Original Article

Preparation of Poly (Lactic-co-glycolic Acid)-Loaded Pentoxyfilline by Nanoparticipation Technique

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Abstract

Background: Nanomaterial concerned to an incidental, natural, or synthesized material containing particles utilized for curing several diseases and keeping for human with animals health; it is either in an unbound condition or as a combination in which one or more external measurements in the size range of 1–100 nm. **Objectives:** The study intended to assess the possible mitigating outcome of the reference pentoxifylline by progressed a new manner of poly (lactic-co-glycolic acid) (PLGA) nanoparticles loading with pentoxifylline on induced thin layer endometrial in female rats. **Materials and Methods:** PLGA nanoparticle was made by employing two procedures included nanoprecipitation technique and double emulsion solvent evaporation method. The experiment firstly was achieved numerous of diagnostic tests to the knowledge of PLGA nanoparticle characteristics that involved ultraviolet (UV) spectrophotometer test. **Results:** UV spectrophotometer test shown for Pentoxyfilline absorbance (0.300124), at wave length (2979) and PLGA- loaded Pentoxyfilline absorbance (3.07877), at wave length 1565), while other tests (scanning electron microscope, cumulative %; encapsulation drug efficiency loading, and zeta) of PLGA-nanoparticle appeared (28.35 nm-35.45 nm, (8.48, 93.3 at maximum wavelength 275 nm, (−13.44 mV), with mobility (−1.05 [μ/s]/[V/cm]) respectively. **Conclusion:** Regarding the characterization of PLGA displayed in this study, it can be concluded that DMSO related organic phase alone gives the shape, particle size with a small diameter, negatively charged in addition to suitable LD, EE with suitable stabilizer. Also, PLGA-Pentoxyfilline regimen treatment with different doses had the ability in recovery of female rats that exposed experimentally induced thin layer endometrium in the uterine horn by ethanol.

Keywords: Nanoparticipation, poly (lactic-co-glycolic acid)-pentoxifylline, scanning electron microscope, ultraviolet spectrophotometer

Introduction

Poly (lactic-co-glycolic acid) (PLGA) is a copolymer manufactured by means of ring-opening copolymerization of two different monomers, the cyclic dimers (1,4-dioxane-2,5-diones) of glycolic acid and lactic acid, and polymers are synthesized as either random or block copolymers herewith cooperating additional polymer properties.[1] The definition for nanotechnology, nanomaterial, nanoscale, or other linked terms are ingredients that have at least one dimension in the size extent about approximately 1 nanometer (nm) to 100 nm. Focused on the current scientific and technical compassion of nanomaterials and their characteristics, the Food and Drug Administration counsels that estimations of effectiveness, safety, public health influence, or regulatory status of nanotechnology products should consider any unique properties and manners that the application of nanotechnology may communicate[2-7] certified that the PLGA nanoparticles supplied. The particle size is more beneficial in the field of medical purposes in which the polydispersity depends on its typical characterization, so the smaller particle size and high polydispersion leading to good interesting in the medical field such as lesser minimum inhibitory concentration, higher entrapment efficiency for augmented drug release, minimum bacterial concentrations meaning that a better antibacterial activity is accomplished with a smaller amount of drug, vaccine delivery systems, and they were progressed as gene carriers. Nanotechnology can be

