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Formulation And Evaluation of Febuxostat Microspheres For The Treatment of Gout

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ABSTRACT

The drug has to be delivered for a prolonged period of time and many medicines have to be taken simultaneously in case of chronic patients. . In order to overcome the above problems, various types of controlled release dosage forms and sustained release dosages forms are formulated and altered. In the present study an attempt has been made to formulate Febuxostat into a microspheres dosage form and prolong its gastric residence time, thus improving the oral bioavailability of the drug and reduces the side effects associated with the other medication of gout treatment. Gout is a painful and potentially disabling form of arthritis that has been around since ancient times. It would be faster and more economical to alter beneficially the properties of the existing drugs than developing new drug. Ethyl Cellulose was used as the wall material due to its safety, stability, hydrophobicity and perfect film forming nature among other polymers. Besides, Ethyl Cellulose has good release retardant property also it can be inferred that Ethyl cellulose can be a good choice to prepared microspheres of poorly water soluble drugs like Febuxostat.

KEYWORDS : Febuxostat, Ethyl Cellulose, microspheres, Gout etc.

Introduction

The drug has to be delivered for a prolonged period of time and many medicines have to be taken simultaneously in case of chronic patients. Frequent administration of drug is necessary when drugs have shorter half-life and all these leads to decrease in patient compliance. In order to overcome the above problems, various types of controlled release dosage forms and sustained release dosages forms are formulated and altered. The term microspheres, Fig. 1, describe as a monolithic spherical structure with the drug or therapeutic agent distributed throughout the matrix either as a molecular dispersion or as a dispersion of particles. Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having a particle size 1-1000nm [1-4].

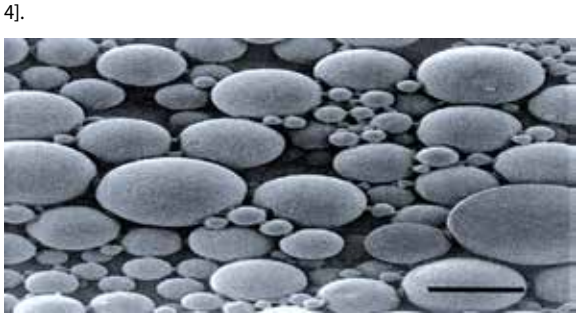


Fig. 1: structure of microspheres

Gout is a painful and potentially disabling form of arthritis that has been around since ancient times. The first symptoms usually are intense episodes of painful swelling in single joints, most often in the feet, especially the big toe. The swollen site may be red and warm. Gout occurs when excess uric acid (a normal waste product) collects in the body, and needle like urate crystals deposit in the joints. Gout is sometimes referred to as the "disease of kings." [5]

Febuxostat (FXT), 2-[3-cyano-4-(2-methylpropoxy) phenyl]-4-methylthiazole-5-carboxylic acid, Fig. 2, is a non-purine compound and a selective inhibitor of both the oxidized and reduced forms of xanthine oxidase. Febuxostat is the first agent marketed in the United States to treat hyperuricemia of gout since allopurinol was approved in 1964. Febuxostat is a novel, potent, non-purine, selective xanthine oxidase inhibitor. It is a weak acid (pKa 3.08) that is practically insoluble in water [6-7].

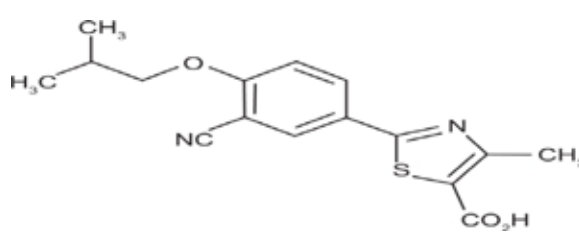


Fig 2. Chemical structure of Febuxostat (FXT)

There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such in Microspheres as carriers of drug become an approach of controlled release dosage form in novel drug delivery system [8-9]. The objective of the present study was to formulate Febuxostat microspheres in order to improve its oral bioavailability by increasing its solubility, dissolution rate and reduces side effect of conventional dosages form of the drug.

