Preparation and Evaluation of Controlled Release Parenteral Nanosuspensions of Anastrazole

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Abstract : Anastrazole is a class of medication called non steroidal aromatase inhibitors. It works by decreasing the amount of estrogen in the body makes this can slow or stop the growth of many types of breast cancer cells that need estrogen to grow. As it is having poor water solubility and poor wettability, anastrazole leads to poor dissolution and hence shows poor bioavailability. The present study is aimed at increasing dissolution rate/solubility of drug and its sustained release property of drug, used as parenteral nanosuspension. Nanosuspension formulations of anastrazole were prepared by anti-solvent solvent precipitation technique. The formulations were characterized by scanning electron microscopy, zeta potential, powder X-ray diffractometry, saturation solubility and in-vitro drug release. The evaluation studies were performed by using optimum formulation based on the particle size and saturation solubility, formulations were selected and further studies with the formulation were conducted. As a result of this method, it was found that the dissolution rate and saturation solubility of the nanosuspension formulation was significantly higher compared to the conventional suspension formulation. Further, the optimum formulation controls the drug release for 24 hours. Therefore, in this study a parenteral nanosuspension formulation of anastrazole were successfully developed and evaluated. The formulation is intended for controlled release delivery of the drug after i.v. administration with enhanced C_{max}.The formulation can be successfully used in breast cancer as it reduces the production of estrogen after intravenous administration.
Keywords : Nanosuspension, anastrazole, tween-80, solvent-antisolvent precipitation method.

Introduction

Anastrazole is a class-IV (Low permeability and low solubility) classified under biopharmaceutical classification system with low solubility and low permeability. Anastrazole is soluble in organic solvents such as ethanol, methanol and completely insoluble in water. Poor solubility leads to poor release rate, therefore to enhance the dissolution rate of the drug, different techniques have been employed in particle size reduction by micronization which is a conventional technique and nanosuspension which is a novel technique where

saturation solubility has been achieved compared with enhanced the dissolution release rate[1,2]. The parenteral administration route is the most effective and common form of delivery for active drug substances with metabolic bioavailabilities drug for which the bioavailability is limited by high first pass metabolism effect of other physicochemical limitation and for drugs with a narrow therapeutic index. For this reason, whatever drug delivery technology that can reduce the total number of injection throughout the drug therapy period will be truly advantageous not only in terms of compliance, but also for potential to improve the quality of the therapy. Such reduction in frequency of drug dosing is achieved, in practice, by the use of specific formulation technologies that guarantee the release of the active drug substance happens in a slow and predictable manner[3,4]. For several drugs, depending on the dose, it may be possible to reduce the injection frequency from daily to once or twice monthly or even less frequently. In addition to improving patient comfort, less frequent injection of drugs in the form of depot formulation smoothes out the plasma concentration time profiles by eliminating the peaks and valleys. Such smoothing out of the plasma profiles has the potential to not only boost the therapeutic benefit but also to reduce unwanted events side effects.The release can either be continuous or pulsatile depending on the structure of the device and the polymer characteristics, continuous release profiles are suitable to generate on 'infusion like' plasma level time profile in the systemic circulation without the necessity of hospitalization. A nanosuspension is a submicron colloidal dispersion of drug particles which are stabilized by surfactants[5,6]. A pharmaceutical nanosuspension is defined as very finely dispersed solid drug particles in an aqueous vehicle for either oral and topical use or parenteral and pulmonary administration. The particle size distribution of the solid particles in nanosuspension is usually less than one micron with an average particle size ranging between 200 and 600nm.In nanosuspension technology, the drug is maintained in the required crystalline state with reduced particle size, leading to an increased dissolution rate and therefore improved bioavailability[7,8]. An increase in the dissolution rate of micronized particles (particle size <10 μm) is related to an increase in the surface area and consequently the dissolution velocity. Nanosized particles can increase solution velocity and saturation solubility because of the vapor pressure effect. In addition, the diffusional distance on the surface of drug nanoparticles is decreased, thus leading to an increased concentration gradient[5]. The increases in surface area and concentration gradient lead to a much more pronounced increase in the dissolution velocity as compared to a micronized product. Furthermore, the saturation solubility is increased as well. Another possible explanation for the increased saturation solubility is the creation of high energy surfaces when disrupting the more or less ideal drug microcrystals to nanoparticles[9,10]. Dissolution experiments can be performed to quantify the increase in the saturation solubility of a drug when formulated into a nanosuspension. The stability of the particles obtained in the nanosuspension is attributed to their uniform particle size which is created by various manufacturing processes. The absence of particles with large differences in their size in nanosuspension prevents the existence of different saturation solubility's and concentration gradients, consequently preventing the ostwald ripening effect[11, 12]. Ostwald ripening is responsible for crystal growth and subsequently formation of micro particles. It is caused by a difference in dissolution pressure/saturation solubility between small and large particles. Molecules diffuse from the higher concentration area around small particles which have higher saturation solubility to an area around larger particles possessing a lower drug concentration. This leads to the formation of a supersaturated solution around the large particles and consequently to drug crystallization and growth of the large particles[13, 14].

