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In-Vitro study of *Anastrozole* loaded PLGA Nanoparticles for the treatment of Breast Cancer

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Abstract

PLGA nanoparticles contains Anastrozole was prepared using the solvent evaporation method. PLGA used as a polymer and PVA as surfactant in this process, the effect of some parameters on particle size and drug loading efficiency was evaluated. SEM photograph typical spherical shape of nanoparticle. The mean particle size was 320nm and was affected homozinization, the maximum drug loading efficiency and pay load was estimated 66.9±0.943% and 17.4±1.142% with drug and PLGA ratio 1:3, this value tend decrease the entraptment of drug proportion in release profile in buffer pH. 7.4, however followed slow release pattern Anasrozole in the medium. The present study provided insight into the significance of PLGA loaded nanoparticles asparenteral delivery of Anastrozole for sustaind release.

Key words: *Anastrozole*, *Anastrozole* Loaded PLGA Nanoparticles, Breast Cancer, Aromatase inhibitors

Introduction

Cancer is a category of ailments in which a group of cells creates unrestrained growth, incursion, and sometimes metastasis (spread to other positions in the body via lymph or blood). These three malignant characteristics of cancers differentiate them from benign tumors, which are self-limited, and do not invade or metastasize. The majority of cancers form a tumor but some, like leukemia, do not. The division of medicine concerned with the study, diagnosis, treatment, and prevention of cancer is termed as oncology. Cancer affects people at all ages with the risk for most types increasing with age. Cancers are caused by a deformity in the genetic material of the transformed cells [1]. Cancer pathogenesis is traceable back to DNA mutations that impact cell growth and metastasis. Substances that cause DNA mutations are known as mutagens and mutagens that cause cancers are known as carcinogens. Particular substances have been linked to specific types of cancer. Tobacco smoking is associated with many forms of cancer [2]. And causes 90% of lung cancer [3]. Long time exposure to asbestos fibers is associated with mesothelioma [4]. There is a number of recognized syndromes whereis an inherited predisposition to cancer, often due to a effective in a gene that protects against tumor formation.

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Certain inherited mutations in the genes BRCA1 (Breast cancer associated gene 1) and BRCA2 (Breast cancer associated gene 2) are associated with an elevated risk of breast cancer and ovarian cancer. Excepting the rare transmissions that occur with pregnancies and only a marginal few organ donors, cancer not only a transmissible disease. The main cause for this is tissue graft rejection caused by MHC incompatibility [5].

Cancers are caused by a number of mutations. Each mutation alters the nature of the cell to some extent. Cancer is fundamentally a disease of continuous of tissue growth. In order for a normal cell to reached into a cancer cell (scheme 1), genes which regulate cell growth and differentiation must be altered [6]. Genetic mutation can occur at many levels, from gain or loss of entire chromosomes to a mutation that affecting single DNA nucleotide. There are two broad types of genes which are affected by these changes. Oncogenes may be normal genes which are expressed at inappropriately high levels, or altered genes which have novel properties. In either case, expression of these genes promotes the malignant phenotype of cancer cells. Tumor suppressor genes are those genes which inhibit cell division, survival, or many other features of cancer cell. Tumor suppressor genes are often disabled by cancer-promoting genetic changes. Typically, changes in a number of genes are needed to transform a normal cell into a effective cancer cell [7].

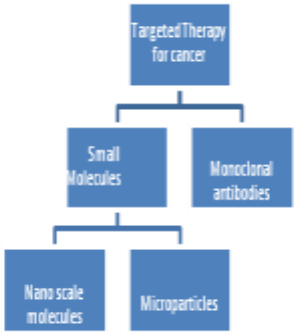
The primary noticeable symptom of breast cancer is typically a lump that goes different from the rest of the

breast tissue. Approx 80 % or more of breast cancer patients are discovered when the woman feels a lump. By the timeof a breast lump is noticeable, it has probably been increasing for years. The earliest breast cancers are detected by a mammogram [6]. Lumps present in lymph nodes located in the armpits can also denoted breast cancer.