MATERIALS AND METHODS

Materials

FXT was collect as a gift sample from Vivi med. Lab, India Ltd. The best quality of entire chemicals were used like Dichloromethane**,** Acetonitrile**,** Methanol, Ortho phosphoric and Hydrochloric Acid (LR grade, Merck), Ethyl cellulose (Ethocel), Span-20, (Thomas Baker Chem.). Instrument were used for characterization are Electronic balance, FTIR (Bruker), UV-Vis spectrophotometer (Thermo scientific), Scanning Electron Microscopy (JEOI Model JSM-6390 LV), DSC (Mettler Toledo DSC 822e), PXRD (Bruker Axs D8 Advance) and Stability Chamber (Medical Equipment, India) etc.

Methods

Preformulation studies of FXT pure drug was carried out using Drug identification test, Determination of Melting point (capillary method) and Solubility analysis of FXT was performed in different solvents. The moisture content was observed and calculated using following formula:

$$\% \text{ moisture content} = \frac{A - B}{B} X100$$

Where, A= Amount of drug before dry

B= Amount of drug after dry

Lambda max (λ_{max}) was measured using Methanol between 200-400nm. Flow properties of pure drug was studies using determination of Angle of Repose. The drug-excipient interaction study was carried out using FTIR Spectrophotometer (Bruker) and spectrum was recorded in the wavelength region of 4000 to 500 cm^{-1}. Samples were prepared in KBr disks (2 mg sample in 200 mg KBr). The pellet was then placed in the light path and the spectra were obtained and interpreted [10-12].

Preparation of microspheres without drug

Different amount of eudragit were dissolved in 8ml ethanol using magnetic stirrer. The resulting dispersion was then poured into 250ml beaker containing the mixture of 100 ml liquid paraffin light & 0.2% span 60 while stirring, stirring at 500 rpm was continued for 4 hrs. until alcohol evaporated. Microspheres filtered by using filter paper, the residue were washed with 4-5 times by 50 ml of dichloromethane. Microspheres were dried 24 hrs. at room temp [13-14].

Preparation of Febuxostat (FXT) microspheres

Febuxostat loaded ethyl cellulose Microspheres was prepared using emulsion solvent evaporation method. Ethyl cellulose & drug were added (1:1, 2:1, 3:1, 4:1 etc.), Table 1, to the mixed solvent system consisting of acetonitrile & dichloromethane in a ratio 1:1 and mixed thoroughly using a magnetic stirrer. The polymeric phase was slowly added to 50/100/150 ml of light liquid paraffin containing 0.25% span 20 as a surfactant while stirring at 500 rpm. After one hour 10 ml n-hexane was added & stirring was continued for a further 2 to 3 hrs. microspheres was collected by filtration and washed with three portion of 50 ml n-hexane. Collected microspheres were air dried for 12 hrs. [15-16]

Table 1. Formula Design for Febuxostat microspheres

S. NO	DRUG (mg)	POLYMR (Ethyl Cellulose)	SPAN 20	n-HEXANE (ml)	ACETONITRILE (ml)	DICHLOROMETHANE (ml)	STIRRIG RATE (rpm)
1.	50	200 mg	0.25%	10	10	10	500
2.	50	400 mg	0.25%	10	10	10	500
3.	50	500 mg	0.25%	10	10	10	500
4.	50	600 mg	0.25%	10	10	10	500
5.	50	700 mg	0.25%	10	10	10	500
6.	50	800 mg	0.25%	10	15	15	800
7.	50	900 mg	0.25%	10	15	15	500
8.	50	1 gm	0.25%	10	15	15	500

Evaluation of Microspheres [17-18]

Percentage yield and drug entrapment efficiency of prepared microspheres was calculated using the following formula:

$$\%\text{yield} = \frac{\text{weight of microspheres}}{\text{weight of polymer} + \text{drug}} X100$$

$$\% \text{ Entrapment efficiency} = \frac{\text{Total drug - Diffuse drug}}{\text{Total drug}} X100$$

Flow property was studies using measuring of angle of repose. Particle size of the microspheres was determined using optical microscopy. The shape and surface morphology of FXT microspheres were examined using scanning electron microscopy (SEM). The microspheres viewed at an accelerating voltage of 10-20kV. The DSC analysis of pure drug, drug-loaded microspheres were carried out to evaluate any possible drug-polymer interaction. The analysis was performed from 20 °C to 210°C temperature range under nitrogen flow of 20 ml min. Drug-polymer interactions were studied using FTIR spectroscopy.