Materials and Methods

Anastrazole is obtained as a gift sample from yucca enterprises, mumbai, india. Ethanol, tween 80 and methanol are from SD fine chemicals limited. All the other ingredients used were of analytical grade.

Preparation of anastrazole nanosuspensions:

Anastrazole nanosuspension was prepared by solvent-antisolvent precipitation method. Required quantity of anastrazole was weighed and dissolved in appropriate quantity of ethanol.Tween 80 in required quantities of distilled water was dissolved in a 250ml Buchner flask.Ethanol was used as a solvent and tween 80 as a surfactant to stabilize the nanosuspension formulation. Media 1 is added to media 2 drop wise with a constant stirring on a magnetic stirrer using a butterfly syringe. Vacuum was applied by placing paper on Buchner flask while stirring till the solvent evaporates. Product was filtered and dried at room temperature in a desiccator over night.Finally the product was filtered and centrifuged at 7000 rpm for 10 min. Particle size of drug nanosuspension after bath sonication mixing was measured. The drug nanosuspension was freeze dried. Depending on the average particle size the selected batches were freeze dried to obtain dry powder. When the dried form is reconstituted in an aqueous system, it may be redispersed to achieve its original particle size. The

redispersibility of dried nanoparticles depends on the parameters of the freeze drying process. In this study, an apparatus with a freezing rate gradient was used to systematically investigate the effect of cryoprotectants on the redispersibility of cryoprotectants on the redispersibility of nanoparticles as a function of freezing rate. Sucrose, lactose, mannitol, and polyethylene glycol were used as cryoprotectants for a nanosuspension. A fast freezing rate and a high cryoprotectant concentration were generally favored[15,16,17,18, 19, 20].

Table no: 1 Different batches of Anastrazole nanosuspension preparations

Formulation	F- 1	F- 2	F- 3	F- 4	F- 5	F- 6	F- 7	F-8
Anastrazole	100mg	100mg	100mg	100mg	100mg	100mg	100mg	100mg
Ethanol	25ml	5ml	5ml	5ml	5ml	5ml	5ml	10ml
Distilled water	50ml	50ml	25ml	10ml	10ml	10ml	10ml	5ml
Tween 80	0.5ml	0.5ml	0.5ml	0.5ml	0.25ml	0.75ml	1.0ml	0.5ml

Characterization of Nanosuspension [21, 22, 23, 24]

Particle size analysis:

The mean particle size was determined by using optical microscope. In this method 250 particles size was determined by using stage micro meter and the average particle size was determined.

Scanning Electron Microscopy:

In order to examine the particle surface morphology and shape, Scanning Electron Microscopy (SEM) was used. A concentrated aqueous suspension was spread over a slab and dried under vacuum. The sample was shadowed in a cathodic evaporator with gold layer 20 nm thick. Photographs were taken using a JSM-5200 Scanning Electron Microscope (Tokyo, Japan) operated at 20 kV.

Zeta Potential:

The zeta potential was used to measure of the electric charge at the surface of the particles, indicating the physical stability of colloidal systems. In this study, the Zeta Potential was assessed by determining the electrophoretic mobility of the particles. The zeta potential was measured using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). Samples were diluted with the respective original dispersion medium which provides information regarding the thickned of the diffuse layer. Diluted nanosuspension was added to the sample cell (quartz cuvette) and was put into the sample holder unit and measured. The higher the measured zeta potential, better was the physical long-term stability of the dispersion.

Saturation Solubility:

Saturation solubility is defined as the maximum quantity of a compound (solute) that can be dissolved in a certain quantity of a specific solvent at a specified temperature.The saturation solubility of the prepared nanosuspension, oral suspension and the pure drug were done by stirring on magnetic stirrer for 48hrs in distilled water. The nanosuspension powder was dispersed in 10ml of distilled water, kept for magnetic stirring for 48hrs. Then 1.5 ml of nanosuspension was filled into centrifugation tube and centrifuged at 25000 rpm for 30 min (Remiindusrties centrifuge). Supernatant was filtered and analyzed spectrophotometrically using UV-visible spectrophotometer (UV- Shimadzu) at 221 nm after suitable dilution with phosphate buffer (pH 7.4) containing Tween 80, which was used as blank. The pure drug was dispersed in 10ml of water. The prepared oral suspension was kept for magnetic stirring for 48hrs. Then 1.5 ml of it was filled into centrifugation tube and centrifuged at 25000 rpm for 30 minutes (Remi indusrties centrifuge). Supernatant was filtered and analyzed spectrophotometrically using UV-visible spectrophotometer (UV-Shimadzu) at 221 nm after suitable dilution with phosphate buffer (pH 7.4) containing tween 80, which was used as blank.

Powder X-Ray Diffraction (PXRD) analysis:

The drug crystalline state in the polymer sample was evaluated by Powder X-Ray Diffraction (PXRD) analysis. X-ray spectra were recorded with X'Pert-PRO multipurpose X-Ray diffractometer (PANalytical,

Tokyo, Japan) using Ni-filtered, Cu Ka radiation, a voltage of 45 kV, and a current of 40 mA with a scintillation counter. The instrument was operated in the continuous scanning speed of 4°/min over a 2θ range of 5° to 40°. The samples were grinded using a wedgwood mortar and pestle, placed into the cavity of an aluminum sample holder and packed smoothly using a glass slide.

