



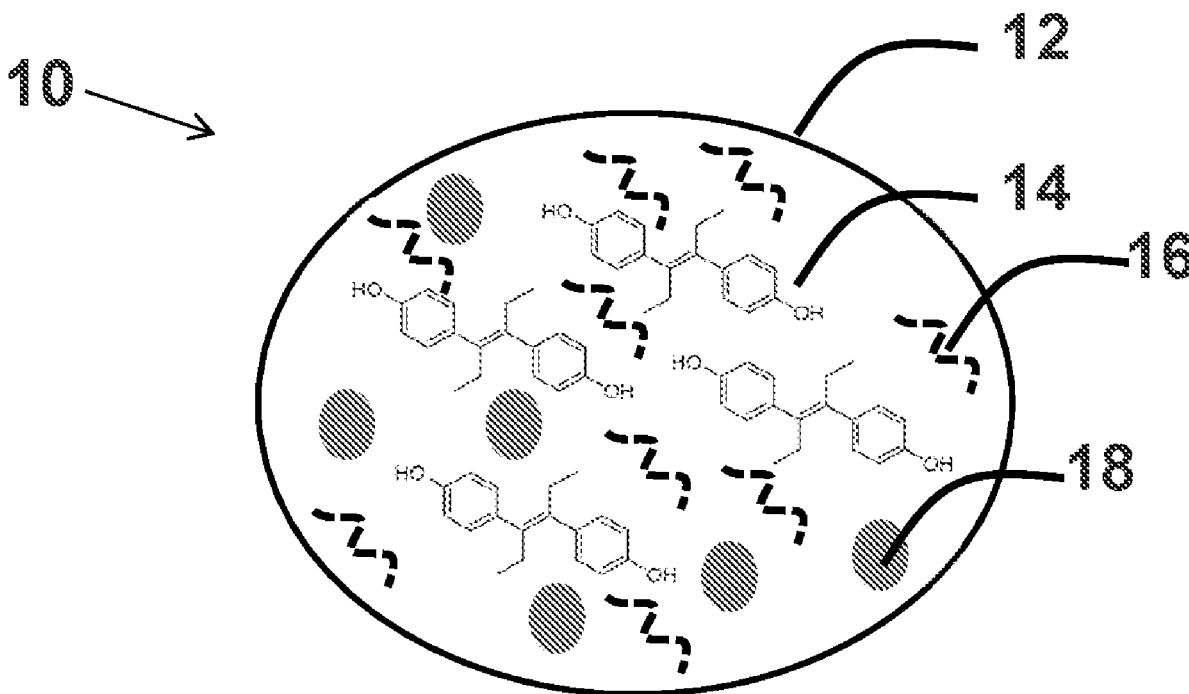
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(19) **United States**(12) **Patent Application Publication**
Chow et al.(10) **Pub. No.: US 2020/0246278 A1**(43) **Pub. Date: Aug. 6, 2020**(54) **INJECTABLE DIETHYLSTILBESTROL
NANOSUSPENSION FORMULATION****Publication Classification**(71) Applicants: **University of Houston System,**
Houston, TX (US); **Board Of Regents
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Austin, TX (US)(51) **Int. Cl.**
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A61K 9/00 (2006.01)
A61P 35/00 (2006.01)
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Houston, TX (US); **Board Of Regents
Of The University Of Texas System,**
Austin, TX (US)(21) Appl. No.: **16/636,478**(22) PCT Filed: **Aug. 6, 2018**(86) PCT No.: **PCT/US2018/045362**

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(2) Date: **Feb. 4, 2020****Related U.S. Application Data**(60) Provisional application No. 62/541,500, filed on Aug.
4, 2017.(57) **ABSTRACT**

Embodiments of the present disclosure pertain to formulations for cancer treatment that include a particle loaded with diethylstilbestrol (DES) and a pharmaceutically acceptable carrier. The pharmaceutically acceptable carrier may also be loaded into the particle. The formulations may also include a stabilizer that is also loaded into the particle. The particles may be dispersed in an aqueous solution to form a nanosuspension that is adapted for subcutaneous administration and sustained release of DES. Additional embodiments pertain to methods of treating cancer in a subject by administering to the subject a formulation of the present disclosure. The subject may be a human suffering from prostate cancer. The administration of the formulations to the subject may bypass or minimize first pass metabolism. The administration may also minimize hepatic exposure of DES. Additional embodiments pertain to methods of making the formulations of the present disclosure by loading a particle with DES.



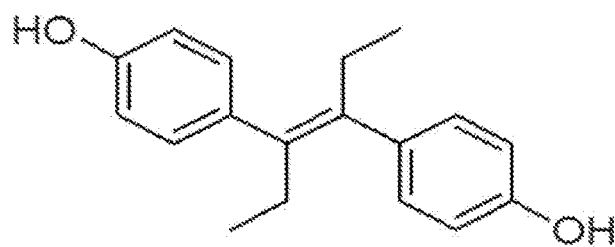


FIG. 1A

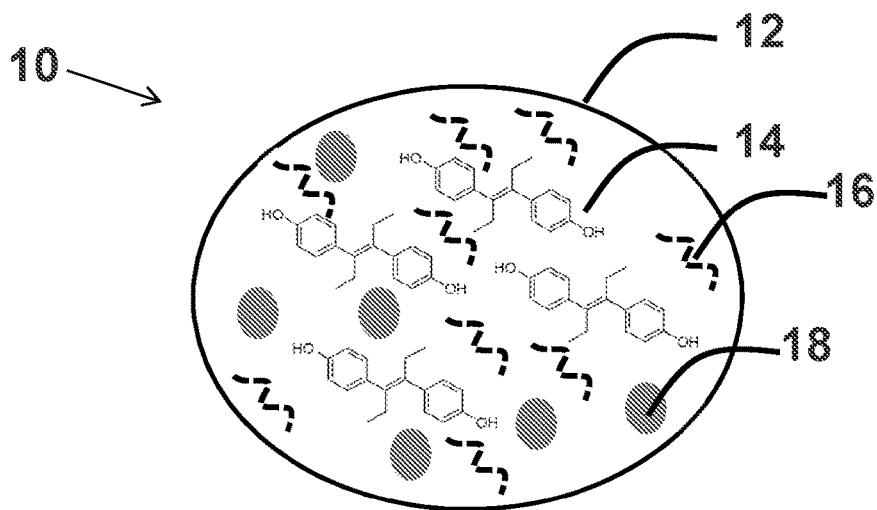


FIG. 1B

FIG. 1

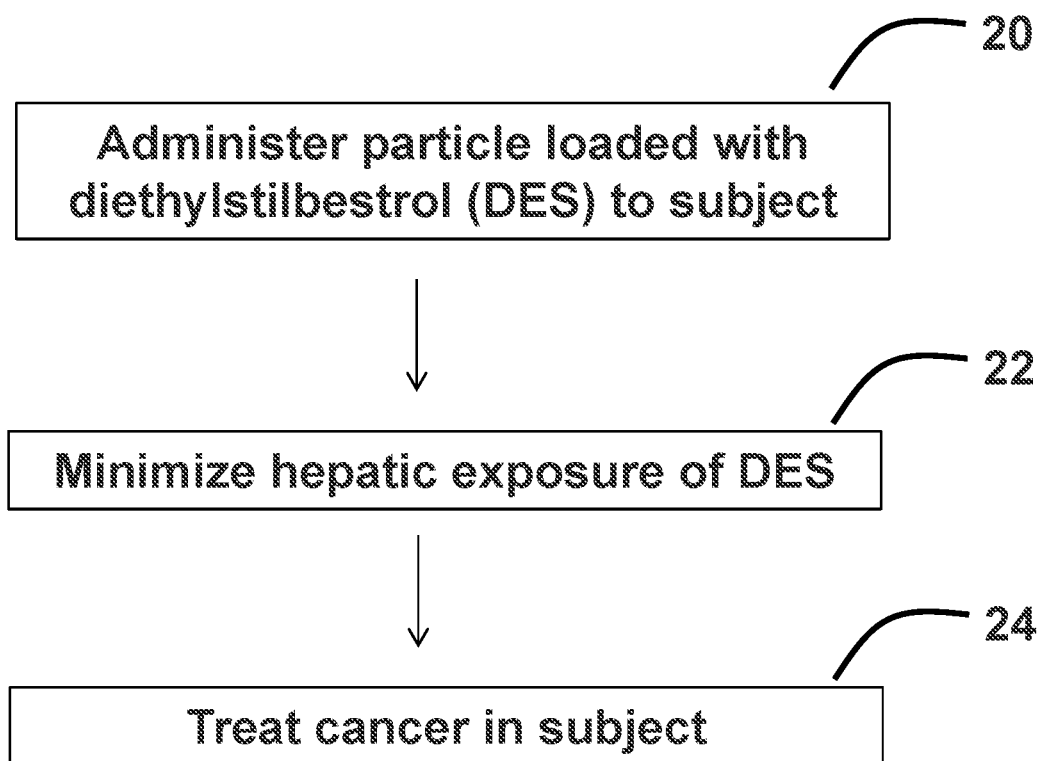


FIG. 1C

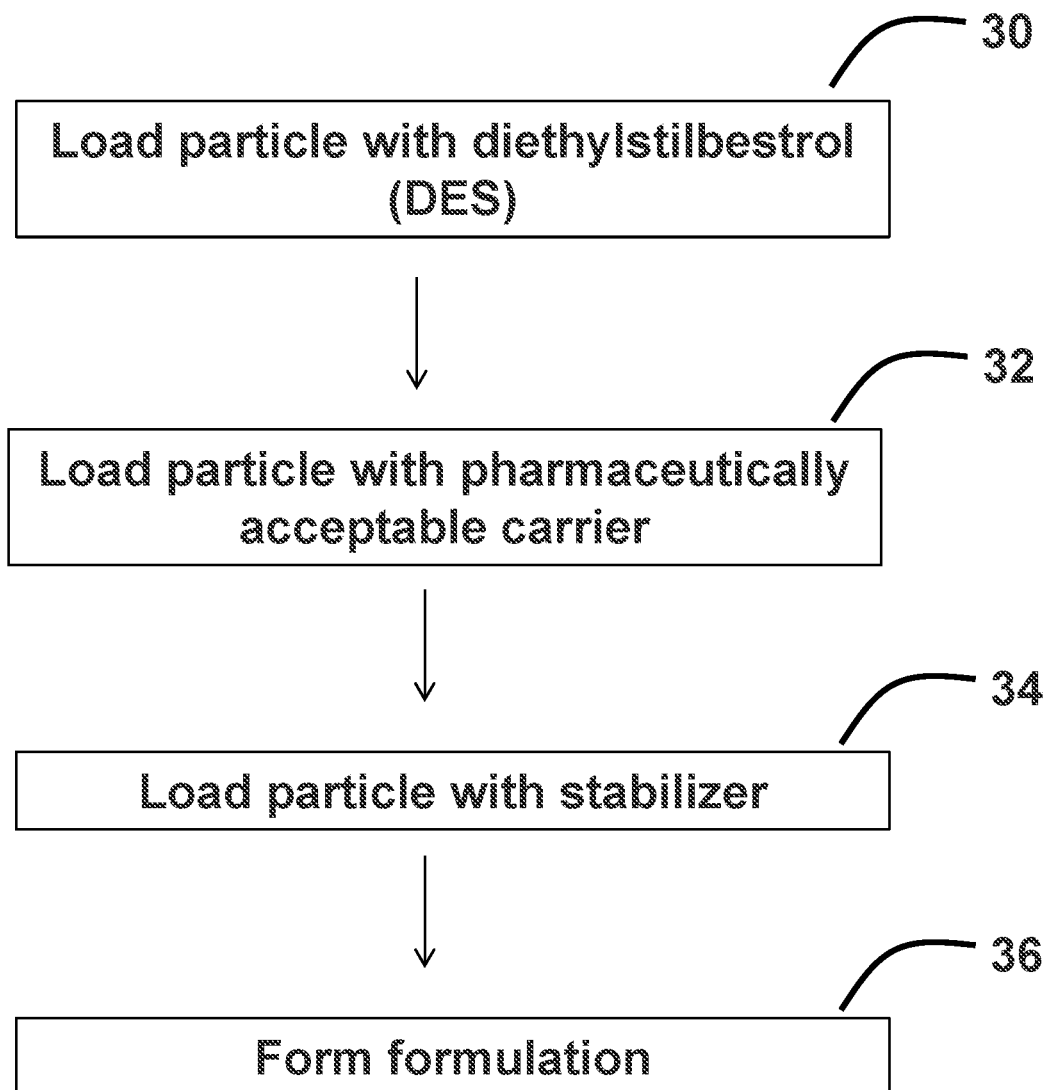


FIG. 1D

Bar magnet 
 Glass bead 
 Drug particle 

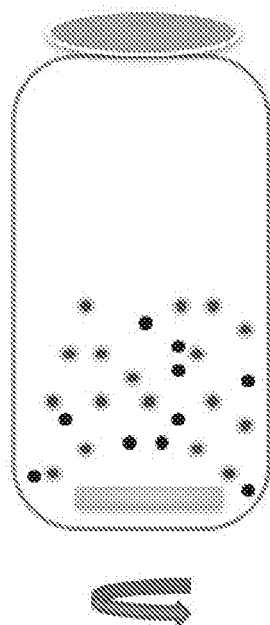


FIG. 2

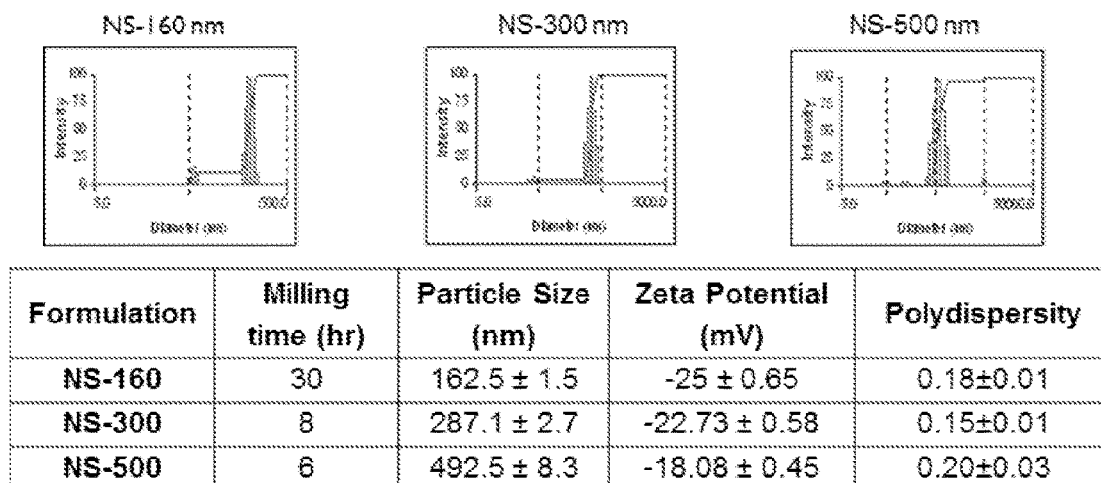


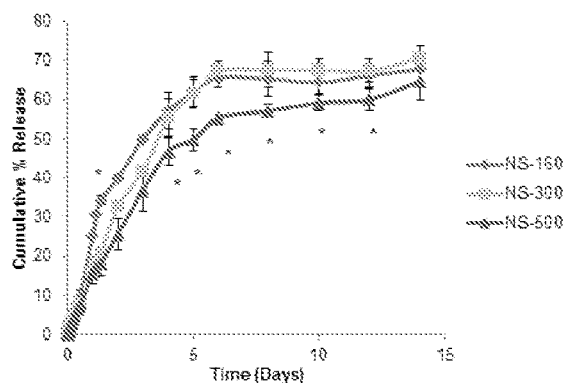
FIG. 3A

Mass balance:

160 nm = 98.9%

300 nm = 99.1%

500 nm = 97.8%

♦ *In vitro* release study (n=3):Figure: *In vitro* release of the nanosuspension formulations. All values are Mean ± SD

Formulation	Initial % Release (1 day)	Extent of release (6 days)	Extent of release (14 days)
NS-160	25.06±0.15*	65.76±3.68	67.77±4.89
NS-300	15.68±2.69	67.38±6.03	70.53±4.80
NS-500	15.51±2.52	55.42±1.57*	64.79±7.78

Initial Release Rate, Extent of Release after 6 days and Extent of Release after 14 Days. n=3 (* denotes a significant difference between the groups with $p < 0.05$). All values are Mean ± SD.

