate and bioavailability of FXT. For instance, tablets [7, 8], ethosomes [9], solid dispersion [10], nanoemulsion [11] and the self-nano emulsifying delivery system (SNEDDS) [12]. Following a review of these works, it was observed that the author improved the limited solubility of FXT without investigating the Physico-chemical and functional characterization. Based on the drawbacks and shortcomings of the existing research work, we report a nanosuspension technology for enhancing the solubility, permeability, and biopharmaceutical parameters of FXT.

Nanosuspension (NS) is the dispersion of fine colloidal solid drug particles. NS is biphasic and is stabilized through the incorporation of surfactants. Pharmaceutical nanosuspensions have an acceptable particle size range of 1 µm [13]. NS has become an integral part of nanocarriers because of their advantages, such as lowered particle size, improved dissolution rate, and delayed absorption. Moreover, the active pharmaceutical ingredients (API) exhibit a high log P value. It can be formulated as NS to enhance its bioavailability, and reduce toxicity, dosing frequency, stability, and accessibility to different routes [14, 15]. However, NSs are unfortunately susceptible to physical instability due to the high surface energy of nanocrystals, which causes NS to be thermodynamically unstable via the risks of agglomeration and crystal growth (Ostwald ripening) [16-18]. The solvent-antisolvent method is the most common and efficient method for preparing NS due to its ease of manufacturing and better stability. Moreover, in association with the homogenization technique, the solvent-antisolvent method could reduce particle size and inhibit nucleation and crystallization processes [19]. In the present study, the solvent-antisolvent method, homogenization, and excipients (i.e., PVPK-30 and sodium lauryl sulfate) produce the febuxostat nanosuspension (FXT NS). PVP K-30 acts as a stabilizer and provides NS stabilization. In contrast, sodium lauryl sulfate (SLS) acts as a surfactant and reduces the interfacial tension between the two phases, forming a stable NS. Moreover, the interaction between the PVP K-30 and SLS could enhance the dispersion of FXT within these polymers and cause amorphization. The amorphized FXT particle enhances the aqueous solubility and dissolution rate and improves oral bioavailability. The lyophilization of FXT NS using mannitol cryoprotectant reduces particle aggregation and enhances the physical and chemical stability of FXT NS [20].

In the present study, we developed lyophilized FXT NS using PVP K-30 and SLS via the solvent-antisolvent method followed by lyophilization. The lyophilized FXT NS was characterized for Physico-chemical and functional particle size parameters, differential scanning calorimetry, Fourier transform infrared spectroscopy, powder x-ray diffractometry, and *in vitro* dissolution studies.

MATERIALS AND METHODS

Febuxostat (1%w/w) was gifted by Metrochem API Private Limited, Andhra Pradesh, India. Polyvinyl pyrrolidone (PVP K-30), ethanol, mannitol, and sodium lauryl sulfate were purchased from Loba Chemic Pvt. Ltd., Mumbai, India.

Preparation of lyophilized febuxostat nanosuspension (FXT NS)

Febuxostat nanosuspension (FXTNS) was prepared by the anti-solvent precipitation technique reported earlier in the literature [21]. FXT NS was developed in two phases. Briefly, febuxostat (FXT) 250 mg was weighed using a digital balance (Model: 335, Systronics, Ahmadabad) and transferred into a 50 ml beaker. The weighed ingredients were dissolved in 1.5 ml of ethanol, forming an organic phase (phase 1). Phase 1 was filtrated through a membrane (0.22-µm) to remove any impurities. The anti-solvent phase (phase 2) was prepared by dispersing polyvinyl pyrrolidone K-30 (stabilizer) and sodium lauryl sulfate (surfactant) in 20 ml of distilled water (kept in

an ice bath). Freshly prepared dispersion was immediately introduced into phase 1 (below 3 °C) under a vigorous stirring speed resulting in a whitish opalescence suspension formation. The developed suspension was homogenized using a homogenizer (Model: RQT-127A RemiMotor, Mumbai, India) at 5000 rpm, forming FXT NS. The prepared FXT NS was lyophilized using a lyophilizer (Penguin classic plus 4 kg lyophilizer) with (1% w/v) mannitol as a cryoprotectant. The obtained lyophilized FXT NS was preserved in amber-coloured (light protected) glass vials, purged with N2 and stored at room temperature until further analysis.