In-vitro drug release:

In-vitro dissolution studies of samples were carried out using USP apparatus II paddle method by dispersed powder technique. Accurately weighed samples were added to 500 ml of buffer media (Phosphate buffer pH 7.4) at 37 + 0.5°C and stirred at 50 rpm. An aliquot of 10ml was withdrawn at different time intervals. The solid particles were prevented from pipetting by withdrawing the sample through a pipette fitted with a cotton plug. An equal volume of fresh dissolution medium was immediately replaced. The filtered samples were assayed spectrophotometrically at 221nm by blank correction method as reported under the section. Phosphate buffer pH 7.4 was used for in-vitro dissolution studies. The dissolution of nanosuspension was compared with the dissolution of equivalent amount of the oral suspension and pure drug.

Results and Discussion

Preformulation study for Anastrazole has been performed to know the drug physical properties so as to design it to a suitable formulation. Anastrazole is a class -IV (Low solubility and low permeability) classified under BCS Classification with low solubility and low permeability. Poor solubility leads to poor dissolution, therefore to enhance the dissolution rate of the drug, different techniques have been employed in the present study such as particle size reduction by nanosuspensions which is a novel technique where saturation solubility has been achieved compared to micronization, and formation of inclusion complexes by cyclodextrins which is a conventional technique, which also proved to enhance the dissolution rate, additionally use of surfactant which is commonly employed technique to enhance the solubility of the drug which improves dissolution rate and thereby enhances the bioavailability.The saturation solubility of the pure drug in phosphate bufferpH7.4 was found to be 0.39mg/ml. The saturation solubility of the nanosuspension F-6 in phosphate buffer pH7.4 was found to be 3.9 µg/ml. The mean particle size was determined by using optical microscopy. The average particle size was found to be 0.34µ, 0.72µ and 0.81µ respectively. It was observed that by increasing the concentration of surfactant reduces the particle size in the formulation.

Table no: 2 Different batches of Anastrozole nanosuspension preparation with particle size

Formulation	F1	F2	F3	F4	F5	F6	F7	F8
Anastrozole	100mg	100mg	100mg	100mg	100mg	**100mg**	100mg	100mg
Ethanol	25ml	5ml	5ml	5ml	5ml	**5ml**	5ml	10ml
Distilled water	50ml	50ml	25ml	10ml	10ml	**10ml**	10ml	5ml
Tween 80	0.5ml	0.5ml	0.5ml	1ml	1.5ml	**2ml**	0.5ml	0.5ml
Average particle size	3.19µ	3.61µ	2.21µ	0.59µ	0.68µ	**0.34µ**	0.72µ	0.81µ

The results of in-vitro release studies were performed and the formulation F-6 is selected for further studies as it has average particle size of 0.34µ given in table 2 and saturation solubility was found to be 3.9 µg/ml.SEMwas used to determine the from the figure 1 and 2surface morphology was visualized it was founed to be spherical, smooth, wavy and free flowing and concluded that the average particle size was found to be in a nano range i.e.,from96.9 nm to 186 nm and that there is a size reduction of particles. Zeta size has been determined by zetasizer and reported as 212.5nm from figure 3.The zeta potential and charge of the nanosuspension were evaluated by measuring the zeta potential of the nanosuspension by the malvern zeta sizer. The zeta potential of the optimized formulation F-6 was found to be-41.9mV from figure 4.XRPD was used to investigate the physical nature of the encapsulated drug, the powder X-ray. XRPD was used for analysis of a variety of transformations during pharmaceutical processing and storage such and degree of hydration. From the figures 5 and 6, XRPD graphs it was observed that the crystallanity of the drug was changed in the nanosuspension. The peaks obtained for pure drug was very clear and sharp the intensity of the peaks was very high when compared to peaks of anastrazole nanosuspension. Reduction in the peak intensity indicates the change in crystal structure. From this we can conclude, that there was reduction in the crystallanity and change