BRCA1 (breast cancer 1, early onset stage) and BRCA2 are human genes, some mutations of which are associated with a significant increase in the risk of breast cancer, as well as other cancers. BRCA1 belongs tumor suppressors, which maintains genomic integrity to restrict dangerous genetic changes. Some variations of the BRCA1 gene lead to an increased risk of breast cancer. Researchers have identified hundreds ofchanges like mutations in the BRCA1 gene, many of which are associated with an increased risk of cancer. Women who have an abnormal BRCA1 or BRCA2 gene have up to an 85% risk of developing breast cancer by age 70; increased risk of developing ovarian cancer is about 55% for women with BRCA1 mutations and about 25% for women with BRCA2 mutations. These mutations can be make changes in one or a small number of DNA base pairs (the building blocks of DNA). Those mutations can be identified by PCR and DNA sequencing. In some cases, large segments of DNA are rearranged, those large segments also called large rearrangements can be a deletion or a duplication of one or several exons in the gene.

Available Strategies for treatment of breast cancer

- Surgery
- Radiation therapy
- Chemotherapy
- Immunotherapy
- Hormonal therapy
- Angiogenesis inhibitors
- New drug delivery system (Targeted therapies)



Scheme: Different approaches for tumour targeting

Small molecule targeted therapy molecules are generally inhibitors of enzymatic domains on mutated, overexpressed or otherwise critical proteins within the cancer cell. Prominent examples are the tyrosine kinase inhibitors imatinib (Gleevec/Glivec) and gefitinib (Iressa). Another strategy is Monoclonal antibody therapy in which the therapeutic agent is an antibody which specifically binds to a protein on the surface of the cancer cells. Examples include the anti-HER2/neu antibody trastuzumab (Herceptin) used in breast cancer, and the anti-CD20 antibody rituximab, used in a variety of malignancies. Targeted therapy can also involve small peptides, for example, radionuclides which are attached to these peptides eventually kill the cancer cell if the nuclide decays in the vicinity of the cell.

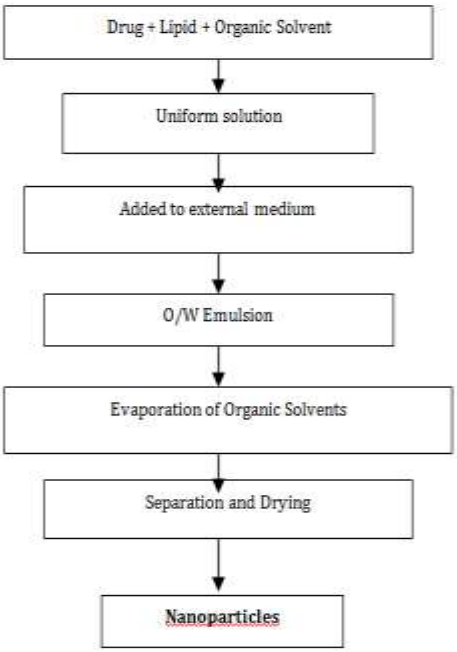
Nanoparticles as potential carrier for management of cancer

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000 nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanoparticles are one of the multi-particulate delivery systems and are prepared to obtain prolonged or controlled drug delivery and to improve bioavailability as well as stability [12]. Nanoparticles can also offer advantages like limiting fluctuations within the therapeutic range, reducing side effects, decreasing dosing frequency, and improve patient compliance [8]. Nanocapsules are systems in which the drug molecule is confined to a cavity surrounded by a unique polymer membrane while nanospheres are matrix systems in which the drug is physically and uniformly dispersed. In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as polyethylene glycol (PEG) also known as long-circulating particles, that have been used as potential drug delivery devices because of their character or ability to circulate such as prolonged period time target for a particular organ, as carriers of DNA in gene therapy, their ability to deliver proteins, peptides and genes [9]. The major aim in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active ingredient in order to achieve the site-specific action of the drug at the therapeutically optimization rate and dose regimen. For suddenly, they help to increase the stability of drugs/proteins and possess useful controlled release properties [10,11].

1. Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration.

2. They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve an increase in drug therapeutic efficacy and reduction in side effects.
3. Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction; this is an important factor for preserving the drug activity.
4. Site-specific targeting can be achieved by attaching targeting ligands to the surface of particles or use of magnetic guidance.
5. The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular, etc.

However, nanotechnology for drug delivery applications may not be suitable for all drugs, especially those drugs that are less potent because the higher dose of the drug would make the drug delivery systemmuch larger, which would be very difficult to administer [12].