Physico-chemical characterization

Particle size and zeta potential

Particle size is the most widely used indicator to determine the release behaviour of FXT formulations in liquid media. In this study, the particle size of FXT within FXT NS was evaluated using Photon Cross-Correlation Spectroscopy (PCCS) equipped with Dynamic Light Scattering (DLS) technology. Briefly, an approximate quantity of FXT NS was weighed and dispersed into 5 ml of distilled water. The resultant dispersion was placed into the analyzer. The placed dispersion was analyzed using a particle size analyzer (Model: Nanophox Sympatec, GmbH, Clausthal-Zellerfeld, Germany) within the sensitivity range of 1 nm to 10 µm. The same dispersion of formulation was analyzed for zeta potential to determine the physical stability of FXT in FXT NS was evaluated using a Nano Particle Analyzer (Model: NanoPlusTM-2, Particulate System, Norcross, GA, USA) within the sensitivity range of–200 to+200 mV [22].

Scanning electron microscopy

The FXT, PM and lyophilized FXT NS samples were analyzed to study their surface characterization using a scanning electron microscope (Model: S-3700N, Hitachi, Japan, Hyderabad). Briefly, the samples (~50 mg) were weighed and spread as a thin layer on double-faced carbon tape and then loaded into the sample chamber of the SEM. After loading, the sample was coated with gold (~400°) via a sputter coating technique. The coated sample was scanned at an accelerating voltage of 10 kV. The scanned image of each sample at various magnifications was analyzed using the instrument attached software (Smart® SEM V05.06).

FTIR

FTIR is an important analytical technique used for drug-polymer interaction studies. FTIR analysis was carried out using a Fourier Transform Infrared Spectrophotometer (Model: 84005, Shimadzu Asia Pacific Pvt, Ltd, Singapore). Briefly, the samples of FXT, PVP K-30, PM, and FXT NS were weighed with the FTIR grade of potassium bromide and compressed into thin transparent discs using a Mini Hand Press Machine (Model: MHP-1, P/N-200-66, 747-91, Shimadzu, Kyoto, Japan). This disc was then scanned at a wave number range of 400 to 4000 cm^{-1} under the scanning resolution of 4 cm^{1}. The scanned image of each FT-IR sample was analyzed and interpreted using the instrument accompanied software (IR Solution, version 1.10) [23].

DSC

The samples of FXT, physical mixture (PM), and FXT NS were analyzed to study their thermal behaviour using a differential scanning calorimeter (Model: UCT/TEQIP/959-07/CFL/DSC, Shimadzu). Briefly, the samples (~2 mg) were weighed and filled into a previously calibrated and N_2 purged analyzing area. The added sample was heated at a rate of 10 °C/min within the heating range of 0–400 °C. Each sample's heated sample-based DSC spectrum was interpreted using instrument accompanied software (Universal Analysis 2000, V4.5A, Build 4.5.0.5).

PXRD

A powder x-ray diffractometer (Model: UCT/TEQIP/552-07/CFL/XRD, Shimadzu) was used to analyze the crystal characteristics of FXT, PM, and FXT NS respectively. Briefly, samples (~50 mg) were loaded into a sample analyzing area and illuminated for 20 min at 25 °C using a Cu radiation source (= 1.5406A°). The illuminated sample was scanned and detected using a dimensional silicon strip-based technology detector (LYNXEYE™). The obtained diffraction spectra on the 2θ angle between the 3 to 60° at a count rate of 5s were interpreted using PXRD accompanied software [24].

Solubility analysis

Solubility is one of the essential evaluation parameters to obtain the desired drug concentration in systemic circulation for the desired pharmacological response. Briefly, the pure FXT and lyophilized FXT NS in excess quantity were dispersed in 5 ml of distilled water in sealed glass vials. The content in the vials was then agitated using a shaker (Model: RSB-12, Remi House, Mumbai, India) for 24 h. After agitation, the developed dispersion was centrifuged at 1500 rpm for 25 min, followed by filtration using a membrane filter (0.45 µ). The filtrate was suitably diluted and analyzed at the maximum wavelength of (ƛ max= 315 nm) against the blank to determine the solubility of the sample in water. The sample absorbance was recorded using a UV-visible spectrophotometer (Model No. UV2401 PC, Shimadzu Corporation, Singapore) [25].

Drug content

In a 50 ml dry volumetric flask, 509.29 mg of lyophilized FXT NS powder (weight equivalent to 40 mg of FXT) was dissolved in 10 ml phosphate buffer (pH 6.8). The flask's content was then stirred using a magnetic stirrer at a speed of 50 rev/min for 24 h at 25±0.5 °C. The resulting solution was then filtered through Whatman filter paper (No. 42). The filtrate was suitably diluted and analyzed at the maximum wavelength of (ƛ max~313 nm) against the blank to determine the solubility of each sample in water [26].