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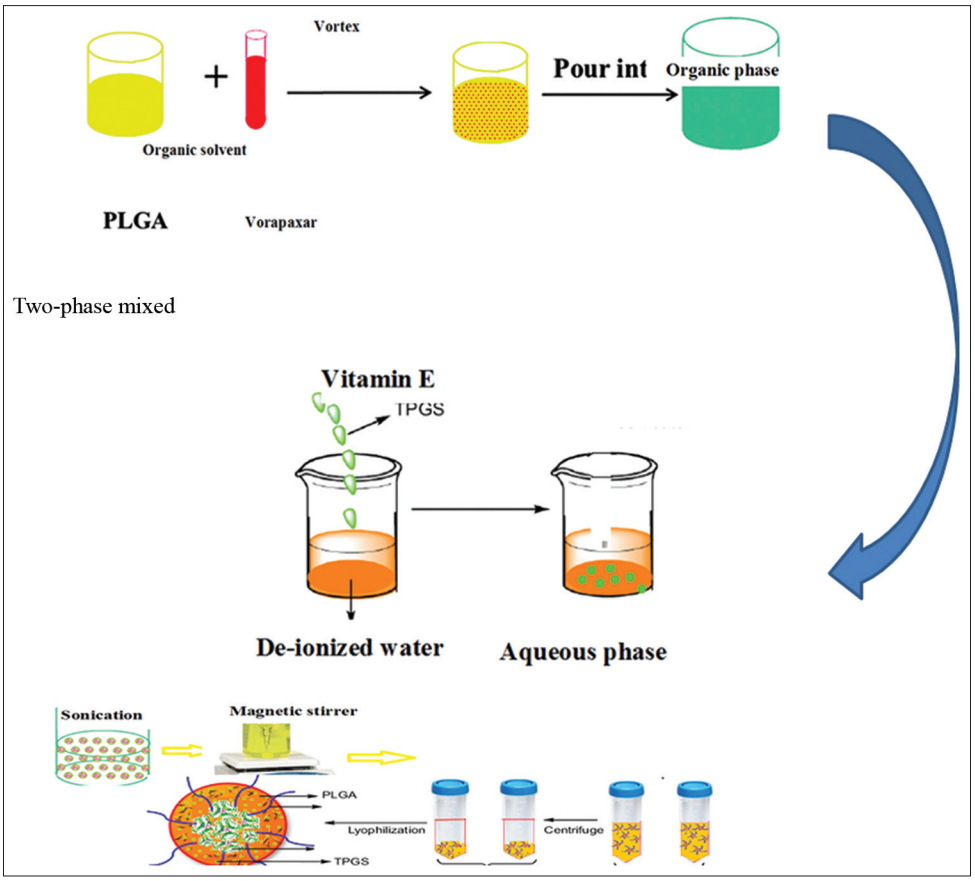

Figure 1: Nanoprecipitation method for preparing poly (lactic-co-glycolic acid) –pentoxifylline polymer nanoparticles

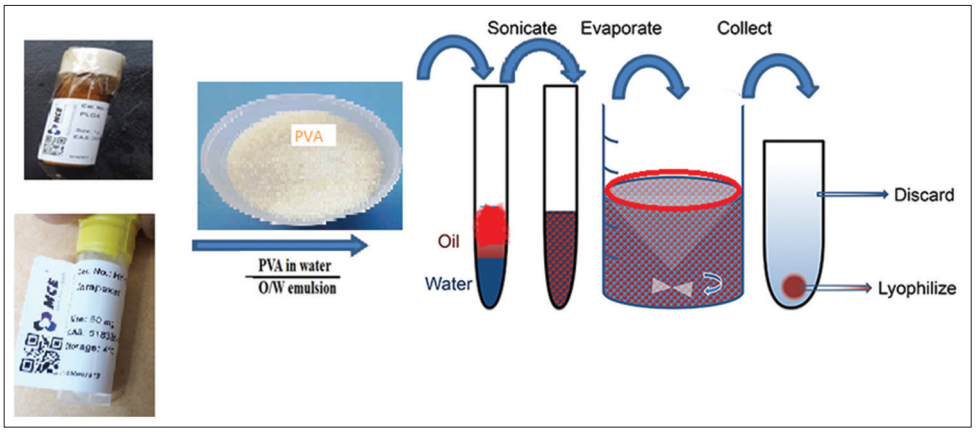

Figure 2: PVA O/ W emulsion for preparing poly (lactic-co-glycolic acid) - Pentoxifylline polymer nanoparticles

planned to progress the pharmacokinetics and biodistribution of the drug.[8,9]

Materials and Methods

Preparation of poly (lactic-co-glycolic acid)-pentoxifylline nanoparticles

Nanoparticles were prepared by the nanoprecipitation method[10] [Figures 1 and 2] according[11-13] with mild modification. All steps including stabilization, organic solvents, sonication, centrifugation, magnetic stirrer, etc.. can be listed by the following points:

1. The organic solution included 10 mg of PLGA was placed in a glass test tube and then transferred 1 ml of each following solvent (ethyl acetate, dichloromethane, dimethyl sulfoxide [DMSO], acetone, and chloroform) to choose the suitable one of the solvents and then each one was added to the PLGA, after that the top of the tube was closed with a small piece of aluminum foil, and parafilm. The parafilm was put tightly opposite to the top edge of the tube
2. The level of each solvent was tagged on the outside of the test tube and the dissolving PLGA was incubated

overnight, and then, the vortex on high speed until all PLGA is entity dissolved (~10 min)

3. Twenty (20 µl) of pentoxifylline 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 it and make it homogenously dispersed as emulsified polymer solution
4. The emulsified polymer solution was put in the small beaker 20 ml and then directly transferred to the ultrasonicator and immersed in the ice water and the emulsion was sonicated for 9 min, pulse on time 15 s with pulse off time 15 s, and 50% amplitude
5. An aqueous phase was prepared by adding 100 ml of 0.03% w/v Vitamin E-tocopheryl polyethylene glycol succinate (TPGS) to a 200 ml glass beaker for overnight, after that it was 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 a glass test tube of the emulsified polymer solution. Then, the test tube was stirred at 500 rpm stirring speed
6. The emulsified polymer solution nanoparticle was put in a beaker 100 ml and then wrapped in aluminum foil; the top of the beaker was left open to facilitate solvent evaporation
7. The suspension obtained was filtered (Whatman filter paper 1, diameter: 9 cm, pore size: 11 µm) to discard any precipitated and then centrifuged at 14,000 rpm at 4°C. The supernatant containing the unbound drug was discarded; the pellet obtained was washed 2–3 times with distilled water
8. The empty nanoparticles were prepared according to the same procedure that was mentioned during the preparation of pentoxifylline – PLCA nanoparticles without added any drug.

Instrumentation

A double beam ultraviolet (UV)-visible spectrophotometer (Shimadzu, model 1800) having two matched quartz cells with 1 cm light path length and loaded with UV probe software was used for the recording of spectra and measuring absorbance for method development and validation study.

Selection of diluents such as methanol, acetone, and DMSO was used as diluent for pentoxifylline sulfate drug substances based on the solubility characteristics of the drug substances; excellent solubility was recorded in DMSO so that chosen as preferable diluent. Each 1 mg of pentoxifylline was dissolved in 0.2 ml of DMSO and completed to 10 ml with deionized water. After that spectra for pentoxifylline sulfate were measured from 100 to 3000 nm for wavelength by recording the UV-visible spectrum of the standard solution. Maximum absorbance (λ max) was shown at 2979 nm for standard solution pentoxifylline sulfate with absorbance 0.300124.

Preparation of standard solutions for pentoxifylline sulfate

Pentoxifylline sulfate (1 mg) exactly was moved for working standard into a 50 ml volumetric flask and then was auxiliary (DMSO) 0.2 ml then complete to 10 ml of de-ionized water and sonicated for 5 min to dissolve with intermittent shaking. Then, 1 ml of diluent DMSO (100 µg) was transferred to 50 ml beaker and the diluent DMSO from 50 µg/ml was made to concentration 1 µg/L, as well as the absorbance was verified at each concentration to fix the unknown concentration occasioned from loading drugs by dimension the first supernate of nanoparticle of pentoxifylline after centefugated at 14,000 rpm for 20 min.

Characterization of pentoxifylline loaded poly (lactic-co-glycolic acid) nanoparticles

This step was conducted using apparatus zeta potential measurement, UV-visible spectrometry, and scanning electron microscope (SEM). The cumulative drug release and drug loading (DL) at different solvent and emulsifier were studied. Characterization of nanoparticles yield (DL) and encapsulation efficiency (EE) were quantified by measuring the absorbance of 275 nm.[14] DL and EE of the prepared nanoparticles were quantified by measuring the absorbance of 275 nm[14] using a SHIMADZU UV-1700 UV-Vis spectrophotometer (EE = 93.3%, DL = 8.48%), respectively. The freeze-dried nanoparticle samples (1 mg) were dissolved in 0.2 ml of DMSO and completed to 10 ml of deionized water, for spectrophotometric measurement.