Scheme 2: preparation of nanoparticles by solvent evaporation [13]

About Anastrozole

Kumar et al. reported that Development and validation of spectrophotometric method for estimation of anastrozole bulk and pharmaceutical dosage formulation. Method was developed and validated by using a simple solvent system for anastrozole bulk and tablet dosage form. In the developed method, water and ethanol are used as solvents and λ-max was determined to be 221 nm. The procedure was validated as per ICH rules for Accuracy, Precision, Detection limit, Linearity, Reproducibility, and Quantitation limit. The linearity concentration range was 40-60µg/ml with the correlation coefficient of 0.9971. The percentage recovery for anastrozole was found to be 98.6 to 100.8 %. Limit of detection and limit of quantitation values were found to be 1µg/ml and 3µg/ml. The method has been successfully used to analyze commercial solid dosage containing 1 mg of anastrozole with good recoveries and proved to be robust. This provides shorter analysis time and conserves the solvent system [4]. Srinivasulu et al. reported that Reverse Phase HPLC method for analysis of anastrozole in Pharmaceutical dosage forms. Simple and precise RP HPLC method was developed and validated for the determination of anastrozole in pharmaceutical dosage forms. Chromatography was carried out on an Inertsil ODS (250 mmX 406 mm) C18 column using a mixture of Buffer: Acetonitrile (60:40) as The mobile phase at a flow rate 1.0 ml/min. The analyte was monitored using UV detector at 215 nm. The retention time of the drug is 6.431 min for anastrozole. The proposed method is found to be having linearity in the concentration range of 0.1 to 0.6 µg/ml with a correlation coefficient of r=0.9999. The mean recoveries obtained for anastrozole are in the range 99.8-100.2%. The developed method has been statistically validated and found simple and accurate. Due to its simplicity, rapidness, high precision and accuracy of the proposed method it may be used for determining anastrozole in bulk and dosage forms [5].

Anastrozole for breast cancer

The trial suggested that anastrozole is the preferred medical therapy for postmenopausal women with localized breast cancer, which is estrogen receptor (ER) positive [14]. Another study found that the risk of recurrence was reduced 40%, but was associated with an increased risk of bone fractures. Anastrozole lowers estrogen levels in postmenopausal women, which may slow the growth of certain types of breast tumors that need estrogen to grow in the body. Anastrozole is used to treat breast cancer in postmenopausal women.

Aromatase inhibitor inactivates the production of estrogen from androgens, by suppressing aromatase enzyme activity. The breast cancer patients treated with aromatase inhibitors show, less level of estrogen secretion in the tumor cells [15].

Aromatase inhibitors effectively prevent breast cancer recurrence and development of new contralateral tumours in postmenopausal women.

We assessed the efficacy and safety of the aromatase inhibitor anastrozole for treatment and prevention of breast cancer in postmenopausal women who have at high risk of the disease

In children increase estrogenshown to help slow this process, and increase adult height prediction in adolescent males treated with protein-based peptide hormones for the treatment of growth hormone deficiency [16].

Pharmacokinetic Data

Bioavailability- 83-85%
Protein binding - 40%
Metabolism - 85% hepatic
Biological half-life- 46.8h
Excretion- 11% renal

Chemical Data

Formula- $C_{17}H_{19}N_5$
Molecular mass- 293.366 gm/mol.

AIM: Formulation and evaluation of Anastrozole loaded PLGA nanoparticles for the treatment of breast cancer

Material and Methods

Material

The following drug, excipients, and chemicals were used for the formulation and evaluation studies of Anastrozole loaded PLGA nanoparticles.

Drug

- Anastrozole-Ranbaxy lab. Ltd (Gurgaon, Haryana)

Excipients

- PLGA- SUN Pharma (SPARC, Vadodara)
- PVA (cold)- Genuine chemical Co. Bombay

Chemicals

- DCM (Dichloromethane)- Qualigens fine chemicals
- Ethanol- Oxford laboratory reagents, Mumbai
- Anhydrous dibasic sodium Phosphate- Oxford laboratory reagents, Mumbai

Equipment

- UV-visible spectrophotometer (1700)-Shimadzu
- Mechanical stirrer- Remi
- Electronic balance- Capsons
- Vortex Shaker- Labcare
- Homogenizer- Remi
- Cooling microfuge- Remi

Distilled water was used throughout the study.