% Drug content = Practical drug content/Theoretical drug content x 100

***In vitro* dissolution test**

A dissolution method was employed to evaluate the release performance of FXT nanosuspension. Briefly, the dissolution of FXT NS was studied in a dissolution test apparatus (Model No. DA-3, Veego Scientific Devices, Mumbai) in two different media: HCl (pH 1.2) and phosphate buffer (pH 6.8). An approximate amount of the above-mentioned sample containing ~2 mg of FXT NS was weighed and dispersed in 2 ml of ethanol. This dispersion was sonicated and then loaded into the dialysis bag. The dialysis bag requires an average diameter of ~21.55 mm, an average flat width of ~32.34, a loading capacity of ~3.63 ml, and a molecular size cut off ~12,000–14,000 kDa was used in the dissolution studies. Moreover, the dialysis bag was rinsed as per the manufacturer's guidelines. The loaded samples in the dialysis bag were suspended vertically in the dissolution flask containing freshly prepared acid buffer (200 ml, pH 1.2) dissolution media. The media in the flask was continuously stirred at 50 RPM with a magnetic stirrer for 12 h at 37 °C. At a predetermined time, the samples were removed from the flask and compensated with the same quantity of fresh dissolution media. The removed samples were diluted suitably, and the solution absorbance at a maximum wavelength (ƛ max ~314 nm) was measured on a UV–visible spectrophotometer (Model No. UV 2401 PC, Shimadzu Corporation, Singapore) against the blank. The experiments were performed in triplicate, and the mean values were reported. The reported absorbance of samples was further used to estimate the cumulative release of FXT, and a graph plotted the cumulative percent of drug versus time in minutes [27].

RESULTS AND DISCUSSION

Preparation of FXT NS

In this study, FXT NS was prepared using the ethanol-based solvent-antisolvent method. FXT is an anti-gout drug of BCS Class-II. A preliminary experimental study was carried out for the preparation of FXT NS. This study demonstrates that FXT is insoluble in water and shows higher solubility in organic solvents. This physicochemical property of FXT was utilized in this study to prepare NS using the solvent-antisolvent method. Following this, PVP K-30 was selected as a stabilizer for the preparation of NS. A preliminary analysis shows that PVP K-30 is a water-soluble polymer enhancing poorly soluble drugs' dissolution rate. Sodium lauryl sulfate was used as a surfactant. A combination of PVP K30 and SLS was selected as the stabilizer for long-term stability. The

rationale for using a combination of PVP K30 and SLS was based on the combined effect of steric and electrostatic stabilization. Previous literature reported using organic solvents such as methanol, N, and N-dimethylformamide as a choice of solvent for preparing stable FXT NS. These solvents were tested and found to show low solubility for FXT. This solubility problem was overcome by using ethanol as a choice of solvent because of its semi-polar nature and provides higher solubility for FXT [28].

Particle size and zeta potential

Particle size distribution and zeta potential are essential indicators of the physical stability of sub-micron particles when dispersed in a liquid medium [29]. The results of the particle size analysis of the prepared FXT NS formulation are shown in fig. 1. The mean particle size of the prepared formulation was observed to be ~ 2170.2±0.03 nm due to the presence of a thick layer of PVP-K30 on the particle surface and diffusion between the solvent and the anti-solvent caused to enhance the particle size of lyophilized FXT NS [30]. The polydispersity index of these particles was found to be 0.6±0.01 nm, indicating a relatively narrow size distribution (table 1). In addition, a zeta potential of ~-1.6±0.6 mV was observed in lyophilized FXT NS (fig. 2). The lower zeta potential could provide electrostatic repulsion to prevent aggregation and agglomeration of drug nanoparticles [31].

Table 1: Particle size optimized lyophilized FXT NS

Batch	*z-Average (nm)	*PDI	*Zeta potential (mV)	S. P. Area ratio	SD (nm)	Dispersion medium viscosity (mPa. s)
B5	2170.2±0.03	0.6±0.01	-1.6±0.6	1.00	1869.3	2.032

*Each value represents mean±SD, (n=3)

