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Poly (lactic-co-glycolic Acid) -loaded

Fadrozole preparation by nanoprecipitation method

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Abstract

Nanoparticles were atoms with smaller than one micron. The size of nanoparticles to 1 to 100 nm, poly (lactic-co-glycolic acid) PLGA had important features including: biocompatibility, biodegradability, drug delivery systems (DDS) and constant release. The study assessed nano-precipitation method of PLGA loaded Fadrozole on induced endometrial periglandular fibrosis (EPF) in female mice compared with the reference Fadrozole. A technology was used to create PLGA nanoparticles, the features of PLGA nanoparticles, included an ultraviolet (UV) spectrophotometer test, which resulted a PLGA-loaded Fadrozole absorbance (4.5452) with a wave length of 336.32 nm, as well as other tests included (scanning electron microscope, transmission electron microscope and zeta potential). According to the description of PLGA revealed in this study, the DMSO associated organic phase alone had specified the form, particle size with a small diameter, charged particles in addition to suitable nanoparticle yield percent, encapsulation efficiency and drug loading with sufficient stabilizer. Finally, we concluded that female mice who were subjected artificially induced endometrial periglandular fibrosis by daily 17 – estradiol S/C injection and H_2O_2 treatment with drinking water for 12 weeks were able to cure with the Poly (lactic-co-glycolic acid) -Fadozole regimen therapy at various doses.

Keywords: Nanoprecipitation, PLGA- loaded Fadrozole.

Introduction:

PLGA, as polymer, (lactic-co-glycolic acid) was a popular eco-friendly polymer for encapsulating a variety of useful substances, including hydrophilic

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and hydrophobic small molecule medicines, DNA, and proteins (1;2). Furthermore, it has a high level of biocompatibility (3). Breakdown of the polymer matrix resulted in complete liberation of contained compounds (4). International regulatory authorities like as the FDA and the EMA had considered PLGA safe for use in medical products supplied to humans via traditional orally administered methods (5). PLGA had been explored broadly for emerging nanoparticles for monitoring release of protein and peptide drugs owing to its biodegradability and biocompatibility (6). The nanoparticle of PLGA formulation had high encapsulation efficiency, loading capacity, sustained and completed releasing of the encapsulated drug with used structural integrity (7).

Furthermore, PLGA nanoparticles exhibited the ability to promote the efficient transfer of protein molecules adsorbed or situated on the interface of the nanoparticles. In another work reported by (8), the breakdown of PLGA during the drug release process resulted in the production of lactic and glycolic acids, lowering the pH of the environment and causing denaturation of the contained proteins (8). Synthesis and breakdown of PLGA ring breaking polymerization and hydrogenation of lactic and glycolic acids were the key reactions employed to make PLGA. The most important PLGA degradation mechanisms include oxidation, hydration, and enzymatic activity (9).

The combinatorial effect of drug-PLGA on endometrial periglandular fibrosis reported, for the first time, in mice model, where two different drugs, curcumin and aromatase inhibitor were enclosed in PLGA, known for its low risk of toxicity and sustained release properties. The dual drug NP therapy exerted its anti-estrogenic, anti-inflammatory, anti-angiogenic and antioxidant effect on endometrial periglandular fibrosis mice in a controlled manner, thereby leading to regression of the disease pre-clinical non-human primate studies for investigating the safety and efficacy of these nanoparticles at smaller doses (10).

Fadrozole is a potent, selective non-steroid aromatase inhibitor, exhibited a specific aromatase-inhibiting mechanism of action leaded to inhibit steroidogenesis as well as androgen receptor signaling (11). Treatment of endometrial fibrosis with aromatase inhibitors could be reduced endometrial thickness. So, aromatase inhibitors deserved attention for the conservative treatment of endometrial fibrosis (12). In rats, Fadrozole treatment of induced endometrial fibrosis provoked a reduction in lesion size (dose-dependent

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with Fadrozole) (13). This study aimed to assess the possible mitigating outcome of the reference Fadrozole by progressed a new manner of PLGA nanoparticles loading with Fadrozole on induced endometrial periglandular fibrosis (EPF) in female mice.