Identification, melting point determination, solubility analysis, absorption Maxima (λ_{max}), calibration curve, and Fourier transform infrared spectroscopy were done by following methods

Solvent evaporation method for nanoparticles

In the classical solvent evaporation method, lipids are first dissolved in an organic solvent and are then emulsified in an aqueous phase containing the emulsifying agent [13]. The resulting O/W emulsion is finally stirred for several hours under ambient conditions in order to allow the solvent evaporation. Obtained nanoparticles filtered, rinsed with water and freeze dried in lyophilizer (fig. 3).

Nanoparticles are useful because of their adaptability; the particles can be loaded with a variety of drugs that will then be directed to growing tumours. Nanoparticle research has the capacity to affect profoundly mankind's future.

By safely researching nanoparticle elements into their very smallest components and then engineering these particles to achieve different functions, researchers can dramatically enhance everything from delicate electronics to life-saving medical techniques. However, critics warn that we need to test and re-test all the effects that various nanoparticles can have on the human body before releasing them in wide numbers as the main components of common products that will come into contact with humans several times a day. Given the latest in nanoparticle research for safety, they may be right. Using nanoparticles, drug doses could be much smaller than doses typically used in chemotherapy, making the procedure potentially much safer. The nanoparticles also may permit more effective follow-up, because a doctor could use them to discern whether a tumor was still growing after radiation or chemotherapy treatments. To be prepared nanoparticles will focus more drugs at the tumour site. Nanoparticles can improve a therapy by increasing the length of time the combination lasts in the body. Nanoparticles also boost the effectiveness of treatment compounds because therapeutic molecules tend to clump around each nanoparticle.

Identification of drug

Identification of drug and polymer were carried out. In this perspective the organoleptic properties like appearance, colour and odour were observed, visual observations discerns that the drug a white coloured powder, crystalline solid in nature, these parameters resembles the properties stated in Pharmacopoeia.

Melting point

Melting point of Anastrozole was measured in melting point apparatus (VEEGO)

Solubility analysis

Solubility analysis was carried out in different solvent media [1]. Nanoparticles equivalent to 2mg of Anastrozoledissolved in 2ml of different solvents and the solubility of Anastrozole in various media was determined.

FTIR studies

Fourier transforms Infrared (FTIR) studies of Anastrozole and PLGA were performed by using KBr pellet method. The samples were mixed with potassium bromide and pressed into pellets and were scanned in the IR range of 400- 5000cm-1at 25 °C. In order to carry out drug-polymer compatibility study (Fig. 6) using UNIVERSAL Q 200 V 23.5 instrument.

Preparation of Anastrozole loaded PLGA NPs

Solvent evaporation technique was used to formulate the Anastrozole loaded PLGA NPs [13].

NP formulation was prepared by incorporating the Anastrozole with PLGA. A homogeneous solution of Anastrozole with PLGA (in variable ratio) was prepared by dissolving both Anastrozole and PLGA in appropriate amount of DCM using a vortex shaker (LabcareTM). This solution was then added slowly to 50 mL of aqueous PVA solution (various dilutions) using a Silverson Mixer Homogenizer (Silverson U.S.) to prepare the oil in water (o ⁄ w) emulsion. The emulsion formed was stirred using a magnetic stirrer (REMI, Remi equipment Ltd, Mumbai, India) for 4 h at 25°C, followed by centrifugation (Research centrifuge, Remi Instruments Ltd) for 10-25 min at 12 000 – 18,000 rpm. The supernatant was decanted, and the remaining pellets were washed using the distilled water, washings were repeated for two times, the obtained NPs were dried and collected.

The nanoparticles processing parameters such as drug /polymer ratio, phase ratio (organic phase/ aqueous phase) and stirring time which may affect size and entrapment efficiency were studied.

The concentration of surfactant was optimized in order to obtain small nanoparticles with maximum percent drug entrapment. There was no significant difference in particle size and percent drug entrapment with PVA concentration 2-4% (w/v). Therefore 4% (w/v) PVA was recorded as optimized surfactant concentration.

Stirring time was also optimized in order to achieve stable formulation with minimum average particle size and maximum per cent drug entrapment. Emulsification is carried out under high-shear stress to reduce the size of the emulsion droplets (directly related to the final size of nanoparticles). A stable Nano particulate formulation was achieved after stirring the formulation for 10-25 minutes in a pulsed manner with minimum average particle size and maximum percent drug entrapment.