Pentoxifylline solutions were dissolved within DMSO at various concentrations (1–100 mg/ml), and the absorbance at 275 nm was measured at different concentrations to generate a standard calibration curve (R^2 = 0.8533). Nanoparticle yield, DL, and EE were calculated from the following three equations.[13,15]

$$\text{Nanoparticle yield} = \frac{\text{amount of nanoparticle}}{\text{weight of polymer and fed initially}} \times 100$$

Encapsulation efficiency (E. E).

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

Drug loading (D. L).

$$(\text{D.L}) = \frac{\text{Amount of drugs used to prepare nanoparticles -amount of drugs in supernate}}{\text{amount of drugs used to prepare nanoparticlee +weight of PLGA}} \times 100$$

Preparation of standard and sample solutions for pentoxifylline sulfate

Pentoxifylline sulfate (1 mg) exactly was moved for working standard into a 50 ml volumetric flask and then was auxiliary (DMSO) 0.2 ml then complete to 10 ml of de-ionized water and sonicated for 5 min to dissolve with intermittent shaking. Then, 1 ml of diluent DMSO (100 µg) was transferred to 50 ml beaker and the diluent DMSO from 50 µg/ml was made to concentration 1 µg/L, as well as the absorbance was verified

at each concentration to fix the unknown concentration occasioned from loading drugs by dimension the first supernate of nanoparticle of pentoxifylline after centefugated at 14,000 rpm for 20 min.

Scanning electron microscope

The SEM of PLGA loading pentoxifylline was done in the Qualitative Research Department of the Ministry of Science and Technology as follows:

1. Samples for SEM were equipped on the day of making images; a slip of double-sided carbon tape was put on an SEM stub. A perpetual marker was done to label the metal percentage of the stub for later reference
2. A metal spatula was used together a small amount of lyophilized nanoparticles and softly feast them through the surface of the stripe, the ram surface was downy with tissue
3. The specimens with gold-palladium were coughed and covered for 30–120 s. A longer splutter time was created a regular surface for imaging
4. The parameters for visualizing particles were done in variety from 5 mm to15 mm, the ray intensity of 5–12 KV, and a spot size of 1–3. Microparticles are exposed at ×100 magnification and nanoparticles were renowned at ×3000 magnification
5. The samples were contained a uniform covering of particles across the ram flat surface.

Zeta sizers

The zeta potential measurement was attained using a Malvern Zetasizer Nano ZS (Malvern Instruments, Worcestershire, United Kingdom); this parameter was done in the Ministry of Science and Technology in the province of Baghdad. Temporarily, I ml of PLGA loading pentoxifylline suspension was equipped in MilliQ water by sonication for 30 s. The previously suspension (10 µl) was completed to 1 ml with MilliQ water and the analysis was done. As well as, the mean diameter of three determinations was calculated for each sample.

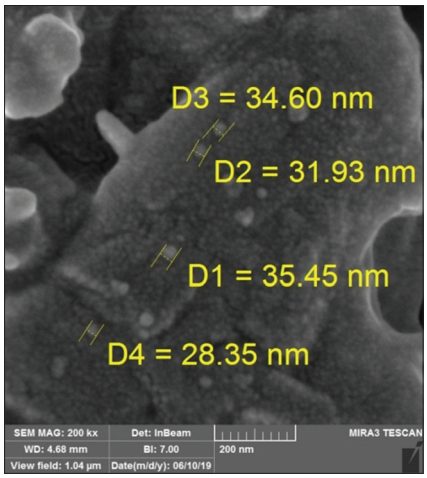

Figure 3: The scanning electron microscope properties and particle sizes of pentoxifylline loaded poly (lactic-co-glycolic acid)

Ethical consideration

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee.

Results

In Figure 3, the SEM test of pentoxifylline loaded PLGA result showed the particle size about (28.35-35-45 nm) with a circle shape, zeta potential measurement was (−13.44 mV) with mobility [(−1.05 (µ/s)/(V/cm)] [Figure 4], herein negatively charged PLGA particles, that was induced a specific zeta potential.[16]

The potential at the slipping plane is called the zeta potential. Zeta potential is an important property of the particle in a dispersion as it has exerted a significant, UV analysis displayed absorbance of pentoxifylline and pentoxifylline loaded PLGA in which it was (3.07877 at wavelength λmax 1565 nm and 0.300124 at wavelength λmax 2979 nm) [Figure 5a and b]. The ratio of 1:10 drug: polymer was used in the current study. The percentage cumulative of it appeared at maximum wavelength 275 nm, the encapsulation (EE = 93.3), and the loading was (LD = 8.48) [Figure 5].