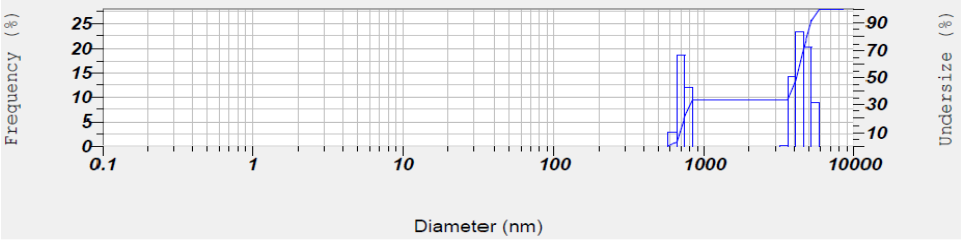


Fig. 1: Particle size of lyophilized FXT NS

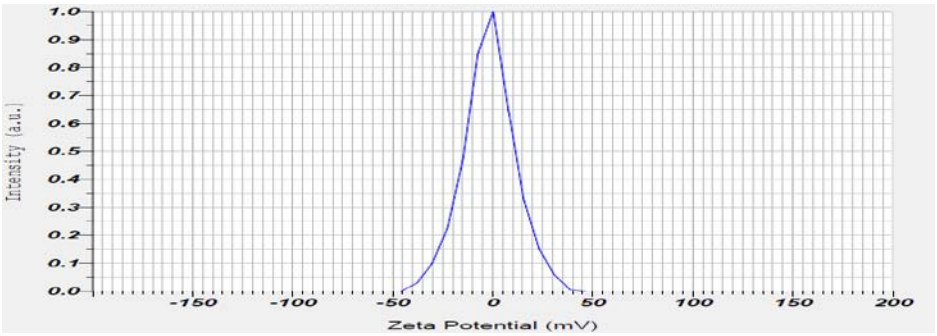
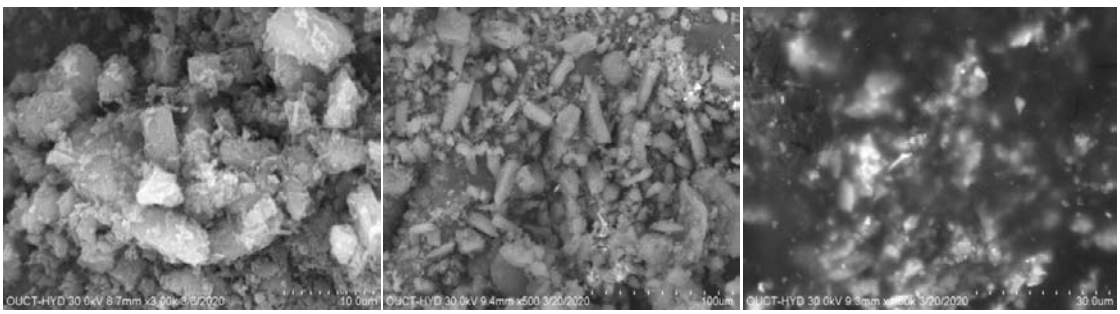


Fig. 2: Zeta potential of lyophilized FXT NS

SEM

SEM photomicrographs obtained for pure FBX, PM, and lyophilized FXT NS are shown in fig. 3. SEM images revealed distinct differences in the morphologies of pure FXT and lyophilized FXT NS. Pure FXT (fig. 3A) showed large and uneven particles with an irregular structure. In the physical mixture, FXT particles were mixed uniformly with polymer, as seen in the crystalline structure. After homogenization of FXT dispersion in the presence of stabilizer (PVP K30 and SLS) (fig. 3B) leads to a change in the morphology of drug particles. This suggests a smaller particle size in the nanometer range with a relatively narrow distribution. Lyophilized FXT NS (fig. 3C) exhibited that the particles were discrete, non-aggregated, homogeneously dispersed and nearly spherical [32].



A **B** **C**

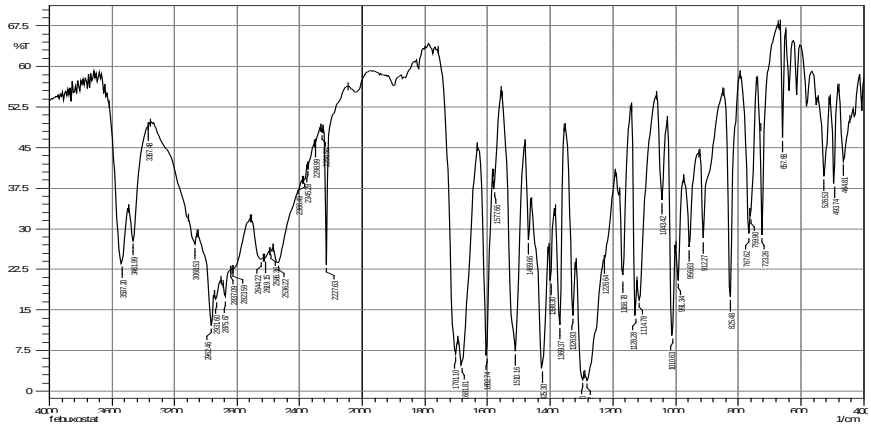
Fig. 3: SEM of A) Febuxostat B) Physical mixture (FXT and PVP K-30) C) lyophilized FXT NS