In vitro drug release studies

Nanoparticles equivalent to 20 mg Anastrozole were suspended in 5 mL phosphate buffered saline (pH 7.4) in a dialysis bag which is suspended in a beaker carrying 100 mL phosphate buffered saline (pH 7.4) as release medium, the contents were kept under mechanical stirring(50r.p.m.), with temperature maintained at 37±1˚C. At selected time intervals over the period 1–48 h, 1.0 mL samples were withdrawn from the beaker and drug content was analyzed after appropriate dilution using a UV spectrophotometer (Pharmaspec-1700; Shimadzu) at a λmax of 240 nm. The experiment was performed in triplicate under sink conditions.

Results and Discussion

Identification of drug

Identification of drug and polymer were carried out. In this perspective the organoleptic properties like appearance, colour and odour were observed, visual observations discerns that the drug a white coloured powder, crystalline solid in nature, these parameters resembles the properties stated in Pharmacopoeia.

Melting point

The melting point of Anastrozole was measured in melting point apparatus (VEEGO), melting point of the pure drug was determined to be 185±1°C (Table 3).

Table 3. Melting point specified and determined

S. N.	Drug	Melting point specified in pharmacopeia	Melting point determined
1	Anastrozole	185-186 ^{O}C	185±1^{O}C

Solubility analysis

Solubility analysis was carried out in different solvents. The drug Anastrozole was found to be soluble in ethanol, methanol, chloroform and Dichloromethane but was not solubilized in water (Table 4). Solubility profile suggests drugs applicability in parenteral formulations.

Table 4. Solubility of Anastrozole in various solvents

S.N.	Drug	Solvent	Inference
1	Anastrozole	Water	-
2	Anastrozole	Ethanol	-+
3	Anastrozole	Methanol	+
4	Anastrozole	Chloroform	+
5	Anastrozole	Dichloromethane	++

Where: (-)=insoluble, (-+)=Sparingly soluble, (+)=Soluble, (++)=Freely soluble

Calibration curve of Anastrozolein phosphate buffer

The calibration curve of Anastrozolewas prepared in pH 7.4 Phosphate buffer (Fig. 2). It gives correlation coefficient of 0.9998 in the concentration range of 2-20µg/ml. A standard curve was prepared for Anastrozole and R^2 value was reported 0.9998.

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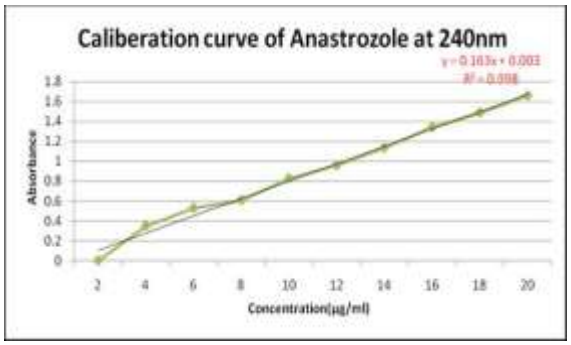

Fig. 2: Calibration curve of Anastrozole in phosphate buffer pH 7.4

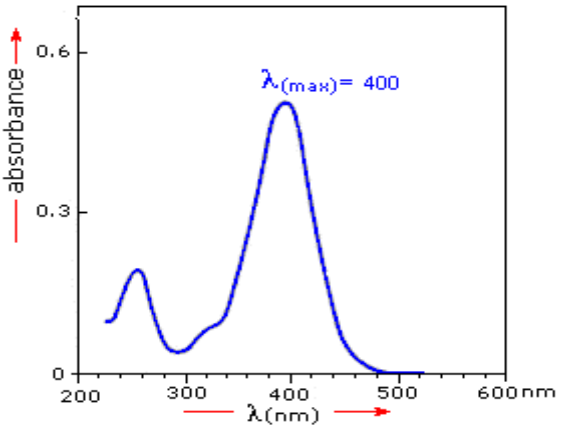

Fig. 1: Absorption maxima of Anastrozole

FTIR studies

Fourier transforms Infrared (FTIR) studies of Anastrozole, PLGA and physical mixture of drug and polymer were performed by using KBr pellet method.