FIG. 3B

FIG. 3

Particle Size	Equations				R ²			
	Zero Order, Q_0/Q_0 vs. t	First Order, $\ln (Q_0 - Q)$ vs. t	Higuchi, Q vs $t^{1/2}$	Weibull, $\ln \ln [1/(1-Q)]$ vs $\ln t$	Zero Order	First Order	Higuchi	Weibull
NS-160	$y = -0.0023x + 0.8655$	$y = -0.0039x - 0.1584$	$y = 0.0484x - 0.0112$	$y = 1.0315x - 5.0963$	0.723	0.7961	0.9013	0.9514
NS-300	$y = -0.0025x + 0.901$	$y = -0.0168x + 0.0491$	$y = 0.0486x - 0.0472$	$y = 0.9514x - 4.8664$	0.804	0.9866	0.9379	0.9866
NS-500	$y = -0.0021x + 0.9147$	$y = -0.0034x - 0.0842$	$y = 0.0421x - 0.0397$	$y = 0.9425x - 4.9991$	0.8427	0.9016	0.9628	0.9817

Q_0 = Drug to be released at zero time (mg)

Q = Drug release at time t (mg)

t = Time in hours

FIG. 3C

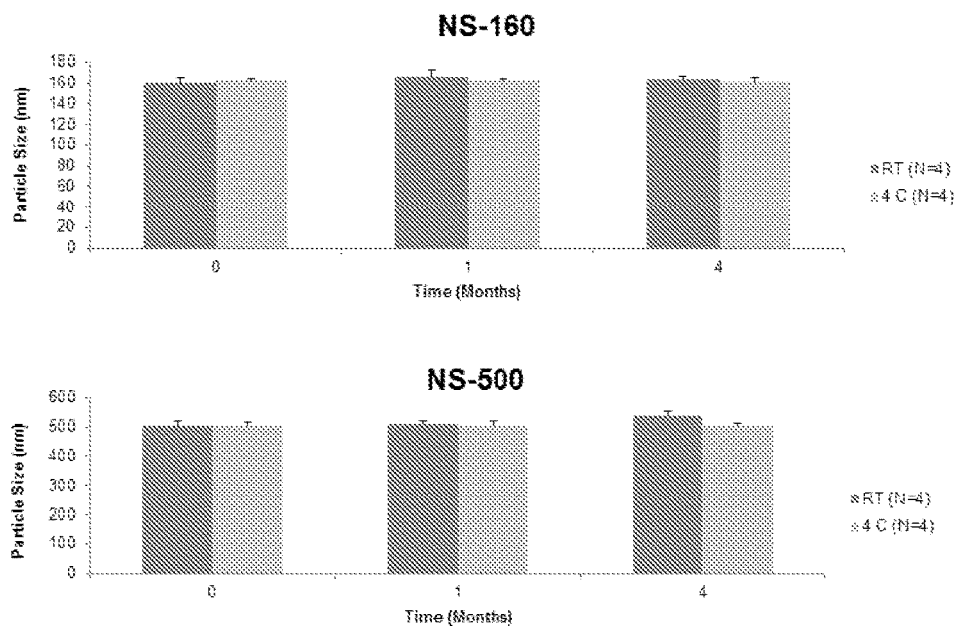


Figure: Physical Stability of 160 nm and 500 nm. * indicates significant increase in particle size ($p < 0.05$). RT is Room Temperature.

FIG. 3D

FIG. 3

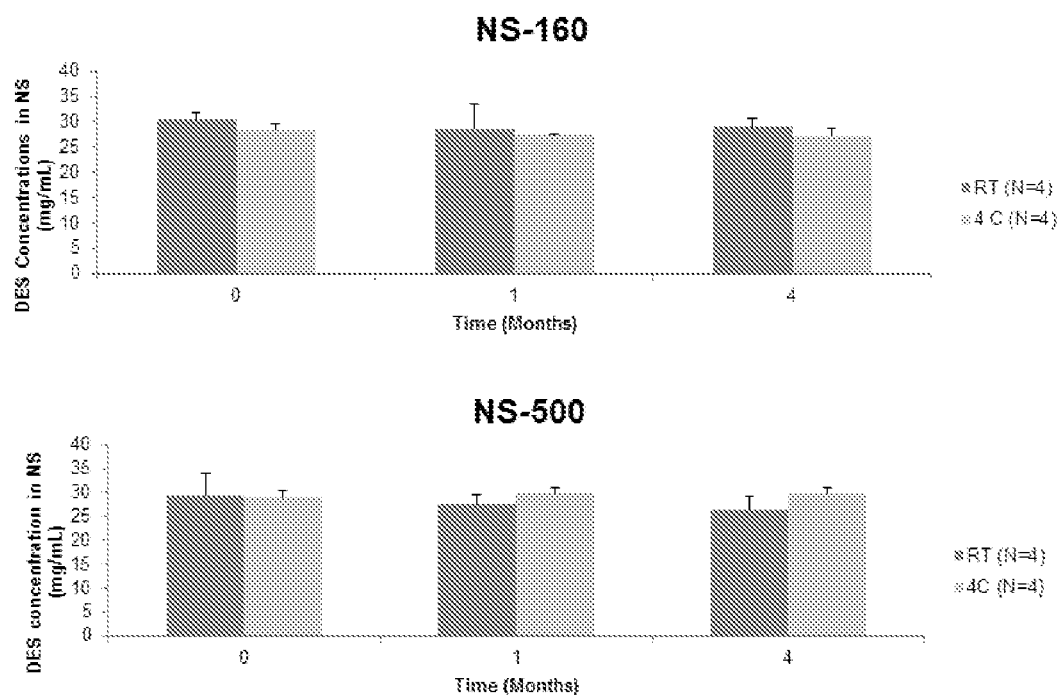


Figure: Chemical Stability of 160 nm and 500 nm. RT is Room Temperature.