FTIR

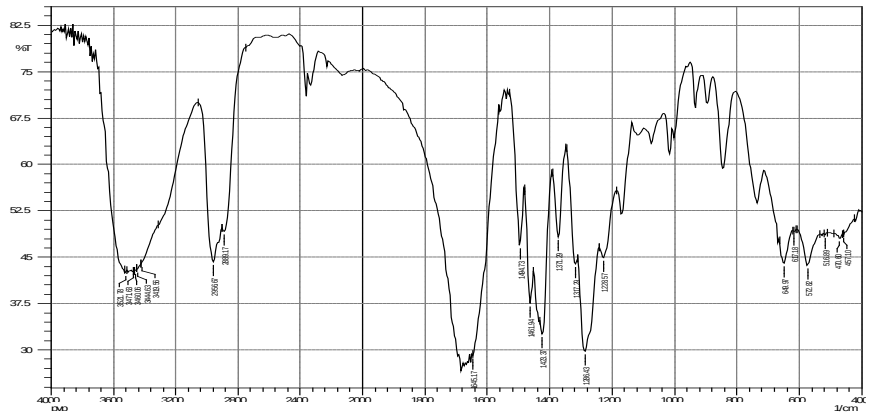
The FTIR spectrum of the drug sample was compared with the reference spectra of Febuxostat. FTIR of Febuxostat (fig. 4A) showed characteristic peaks at 3450.40 cm-1 due to O-H stretching of a free hydroxyl group, 2952.81, 2914.24, 2873.74 cm-1 due to C-H stretching of alkane, 1695.31 cm-1 due to C-O stretching of carboxylic acid, 1577.66, 1473.51 cm-1 due to C-C stretching of an aromatic ring, and 1429.15, 1398.3 cm-1 due to C-H bonding of alkane. All these prominent peaks were identified and matched with reference spectra, which confirm the authenticity of the procured FXT [33]. The spectra of the physical mixture and lyophilized FXT NS (fig. 4C, 4D) showed the same absorbance pattern, indicating the compatibility of drugs and polymer. Therefore, no shifting of positions of the functional groups and no significant interaction between FXT and polymer has been observed.

DSC

DSC is a valuable tool used to determine the physical interaction between the components of formulations. The DSC curve of FXT, PM, and FXT NS are shown in fig. 5. The DSC thermogram of FXT (fig. 5A) showed two peaks. The first peak was obtained at ~213.37 °C, indicating the melting point of FXT, while the second peak appeared at ~248.29 °C, indicating its crystalline behaviour. The thermograph of the PM (fig. 5B) shows the absence of the FXT peak, suggesting that the high concentration of SLS and PVP-K30 could shield the peaks of drugs, leading to the complete disappearance of FXT peaks. The DSC thermogram of FXT NS (fig. 5C) showed a straight line, indicating the complete disappearance of the pure FXT peak. Results suggest that interaction between SLS and PVP-K30 could increase the dispersion of FXT within the polymer, causing amorphization and lowering drug crystallinity, thereby forming FXT NS formulations [34].



A)



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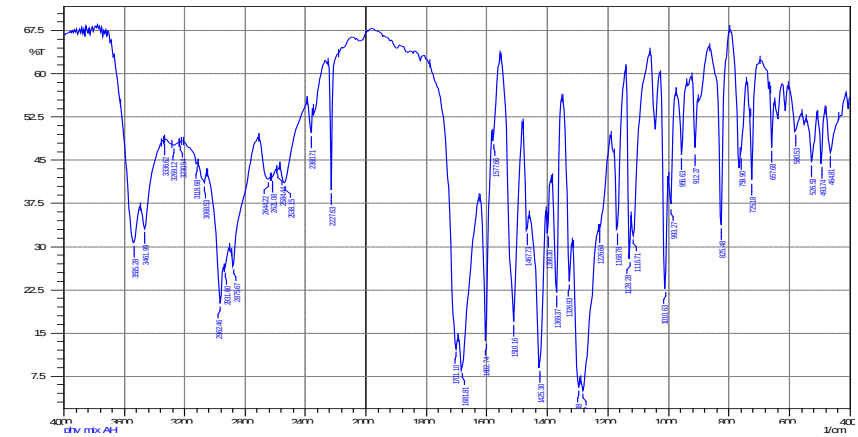
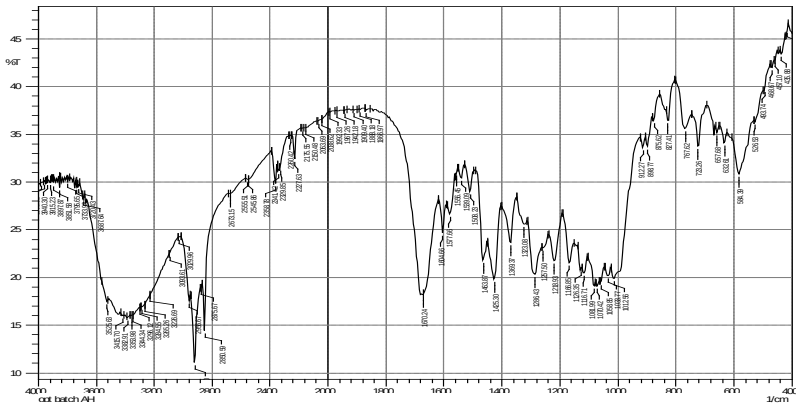
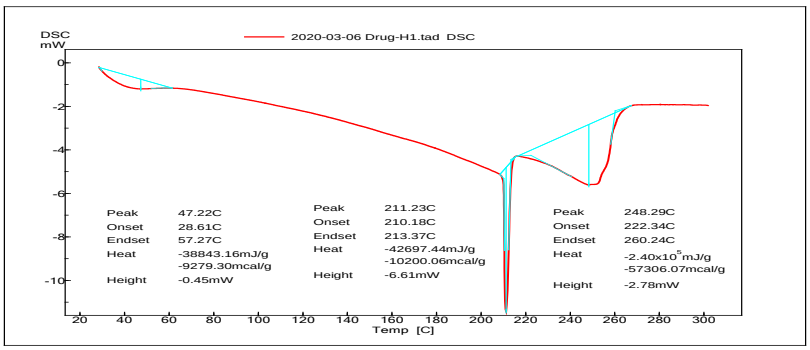