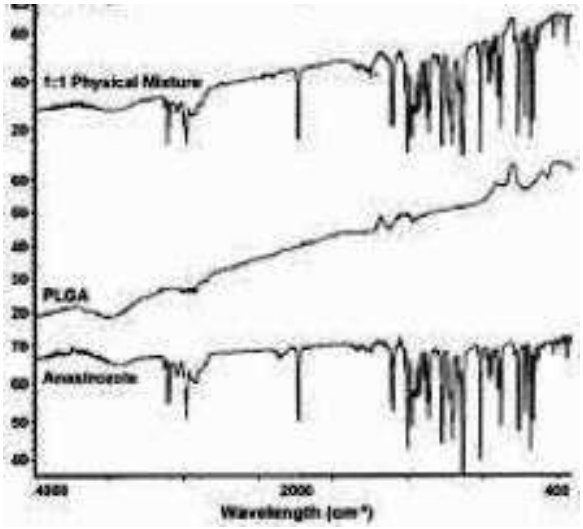

Fig. 3: FTIR of Anastrozole

Drug entrapment efficiency and pay load

Drug entrapment efficiency was determined (optimized) by centrifuging (Centrifuge Remi India) dispersion of Anastrozole -PLGA-NPs at 15,000 rpm, 25^0C for 20 min in PBS pH 7.4 buffer and measuring the amount of Anastrozole in the supernatant with the help of UV-VIS Spectrophotometer (Shimadzu, Japan), set at 240 nm. The amount of Anastrozole was subtracted from theinitial amount of Anastrozole taken in the preparation of NPs to calculate drug entrapment efficiency of NPs. The experiment was performed in triplicate for each batch and average drug entrapment efficiency (66.9±0.943%) was calculated.

Table 5. Percentage of drug entrapment efficiency, drug pay loadand % yield

Efficiency	Mean±S.D.
Entrapment efficiency	66.9±0.943%
Pay load	17.4±1.142%
% yield	96.06±1.25%

N=3, ±standard deviation (S.D.)

Table 6. Composition (%W) of the investigated formulation from F1 to F15

Component → / Formulation ↓	Anastrozole (mg)	PLGA (mg)	PVA (%w/v)	DCM
F1	10	50	2	5ml
F2	10	40	2	5ml
F3	10	30	2	5ml
F4	10	20	2	5ml
F5	10	10	2	5ml
F6	10	50	3	5ml
F7	10	40	3	5ml
F8	10	30	3	5ml
F9	10	20	3	5ml
F10	10	10	3	5ml
F11	10	50	4	5ml
F12	10	40	4	5ml
F13	**10**	**30**	**4**	**5ml**
F14	10	20	4	5ml
F15	10	10	4	5ml

Table 7: Optimization of Drug/Polymer ratio and surfactant % with regard to per cent drug entrapment &average particle size

Formulation Code	Drug/ Polymer Ratio (w/w)	PVA %	% Drug Entrapment	Avg Particle Size (nm)
F1	1:5	2	71.7	759
F2	1:4	2	69.2	742
F3	1:3	2	66.2	736
F4	1:2	2	61.1	715
F5	1:1	2	48.6	705

F6	1:5	3	68.3	710
F7	1:4	3	63.1	685
F8	1:3	3	60.2	640
F9	1:2	3	46.3	525
F10	1:1	3	40.7	486
F11	1:5	4	69.2	425
F12	1:4	4	68.1	405
F13	**1:3**	**4**	**66.9**	**320**
F14	1:2	4	58.3	240
F15	1:1	4	38.2	180

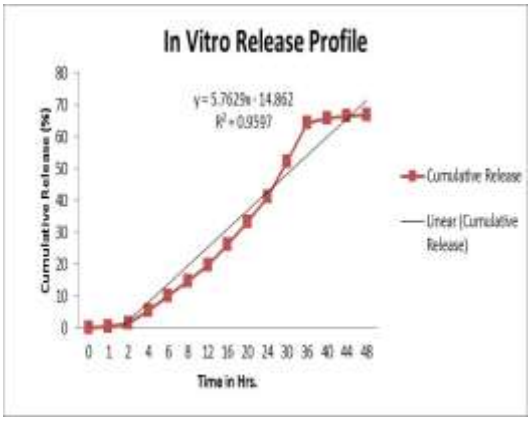

Fig. 4: In-Vitro release curve of nanoparticle (N = 3, ±S.D.)