into amorphous structures as polymorphic transformations, alterations in crystallanity, changes in state. The state of drug has changed from one form to another form so there is change in drug solid state property from crystalline to amorphous. Drug-excipient interaction studies were performed for pure drug and optimed formulations were determined based on the FTIR spectra given in the figures 7 and 8. The FTIR spectra of pure drug, physical mixture and nanosuspensions were compared to check any interactions between the drug and polymer or change in the positions of the functional group of the drug. There is no shift in peak of dug in the formulation. Hence there is no drug and excipient incompatibility problem. From the figures 9and 10, shows DSC thermograms of pure drug powder and optimized nanosuspension formulation respectively. Pure drug powder showed melting thermogram at which corresponds to its melting point and its thermogram in formulation was observed. From thermograms, it was concluded that the drug has been changed its physical structure in formulation thus concluded that there is change in physical state of drug in the formulation and change in drug solid state property from crystalline to amorphous. The results of drug release were expressed in terms of % drug dissolved as the function of time. The data obtained revealed that the onset of dissolution of pure anastrazole was (1.0% in 5 min) and (45%in 24 hrs). From the data obtained it was observed that there was an increase in % drug release (3.2 %in 5 min) and (78.8 in 24^{th}hr) with the optimized nanosuspension. It clearly indicates that nanosuspension formulation has been successful technique to improve the dissolution rate thereby enhancing the controlled release after intravenous (i.v.) administration with enhanced $C_{max.}$ The optimized formulation (F-6) has an average particle size of 0.345µ and saturation solubility was found to be 3.9µg/ml. The nanosuspension formulation is good for breast cancer.
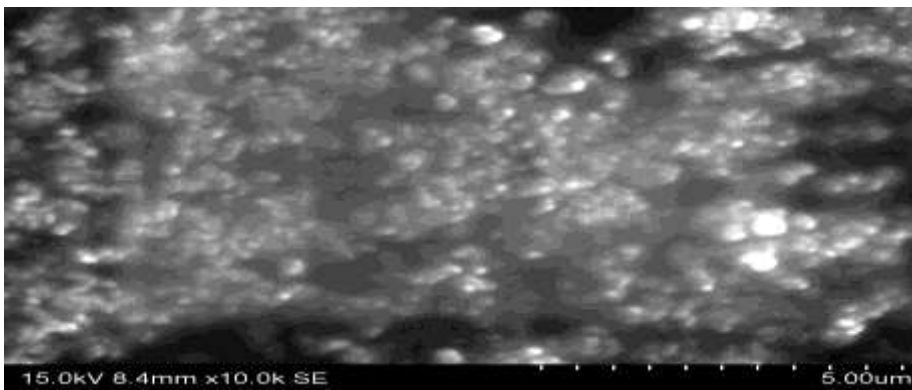

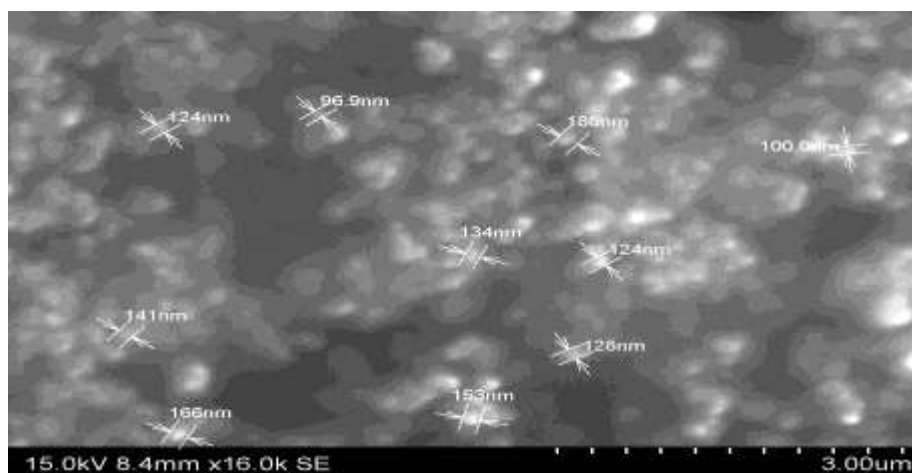**Figure no: 1 SEM picture and surface mrphology anastrazole nanosuspensions in spherical shape**

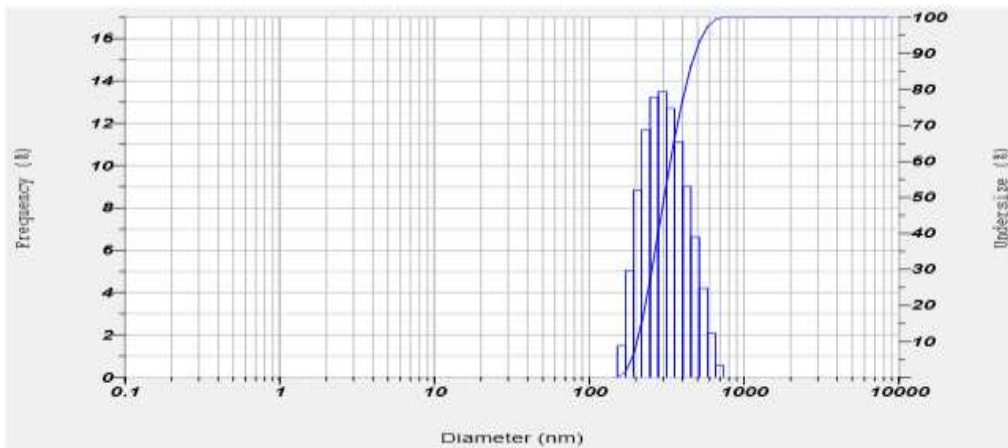**Figure no: 2 SEM picture of anastrazole particles in nano range**