Materials and Methods:

Preparation of poly (lactic-co-glycolic acid)- Fadrozole nanoparticles:

Nanoparticles were prepared by the nanoprecipitation method figure (1) according to (14;15) with mild modification. All steps included: solvents, dissolved or emulsified polymer were done in a chemical fume hood, by following points: The an organic solution included 10 mg of poly (lactic-co-glycolic acid) (PLGA) was placed glass test tube and then transferred 1 ml of each following solvent (Dimethylsulfoxide (DMSO), Acetone and chloroform) to choose the suitable one of them solvents ,and then (0.2) ml DMSO was added to the PLGA (chosen owing to best solvent) then complete to (10) ml of de-ionized water and sonicated for (5) min to dissolve with recurrent shaking.

Then (1) ml of diluent DMSO (100 microgram) was transferred to (50) ml beaker and the diluent DMSO from (50) microgram / ml was made to concentration (1) microgram / L, after that the upper of the tube was closed with a parafilm the foil strongly, the parafilm was put tightly opposite to the top edge of the tube. The level of each solvent was tagged on the outside of the test tube and the dissolved PLGA was incubated overnight, and then vortex on high speed until all PLGA was entirely dissolved (~10 min).

Twenty (20 µl) of Fadrozole was immediately added to the polymer solution (PLGA), the tube was put in the vortex at (800) rpm for 5 min, in order to encapsulate was homogenously dispersed as emulsified polymer solution. The emulsified polymer solution was put in the small beaker (20 ml) and then directly transferred to the ultra-sonicator and immersed in the ice water and the emulsion

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was sonicated for 9 min, pulsed on time 15 seconds with pulse off time 15 seconds and (50%) amplitude.

An aqueous phase was prepared by adding (100) ml of 0.03% w/v (Vitamin E-TPGS) to a (200) ml glass beaker for overnight, after that it put on a magnetic stir bar at stirring speed (500) rpm, and then (10) ml of 0.03 % w/v (Vitamin E-TPGS) was added to glass test tube of emulsified polymer solution. Then the test tube was stirred at (500) rpm stirring speed. The emulsified polymer solution nanoparticle was put in beaker (100) ml and then wrapped in aluminum foil, the top of beaker was leaved open to facilitate solvent evaporation. The suspension gained was filtered (What man filter paper 1, diameter: 9 cm, pore size:11µm) to discard any precipitated and then centrifuged at (14,000) rpm at 4 °C. The supernatant contained the unbound drug was discarded; the pellet obtained was washed 2–3 times with D.W. The empty nanoparticles were equipped according to the similar procedure that mentioned during preparation of Fadrozole– PLGA nanoparticles without added any drug.