Size and shape morphology of nanoparticles

Shape and surface morphology of prepared nanoparticles were evaluated by SEM. The study revealed that most of the nanoparticles were fairly spherical in shape. The surface of the particles showed a characteristic smoothness (Fig.). The polydispersity index is a measure of the distribution of nanoparticles. Laser particle size analyzer yields the diameter of the bulk population (average) and a polydispersity index gives the distribution range from 0.000 to 0.300. Polydispersity index greater than 0.3 indicate the aggregation of particles.

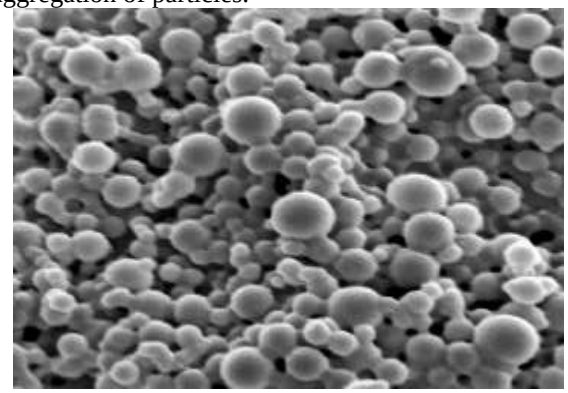

Fig. 5: SEM for Size and shape morphology of nanoparticles

Drug release

An *in vitro* release study was performed in phosphate buffer, pH 7.4 at and the aliquots were withdrawn at time intervals 1, 2, 4, 6, 8, 12, 16, 20, 24, 30 and 36 hours using UV spectrophotometer at 240nm. Drug release from formulation was observed at 1 hour, 4 hours and 12, 24, 36 hours as 0.615±0.099%, 5.337±0.456 and 19.880±0.815, 41. 143±0.415, 64.27±0.820 followed by a significant sustained release rate. Further profiling of release indicated the cumulative percentage drug release of 64.27±0.820% after 36 hours.

Table 9. In-vitro release of Anastrozole from the nanoparticles in pH 7.4 Buffer

S.N.	Time (hrs)	Cumulative release (%) Mean ±SD
1	0	0
2	1	0.615±0.099
3	2	1.480±0.075
4	4	5.337±0.456
5	6	10.027±0.140
6	8	14.910±0.026
7	12	19.880±0.815
8	16	26.022±0.310
9	20	33.413±0.541
10	24	41. 143±0.415
11	30	52.373±0.465
12	**36**	**64.270±0.820**
13	40	65.880±0.654
14	44	66.360±0.231
15	48	66.910±0.389

The result of the present study provided insight into the significant of PLGA loaded nanoparticles as intravenous delivery for Anastrozole. Anastrozole was selected as the suitable drug for being loaded with PLGA nanoparticles for its immense potential in diverse breast cancer. Further, its, low dose(2.5mg-5mg), high frequency of administration (1 to 2 times a day), local or systemic disturbance in the G.I. tract leads to withdrawal of treatment, feasible analytical methodology for its in-vitro studies (UV Spectrophotometry), high physicochemical stability, etc makes it an ideal drug for intravenous (IV) Controlled Release dosage form.

Conclusion

Preformulation studies were carried out; visual observations discerns that the drug a white coloured powder, amorphous solid in nature and a melting point of the pure drug was determined to be 185±1°C; these parameters resembles the properties stated in Pharmacopoeia. The drug Anastrozole was found to be soluble in ethanol, methanol, chloroform and

Dichloromethane but was not solubilized in water, proves that the drug is suitable for the parenteral preparation. FTIR of Anastrozole and mixture of Anastrozole and PLGA shows no interaction between drug and polymer. The calibration curve of Anastrozole was prepared in pH 7.4 Phosphate buffer, and the absorption Maxima (λmax) of Anastrozole was found to be 240nm. It gives correlation coefficient of 0.9998 in the concentration range of 2-20µg/ml. The NPs were prepared by solvent evaporation method. Encapsulation efficiency and pay load of prepared nanoparticles are calculated; (66.9±0.943%)and (17.4±1.142%). The in vitro drug release study of Anastrozole PLGA NPs was conducted in phosphate buffer, pH 7.4. Drug release from formulation was observed at 1 hour, 4 hours and 12, 24, 36 hours as 0.615±0.099%, 5.337±0.456 and 19.880±0.815, 41. 143±0.415, 64.27±0.820 followed by a significant sustained release rate. Further profiling of release indicated the cumulative percentage drug release of 64.27±0.820% after 36 hours.The in vitro drug release showed the regression coefficient values for Higuchi's model (R^2 = 0.973) and Zero-order release kinetics (R^2 = 0.977).R^2 values were calculated for the linear curves obtained by regression analysis of the above plots. It was found that the in vitro drug release of Anastrozole loaded PLGA nanoparticles was best explained by Zero order equation and Higuchi's model as the plot showed the highest linearity for both.