FIG. 3E

FIG. 3

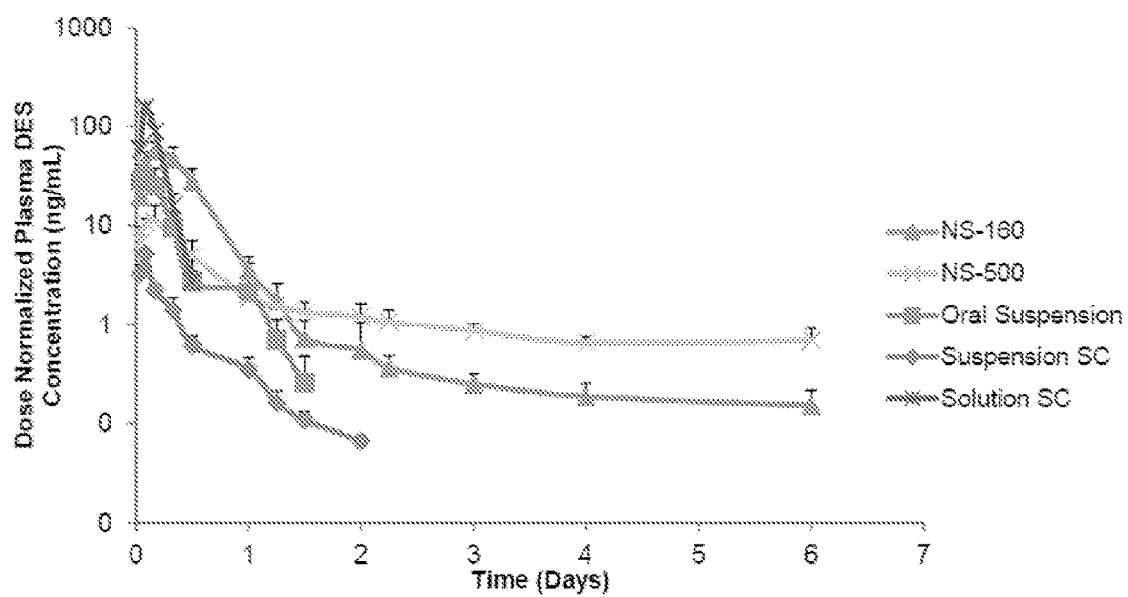


FIG. 4

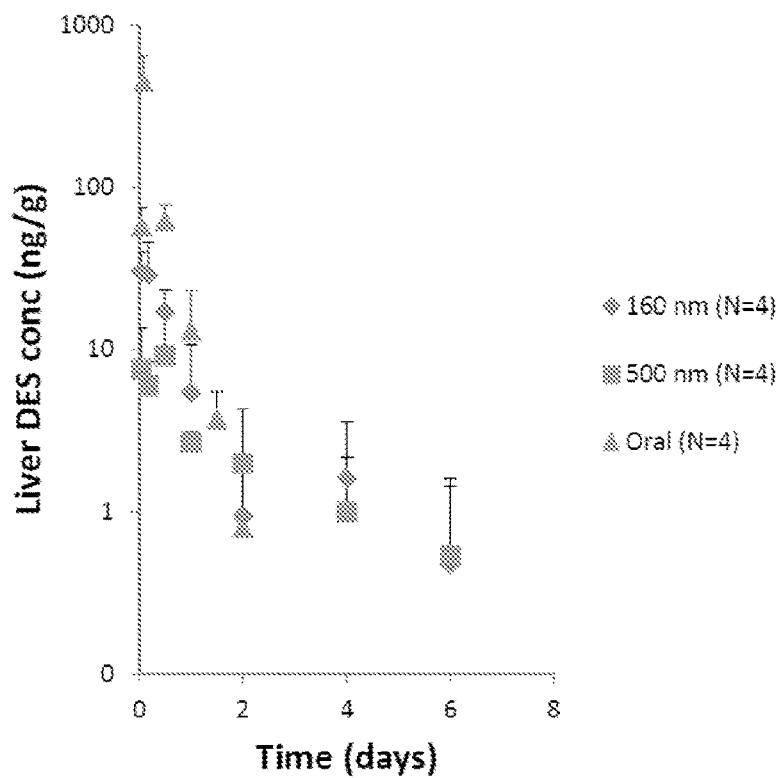


FIG. 5A

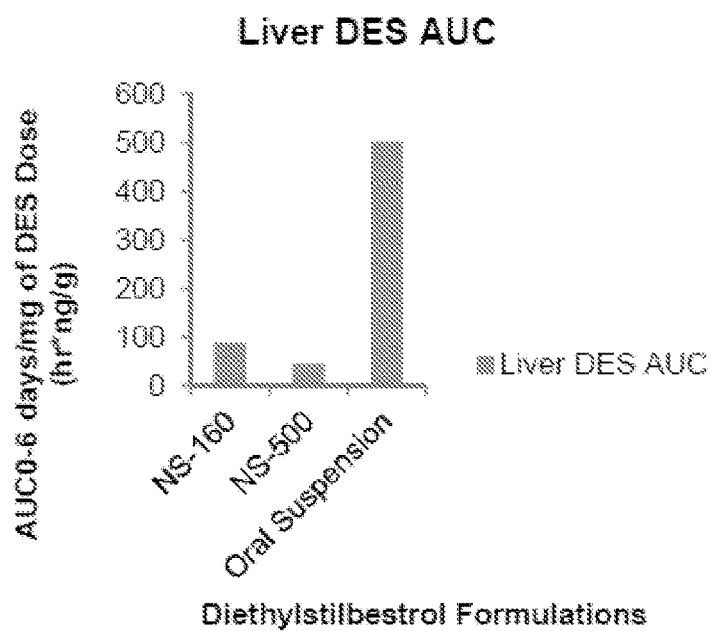


FIG. 5B

FIG. 5

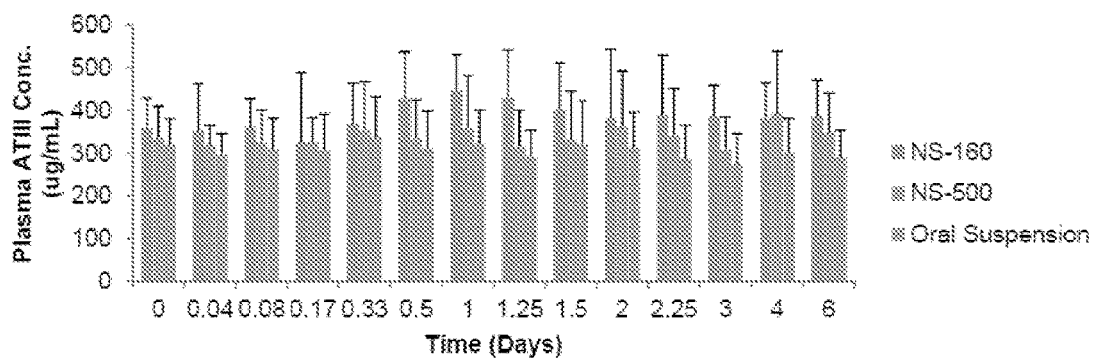


FIG. 6A

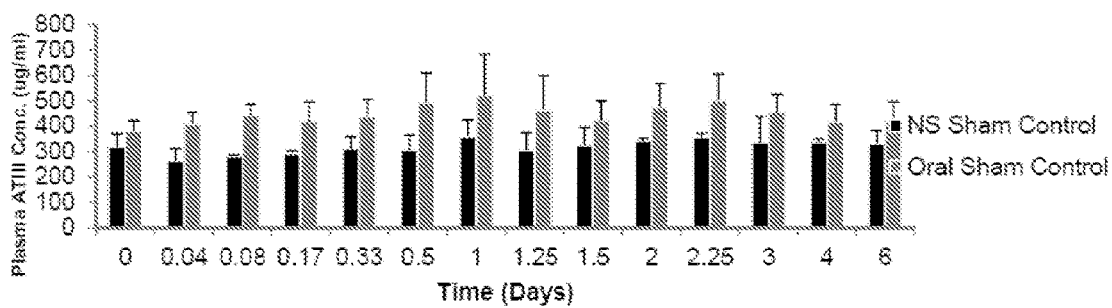


FIG. 6B

FIG. 6

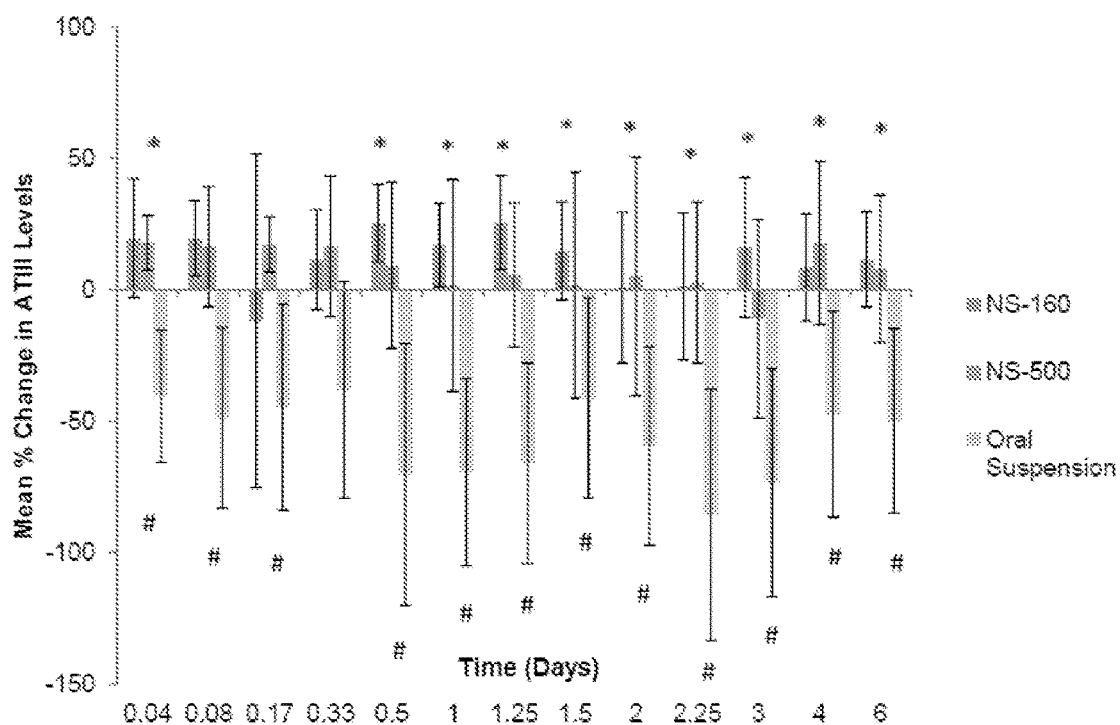


FIG. 6C

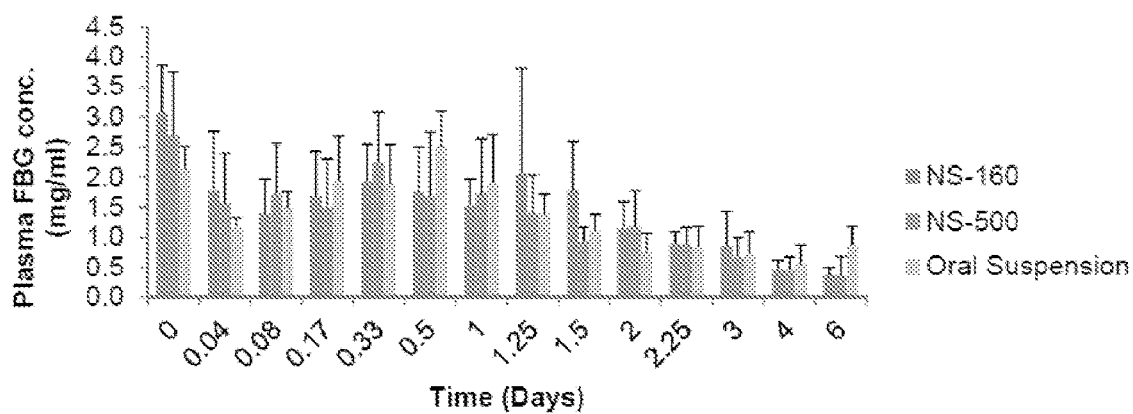


FIG. 6D

FIG. 6

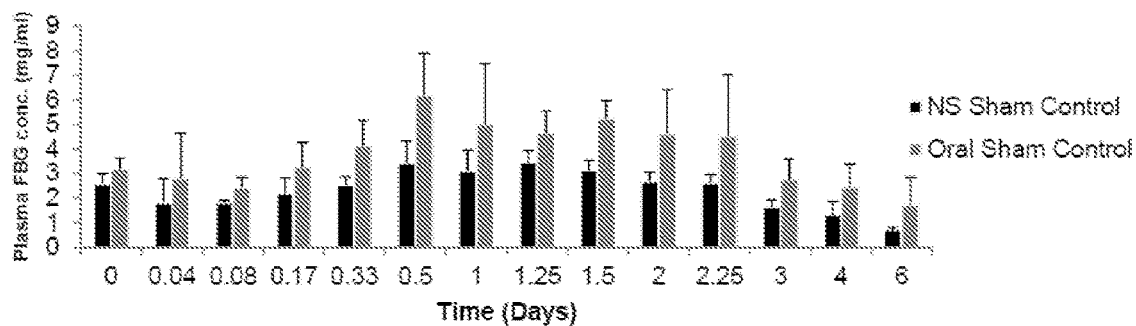


FIG. 6E

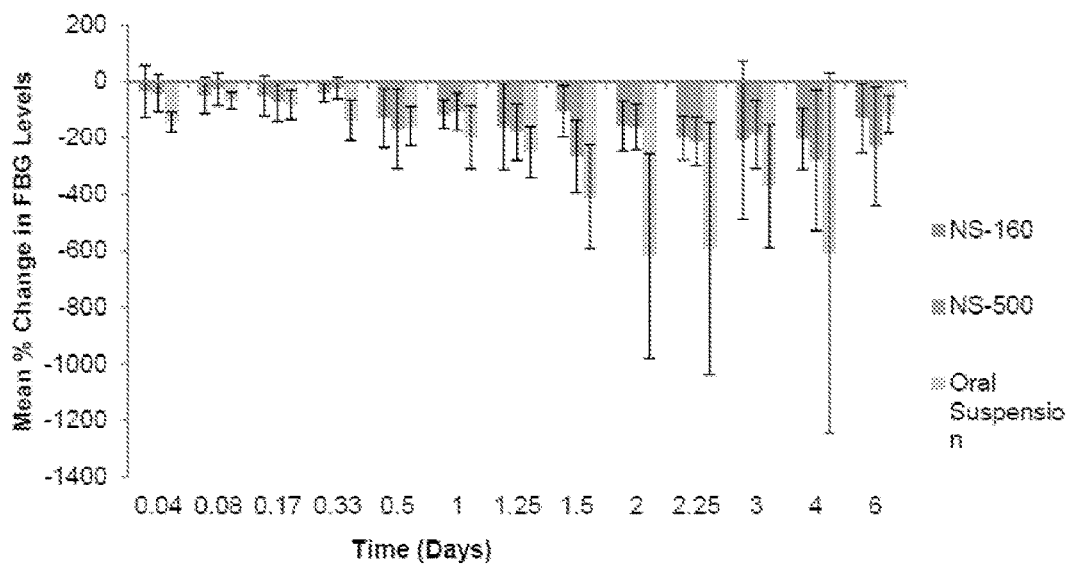


FIG. 6F

FIG. 6

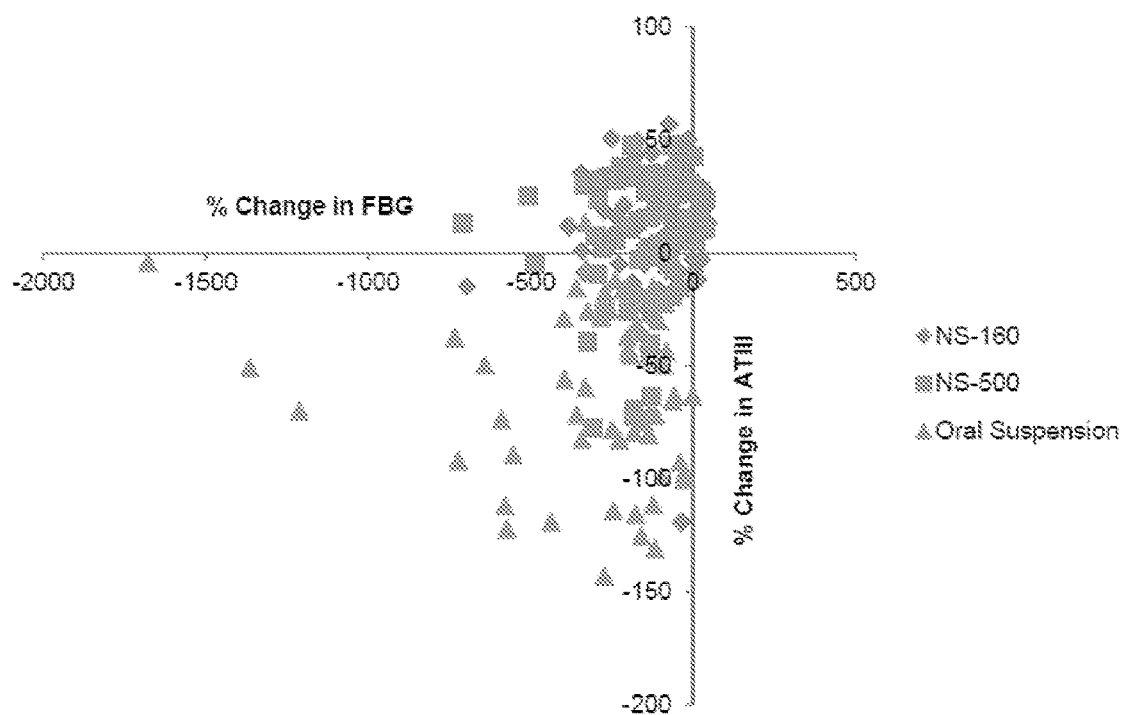


FIG. 6G

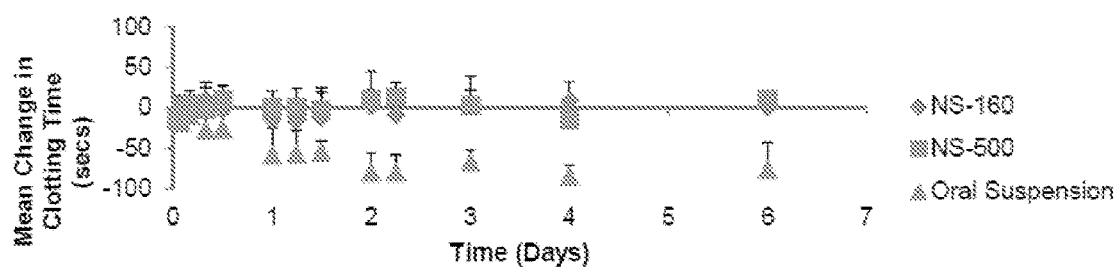


FIG. 6H

FIG. 6

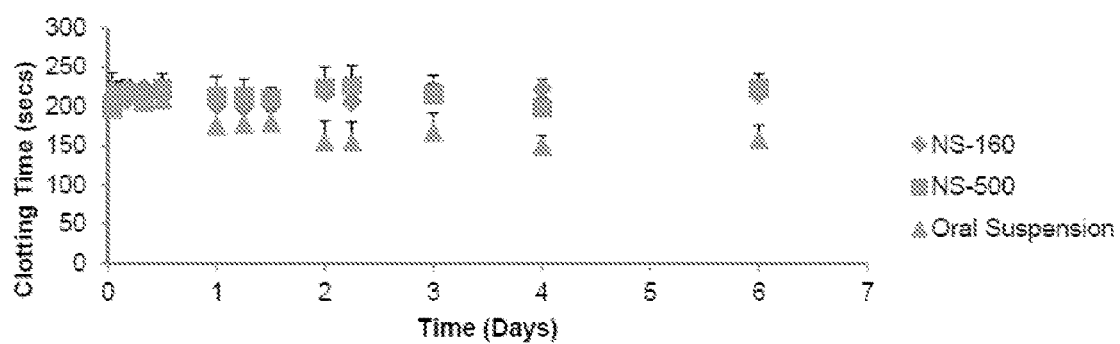


FIG. 6I

FIG. 6

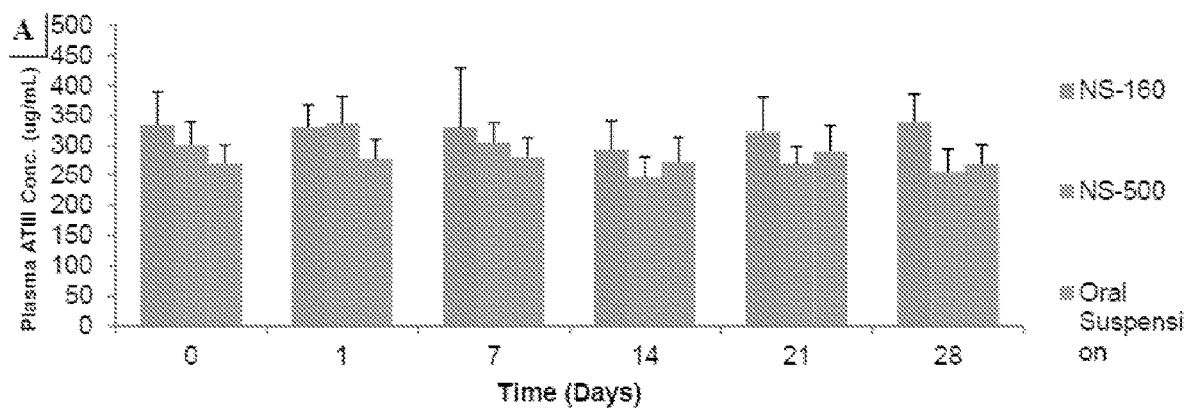


FIG. 7A

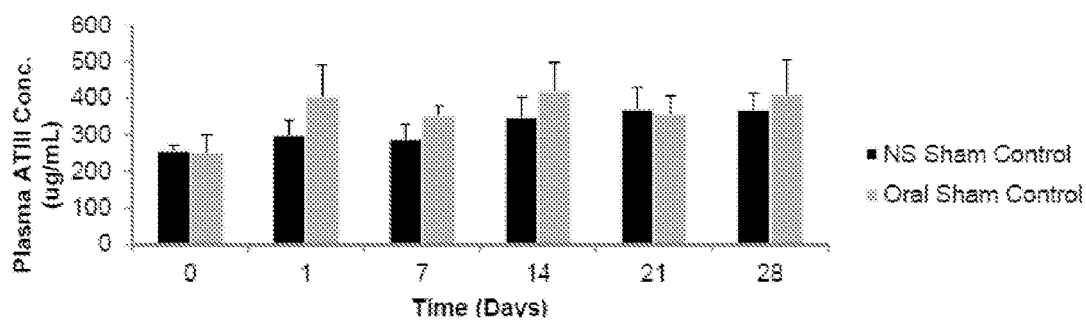


FIG. 7B

FIG. 7

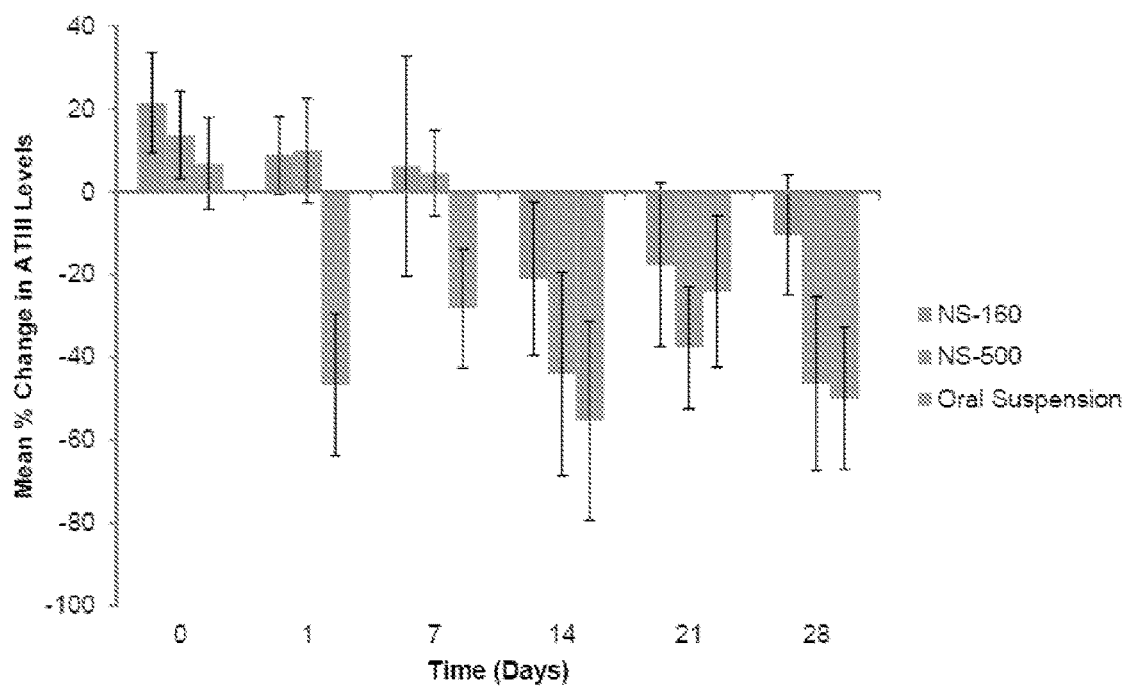


FIG. 7C

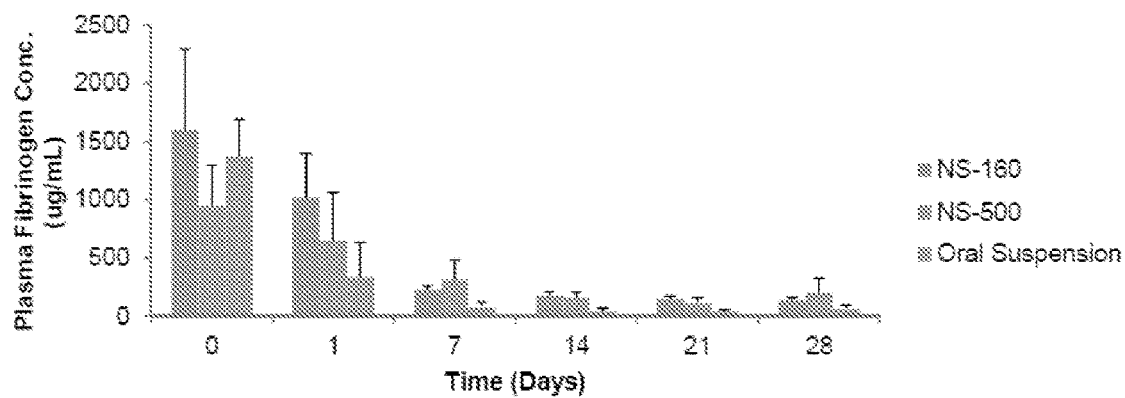


FIG. 7D

FIG. 7

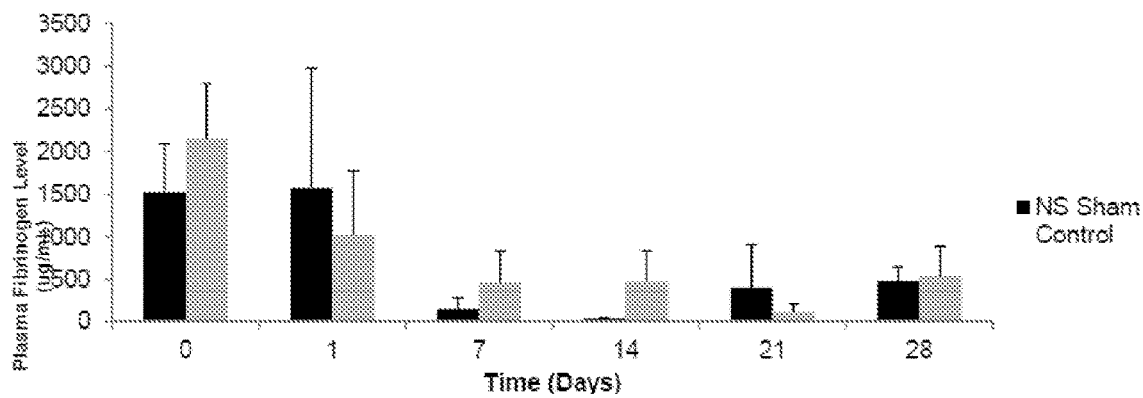


FIG. 7E

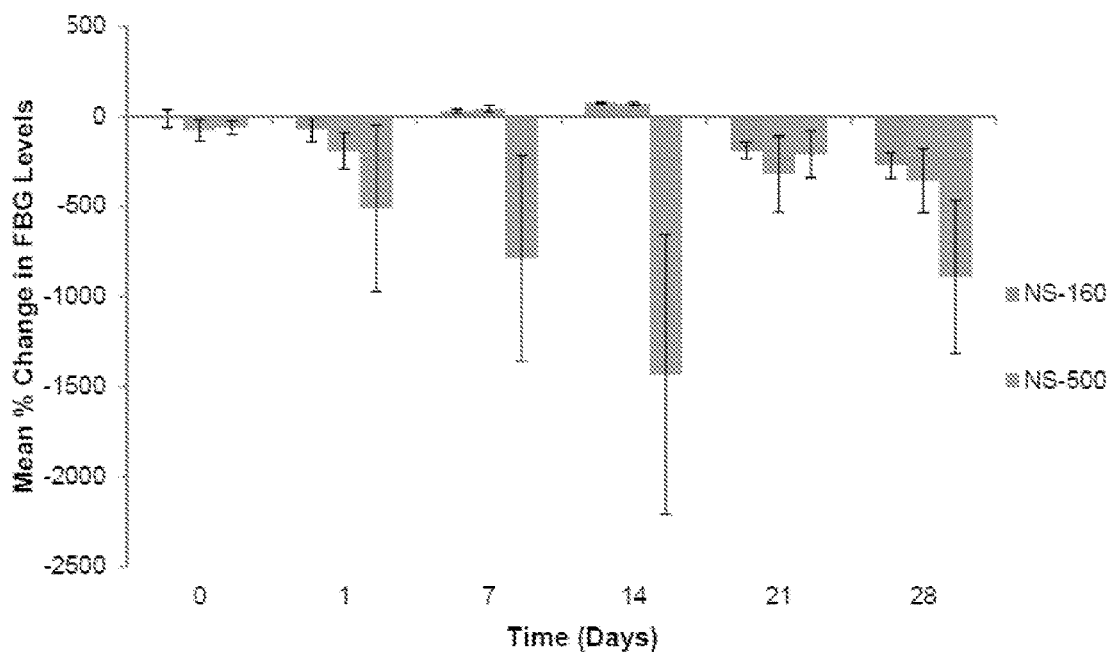


FIG. 7F

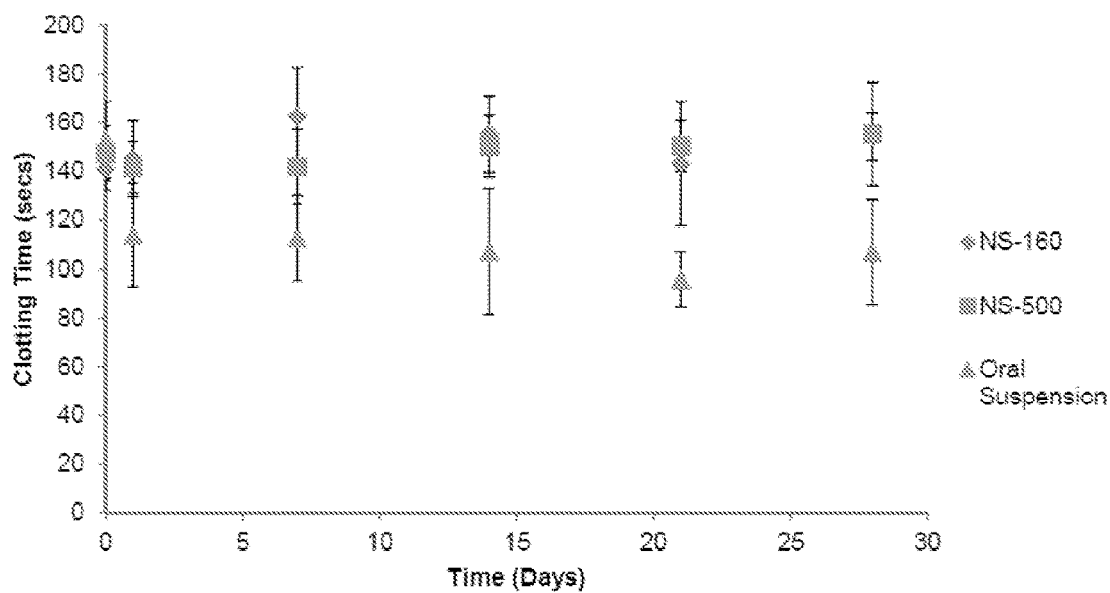


FIG. 7G

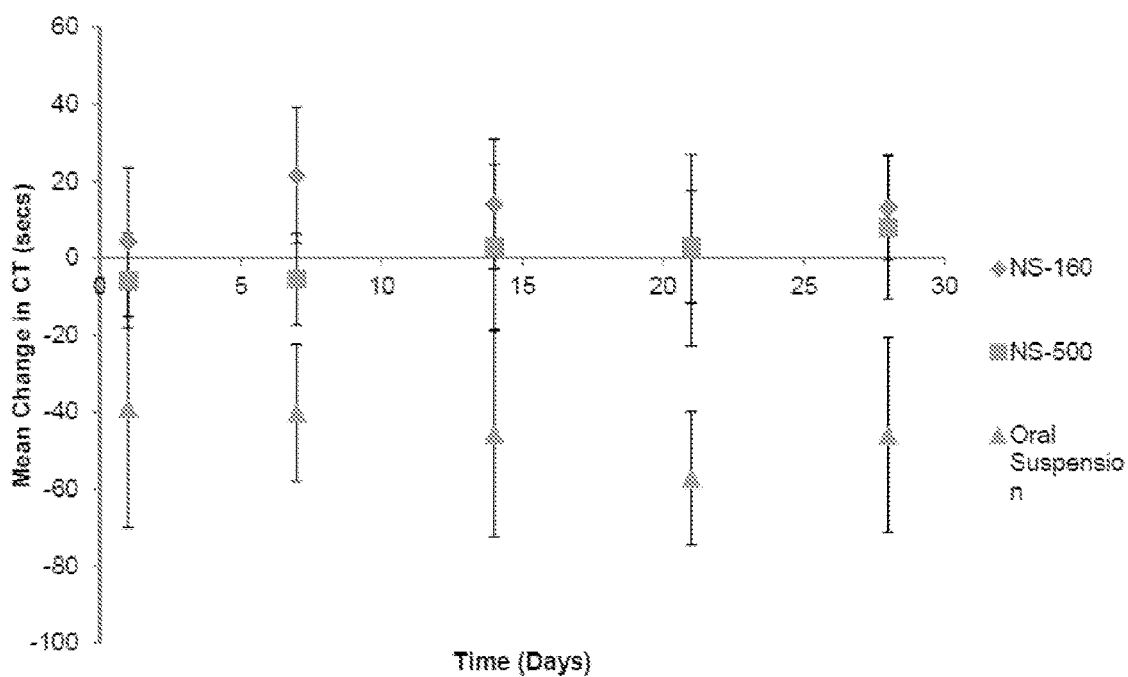


FIG. 7H

FIG. 7

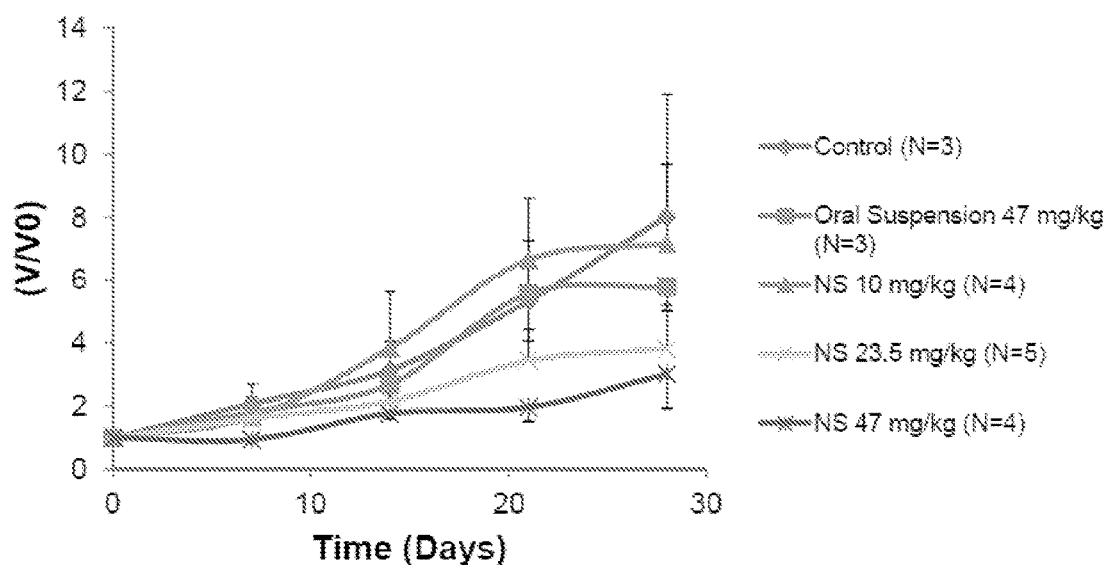


FIG. 8A

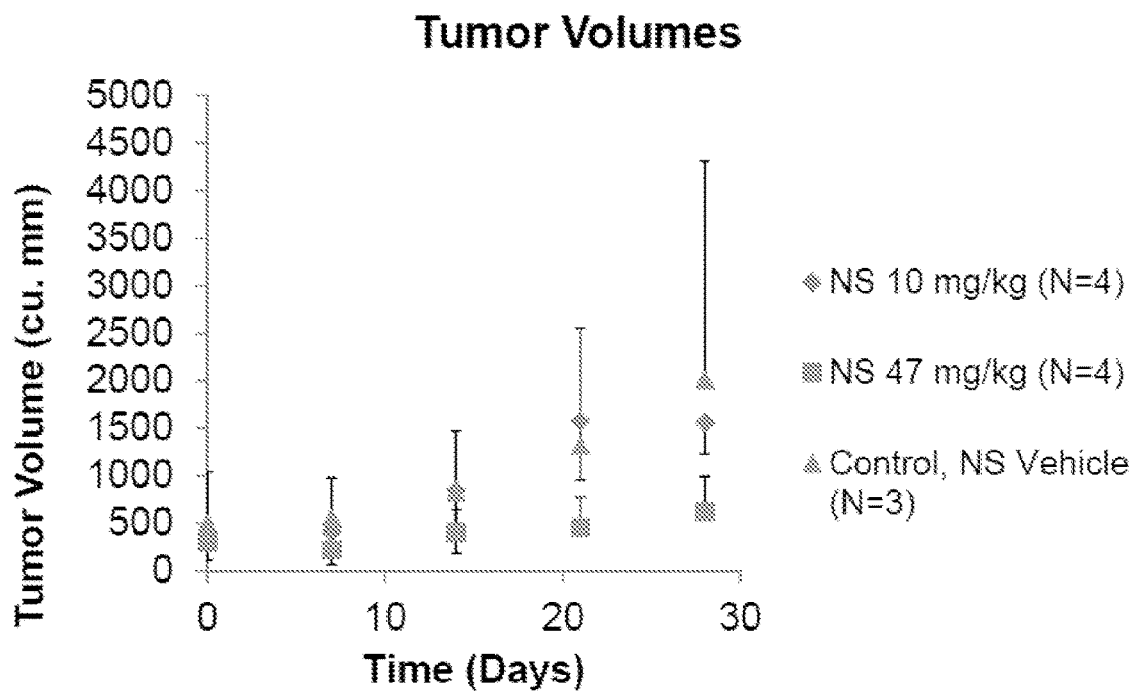


FIG. 8B

FIG. 8

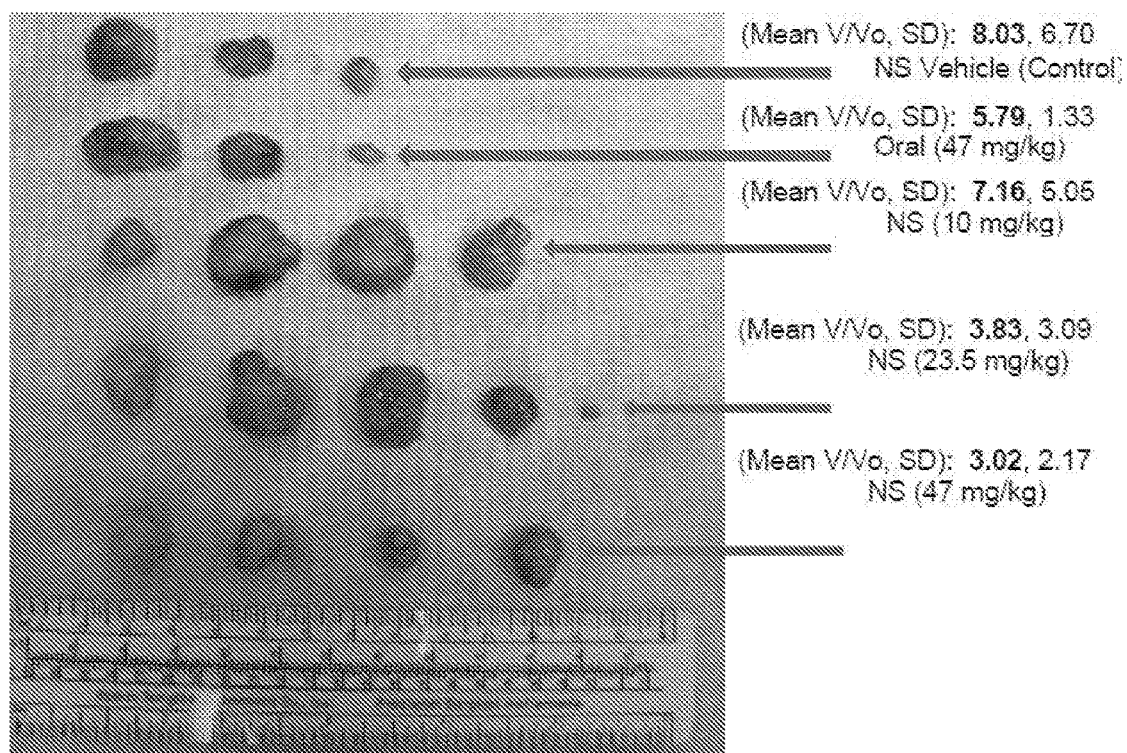


FIG. 8C

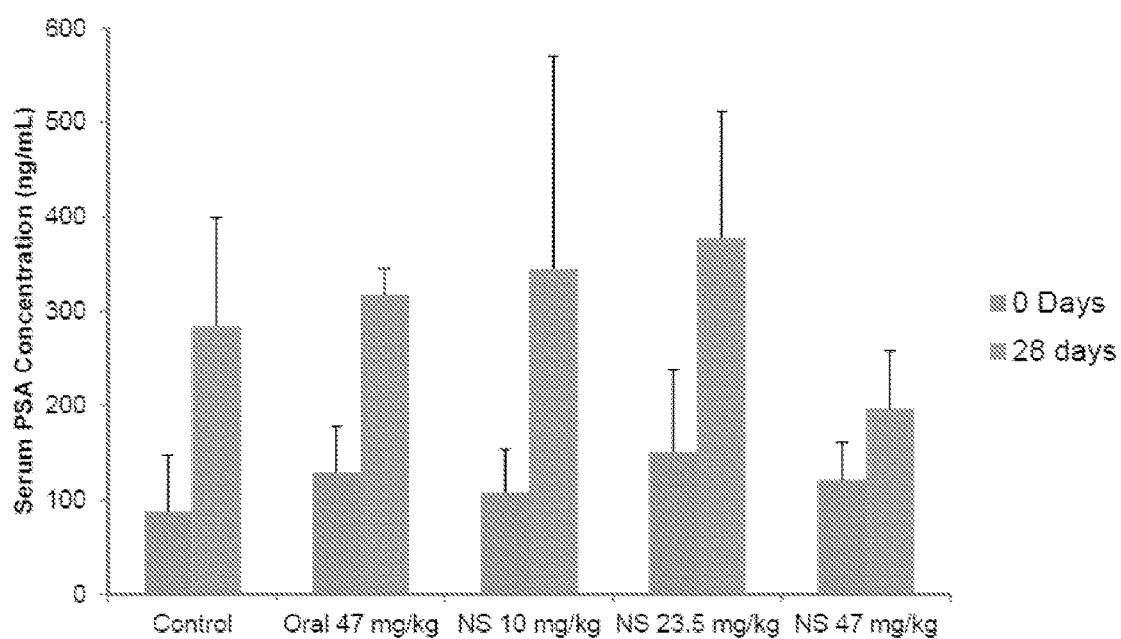


FIG. 8D

FIG. 8

INJECTABLE DIETHYLSTILBESTROL NANOSUSPENSION FORMULATION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/541,540 filed on Aug. 4, 2017. The entirety of the aforementioned application is incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] Not applicable.

FIELD OF THE INVENTION

[0003] The present disclosure relates to formulations of diethylstilbestrol.

BACKGROUND OF THE DISCLOSURE

[0004] Prostate cancer is the most common type of cancer and the second leading cause of cancer related deaths in men in the United States. According to the National Cancer Institute, 238,590 new cases with deaths of 29,720 from prostate cancer have been estimated for 2013.

[0005] Diethylstilbestrol (DES) was mainstay treatment for metastatic and castrate resistant prostate cancer for half a century. In the mid-1980s, the use of DES was drastically reduced because of its severe cardiovascular toxicity and thromboembolic complications, such as deep vein thrombosis, pulmonary embolism and heart attack. At the same time, luteinizing hormone releasing hormone (LHRH) agonists were found to have a similar efficacy profile to that of DES but with significantly lower toxicities. Thus, LHRH agonists replaced DES.