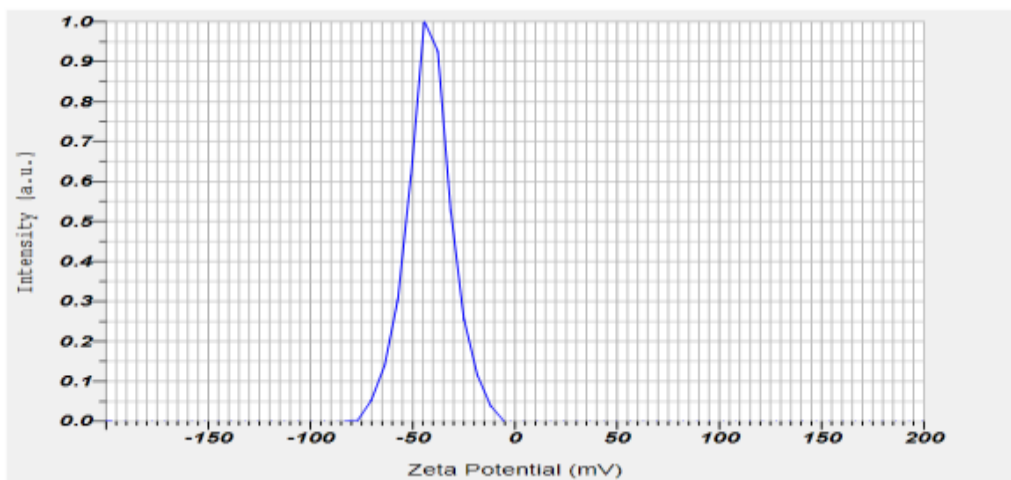**Figure no: 3 Zeta size of anastrazolenanosuspension**

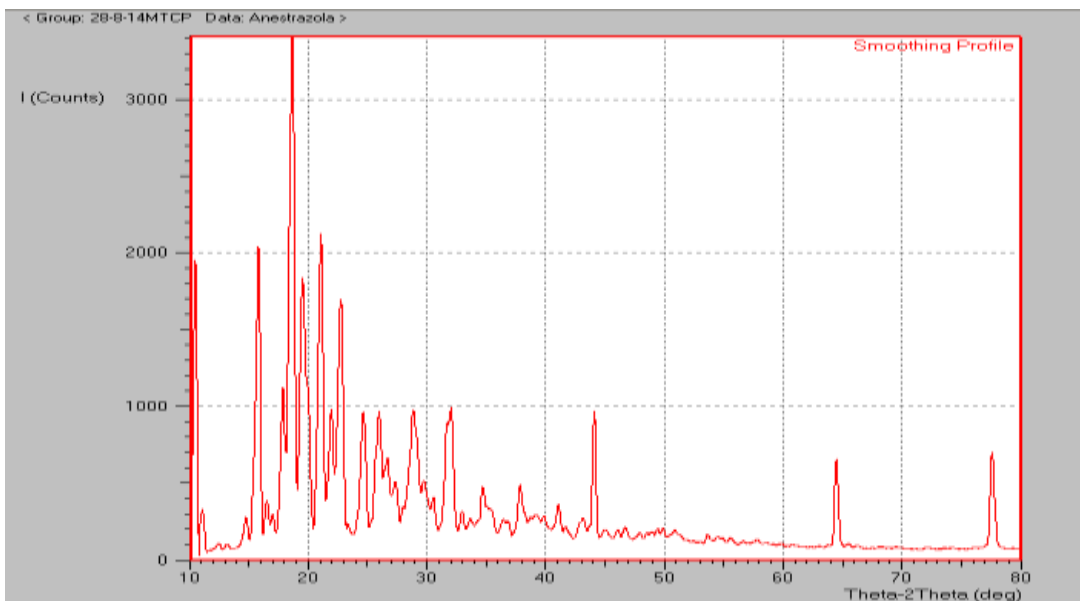

Figure no: 4 Zeta potential of anastrozole nanosuspension
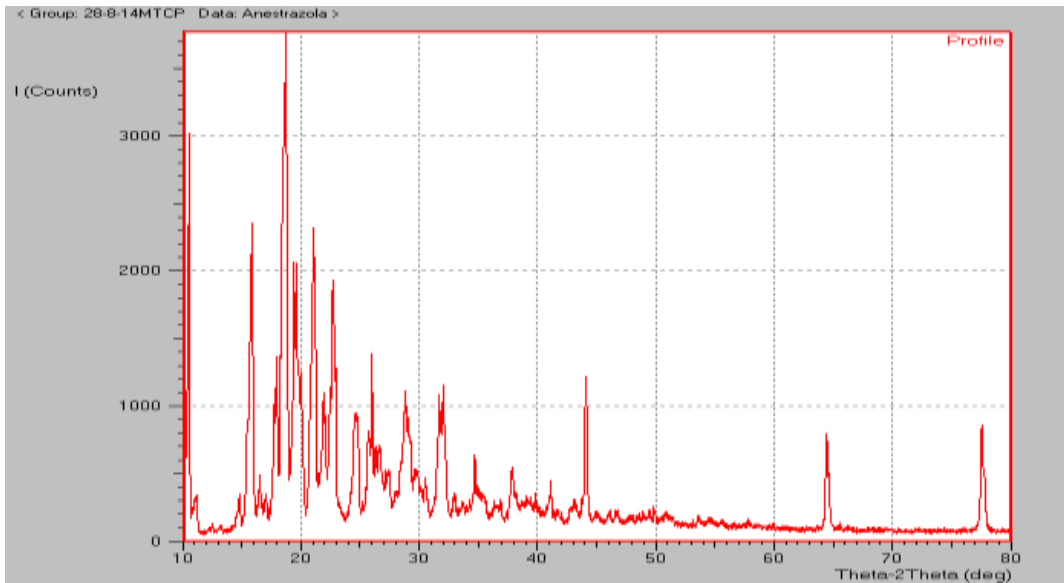