C)

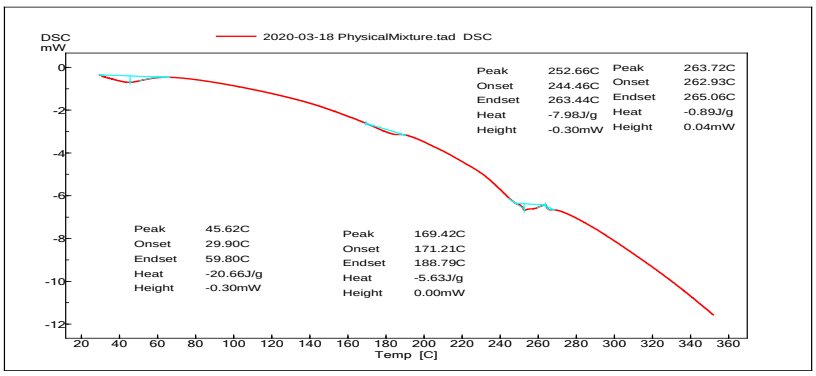


D)

Fig. 4: FTIR of A) FXT B) PVP K-30 C) Physical Mixture D) Lyophilized FXT NS



A)



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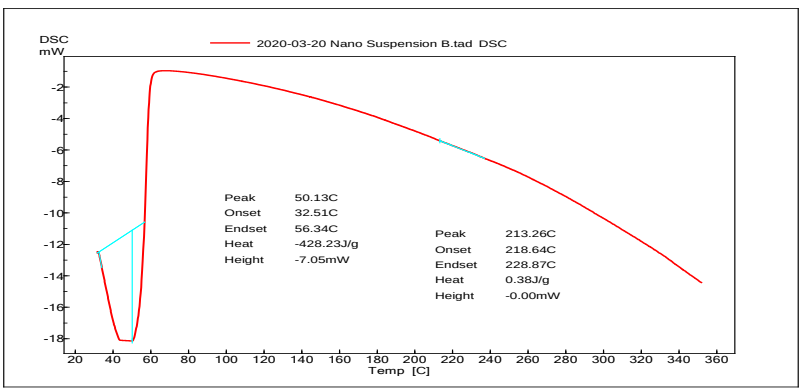
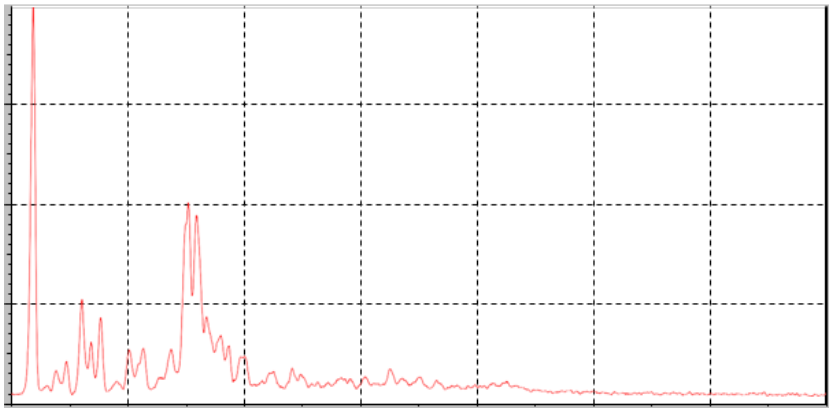