***In-vitro* studies (drug dissolution test)**

The drug dissolution test of microspheres was performed using the Paddle method. Microspheres (50mg) were weighed accurately and filled into tea bags. The tea bags were tied using thread with paddle and loaded into the basket of the dissolution apparatus. The basket was filled with 900 ml of 6.8 pH phosphate buffer (dissolution medium). The content was rotated at 100 rpm at 37°C±0.5°C. The pH of dissolution media was adjusted to 6.8 and maintained for 8 hours. The sample of 10 ml were withdrawn at time interval of 0, 30, 90, 150, 210, 270, 330, 390, 450 and 510 min and replaced by an equal volume of fresh dissolution medium at each time of sample withdrawal. After filtration of each sample and suitable dilution, the samples were analyzed spectrophotometrically at 313 nm. The concentration of FXT in sample was calculated based on calibration curves at desired pH. The cumulative amounts of drug diffused from microspheres were plotted against time [19-20].

***In-Vitro* drug release kinetics using mathematical model**

Various mathematical models were tested for investigate the model of release explaining the kinetics of drug release from FXT microsphere. To analyze the mechanism of the drug release rate kinetics of microspheres, the obtained data were fitted in to Zero-order, First –order, Higuchi's and Korsmeyer-pappas release model [21-22]. Drug release rate kinetics was calculated by using A Microsoft Excel Add-in. Stability studies were carried out as per ICH guidelines.

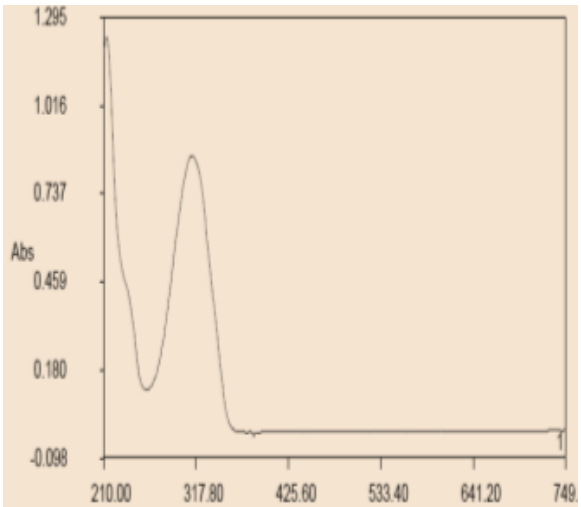
Results and discussion

The organoleptic characters of drug was found same as with the standard value given in the Pharmacopeia. Solubility analysis of the drug indicated that it practically insoluble in water and soluble in Acetone results are reported in Table 2. The melting point of the drug was found to be 202°C-210°C, which corresponds to the literature value of 205°C-208°C and proves the identity and purity of the drug. The moisture content was found to be 0.806%, 0.404% and 0.202% respectively which complies with the standard values which was not more than 1%. Partition coefficient of FXT was found to be 2.90, which indicated that drug is lipophilic in nature.

The absorbance maxima of FXT was found to be 313 nm in methanol and calibration curve was prepared, Fig. 3. The spectrum of pure Febuxostat was equivalent to the spectra obtained by the addition of excipients which indicated that there was no physical and chemical interaction between drug and excipients, Fig. 4 & 5.

Table 2: Solubility Analysis of pure drug FXT

S. No.	Solvents	Solubility	S. No.	Solvents	Solubility
1.	Water	Not soluble	7.	Di ethyl ether	Sparingly soluble
2.	Methanol	Sparingly Soluble	8.	Acetonitrile	Soluble
3.	Ethanol	Soluble	9.	Dichloromethane	Soluble
4.	Acetone	Soluble	10.	Phosphate buffer 6.8 pH	Slightly soluble
5.	n-hexane	Not soluble	11.	Chloroform	Soluble
6.	Dimethyl formamide	Freely Soluble			