Figure no: 5 XRPD graph of anastrazole drug

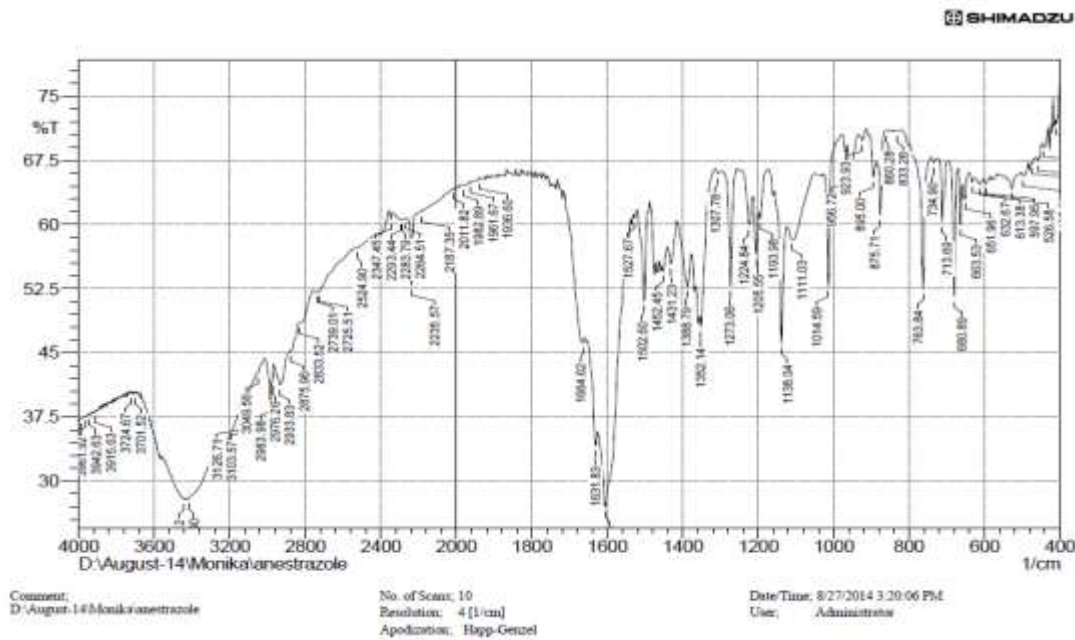**Figure no: 6 XRPD graph of anastrazole nanosuspension (F6)**