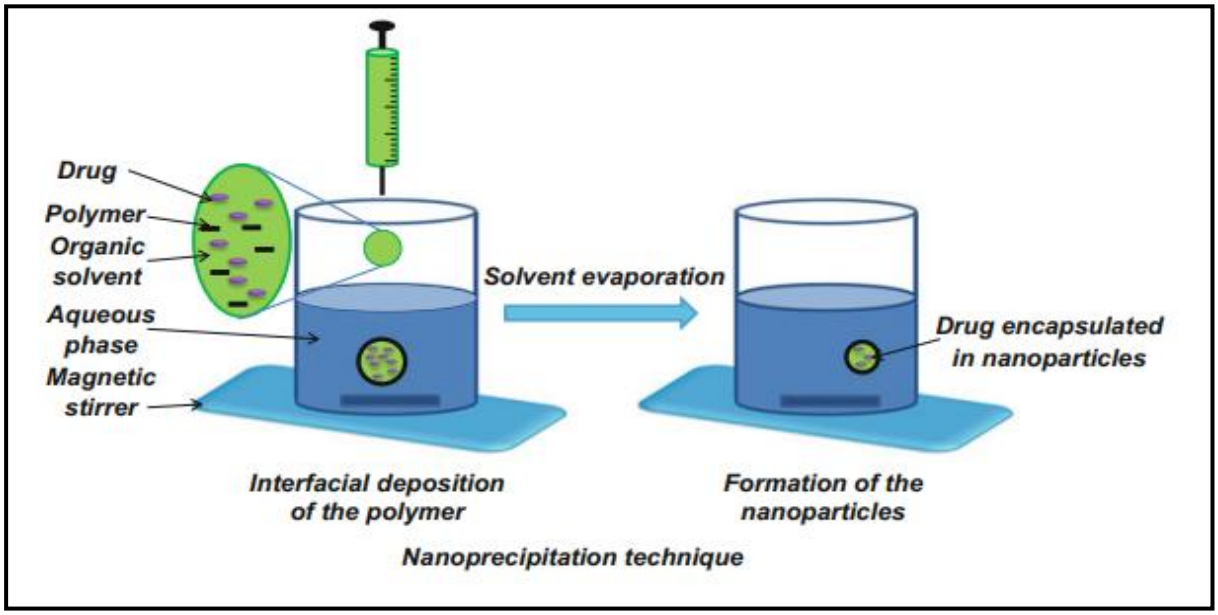

Figure (1): Nanoprecipitation method for preparing poly (lactic-co-glycolic acid) – Fadrozole polymer nanoparticles.

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Characterization of Fadrozole Loaded PLGA Nanoparticles:

This step was conducted using apparatus particle size and zeta potential measurement, UV-Visible Spectrometry, (SEM), (TEM), % nanoparticle yield, % Encapsulation Efficiency, % drug loading at different solvent and emulsifier. A UV-Vis spectrophotometer was used to measure the absorbance at 336.32 nm (16), which was used to characterize (NY), (DL), and (EE) of the produced nanoparticles. For spectroscopy analysis, freeze-dried nanoparticle specimens (1 mg) were dissolved in (0.2) ml DMSO and finished in 10 ml deionized water. The solubility at (336.32) nm was observed at various doses to create a standard calibration curve (R2= 0.5063). Fadrozole solutions were dissolved in DMSO at different concentration (2, 4, 8, 16, 30, 60, 90, and 100 mg/ml), and the absorbance at (336.32) nm was measured at various levels to produce a standard calibration curve (R2= 0.5063). The three main equations were used to determine NY, DL, and EE as the following (14, 15).

$$\text{Nanoparticle yield} = \frac{amount\ of\ nanoparticle}{weight\ of\ polymer\ and\ fed\ initially} \text{x}100 \ldots\ldots\ldots\ldots\ldots\ldots..(1)$$

Encapsulation efficiency (E.E).

$$(\text{E.E}) = \frac{Amount\ of\ drugs\ used\ to\ prepare\ nanoparticles - amount\ of\ the\ drugs\ in\ supernate}{amount\ of\ drugs\ used\ to\ prepare\ nanoparticlee} \text{x}100 \ldots.(2)$$

Drug loading (D.L).

$$(\text{D.L}) = \frac{Amount\ of\ drugs\ used\ to\ prepare\ nanoparticles - amount\ of\ drugs\ in\ supernate}{amount\ \ of\ drugs\ used\ to\ prepare\ nanoparticlee + weight\ of\ PLGA} X100 \ldots..(3)$$

UV-visible spectrophotometer:

For technique verification and application, a dual beam ultraviolet-visible (UV-visible spectrometer) with two associated quartz cells and a 1 cm optical path length loaded with UV probe application was used to monitor spectra and measure absorbance. Based on the stability qualities of the pharmaceutical formulations, a variety of diluents such as methanol, acetone, and DMSO were utilized as emulsifier for Fadrozole hydrochloride (choice of diluent based on the solubility characteristics of the drug substances, good solubility was shown in

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DMSO so that chosen as preferable diluent). DMSO had high solubility; thus, it was selected as the preferred dilution. Each 1 mg of Fadrozole was soaked in (0.2) ml DMSO before being diluted to 10 ml with deionized water. After that spectra for Fadrozole hydrochloride were measured from (100 - 1100) nm with wave length by recording UV-visible spectrum of standard solution. Maximum absorbance (λ max) was shown at (336.32) nm for standard solution Fadrozole hydrochloride with absorbance (4.5452).