Discussion

In this study, the characteristics of pentoxifylline loaded PLGA gave it the ability to increase drug-releasing which may be caused by (an extensive) polymer degradation, resulting in increased permeability of the drug in the polymer matrix. These results were compatible with the previous study reported by Stevanovi *et al*.[16] As well as, these properties indicated to physicochemical properties of PLGA included degradation by hydrolysis of its ester in the presence of water.[17] In addition, these results are in agreement with[18] who proved the flexibility of PLGA leading to use of it in medical devices such as prosthetic devices, surgical sealant films, grafts, sutures, implants, micro- and nanoparticles, and also leading to minimizing the side effect to treatment with any drug that loaded with PLGA.[19] Furthermore, The PLGA and its characterization and correlation with the mechanism of action had proved by,[20] and[21] that demonstrated the control release of PLGA and classified it according to the mechanism of action and employed it as a therapeutic agent due to practical solubilization of drugs, easy fabrication of nano-sized drug delivery systems, and intelligent manipulation of drug release. On the other hand, the properties of PLGA were compatible with previous studies that showed the principle characteristics of nanoparticles in numerous of applications in medicine.[22,23] They also used the term nanomedicine for describing the nanotechnology for medical purposes and defined this science as the use of nanomaterials for diagnosis, monitoring, control, prevention, and treatment of diseases. So, using the PLGA came from the concept of its specific possession as polymer established continued-release delivery systems to keep

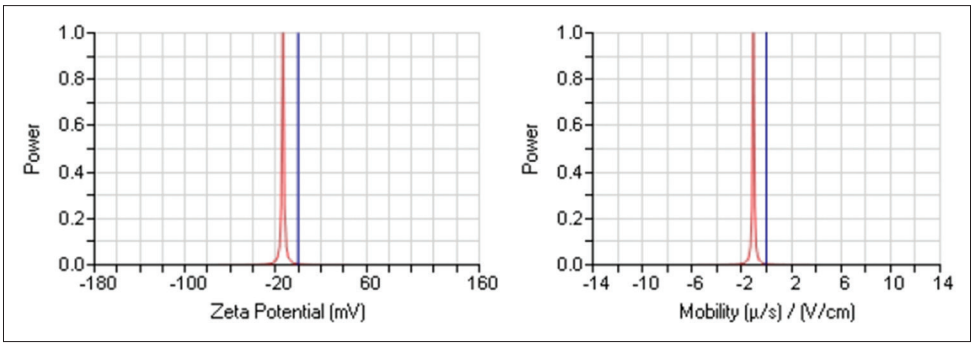

Figure 4: The zeta properties (–13.44 mV) and mobility ((–1.05 [µ/s]/[V/cm]) of poly (lactic-co-glycolic acid)-pentoxifylline