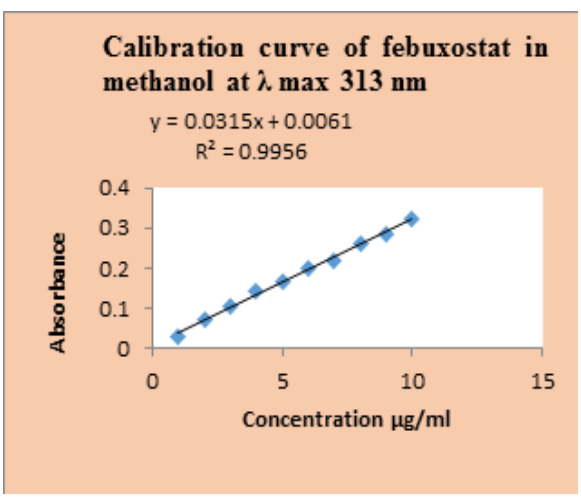


Fig. 3. UV spectrum and calibration curve of FXT

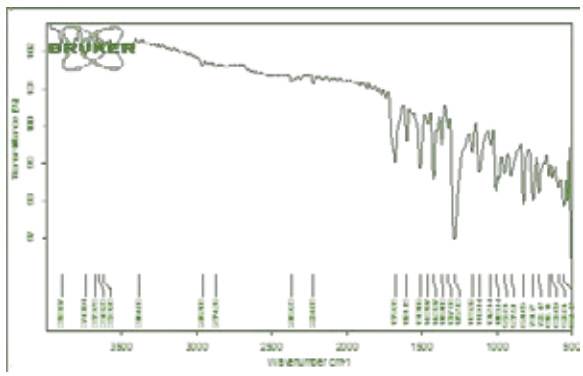


Fig 4. FTIR spectra of pure drug FXT

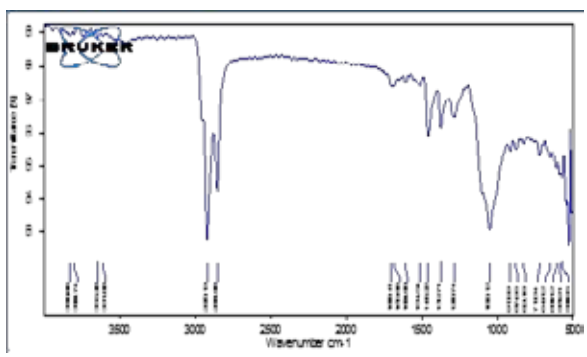


Fig 5. FTIR Spectra of FXT and ethyl cellulose

The percentage yield of all formulations was ranging from 72% to 85.7%, Table 3 and Fig. 6.There was no significant difference observed using solvent mixture therefore F3 showed highest % yield. Entrapment efficiency was found ranging from 49% to 61.7% therefore F2 and F3 showed best entrapment efficiency, Table 3 and Fig. 6. The flow properties of all the formulations were found out by measuring the angle of repose in which F1 and F3 showed excellent flow this indicated that particles were mostly spherical in shape, Table 3.

Table 3: Physical Evaluation of Febuxostat Microspheres

S. No.	Evaluation Parameters	F1	F2	F3	F4	F5	F6	F7	F8
1.	% yield	81.2%	84.1%	85.7%	83.6%	84.95%	78.3%	74.5%	72%
2.	(%)Entrapment efficiency	58.2%	61.7%	60.4%	56%	58.8%	55.3%	52.6%	49%
3.	Angle of repose	27.42	34.12	30.12	31.16	31.16	32.21	33.78	32.83
4.	Particle size (µm)	468.39	328.16	592.62	428.15	516.23	897.30	616.42	780.18

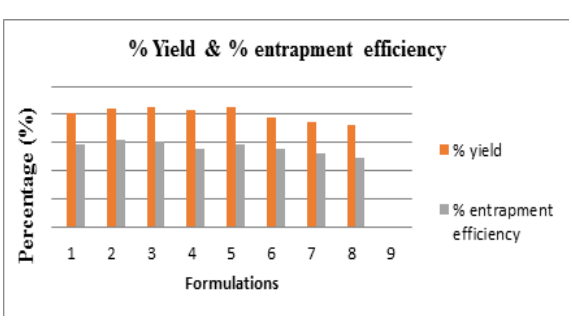
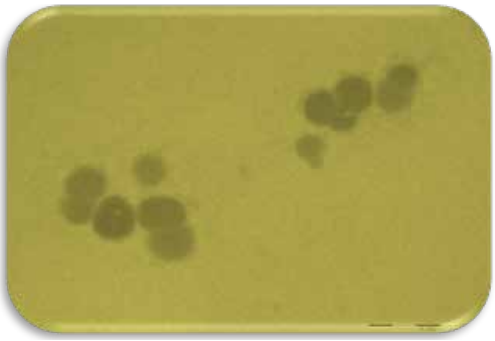


Fig. 6: % yield and % EE of different formulation

From the scanning electron microscopy (SEM), it was observed that particles were spherical, Size range for the formulation was 200µm -500µm and formulation F2 and F4 showed best particle size in range 200µm and 310µm, Fig. 7. DSC studies were performed on drug loaded microspheres, formulation showed sharp peak 190°C which corresponds to its melting point, Fig. 8.