[0006] Recently, there has been a renewed interest in the treatment of prostate cancer with DES since it has been shown to be efficacious in both androgen dependent prostate cancer and castrate resistant prostate cancer. Moreover, LHRH agonists have started to show side effects with extensive use.

[0007] Therefore, there remains a need in the art for new formulations of DES that reduce complications while sustaining its systemic exposure for treatment of prostate cancer. Various embodiments of the present disclosure address this need.

SUMMARY OF THE DISCLOSURE

[0008] In some embodiments, the present disclosure pertains to formulations for cancer treatment that include: a particle loaded with diethylstilbestrol (DES); and a pharmaceutically acceptable carrier. In some embodiments, the particle encapsulates DES. In some embodiments, the particle is a glass-based or metal-based particle with a size of less than about 500 nm in diameter.

[0009] In some embodiments, the pharmaceutically acceptable carrier is also loaded into the particle. In some embodiments, the formulations of the present disclosure also include a stabilizer that is also loaded into the particle at low concentrations, such as concentrations ranging from about 5 wt % to about 15 wt %.

[0010] In some embodiments, the particle is dispersed in an aqueous solution to form a nanosuspension. In some

embodiments, the nanosuspension is adapted for subcutaneous administration and sustained release of DES from the particles.

[0011] In additional embodiments, the present disclosure pertains to methods of treating cancer in a subject by administering to the subject a formulation of the present disclosure. In some embodiments, the subject is a human being suffering from prostate cancer.

[0012] In some embodiments, the administration of the formulations of the present disclosure to the subject occurs in a manner that bypasses or minimizes first pass metabolism. In some embodiments, the administration occurs in a manner that minimizes hepatic exposure of DES. In some embodiments, the administration minimizes hepatic exposure of DES by maintaining the liver-to-plasma DES concentration ratio to less than 2 or less than 0.5 after 24 hours of administration. In some embodiments, the administration of the formulations of the present disclosure to the subject occurs through non-oral routes of administration, such as through subcutaneous administration.

[0013] In additional embodiments, the present disclosure pertains to methods of making the formulations of the present disclosure by loading a particle with DES. In some embodiments, the loading enhances the solubility of DES.