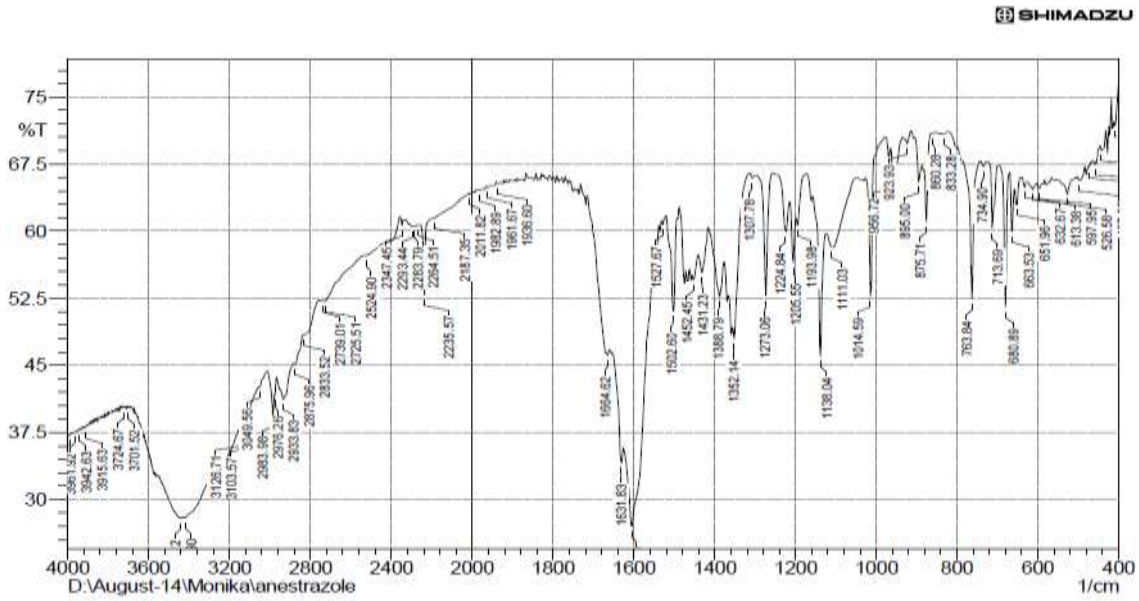

Figure no: 7 FTIR graph of anastrazole drug

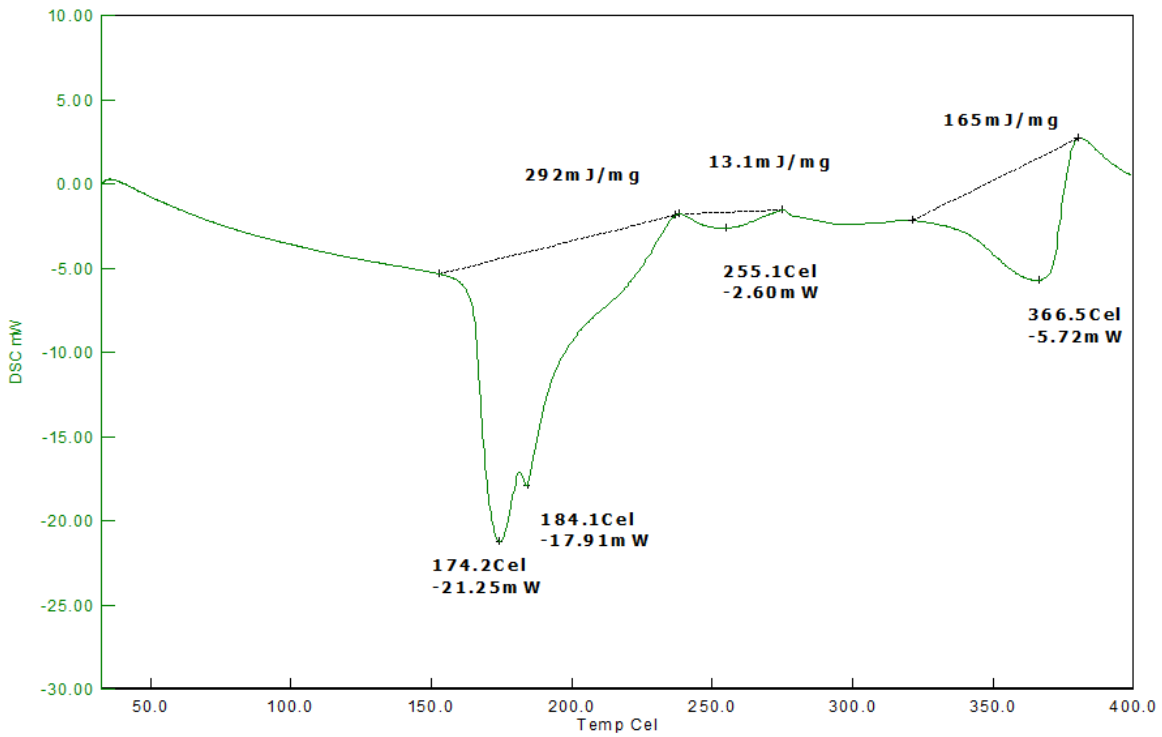**Figure no: 8 FTIR graph of anastrazole nanosuspension (F6)**
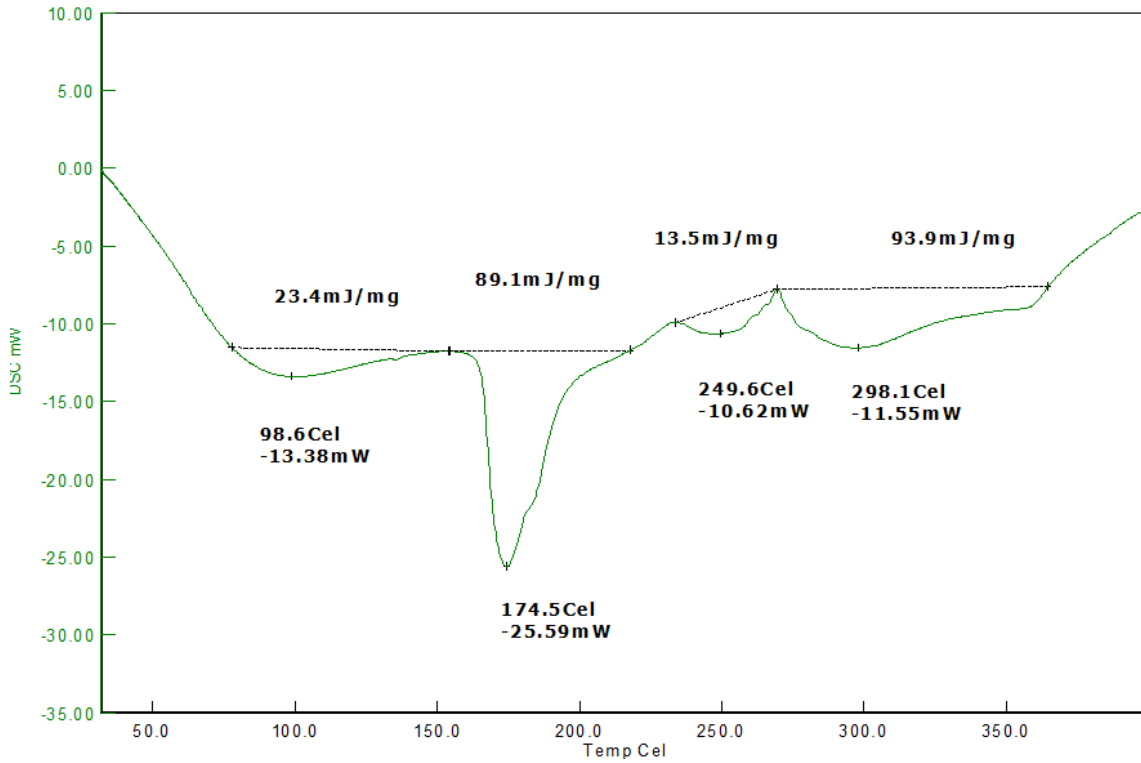