C)

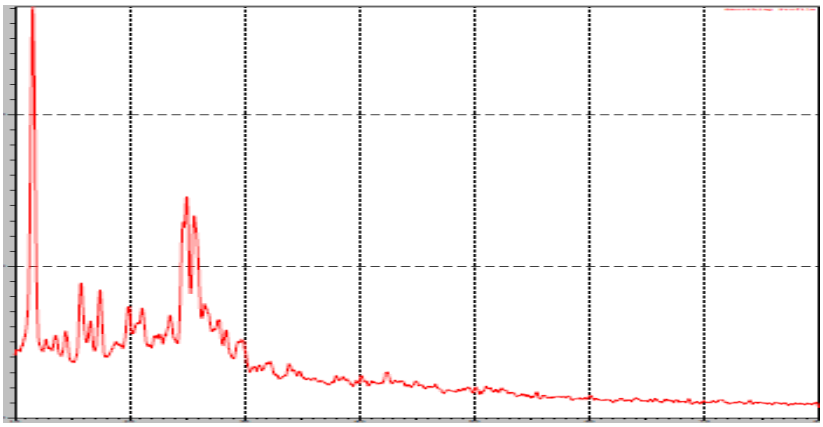
Fig. 5: DSC Thermogram of A) FXT B) Physical Mixture C) Lyophilized FXT NS

PXRD

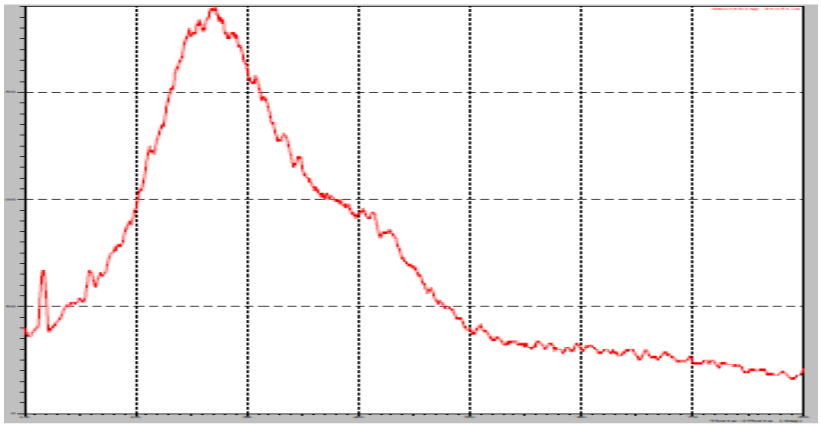
The fig. depicts the comparative structural characteristics of pure FXT, PM, and prepared FXT NS as determined by PXRD analysis in the form of X-ray diffractograms. The X-ray diffractogram of pure FXT (fig. 6A) showed sharp and intense peaks at diffraction angles (2θ) of 12.082, 25.360°, and 26.220° indicating a typical crystalline pattern [35]. These peaks disappeared in the formulation. The PXRD spectra of the FXT NS (fig. 6C) formulation showed a less intense hollow peak that indicated an increase in the amorphous nature of the drug compared to the pure drug. This shows that FXT is dispersed at a molecular level in the polymer matrix. No crystals were found in the lyophilized FXT NS.



A)



B)



C)

Fig. 6: Diffractogram of A) FXT B) Physical mixture C) Lyophilized FXT NS

Solubility analysis

The solubility analysis of pure FXT and lyophilized FXT NS in water, pH 1.2 and 6.8, is shown in table 3. The aqueous solubility of pure FXT is approximately 12.9±0.003 mg L^{-1}. The obtained result is not surprising because FXT is a BCS II drug with low solubility and high permeability. FXT NS revealed a higher aqueous solubility of around ~124±2.5 mg/ml. This improved aqueous solubility could be explained by the following mechanism, i.e., drug-polymer interaction, dispersion, and amorphization of FXT due to the amphiphilic nature of SLS [36].

Table 3: Saturated solubility of FXT NS in different media

S. No.	Media used	Drug solubility *(mg/ml) at 25±0.5 °C	NS solubility *(mg/ml) at 25±0.5 °C
1	Water	12.9±0.003	124±2.5
2	Acid buffer pH 1.2	0.03±0.001	0.69±1.3
3	Phosphate buffer pH 6.8	0.297±0.004	11.9±1.2

*Each value represents mean±SD, (n=3)