[0014] In some embodiments, the loading occurs in the presence of a stabilizer and therefore results in the loading of the stabilizer into the particle. In some embodiments, the loading occurs in the presence of a pharmaceutically acceptable carrier and therefore results in the loading of the pharmaceutically acceptable carrier into the particle. In some embodiments, the loading occurs by methods that include, without limitation, media milling, wet milling, stirring, high pressure homogenization, and combinations thereof.

DESCRIPTION OF THE DRAWINGS

[0015] FIG. 1 provides depictions and illustrations of formulations for cancer treatment. FIG. 1A shows the chemical structure of diethylstilbestrol (DES). FIG. 1B provides a depiction of a formulation where DES is loaded into particles along with pharmaceutically acceptable carriers and stabilizers. FIG. 1C illustrates a method of treating cancer in a subject by administering to the subject a particle loaded with DES. FIG. 1D illustrates a method of making a formulation for cancer treatment by loading a particle with DES.

[0016] FIG. 2 depicts a method of making a formulation for cancer treatment by loading a particle with DES through a wet milling technique.

[0017] FIG. 3 summarizes the results of stability studies for various formulations, including 160 nm particles loaded with DES (NS-160), 300 nm particles loaded with DES (NS-300), and 500 nm particles loaded with DES (NS-500). The particle size and polydispersity of the NS formulations are illustrated in FIG. 3A. The in vitro release of the nanosuspension formulations are shown in FIGS. 3B and 3C, respectively. The physical and chemical stability of the nanosuspensions are shown in FIGS. 3D and 3E, respectively.

[0018] FIG. 4 depicts plasma DES concentration versus time profiles following a single subcutaneous dose of DES nanosuspensions (i.e., NS-160 and NS-500) and a DES oral suspension in vivo (n=7 for NS-160 and NS-500, n=6 for the

oral suspension, and n=3 for the SC solution and SC suspension). All values are Mean $\pm$ SD.

[0019] FIG. 5 depicts the liver bio-distribution of DES nanosuspensions (i.e., NS-160 and NS-500) and DES oral suspensions. FIG. 5A shows that the liver concentrations of DES from the oral suspension were significantly higher than those of the two nanosuspension formulations (NS-160 and NS-500) delivered subcutaneously on the same dose basis. FIG. 5B shows that the hepatic exposure (AUC_{0-6 days}/mg of DES dose) of DES was about 5 times higher for the oral route as compared to the subcutaneous nanosuspension.