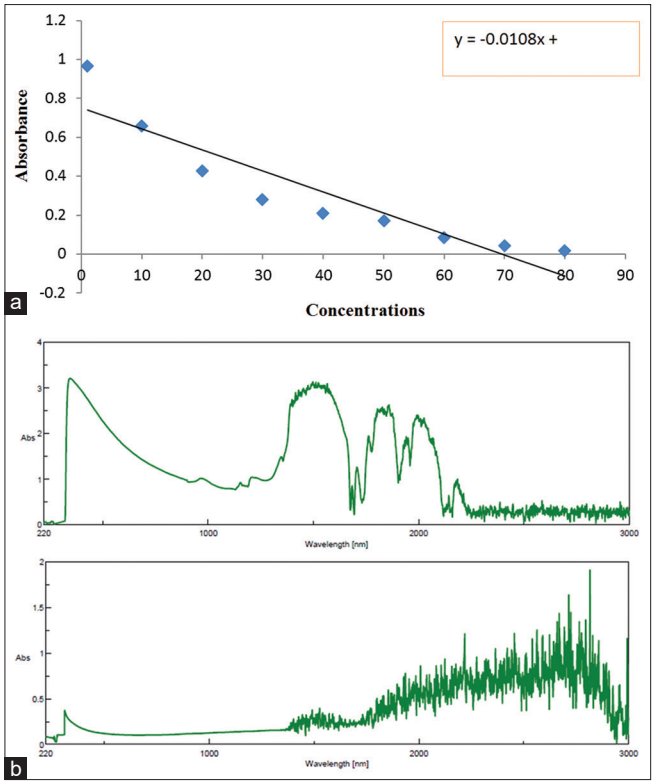

Figure 5: Calibration curve of determination reference pentoxifylline against balance, encapsulation efficiency = 93.3, loading = 8.48 at maximum wavelength of 275 nm. (a) The ultraviolet absorption spectrum of reference pentoxifylline dissolved in dimethyl sulfoxide. The curve referred to the maximum wavelength of pentoxifylline at 1565. Nm. (b) Ultraviolet absorption spectrum of reference of free pentoxifylline. The curve referred to the maximum wavelength of pentoxifylline at 2979. nm

therapeutic drug concentrations for longer periods of time is accepted for decades.[24] These results were noticed as same as with the study of Kashi *et al*.,[5] who demonstrated the safety in using of nanoparticles in treatment of groups that induced with alcohol (ethanol) for 10 days and then treated with it (empty PLGA, pentoxifylline loaded PLGA) for 20 days and compare them with other groups documented the many of properties that included providing a wide range of advantages such as smaller particle size which facilitates the penetration into the cells, higher entrapment efficiency for increased drug release, lower minimum inhibitory concentration, and minimum bacterial concentrations meaning that a better antibacterial activity is achieved with a smaller amount of drug. The idea of performing this trail was unique about using of type of nanoparticle in the surgery and reproductive behavior disorder in the female reproductive system and presenting of varying troubles that were caused infertility that involved thin layer in the endometrium of uterine horn in rats by taking advantage of the properties of nanoparticles and this study had been well matched with previous projects earlier proven capability of PLGA in treatment of endometriosis where it was and still a common gynecological disorder affecting almost 10% of the women in their reproductive age.[25]

Conclusion

Regarding the characterization of PLGA displayed in this study, it can be concluded that DMSO related organic phase alone gives the shape, particle size with a small diameter, negatively charged in addition to suitable LD, EE with suitable stabilizer. Also, PLGA-Pentoxyfilline regimen treatment with different doses had the ability in recovery of female rats that exposed experimentally induced thin layer endometrium in the uterine horn by ethanol.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Huang Y, Ren J, Ren T, Gu S, Tan Q, Zhang L, *et al*. Bone marrow stromal cells cultured on poly(lactide-co-glycolide)/nano-hydroxyapatite composites with chemical immobilization of Arg-Gly-Asp peptide and preliminary bone regeneration of mandibular defect thereof. J Biomed Mater Res A 2010;95:993-1003.
2. FDA (2014). Guidance for Industry Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology. Food and Drug Administration. Available from: https://www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM401695.pdf. [Last accessed on 14 Jan 2020].
3. Kwon IK, Lee SC, Han B, Park K. Analysis on the current status of targeted drug delivery to tumors. J Control Release 2012;164:108-14.
4. Cruz LJ, Tacken PJ, Zeelenberg IS, Srinivas M, Bonetto F, Weigelin B, *et al*. Tracking targeted bimodal nanovaccines: Immune responses and routing in cells, tissue, and whole organism. Mol Pharm 2014;11:4299-313.