Preparation of standard and sample solutions for Fadrazole hydrochloride:

Fadrozole hydrochloride 1 mg exactly was moved for the research standard into 50 ml measuring flask (a volumetric) and then was auxiliary (DMSO) 0.2 ml then complete to 10 m (l90.1 mg/ml) of de-ionized water and sonicated for 5 min to dissolve with intermittent shaking. Then 1 ml of diluent DMSO (100 microgram) was transferred to (50) ml beaker and the diluent DMSO from (50) microgram / ml was made to concentration (1) microgram / L, and the absorbance was verified at each concentration to fix the unknown concentration occasioned from loading drugs by dimension the first supernatant of nanoparticle of Fadrozole after centifugated at (14000) rpm for 20 min.

Scanning Electron Microscopy (SEM):

The SEM was used to examine of morphology of the sample and producing images of it's by scanning the surface with a focused beam of electrons (17). The SEM of PLGA loading Fadrozole was done in Al- Al-M´aarfa center for development in karada/ Baghdad province as following: A slip of dual carbon tape was placed on a SEM stub the day before picture creation to prepare samples for SEM. The metallic proportion of the stubs was labeled with a permanent marker for ready review. A metallic spatula was also used to delicately feast a little quantity of solubilized nanoparticles through the stripe's side, the ram layer being downy with tissues. The gold-palladium specimens were coughed and covered for (30-120) seconds. A regular photographic area was generated by creating a prolonged sputter time. The parameters for visualizing particles were done in variety from 5 mm to 15 mm, the ray intensity of (5-12) Kilo volt, and a spot size of (1-3). Micro-particles were exposed at (100X) magnitude and nanoparticles were distinguished at (3,000X) magnification. The samples were being contained of an identical covering of particles across the ram flat surface.

Transmission electron microscopy (TEM)

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The PLGA loading Fadrozole (nanoparticles) was evaluated by estimation of TEM in Al-M´aarfa center for development. The internal structural of Fadrozole - nanoparticles was set by TEM. A drop (10 µl) of Fadrozole -NPs suspension (1 mg/ml) was laid precisely on a (300) mesh carbon coated copper TEM grid. The superfluous, solution on the grid was extracted utilized a filter paper and the specimen were air-dried for ten hours. The dried specimen was then being tested at 120 Kilo volt under a microscope (15;16).

Zeta Sizers:

The zeta potential measurement was attained by using a Zeta-sizers to measure the particle size of dispersed systems from sub-monomers to several micrometers to diameters, stability of colloidal dispersions and the degree of electrostatic repulsion between adjacent (18). This parameter, was applied in Central Laboratory in Tehran. Temporarily, 1 ml of PLGA loading Fadrozole suspension was equipped in Milli-Q water by sonication for (30) seconds. The previously suspension (10 µl) was completed to 1 ml with Milli-Q water and the analysis was done. As well as, the mean diameter of three determinations was calculated for each sample.

Results:

In figure (2), the SEM test of Fadrozole loaded PLGA result showed the particle size about (27.13 – 49.42 nm) with a circle shape, the TEM images had a distinct spherical form and mono scatter size distribution of NPs with crystallite size varied from (19 – 200 nm) as figure (3) , zeta potential measurement was :(-26.8 mV) with mobility (-0.000208 cm2/Vs) as figure (4) , PLGA particles negatively charged, that was induced a specific zeta sizer , the sloping plane was termed the zeta potential , an important property of the particle in a dispersal as it had exerted a substantial, UV analysis displayed absorbance of Fadrozole loaded PLGA in which it was (4.5452) at wavelength λmax 336.32 nm as figure (5). The ratio of 1:5 drug: polymer was used in the current study. The percentage cumulative of it appeared at maximum wavelength 336.32 nm, nanoparticle yield (NY=90), the encapsulation efficiency (EE = 88), and the loading drug was (LD = 14) as figure (6).