[0020] FIG. 6 depicts results of a short term toxicity study. Plasma ATIII levels for DES formulations (n=5) and a sham control (n=3) are shown in FIGS. 6A and 6B respectively, while the ATIII mean percentage change is shown in FIG. 6C. Plasma FBG levels for DES formulations (n=5) and a sham control (n=3) are shown in FIGS. 6D and 6E, respectively, while the FBG mean percentage change is shown in FIG. 6F. The correlation between ATIII and FBG is illustrated in FIG. 6G. The clotting time is depicted as actual clotting time in FIG. 6H, and mean change in clotting time in FIG. 6I. All values are Mean $\pm$ SD.

[0021] FIG. 7 depicts results of a long term toxicity study. Plasma ATIII levels for DES formulations (n=6) and sham control (n=4) are shown in FIGS. 7A and 7B respectively, while the ATIII mean percentage change is shown in FIG. 7C. Plasma FBG levels for DES formulations (n=6) and sham control (n=4) are shown in FIGS. 7D and 7E respectively, while the FBG mean percentage change is shown in FIG. 7F. The clotting time is depicted as actual clotting time in FIG. 7G and mean change in clotting time in FIG. 7H. All values are Mean $\pm$ SD.

[0022] FIG. 8 depicts the efficacy of DES nanosuspensions in a PCa 183 xenograft prostate tumor mouse model. FIG. 8A illustrates tumor progression by showing changes in V/V₀ over 28 days. V=Volume at time t, V₀=initial volume before the start of treatment. Dose at Time=0, 14 days. NS 160 nm is used. All values are Mean $\pm$ SE. *Significant Tumor Suppression for NS 47 mg/kg as compared to Oral. Unpaired T-Test (p<0.05). FIG. 8B illustrates the effect of NS 10 mg/kg vs NS 47 mg/kg. Significant suppression in tumor volume was seen in 21 days and 28 days of NS 47 mg/kg as compared to NS 10 mg/kg (p<0.05). Unpaired T-Test values were in Mean $\pm$ SD. NS-160 was used. FIG. 8C illustrates the size of the tumors. FIG. 8D illustrates changes in Plasma PSA levels at 0 days and 28 days. * indicates a significant increase in PSA Levels (p<0.05). Unpaired T-Test. All values Mean $\pm$ SD.

DETAILED DESCRIPTION

[0023] All references, publications, and patents cited herein are incorporated by reference in their entirety as though they are fully set forth. Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Hornyak, et al., Introduction to Nanoscience and Nanotechnology, CRC Press (2008); Singleton et al., Dictionary of Microbiology and Molecular Biology 3rd ed., J. Wiley & Sons (New York, N.Y. 2001); March, Advanced Organic Chemistry Reactions, Mechanisms and Structure 7th ed., J. Wiley & Sons (New York, N.Y. 2013); and Sambrook and Russel, Molecular Cloning: A Laboratory Manual 4th ed., Cold Spring Harbor Laboratory Press (Cold Spring Harbor, N.Y. 2012),

provide one skilled in the art with a general guide to many of the terms used in the present application. One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. The present invention is in no way limited to the methods and materials described.

[0024] In the current state of the pharmaceutical industry, the number of drugs that are synthesized and poorly water soluble are significantly increasing. Approximately 60% of the drugs have poor water solubility. The problem of formulating these drugs is exacerbated as they are poorly soluble in both aqueous and organic phase. Such drugs have erratic absorption profiles and a highly variable bioavailability. There have been many methods to formulate such drugs so as to increase the solubility and for more predictable absorption. These formulation approaches include co-solvent solubilization, oily solutions, liposomes, micro-emulsions and solid dispersions. However, there is still a growing need for devising a unique strategy that can not only overcome the problem of formulating hydrophobic drugs but also be feasible to manufacture commercially to improve the pharmacoeconomics.

[0025] Diethylstilbestrol (DES) is a synthetic non-steroidal estrogen with the chemical formula shown in FIG. 1A. Although DES was previously used to treat recurrent prostate cancer, such use was stopped due to severe cardiovascular toxicity and thromboembolic complications resulting from changes in the coagulation cascade.

[0026] Traditionally, DES was administered through the oral route of administration. Estrogen mediated coagulation has been ascribed to multiple abnormalities because of the orally administered DES. These abnormalities arise because of the hepatic exposure of DES. The liver is the main organ that is responsible for the synthesis of the proteins that are needed in the coagulation cascade. Thus, the exposure of DES to the liver leads to various changes in the coagulation proteins, thereby leading to thrombosis and cardiovascular complications.

[0027] Recent clinical trials suggest that circumventing the first pass effect via the parenteral route (injection) for the synthetic estrogens may significantly reduce their cardiovascular toxicity. However, it was unclear if these beneficial effects could be achieved with DES by sustaining its action without the cardiovascular toxicity.

[0028] In some embodiments, the present disclosure pertains to formulations for cancer treatment. In some embodiments, the formulations of the present disclosure include a particle loaded with DES and a pharmaceutically acceptable carrier. In some embodiments, the formulations of the present disclosure also include one or more stabilizers for stabilizing DES. In some embodiments, the formulations of the present disclosure also include one or more other therapeutic agents that are suitable for the treatment of cancer.

[0029] In more specific embodiments, the formulations of the present disclosure can be depicted as formulation 10 in FIG. 1B. In this embodiment, formulation 10 includes particle 12 loaded with DES 14, pharmaceutically acceptable carrier 16, and stabilizer 18.

[0030] In additional embodiments, the present disclosure pertains to methods of treating cancer in a subject in need thereof by administering to the subject a formulation of the present disclosure. In some embodiments, the formulations of the present disclosure are administered in such a manner as to minimize hepatic exposure of DES. In more specific

embodiments illustrated in FIG. 1C, the cancer treatment methods of the present include a step of administering a particle loaded with DES to a subject (step 20) such that the administration minimize hepatic exposure of DES (step 22) and results in the treatment of cancer in the subject (step 24).

[0031] In additional embodiments, the present disclosure pertains to methods of making the formulations of the present disclosure by loading a particle with DES. In some embodiments, the particles may also be loaded with pharmaceutically acceptable carriers and stabilizers. In more specific embodiments illustrated in FIG. 1D, the methods of making the formulations of the present disclosure include: loading a particles with DES (step 30), loading the particle with a pharmaceutically acceptable carrier (step 32), and loading the particle with a stabilizer (step 34) to form a formulation (step 36).

[0032] As set forth in more detail herein, the formulations and methods of the present disclosure can have numerous embodiments. In particular, the formulations of the present disclosure can include various types of particles, pharmaceutically acceptable carriers, stabilizers, and DES forms. Moreover, various methods may be utilized to administer the formulations of the present disclosure to various subjects in order to treat various types of cancers. In addition, various methods may be utilized to make the formulations of the present disclosure.

[0033] Particles

[0034] The formulations of the present disclosure can include various types of particles. In some embodiments, the particles include structures that can encapsulate DES. In some embodiments, the particles include, without limitation, glass-based particles, metal-based particles, lipid-based particles, liposomes, carbon-based particles, polymer-based particles, silica-based particles, and combinations thereof.

[0035] In some embodiments, the particles of the present disclosure include glass-based particles. In some embodiments, the glass-based particles include glass beads.

[0036] In some embodiments, the particles of the present disclosure include metal-based particles. In some embodiments, the metal-based particles include, without limitation, zirconium particles, gold particles, and combinations thereof. In some embodiments, the metal-based particles include zirconium beads.

[0037] In some embodiments, the particles of the present disclosure include lipid-based particles. In some embodiments, the lipid-based particles include liposomes.

[0038] In some embodiments, the particles of the present disclosure include carbon-based particles. In some embodiments, the carbon-based particles include, without limitation, fullerenes, carbon nanotubes, quantum dots, and combinations thereof.

[0039] In some embodiments, the particles of the present disclosure include polymer-based particles. In some embodiments, the polymer-based particles include dendrimers.

[0040] The particles of the present disclosure can have various shapes. For instance, in some embodiments, the particles of the present disclosure are in the form of beads, circular shapes, oval shapes, shells, nanoshells, and combinations thereof. In some embodiments, the particles of the present disclosure are in the form of beads. In some embodiments, the particles of the present disclosure are in the form of nanoshells.

[0041] The particles of the present disclosure can also have various sizes. For instance, in some embodiments, the particles of the present disclosure have sizes of less than about 1,000 nm in diameter. In some embodiments, the particles of the present disclosure have sizes of less than about 500 nm in diameter. In some embodiments, the particles of the present disclosure have sizes ranging from about 100 nm to about 500 nm in diameter. In some embodiments, the particles of the present disclosure have sizes of 160 nm, 300 nm, or 500 nm in diameter.

[0042] The particles of the present disclosure can also have various porosities. For instance, in some embodiments, the particles of the present disclosure can have a plurality of micropores, mesopores, macropores, and combinations thereof.

[0043] Diethylstilbestrol

[0044] DES may be loaded into the particles of the present disclosure in various manners. For instance, in some embodiments, DES may be encapsulated within the particles. In some embodiments, DES may be dispersed within the particles. In some embodiments, DES may be suspended with the pharmaceutically acceptable carrier within the particles. In some embodiments, DES may be suspended with one or more stabilizers within the particles.

[0045] DES may also be loaded into the particles of the present disclosure in various states. For instance, in some embodiments, DES may be in a solid state. In some embodiments, DES may be in a liquid state. In some embodiments, DES may be in a solid state and a liquid state.

[0046] The formulations of the present disclosure can include various concentrations of DES. For instance, in some embodiments, DES is present in a concentration ranging from about 1 wt % to about 50 wt %. In some embodiments, DES is present in a concentration ranging from about 5 wt % to about 20 wt %. In some embodiments, DES is present in a concentration ranging from about 5 wt % to about 15 wt %. In some embodiments, DES is present in a concentration ranging from about 10 wt % to about 15 wt %. In some embodiments, DES is present in a concentration ranging from about 5 wt % to about 10 wt %. In some embodiments, DES is present in a concentration of about 10 wt %. In some embodiments, DES is present in a concentration of about 15 wt %.

[0047] Pharmaceutically Acceptable Carriers

[0048] The formulations of the present disclosure can also include various types of pharmaceutically acceptable carriers. In some embodiments, suitable pharmaceutically acceptable carriers include one or more compounds that can facilitate the administration of DES to a subject. In some embodiments, the pharmaceutically acceptable carrier includes, without limitation, excipients, solvents, fillers, binders, disintegrants, lubricants, buffers, oils, and combinations thereof. In some embodiments, the pharmaceutically acceptable carrier includes, without limitation, water, phosphate buffered saline, alcohol, glycerol, chitosan, alginate, chondroitin, Vitamin E, mineral oil, dimethyl sulfoxide (DMSO), and combinations thereof.

[0049] In some embodiments, the pharmaceutically acceptable carrier includes an excipient. In some embodiments, the excipient is an excipient that excludes solvents, water or other diluents. In some embodiments, the excipient includes, without limitation, antiadherents (e.g., magnesium stearate), binders (e.g., gelatin and/or polysaccharides), coatings (e.g., hydroxypropyl methylcellulose), disinte-

grants (e.g., modified starches), glidants (e.g., magnesium carbonate), lubricants, preservatives (e.g., anti-oxidants), delivery vehicles (e.g., mineral oil), and combinations thereof.

[0050] Pharmaceutically acceptable carriers may be associated with the formulations of the present disclosure in various manners. For instance, in some embodiments, the pharmaceutically acceptable carrier is loaded into a particle. In some embodiments, the pharmaceutically acceptable carrier is embedded with the DES.

[0051] Stabilizers

[0052] In some embodiments, the formulations of the present disclosure can also include one or more stabilizers. The formulations of the present disclosure can also include various types of stabilizers. For instance, in some embodiments, suitable stabilizers include one or more compounds that can prevent, mitigate or reduce the degradation of DES contained in the formulations. In some embodiments, suitable stabilizers include one or more compounds that can enhance or maintain the stability of DES contained in the formulations.

[0053] In some embodiments, suitable stabilizers include, without limitation, surfactants, anti-oxidants, antifoaming agents, wetting agents, dispersants, thickeners, emulsifiers, binders, and combinations thereof. In some embodiments, suitable stabilizers include, without limitation, Pluronic 108 (F-108), Pluronic 68 (F-68), Polysorbate 80 (Tween 80), Polyvinylpyrrolidone 40 (PVP-40), and combinations thereof. In some embodiments, suitable stabilizers include, without limitation, Pluronic F108, Tween 80, and combinations thereof.

[0054] The formulations of the present disclosure can include various concentrations of stabilizers. For instance, in some embodiments, the stabilizers are present in a concentration ranging from about 1 wt % to about 50 wt %. In some embodiments, the stabilizers are present in a concentration ranging from about 5 wt % to about 20 wt %. In some embodiments, the stabilizers are present in a concentration ranging from about 5 wt % to about 15 wt %. In some embodiments, the stabilizers are present in a concentration ranging from about 10 wt % to about 15 wt %. In some embodiments, the stabilizers are present in a concentration ranging from about 5 wt % to about 10 wt %. In some embodiments, the stabilizers are present in a concentration of about 10 wt %. In some embodiments, the stabilizers are present in a concentration of about 15 wt %.

[0055] In some embodiments, the stabilizer is pluronic F108 and present in a concentration of about 10 wt %. In some embodiments, the stabilizer is Tween 80 and present in a concentration of about 10 wt %.

[0056] The stabilizers of the present disclosure may be associated with the formulations of the present disclosure in various manners. For instance, in some embodiments, the stabilizer is loaded into a particle. In some embodiments, the stabilizer is embedded with the DES.

[0057] Additional Therapeutic Agents

[0058] The formulations of the present disclosure can also include one or more additional therapeutic agents. In some embodiments, the one or more therapeutic agents are also suitable for treating cancer. In some embodiments, the one or more therapeutic agents include, without limitation, abiraterone acetate, apalutamide, bicalutamide, cabazitaxel, degarelix, docetaxel, enzalutamide, flutamide, goserelin

acetate, leuprolide acetate, mitoxantrone hydrochloride, nilutamide, radium 223 dichloride, sipuleucel-T, and combinations thereof.

[0059] Formulation Forms

[0060] The formulations of the present disclosure can be in various forms. For instance, in some embodiments, the formulations of the present disclosure may be in the form of nanosuspensions. In some embodiments, the nanosuspensions include suspensions where the particles of the present disclosure are dispersed in an aqueous solution.