The data of cumulative percentage release of FXT microspheres are reported in Table 4 and Fig. 9. Among the eight formulations, formulation F2, F3 and F6 showed higher % cumulative release and F5 & F7 showed lowest % cumulative release,. The *in-vitro* studied was performed for 8 hrs. The stability study revealed that there was no significant change in the drug content at the end of 45 days, so the formulation is said to be stable at different atmospheric conditions, Table 5.

SEM of F2



SEM F4

Fig. 7: SEM micrograph of F2 and F4

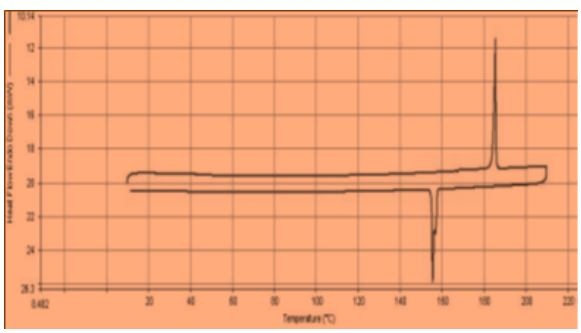


Fig. 8: DSC spectra of optimized formulation F2

Table 4: Cumulative % drug releases of prepared formulation

Time (min.)	Cumulative % Drug Release							
	F1	F2	F3	F4	F5	F6	F7	F8
0	00	00	00	00	00	00	00	00
30	17.4	9.3	17.50	17.52	17.4	35.12	8.4	7.52
90	21.49	23.8	31.09	30.2	21.48	36.26	17.49	9.62
150	23.82	29.86	34.68	34.51	23.76	37.36	30.2	23.8
210	26.73	32.41	35.18	35.26	26.28	39.68	34.61	29.81
270	26.87	33.45	36.23	36.11	26.81	40.1	35.18	32.4
330	29.11	34.49	36.93	36.67	28.89	40.76	36.12	33.46
390	32.4	35.56	38.48	38.17	29.37	41.94	36.62	34.48
450	33.61	40.12	40.16	38.94	31.16	43.07	37.28	35.43
510	34.18	43.28	44.12	40.12	33.51	45.46	38.31	39.81

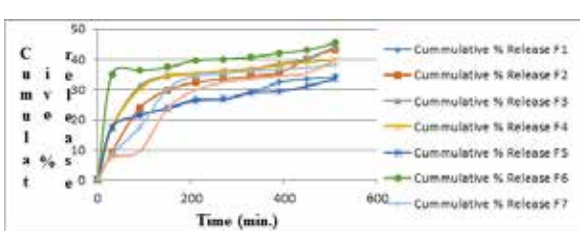


Fig. 9. % cumulative release profile of prepared formulation

Table 5: Stability Analysis during accelerated condition

Days	Ambient conditions (F2)		Accelerated condition 40°C±2°C	
	Physical appearance	% Entrapment efficiency	Physical appearance	% Entrapment efficiency
0	No change	No change	No change	No change
15	No change	60.87%	No change	60.36%
30	No change	60.25%	No change	60.12%
45	No change	60.22%	No change	59.27%

Conclusion

The *in vitro* dissolution studies showed that Febuxostat microspheres showed better controlled release effect over a period of 8 hours than other formulations and also indicates that microspheres remain in the stomach for more than 8 hours. The particle size and surface morphology of a microsphere by SEM, show uniform size distribution. The average particle size was found to be in the range of 328.16 to 897.30µm. IR Spectra and DSC thermograph of the Febuxostat, ethyl cellulose and physical mixture, indicated that there was no interaction between the drug and the polymer and confirmed the drug stability. So, the ethyl cellulose microspheres of Febuxostat were successfully prepared using solvent evaporation technique and confirm it as best method for preparing microspheres from its size uniformity and spherical shape.

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