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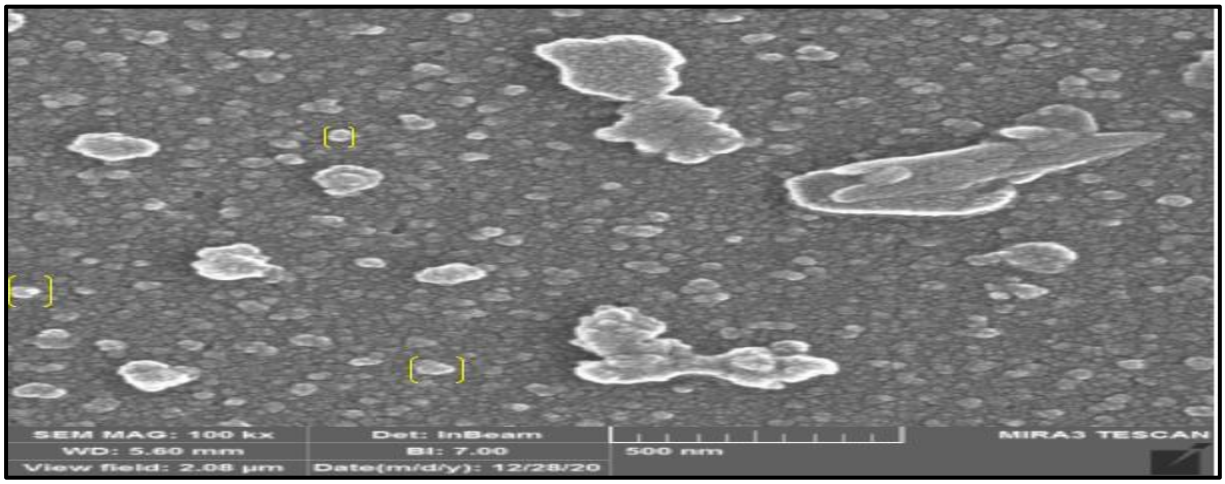

Figure (2): The scanning electron microscope (SEM) properties and particle sizes of Fadrozole loaded PLGA.

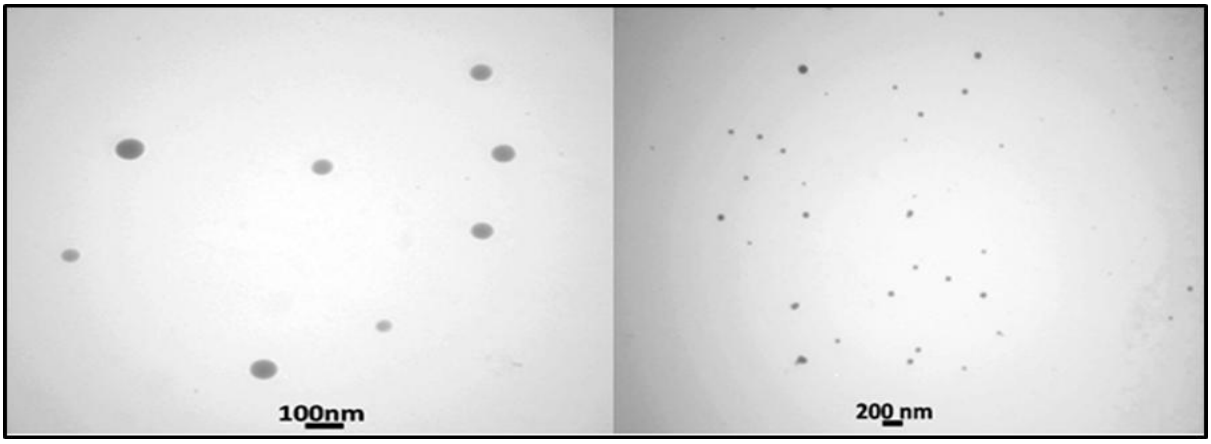

Figure (3): The Transmission electron microscope (TEM) properties of Fadrozole loaded PLGA micelles