[0061] The formulations of the present disclosure may be adapted for various purposes. For instance, in some embodiments, the formulations of the present disclosure may be adapted for subcutaneous administration. In some embodiments, the formulations of the present disclosure may be adapted for the sustained release of DES from the particles.

[0062] Administration of Formulations to Subjects

[0063] The formulations of the present disclosure may be administered to subjects in various manners. For instance, in some embodiments, the formulations of the present disclosure may be administered to subjects in a manner that bypasses or minimizes first pass metabolism. In some embodiments, the formulations of the present disclosure may be administered to subjects in a manner that minimizes hepatic exposure of DES.

[0064] In some embodiments, the administration of the formulations of the present disclosure minimizes hepatic exposure of DES by maintaining the liver-to-plasma DES concentration ratio to less than 2 after 24 hours of administration. In some embodiments, the administration minimizes hepatic exposure of DES by maintaining the liver-to-plasma DES concentration ratio to less than 1.5 after 24 hours of administration. In some embodiments, the administration minimizes hepatic exposure of DES by maintaining the liver-to-plasma DES concentration ratio to less than 1 after 24 hours of administration. In some embodiments, the administration minimizes hepatic exposure of DES by maintaining the liver-to-plasma DES concentration ratio to less than 0.5 after 24 hours of administration. In some embodiments, the administration minimizes hepatic exposure of DES by maintaining the liver-to-plasma DES concentration ratio to less than 0.2 after 24 hours of administration. In some embodiments, the administration minimizes hepatic exposure of DES by maintaining the liver-to-plasma DES concentration ratio to less than 0.15 after 24 hours of administration.

[0065] In some embodiments, the administration of the formulations of the present disclosure minimizes hepatic exposure of DES by maintaining the liver-to-plasma DES concentration ratio to about 0.16 after 24 hours of administration. In some embodiments, the administration minimizes hepatic exposure of DES by maintaining the liver-to-plasma DES concentration ratio to about 0.12 after 24 hours of administration.

[0066] The formulations of the present disclosure may be administered to subjects through various routes. For instance, in some embodiments, the formulations of the present disclosure may be administered to subjects through non-oral routes of administration (i.e., routes of administration other than ingestion by mouth and into the gastrointestinal tract). In some embodiments, the non-oral routes of administration include, without limitation, inhalation, subcutaneous administration, intravenous administration, intraperitoneal administration, intramuscular administration,

intrathecal injection, topical administration, central administration, peripheral administration, and combinations thereof.

[0067] In some embodiments, the administration occurs by intravenous administration. In some embodiments, the administration occurs by subcutaneous administration. In some embodiments, the subcutaneous administration results in the formation of a depot of the formulation in vivo (e.g., under the skin of a subject). In some embodiments, the formed depot helps sustain the plasma DES concentration and minimize the hepatic exposure of DES, thereby reducing the cardiovascular toxicity of DES while maintaining its efficacy.

[0068] The administration of the formulations of the present disclosure can result in the sustained release of DES from the particles of the present disclosure in various manners. For instance, in some embodiments, at least 10% of DES molecules is released from the particles within 1 day of administration. In some embodiments, at least 40% of DES molecules are released from the particles within 5 days of administration. In some embodiments, at least 50% of DES molecules are released from the particles within 10 days of administration. In some embodiments, at least 60% of DES molecules are released from the particles within 15 days of administration.

[0069] The administration of the formulations of the present disclosure can also have various physiological effects in a subject. For instance, in some embodiments, the administration maintains the levels of coagulation factors. In some embodiments, the administration maintains plasma ATIII levels at a constant level (e.g., an ATIII level that does not change more than 15% after 24 hours of administration, more than 10% after 24 hours of administration, or more than 5% after 24 hours of administration). In some embodiments, the administration maintains plasma FBG levels at a constant level (e.g., an FBG level that does not change more than 15% after 24 hours of administration, more than 10% after 24 hours of administration, or more than 5% after 24 hours of administration). In some embodiments, the administration does not substantially affect the blood clotting time of subjects.

[0070] Subjects

[0071] The pharmaceutical compositions of the present disclosure may be administered to various subjects. For instance, in some embodiments, the subject is a human being. In some embodiments, the subject is a non-human animal. In some embodiments, the non-human animal includes, without limitation, mice, rats, rodents, mammals, cats, dogs, monkeys, pigs, cattle and horses.

[0072] In some embodiments, the subject to be treated is suffering from cancer, such as prostate cancer. In some embodiments, the subject to be treated is a human being suffering from cancer.

[0073] Treatment of Cancer

[0074] The pharmaceutical compositions of the present disclosure may be utilized to treat various types of cancer. For instance, in some embodiments, the cancer to be treated include prostate cancer. In some embodiments, the prostate cancer may include recurrent prostate cancer, such as Stage III or Stage IV prostate cancer.

[0075] Methods of Making Formulations

[0076] Various methods may also be utilized to make the formulations of the present disclosure. For instance, the methods of the present disclosure may utilize various meth-

ods to load particles with DES, stabilizers, pharmaceutically acceptable carriers, additional therapeutic agents, and combinations thereof. In some embodiments, the loading occurs by methods that include, without limitation, media milling, wet milling, stirring, high pressure homogenization, and combinations thereof.

[0077] In some embodiments, the loading occurs by media milling. In some embodiments, media milling occurs by placing particles, DES, stabilizers, pharmaceutically acceptable carriers, and/or additional therapeutic agents in a medium. Thereafter, the medium can be exposed to high shear and attrition forces, which can in turn lead to a reduction in particle size.

[0078] The loading steps of the present disclosure can have various incubation times. For instance, in some embodiments, the loading steps of the present disclosure have incubation times that range from about 30 minutes to about 40 hours. In some embodiments, the loading steps of the present disclosure have incubation times that range from about 1 hour to about 40 hours. In some embodiments, the loading steps of the present disclosure have incubation times that range from about 1 hour to about 32 hours. In some embodiments, the loading steps of the present disclosure have incubation times of about 30 hours. In some embodiments, the loading steps of the present disclosure have incubation times of about 8 hours. In some embodiments, the loading steps of the present disclosure have incubation times of about 6 hours.

[0079] The loading steps of the present disclosure can have various effects. For instance, in some embodiments, the loading steps of the present disclosure can enhance the solubility of DES.

[0080] In some embodiments, the loading of particles with DES occurs in the presence of a stabilizer, thereby resulting in the loading of the stabilizer into the particles. In some embodiments, the loading of particles with DES occurs in the presence of a pharmaceutically acceptable carrier, thereby resulting in the loading of the pharmaceutically acceptable carrier into the particles. In some embodiments, the loading of particles occurs in the presence of DES, a pharmaceutically acceptable carrier, and a stabilizer.

[0081] Different concentrations of DES may be loaded into particles. For instance, in some embodiments, the loading media may have DES concentrations that range from about 1 mg/ml of DES to about 10 mg/ml of DES. In some embodiments, the loading media may have DES concentrations that range from about 1 mg/ml of DES to about 5 mg/ml of DES. In some embodiments, the loading media may have DES concentrations that range from about 1.4 mg/ml of DES to about 4.3 mg/ml of DES.

[0082] In some embodiments, the DES concentration in the loading media may be used to control the size of the loaded particles. For instance, in some embodiments, an increase in the DES concentration of the loading media may result in a decrease in the size of the loaded particles.

[0083] Applications and Advantages

[0084] The methods and formulations of the present disclosure can have numerous applications and advantages. For instance, in some embodiments, the methods and formulations of the present disclosure provide sustained release nanosuspension formulations of DES for reducing hepatic exposure of DES by circumventing the first pass metabolism while maintaining efficacy by sustaining plasma DES concentrations. The sustained release of DES can also enable

less frequent dosing, thereby improving patient compliance. In some embodiments, the methods and formulations of the present disclosure provide a DES formulation that retains its high efficacy and reduced toxicity (e.g., cardiovascular toxicity).

[0085] Moreover, the methods and formulations of the present disclosure can be utilized to increase the solubility and bioavailability of DES. The methods and formulations of the present disclosure also have low toxicity, high DES loading, and a significantly higher DES stability as compared to conventional drug solutions. In addition, the fabrication methods of the present disclosure can be readily scaled up while achieving narrow size particle distribution.

[0086] As such, the methods and formulations of the present disclosure may be useful for the treatment of various types of cancers, especially recurrent and advanced cancers. For instance, in some embodiments, the methods and formulations of the present disclosure may be useful for the treatment of advanced prostate cancer, such as Stage III and Stage IV prostate cancer. In some embodiments, the formulations of the present disclosure may be utilized as part of an overall treatment regimen for a type of cancer.

Additional Embodiments

[0087] Reference will now be made to more specific embodiments of the present disclosure and experimental results that provide support for such embodiments. However, Applicants note that the disclosure below is for illustrative purposes only and is not intended to limit the scope of the claimed subject matter in any way.

Example 1. Nanosuspension Formulations of Diethylstilbestrol for the Treatment of Prostate Cancer

[0088] In this Example, Applicants describe a new nanosuspension formulation of diethylstilbestrol (DES) for the treatment of prostate cancer. The overall objective in this Example is subcutaneous delivery of DES by developing a nanosuspension formulation. The central hypothesis is that compared to the oral route, the subcutaneous nanosuspension formulation will, by forming a depot, sustain the plasma DES concentration and minimize the hepatic exposure by circumventing the first pass effect. Applicants propose that this change in the formulation and route of administration would potentially reduce the thromboembolic complications and cardiovascular toxicity.

[0089] Applicants demonstrate here that DES with its physicochemical properties and chemistry can be successfully formulated as a nanosuspension formulation. The DES nanosuspension with different particle sizes can be developed. The nanosuspension formulations were characterized based on particle size, zeta potential, polydispersity and in vitro release.

[0090] Injectable nanosuspension formulations of various particle sizes of 160 nm (NS-160), 300 nm (NS-300) and 500 nm (NS-500) were successfully formulated and extensively characterized. The in vitro release study demonstrated a sustained release of DES from the nanosuspensions as compared to that of the DES co-solvent preparation. Also, the in vitro release of DES was inversely dependent on the particle size of the nanosuspensions. The pharmacokinetic studies in the Sprague Dawley rats demonstrated that, as compared to the oral DES suspension and subcutaneous

co-solvent (solution), the subcutaneously administered DES nanosuspensions significantly sustained the release of DES and prolonged the circulation of DES in blood.

[0091] In addition, the DES nanosuspensions showed a longer elimination half-life and slower clearance than the oral DES suspension. The DES nanosuspensions, with higher systemic exposure, also showed a 20 times reduction of the hepatic exposure as compared to the oral DES suspension on the same dose basis. The subcutaneous DES nanosuspensions showed significantly lesser changes in the levels of the coagulation factors such as fibrinogen and anti-thrombin III, and the rat blood clotting time as compared to those of oral DES suspension in the short term and long term toxicity studies.

[0092] The aforementioned effects could potentially decrease the cardiovascular toxicity and thromboembolic complications of DES. In the efficacy study in a prostate cancer tumor mouse model, the DES nanosuspension (NS-160) exhibited significant tumor suppression as compared to the oral DES suspension on the same dose basis. Also, the prostate specific antigen (PSA) levels elevated significantly with the progression of the tumors for treatment with oral DES but were maintained stable for the NS-160 treatment group over a period of 28 days.

[0093] As such, this Example demonstrates the viability of formulating nanosuspensions of DES that achieve sustained release of DES, low hepatic exposures and potential reduction of oral DES complication with enhanced efficacy when the formulation was administered by subcutaneous injection. This Example also illustrates that, DES, which has lost its primary place for the treatment of prostate cancer because of its cardiovascular toxicity and thromboembolic complications, needs to be reconsidered as a treatment option.

Example 1.1. Developing a DES Nanosuspension Formulation for Subcutaneous Administration

[0094] A top down milling approach was used in the formulation of the DES nanosuspensions. The DES nanosuspension formulations were prepared by the wet milling technique shown in FIG. 2. A mixture of DES powder (20 mg), stabilizers (i.e., 10% w/w Pluronic F108 (130 μ L) and 10% w/w Tween 80 (30 μ L)) and double distilled water (200 μ L) was added to a 7 mL amber colored scintillation vial pre-filled with a mixture of glass beads and magnetic stir bars. The glass beads added were in a definite ratio of 1:1:2 (0.8 g) for the sizes of 0.5-0.75 (S), 0.75-1 (M), and 1-1.3 (L) μ m, respectively. The mixture was stirred at 1,600 rpm. Intermittent sampling was performed to evaluate the particle size distribution of DES nanosuspension formulations by using Brookhaven ZetaSizer with Zeta Plus Particle Sizing software Ver.3.85 (Brookhaven Instrument Corporation, NY, USA).

[0095] In order to evaluate the best conditions and factors for the formulation of the DES nanosuspension formulations, the different types of stabilizers, concentration of stabilizers, amount of drug in the formulation, milling time, and varying ratios of glass beads were used. The stirring rate was kept constant at 1,600 rpm. The various factors were screened one factor at a time keeping the other factors constant.

[0096] To select the stabilizer candidates for the DES nanosuspension formulations, different stabilizers such as Pluronic® 108 (F-108), Pluronic® 68 (F-68), Polysorbate 80 (Tween 80), and Polyvinylpyrrolidone (PVP) 40 were

used at a concentration of 10% w/w. From the different stabilizers, Tween 80 was used in combination with F-108, F-68 and PVP 40. The F-68 and PVP 40 failed to stabilize the DES nanosuspension formulations. The combination of F-108 and Tween 80 stabilized the DES nanosuspension formulations effectively and led to lowering of the particle size of the formulation.

[0097] Tween 80 (30 μ L of 10% w/w) was used at a lower amount than F-108 (130 μ L of 10% w/w). This combination led to efficient stabilization and effective lowering of the particle size of DES nanosuspension formulation. F108 used alone did not result in adequate stabilization and wetting of the nanosuspensions. Hence, from the different stabilizers, a combination of the F-108 and Tween 80 was selected.

[0098] The different concentrations of F-108 and Tween 80 were evaluated at 5%, 10% and w/w. The 10% and 15 w/w selections resulted in comparable or significant lowering of the particle size as compared to 5 w/w of the stabilizers. The 10% w/w group was selected as it has a lower concentration of the stabilizers in the formulation as compared to the 15% w/w group.

[0099] Different sizes of glass beads, S, M and L, were used in the ratios of 50:1, 40:1 and 30:1 (Beads:Drug amount) while keeping the other factors constant (i.e., at 20 mg of DES, and 10% w/w of F 108 and Tween 80). The 50:1 ratio had 0.5 g of L, 0.3 g of M and 0.2 g of S beads. The 40:1 ratio had 0.4 g of L, 0.2 g of M and 0.2 g of S beads. The 30:1 ratio had 0.3 g of L, 0.2 g of M and 0.1 g of S beads. The effect of above mentioned ratios were evaluated over a duration of 32 hours. The particle size was not reduced effectively by using a 30:1 ratio while the 50:1 ratio caused too much packing in the vial and the milling was not efficient. The 40:1 ratio reduced the particle size efficiently and faster as compared to that of the 50:1 ratio.