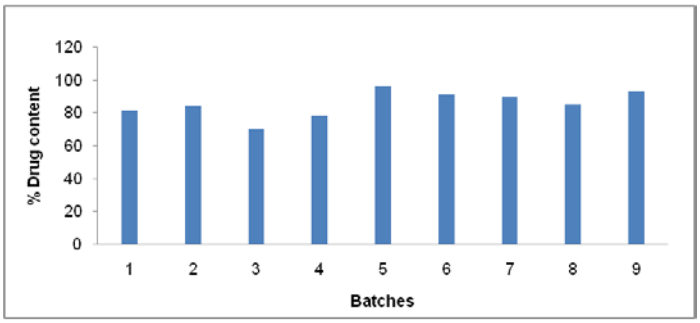


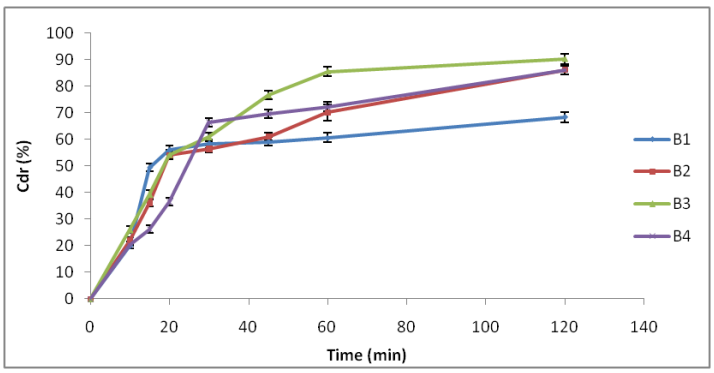
Fig. 7: Febuxostat content in lyophilized nanosuspension. Error bars were omitted, (n=3)

Drug content

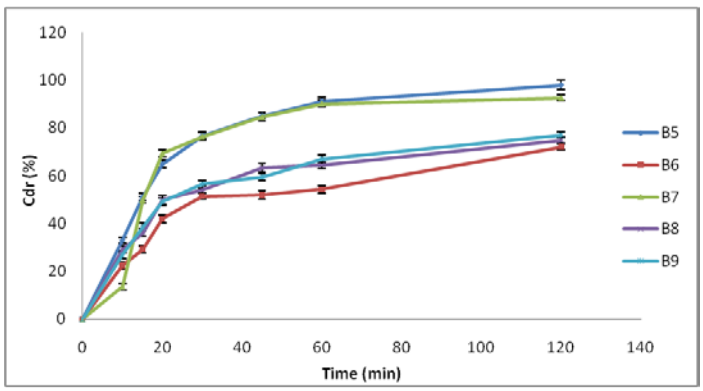
All the formulation batches were assessed to determine the amount of FXT present in the lyophilized FXT NS. A UV spectrometer determined the assay of the drug at 313 nm. The percent FXT content of FXT NS in the optimized batch (B5) was approximate ~ 97.97±1.21% w/w (fig. 7).

***In vitro* dissolution test**

The *in vitro* release of lyophilized FXT NS was released in a USP dissolution test apparatus. The dissolution profile was carried out in the freshly prepared acidic buffer (pH 1.2) and phosphate buffer (pH 6.5). Optimized NS exhibited a significant enhancement in dissolution rate in both media. There is an increase in the dissolution rate of lyophilized FXT NS with a decrease in the particle size because of the resultant enhancement in the surface area and a decrease in the diffusion layer thickness [37, 38]. Batch B1 to B4 (fig. 8A) *in vitro* drug release % CDR was found to be 68.4±0.48%, 86.16±0.69%, 90.4±0.45%, 86.25±0.47%. Batch B5 to B9 (fig. 8B)% CDR was found to be 97.99±0.39%, 72.0±0.74%, 92.66±0.49%, 74.8±0.32%,and 76.92±0.39%. The cumulative percent drug release of all batches was 68.4%–97.99% in 120 min. The higher release of FXT NS is up to 120 min for the B5 batch. This could be attributed to the enhanced wettability and solubility of surface stabilizers.



A). Each value represents mean±SD, (n=3)



B). Each value represents mean±SD, (n=3)

Fig. 8: *In vitro* dissolution profile of febuxostat from batches A) B1 to B4 B) B5 to B9

CONCLUSION

Nanosuspension has proven to be a promising method for increasing hydrophobic drug's saturation solubility, dissolution rate, and bioavailability. Significant enhancement was observed in the solubility, dissolution, and bioavailability of FXT, a BCS class II drug. Improvement of solubility of the drug was observed, which could explain the considerable enhancement in FXT bioavailability. Biopharmaceutical characterization of FXT indicated that both solubility and dissolution were limiting the absorption of FXT, and nanonization could significantly improve the dissolution of FXT.

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Nil

AUTHORS CONTRIBUTIONS

All the authors contributed equally.

CONFLICT OF INTERESTS

There is no conflict of interest among authors.

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