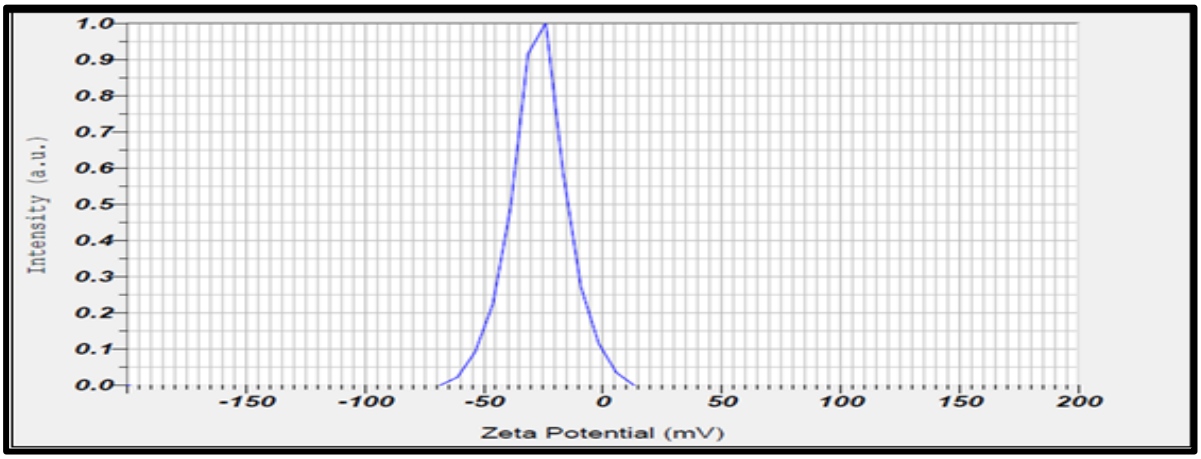

Figure (4): Zeta Potential (Mean): (-26.8 mV), and Electrophoretic Mobility Mean: (-0.000208 cm2/Vs) of PLGA-Fadrozole.

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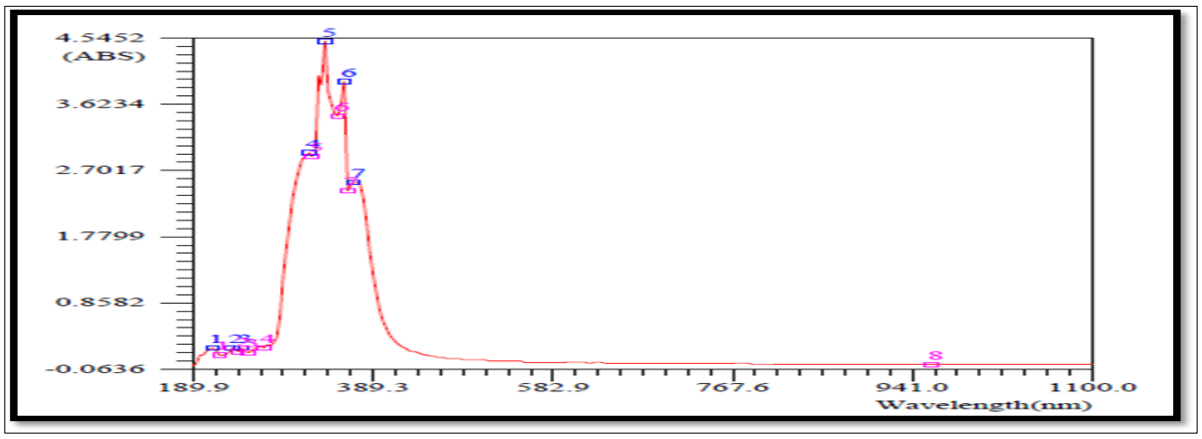

Figure (5): UV absorption spectrum of PLGA- Fadrozole dissolved in DMSO. The curve referred to the maximum wavelength of fadrozole at 336.32nm.