Figure no: 9 DSC graph of anastrazole drug

Figure no: 10 DSC graph of anastrazole nanosuspension (F6)

Table no: 3 Dissolution profile of nanosuspension formulations of F1-F8 formulations

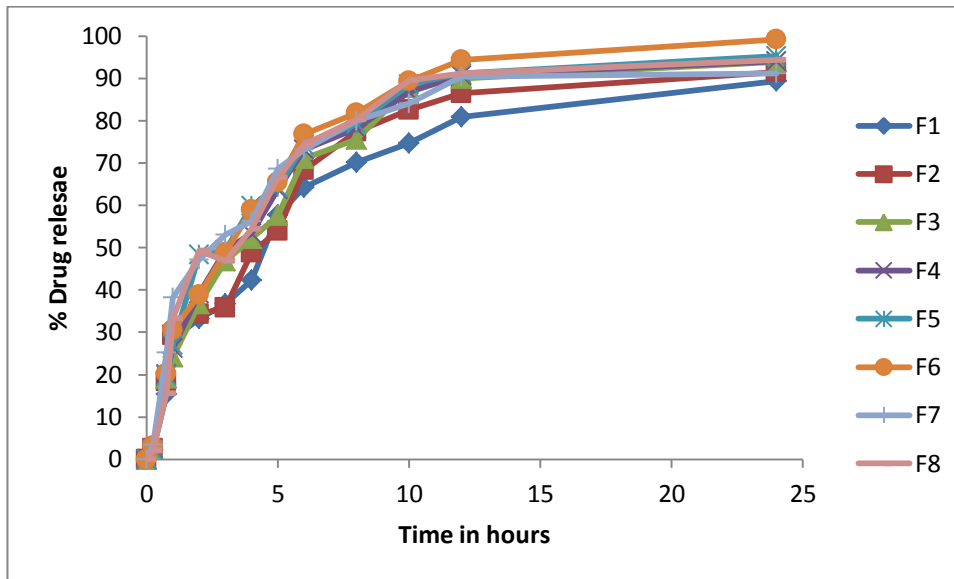| TIME(hours)	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	**0**	0	0
0.25	2.3	2.5	2.4	2.6	2.2	**3.2**	3.45	2.1
0.75	15.4	18.4	19.2	20.1	19.8	**20.2**	25.2	15.6
1	28.4	29.3	24.2	26.3	27.1	**30.5**	38.23	33.2
2	33.2	34.3	36.7	39.2	48.3	**38.9**	47.12	49.3
3	36.7	35.8	46.8	49.4	49.3	**48.8**	53.14	46.9
4	42.3	48.9	52.1	53.4	59.9	**58.9**	56.2	54.4
5	57.8	53.9	57.6	63.8	64.2	**65.4**	68.7	66.5
6	64.2	68.4	71.1	73.2	74.3	**76.8**	73.2	74.4
8	70.21	77.47	75.64	78.12	79.21	**81.76**	79.99	80.23
10	74.65	82.63	87.82	86.73	88.63	**89.34**	84.12	89.47
12	80.85	86.45	89.97	90.79	91.23	**94.36**	90.45	91.12
24	89.34	91.56	93.98	93.99	95.21	**99.25**	91.23	94.32

Figure no: 11 Graphical representation of dissolution profile of nanosuspension formulations of F1-F8

Conclusion

The preparation of parenteral nanosuspension for anastrazole was successfully prepared by solvent-antisolvent precipitation method. The whole of the eight nanosuspension formulations were determined for average particle size and saturation solubility. The optimum formulation based on less particle size and increased saturation solubility was selected. The nanosuspension (F-6) formulation has an average particle size of 0.345µ and saturation solubility was found to be 3.9µg/ml. The optimized sustained release nanosuspension formulation was characterized by SEM, zeta potential and XRPD studies. It is further evaluated for in vitro drug release rate studies. The particles surface of Anastrazole nanosuspension was spherical in shape. In particle size analysis, Anastrazole nanosuspension obtained comparatively nanonized particles range in 96nm-190nm. The 24th hour percentage drug release of optimized nanosuspension formulation was found to be 78.8%. XRPD studies showed that the peaks obtained for pure drug is very clear and sharp the intensity of peaks are very high when compared to peaks of optimized Anastrazole nanosuspension formulation. Reduction in the peak indicates the changes in the crystal structure. From this we can conclude that there was reduction in crystallinity and change into amorphous structure. Therefore, in this study a parenterals controlled release of Anastrazole nanosuspension formulation of Anastrazole was successfully developed. The formulation is intended for controlled release delivery of the drug after i.v. administration with enhanced C_{max}. Hence it is good for breast cancer. The formulation can be good for adjuvant treatment of hormone receptor positive breast cancer, as well as hormonal treatment of advanced breast cancer in post-menopausal women because of the natural tendency of nanosuspension to be taken up into systemic circulation after intravenous administration.

Acknowledgements

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