5. Kashi TS, Eskandarion S, Esfandyari-Manesh M, Marashi SM, Samadi N, Fatemi SM, *et al*. Improved drug loading and antibacterial activity of minocycline-loaded PLGA nanoparticles prepared by solid/oil/water ion pairing method. Int J Nanomedicine 2012;7:221-34.
6. Hamdy S, Haddadi A, Shayeganpour A, Samuel J, Lavasanifar A. Activation of antigen-specific T cell-responses by mannan-decorated PLGA nanoparticles. Pharm Res 2011;28:2288-301.
7. Kumar MN, Mohapatra SS, Kong X, Jena PK, Bakowsky U, Lehr CM. Cationic poly(lactide-co-glycolide) nanoparticles as efficient *in vivo* gene transfection agents. J Nanosci Nanotechnol 2004;4:990-4.
8. Walsh MD, Hanna SK, Sen J, Rawal S, Cabral CB, Yurkovetskiy AV, *et al*. Pharmacokinetics and antitumor efficacy of XMT-1001, a novel, polymeric topoisomerase I inhibitor, in mice bearing HT-29 human colon carcinoma xenografts. Clin Cancer Res 2012;18:2591-602.
9. Allen TM, Cullis PR. Drug delivery systems: Entering the mainstream. Science 2004;303:1818-22.
10. Al-Rubaee SH, AL-Azawee TS, Taha AA. Duodenal histomorphological changes in broilers administered poly d, l-lactic-coglycolic acid (PLGA) nanoparticles encapsulated with peptide. Iraqi J Vet Med 2020;44:80-8.
11. McCall RL, Sirianni RW. PLGA nanoparticles formed by single- or double-emulsion with vitamin E-TPGS. J Vis Exp 2013;82:51015.
12. Gaonkar RH, Ganguly S, Dewanjee S, Sinha S, Gupta A, Ganguly S, *et al*. Garcinol loaded vitamin E TPGS emulsified PLGA nanoparticles: Preparation, physicochemical characterization, *in vitro* and *in vivo* studies. Sci Rep 2017;7:530.
13. Jasim MA, Hasan HF, Awady JM. Preparation of vorapaxar loaded with vitamin ETPGS and PVA emulsified PLGA nanoparticles *in vitro* studies. Research J. Pharm. and Tech 2019;12:4503-10.
14. Rao PL, Doredla N, Subhani SH, Narsu KK. UV spectrophotometer Estimation of Pentoxyfilline in Bulk Drug and Its Pharmaceutical Formulation. Int J Pharm Tech Res 2013;5:790-9.
15. Madani F, Goodarzi A, Hashemi M, Mujokoro B, Khosravani M, Adabi M. Preparation of Methotrexate loaded PLGA nanoparticles coated with PVA and Poloxamer188. Nanomed Res J 2018;3:19-24.
16. Stevanovi M, Radulovi A, Jordovi B, Uskokovi D. Poly(DL-lactide-co-glycolide) Nanospheres for the Sustained Release of Folic Acid. J Biomed Nanotechnol 2008;4:1-10.
17. Lin G, Cosimbescu L, Karin NJ, Tarasevich BJ. Injectable and thermosensitive PLGA-g-PEG hydrogels containing hydroxyapatite: Preparation, characterization and *in vitro* release behavior. Biomed Mater 2012;7:024107.
18. Pavot V, Berthet M, Rességuier J, Legaz S, Handké N, Gilbert SC, *et al*. Poly(lactic acid) and poly(lactic-co-glycolic acid) particles as versatile carrier platforms for vaccine delivery. Nanomedicine (Lond) 2014;9:2703-18.
19. Marin E, Briceño MI, Caballero-George C. Critical evaluation of biodegradable polymers used in nanodrugs. Int J Nanomedicine 2013;8:3071-91.
20. Langer R, Peppas NA. Advances in biomaterials, drug delivery, and bionanotechnology. AIChE J 2003;49:2990-3006.
21. Neuse EW. Synthetic polymers as drug-delivery vehicles in medicine. Met Based Drugs 2008;2008:469531.
22. Bleeker EA, de Jong WH, Geertsma RE, Groenewold M, Heugens EH, Koers-Jacquemijns M, *et al*. Considerations on the EU definition of a nanomaterial: Science to support policy making. Regul Toxicol Pharmacol 2013;65:119-25.
23. Tinkle S, McNeil SE, Mühlebach S, Bawa R, Borchard G, Barenholz YC, *et al*. Nanomedicines: Addressing the scientific and regulatory gap. Ann N Y Acad Sci 2014;1313:35-56.
24. Wang Y, Yang X, Liu W, Zhang F, Cai Q, Deng X. Controlled release behaviour of protein-loaded microparticles prepared via coaxial or emulsion electrospray. J Microencapsul 2013;30:490-7.
25. Jana SK, Banerjee P, Mukherjee R, Chakravarty B, Chaudhury K. HOXA-11 mediated dysregulation of matrix remodeling during implantation window in women with endometriosis. J Assist Reprod Genet 2013;30:1505-12.