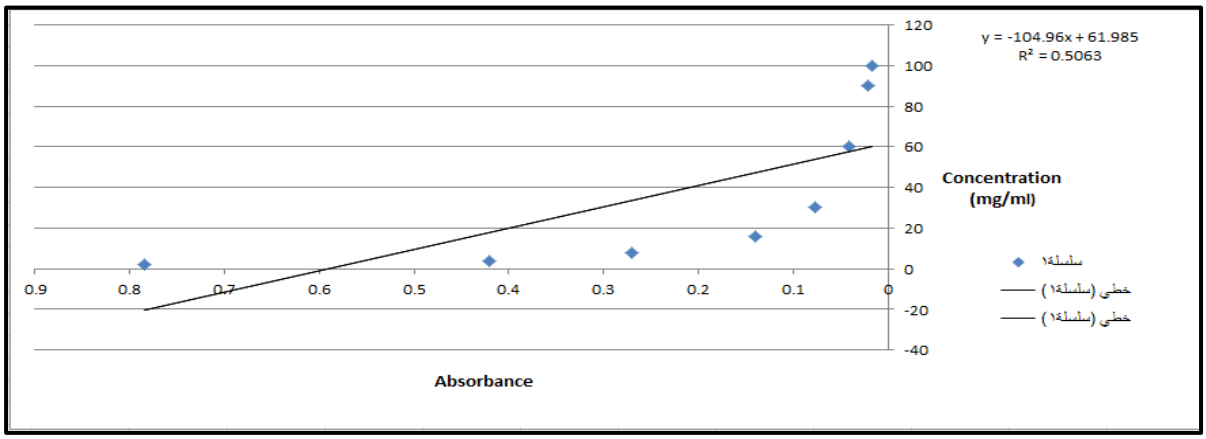

Figure (6): Calibration curve of determination reference Fadrozole against balance NY=90 , EE= 88, DL= 14.5 at maximum wave length 336.32nm.

Discussion:

These Characterization might be given the Fadrozole loaded PLGA the ability to enhance drug releasing which might be caused by an extensive polymer degradation, leading to enhance penetrability of the drug in the polymer matrix, these results were in an agreement with previous studies reported by (19). These characteristics indicated to physicochemical properties of PLGA included: degradation by hydrolysis of its ester in the presence of water (20). In addition, these results were in agreement with (21) who proved the flexibility of PLGA nanoparticles and leading to be minimized the side effect to treatment with any drug that loaded with PLGA (8). The PLGA and its characterization and correlation with mechanism of action had proved by (22), who demonstrated the

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control release of PLGA and classified it upon mechanism of action and used it as therapeutic agent due to practical solublization of drugs, easy production of nano-sized drug delivery systems (DDS), and drug release.

In other hand, these properties of PLGA had on compatibility with previous studies that could the principle characteristics of nanoparticles in various medical applications (23, 24) in which they were used of nanotechnology for medical purposes (nano-medicine), that defined as the nano-materials using for diagnosis, monitoring, control, prevention and therapy of diseases. So, the using the PLGA came from the concept of its specific possession as polymer established continued-release DDS to have therapeutic drug concentrations for extended periods of time (25).

Drug loaded PLGA by nanopreciptation methods had documented many properties that included provided that an extensive range of advantages such as: 1) smaller particle size that assisted the infiltration into the cells, 2) higher encapsulation efficiency for improved drug release, 3) lower minimum inhibitory concentration 4) minimal bacterial concentrations meaning that a better antibacterial activity was achieved with a smaller amount of drug (26).

An idea of performing this trail was unique about using of type of nanoparticle in the reproductive behavior disorder in female reproductive system and presenting of varying troubles that was caused infertility that included endometrial periglandular fibrosis in mice by taking advantage of the properties of nanoparticles and this study had been well corresponding with previous projects earlier proven capability of PLGA in treatment of endometrial periglandular fibrosis EPF where it was a common gynecological disorder affecting almost animals in their reproductive age (27).

As well as, the nanopreciptation technique of the PLGA-Fadrozole in the presence of (Vitamin E - TPGS) had an enhancing effect because of its antioxidant action, protecting cell membrane from ROS as radical scavenger delivering of hydrogen (H) atom to free radicals (28). In addition, vitamin E affected estrogen metabolism by down-regulating aromatase expression, as seen in the vitamin E treated group, affected the local synthesis of estrogenic steroids. A complete block of estrogen signaling leaded to atrophic epithelial cells with no secretory activity (29).

Based on the statement of PLGA proposed in this trial, it could be inferred that the (DMSO) associated organic phase

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alone provided the form, particle size with a thin wall, and negatively charged, in addition to the proper NY, EE and LD with appropriate stabilizer. Female mice that were clinically subjected to cause induced endometrial periglandular fibrosis by frequent 17- β – estradiol (S/C) injections and H_2O_2 delivery with drinking water for 12 weeks were also able to recover with PLGA-Fadrozole regimen therapy at different doses.

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