Therefore, the release seems to fit the zero order and Higuchi's model. So, the diffusion of the drug from the nanoparticles was the main mechanism for the drug release.

The results of the present study provided insight into the significant of PLGA loaded nanoparticles as parentral delivery for Anastrozole. Anastrozole was selected as the suitable drug for being loaded with PLGA nanoparticles for its immense potential in diverse breast cancer. Further, its, low dose(2.5mg-5mg), high frequency of administration (1 to 2 times a day), local or systemic disturbance in the G.I. tract leads to withdrawal of treatment, feasible analytical methodology for its in-vitro studies (UV Spectrophotometry), high physicochemical stability, etc makes it an ideal drug for Controlled Release dosage form.

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References

1. Kinzler KW, Vogelstein B. The genetic basis of human cancer. 2nd illustrated revised. ed. New York: McGraw-Hill, Medical Pub. Division; 2002. p. 5.
2. Merlo LM, Pepper JW, Reid BJ, Maley CC. Cancer as an evolutionary and ecological process. Nat Rev Cancer 2006;6:924–35.
3. Sasco AJ, Secretan MB, Straif K. Tobacco smoking and cancer: a brief review of recent epidemiological evidence. Lung Cancer2004;45:3–9.
4. Biesalski HK, Mesquita BB de, Chesson A, Chytil F, Grimble R, Hermus RJ, *et al.*European consensus statement on lung cancer: risk factors and prevention. Lung Cancer Panel CA: A Cancer J Clin 1998;48:167–76.
5. Tolar J, Neglia JP. Transplacental and other routes of cancer transmission between individuals. J Pediatr Hematol Oncol 2003;25:430–4.
6. Croce CM. Oncogenes and cancer. N Engl J Med 2008;358:502–11.
7. Knudson AG. Two genetic hits (more or less) to cancer.Nat Rev Cancer 2001;1:157–62.
8. Jain S, Saraf S. Influence of processing variables and*in vitro* characterization of glipizide loaded biodegradable nanoparticles. Diabetes Metab Syndrome: Clin Res Rev 2009;3:113–7.
9. Lee M, Kim SW. Polyethylene glycol-conjugated copolymers for plasmid DNA delivery. Pharm Res 2005;22:1-10.
10. Vila A, Sanchez A, Tobio M, Calvo P, Alonso MJ.Design of biodegradable particles for protein delivery. J Controlled Release 2002;78:15-24.
11. Mu L, Feng SS. A novel controlled release formulation for the anticancer drug paclitaxel (Taxol (R)): PLGA nanoparticles containing vitamin E. J Controlled Release 2003;86:33-48.
12. Jain S, Shukla K, Jain V, Saraf S, Saraf S. Nanoparticles: emerging carriers for delivery of bioactive agents. Pharm Times 2007;39:30–5.
13. Reithmeier H, Herrmann J, Gopferich A. Development and characterization of lipid microparticles as a drug carrier for somatostatin. Int J Pharm 2001;218:133-43.
14. Howell A, Cuzick J, Baum M. "Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years'

adjuvant treatment for breast cancer". Lancet 2005;365(9453):60–2.

15. Jaynthy Chellam, Rohini Deepa I. Computational biological study of aromatase inhibitors docking with human placental aromatase cytochrome P450. Int J Pharm Pharm Sci 2016;8(7):93-7.
16. Abhinesh Kumar & Krutika K. Sawant Application of multiple regression analysis in the optimization of Anastrozole-loaded PLGA nanoparticles, Journal of Microencapsulation: Micro and Nano Carriers, Volume 31, Issue 2, 2014, pages 105-114.

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