[0100] Three different amounts of DES at 10 mg, 20 mg and 30 mg were also used to test the effect of the amount of drug in the formulation to the reduction in the particle size while keeping the other factors constant. The particle size was effectively reduced for 10 and 20 mg of DES but 30 mg of DES did not reach lower particle sizes.

[0101] Milling times of 1 to 32 hours were evaluated for reduction in particle size by fixing all the other factors. The particle size decreased with the milling time. After 30 hours, the mean particle size reached a plateau. Hence, 30 hours of milling was selected as total time of milling for the NS-160

group. The NS-500 group was achieved in 6 hours and NS-300 was achieved after 8 hours milling.

Example 1.2. Release and Stability of DES Nanosuspensions

[0102] Beads of different sizes: 160 nm (NS-160), 300 nm (NS-300) and 500 nm (NS-500), were evaluated for DES release. The beads were added to a dialysis bag (M.W. cut-off 6000-8000). The release medium was PBS, pH 7.4 at 37° C. with 0.2% Tween 80 to maintain sink conditions. The stirring rate was 100 rpm. Samples were collected in the time frame of 15 min to 14 days. Drug release and stability of DES was monitored for 14 days by validated HPLC assay (LLOQ 198 ng/ml).

[0103] The particle size and polydispersity of the NS formulations are illustrated in FIG. 3A. The in vitro release of the nanosuspension formulations are shown in FIGS. 3B and 3C, respectively. The physical and chemical stability of the nanosuspension are shown in FIGS. 3D and 3E, respectively.

Example 1.3. Determining the Pharmacokinetic Parameters and the Liver Bio-Distribution from the DES Nanosuspension Formulations In Vivo

[0104] In one embodiment, Applicants found that DES nanosuspension formulations, when delivered subcutaneously, sustain the plasma DES concentration with good bioavailability and low hepatic exposure, by forming a depot in vivo.

[0105] In a pharmacokinetics (PK) study, Applicants prepared desired formulations comprising NS-160, NS-500, an SC solution, an oral suspension, and/or an SC suspension. These formulations were administered to SD rats (300-350 g) anesthetized with a Ketamine cocktail. The dosing was 7 mg, administered subcutaneously and/or orally. Blood sampling of the SD rats were done in the tail vein at the following times: 0.04, 0.08, 0.17, 0.33, 0.5, 1, 1.25, 1.5, 2, 2.25, 3, 4, and 6 days. The samples were stored at -80° C., until further analysis by solvent extraction (ethyl acetate), and validation by LC-MS/MS. LLOQ of 0.78 ng/ml was used. The in vivo PK results in SD rats are shown in FIG. 4, and the PK parameters are shown in Table 1.

TABLE 1

PK parameters					
Parameter	Unit	160 nm (SC) [◇] Mean $\pm$ SD	500 nm (SC) [◇] Mean $\pm$ SD	Solution (SC) [€] Mean $\pm$ SD	Solution (SC) [€] Mean $\pm$ SD
C _{max} /dose	(ng/mL)/ mg	58.35 $\pm$ 15.05* ^{\$^} [^]	13.49 $\pm$ 2.29* [^]	35.03 $\pm$ 12.39	157.24 $\pm$ 10.47
T _{max}	hr	4.28 $\pm$ 0.054* [^]	3.06 $\pm$ 1.74	1.67 $\pm$ 0.97	2.00 $\pm$ 0.07
AUC/Dose	(hr*ng/ mL)/mg	736.70 $\pm$ 210.12* ^{\$^}	365.12 $\pm$ 99.90* [^]	207.06 $\pm$ 73.41	600.34 $\pm$ 57.09
Rel. F		1.23	0.61		
K ₀₁	1/hr	0.38 $\pm$ 0.16*	4.18 $\pm$ 4.99	3.10 $\pm$ 3.96	1.44 $\pm$ 0.30
K ₁₀	1/hr	0.18 $\pm$ 0.03	0.08 $\pm$ 0.04* ^{\$^}	0.29 $\pm$ 0.12	0.46 $\pm$ 0.09
K ₁₂	1/hr	0.015 $\pm$ 0.006 ^{\$^}	0.17 $\pm$ 0.10		
K ₂₁	1/hr	0.010 $\pm$ 0.004	0.04 $\pm$ 0.05		
K ₀₁ HL	hr	2.11 $\pm$ 0.71* [^]	1.63 $\pm$ 0.51	0.71 $\pm$ 0.07	0.50 $\pm$ 0.11
K ₁₀ HL	hr	3.90 $\pm$ 0.59* ^{\$^} [^]	11.54 $\pm$ 7.19* [^]	2.66 $\pm$ 0.84	1.55 $\pm$ 0.27
Alpha HL	hr	3.59 $\pm$ 0.56	3.24 $\pm$ 2.21		
Beta HL	hr	89.18 $\pm$ 31.88* [^]	146.22 $\pm$ 99.12* [^]		

TABLE 1-continued

PK parameters					
Parameter	Unit	160 nm (SC) [◇] Mean $\pm$ SD	500 nm (SC) [◇] Mean $\pm$ SD	Solution (SC) ^ε Mean $\pm$ SD	Solution (SC) ^ε Mean $\pm$ SD
V ₁ F	L	8.71 $\pm$ 2.90* ^{\$}	48.02 $\pm$ 28.15* [^]	20.81 $\pm$ 11.21	3.73 $\pm$ 0.60
CL	L/hr	1.57 $\pm$ 0.54* ^{\$}	2.98 $\pm$ 1.05* [^]	5.37 $\pm$ 1.86	1.67 $\pm$ 0.15
V ₂ F	L	15.07 $\pm$ 8.80 ^{\$}	322.58 $\pm$ 160.94		

*significant difference 160 nm or 500 nm with Oral as reference.

[^]significant difference between the 160 nm or 500 nm with solution as reference.

^{\$}significant difference between 160 nm and 500 nm. Unpaired t test (p <0.05).

[◇] two compartment model while one compartment model.

[0106] Liver bio-distribution is shown in FIG. 5 and Table 2. FIG. 5 compares NS-160, NS-500, and an oral suspension. At each time point, dosage was 7 mg, and N=4. All values are mean $\pm$ -SD.

TABLE 2

Liver biodistribution of DES nanosuspensions.			
AUC ₀₋₆ /mg of DES Dose (hr*ng/mL)			
Formulation	Liver	Plasma	L/P ratio
NS-160	87.66	735.84	0.12
NS-500	45.97	290	0.16
Oral Suspension	502.60	243.18	2.07

[0107] Table 3 illustrates the in vivo dissolution rate using non-compartmental analysis, where $MDT_{NS} = MRT_{NS} - MRT_{solution}$.

TABLE 3

In vivo dissolution rates of DES nanosuspensions using non-compartmental analysis.				
Formulation	MDT _{in vivo} (hr)	MDT _{in vitro} (hr)	In vivo dissolution rate (1/hr)	In vitro dissolution rate (1/hr)
Solution	3.44	4.74	0.29	0.211
NS-160	7.90	46.04	0.12	0.021
NS-500	33.55	56.53	0.03	0.017

[0108] Based on the above experiments and results, Applicants found that sustained plasma DES concentrations were achieved from the 160 nm and 500 nm nanosuspension formulations for 6 days. In one embodiment, significantly higher initial plasma DES concentrations were achieved from 160 nm than 500 nm. Applicants found higher relative F for nanosuspension formulations as compared to the oral route.

[0109] It was found that the 160 nm formulation has a significantly higher AUC and slower CL than the 500 nm and oral formulations. Moreover, elimination half-lives were significantly prolonged for 160 nm and 500 nm as compared to that of the oral solution. The NS formulations were also found to have a significantly lower hepatic exposure as compared to that of the oral formulations. Good linear IVIVC for the dissolution time of the subcutaneous DES formulations were observed.

Example 1.4. Evaluating the Performance of the Subcutaneous DES Nanosuspension Formulations Based on Toxicity and Efficacy Profiles-Pharmacodynamics Evaluation

[0110] Applicants demonstrate in this Example that subcutaneous sustained delivery of DES decreases the changes in the coagulation factors while maintaining the efficacy. Applicants conducted both a short term toxicity study as well as a long term toxicity study. In the short term toxicity study, the dosing was kept the same as in the PK study. Toxicity was tested for groups NS-160, NS-500, and oral suspension. Toxicity was evaluated in Fibrinogen (FBG), Anti-thrombin III (ATIII), and Rat blood clotting time (RBCT). Sham Control was done for NS and oral suspensions. In the long term toxicity study, with a duration of 28 days, the groups were NS-160, NS-500 and Oral Suspension. The dosing was 7 mg of DES/14 days for NS and 7 mg of DES/weekly for Oral Suspension.

[0111] Toxicity was evaluated in Fibrinogen (FBG), Anti-thrombin III (ATIII), and Rat blood clotting time (CT). Sham Control was done for NS and oral suspensions. The rat clotting time was measured using the following procedure: 25 μ L blood (tail) was collected in microhematocrit glass capillary; chronometer was started with first blood contact; flow by gravity was monitored between two marks on tube Tilting capillary at -60 degrees and $+60$ degrees; and finally chronometer was stopped as blood stops to flow.

[0112] The results for the short term study are shown in FIG. 6. The results for the long term study are shown in FIG. 7.

[0113] In conclusion, from this data, Applicants found that plasma ATIII levels remain constant with NS DES formulations (SC) but decrease with Oral DES. Plasma FBG levels were found to remain constant with NS DES formulations (SC) and decrease with Oral DES. Excessive utilization of FBG was found to form fibrin (clot). The clotting time was found to remain constant with NS DES formulations and decrease significantly with Oral DES. Thus, Applicants found that NS formulations of DES may reduce the thromboembolic and CV complications.

Example 1.5. Evaluating the Efficacy of Nanosuspensions with the PCA 183 Xenograft Prostate Tumor Mouse Model as Compared to the Oral DES

[0114] MDA PCa 183 xenograft was derived from androgen-dependent prostate carcinoma from drug naïve patients. The method comprised rinsing tumor tissue 6 times by sterile PBS, adding DMEM+10% FBS medium to tumor and

mince into 1 mm pieces, incubating with $1 \times$ Accumax at 37° C. for 20 mins at rotating conditions and filtering single cells ($70 \mu\text{m}$), injecting 50 μL of tumor cells into the rear flank of SCID mice, and finally measuring and evaluating PSA levels and tumor volume. The PSA mouse model was chosen because PSA levels rise during the progression of prostate cancer, FDA approval, and typical biomarker in humans. The mice used in this study were divided into 5 groups: (1) Group A (4 Mice)—Control, NS vehicle; (2) Group B (4 Mice)—Diethylstilbestrol, 47 mg/kg, 100 μL , Oral Suspension; (3) Group C (4 Mice)—Diethylstilbestrol, 10 mg/kg, 100 μL , SC, 26 5/8G, NS-160; (4) Group D (5 Mice)—Diethylstilbestrol, 23.5 mg/kg, 100 SC, 26 5/8G, NS-160; and (5) Group E (4 Mice)—Diethylstilbestrol, 47 mg/kg, 100 SC, 26 5/8G, NS-160.

[0115] The tumor progression and plasma PSA levels are illustrated in FIG. 8. This efficacy study was the first report of DES efficacy in the PCA 183 xenograft prostate tumor mouse model. In this study, Applicants found that subcutaneous 160 nm NS formulation with two doses of 47 mg/kg maintains the PSA levels and controls the tumor progression over a period of 28 days. 160 nm NS formulation was shown to have a better efficacy profile as compared to oral DES on the same dose basis. Thus, Applicants have shown that 160 nm DES NS formulation with sustained exposure maintains DES efficacy. In conclusion, subcutaneous sustained delivery of DES is likely to decrease the changes in the coagulation factors while maintaining the efficacy.

[0116] Without further elaboration, it is believed that one skilled in the art can, using the description herein, utilize the present disclosure to its fullest extent. The embodiments described herein are to be construed as illustrative and not as constraining the remainder of the disclosure in any way whatsoever. While the embodiments have been shown and described, many variations and modifications thereof can be made by one skilled in the art without departing from the spirit and teachings of the invention. Accordingly, the scope of protection is not limited by the description set out above, but is only limited by the claims, including all equivalents of the subject matter of the claims. The disclosures of all patents, patent applications and publications cited herein are hereby incorporated herein by reference, to the extent that they provide procedural or other details consistent with and supplementary to those set forth herein.

What is claimed is:

1. A formulation for cancer treatment comprising:
a particle loaded with diethylstilbestrol; and
a pharmaceutically acceptable carrier.
2. The formulation of claim 1, wherein the particle encapsulates diethylstilbestrol.
3. The formulation of claim 1, wherein the particle is selected from the group consisting of glass-based particles, metal-based particles, lipid-based particles, liposomes, carbon-based particles, polymer-based particles, silica-based particles, and combinations thereof.
4. The formulation of claim 1, wherein the particle comprises glass-based particles or metal-based particles.
5. The formulation of claim 1, wherein the particle has a size of less than about 1,000 nm in diameter.
6. The formulation of claim 1, wherein the particle has a size ranging from about 100 nm to about 500 nm in diameter.
7. The formulation of claim 1, wherein the pharmaceutically acceptable carrier is loaded into the particle.

8. The formulation of claim 1, wherein the pharmaceutically acceptable carrier is selected from the group consisting of excipients, solvents, fillers, binders, disintegrants, lubricants, buffers, oils, and combinations thereof.

9. The formulation of claim 1, wherein the diethylstilbestrol is in solid state.

10. The formulation of claim 1, wherein the formulation further comprises a stabilizer.

11. The formulation of claim 10, wherein the stabilizer is loaded into the particle.

12. The formulation of claim 10, wherein the stabilizer is selected from the group consisting of surfactants, antioxidants, antifoaming agents, wetting agents, dispersants, thickeners, emulsifiers, binders, and combinations thereof.

13. The formulation of claim 10, wherein the stabilizer is present in a concentration ranging from about 5 wt % to about 15 wt %.

14. The formulation of claim 1, wherein the cancer is prostate cancer.

15. The formulation of claim 1, further comprising one or more additional therapeutic agents, wherein the one or more additional therapeutic agents are suitable for the treatment of cancer.

16. The formulation of claim 1, wherein the particle is dispersed in an aqueous solution.

17. The formulation of claim 1, wherein the formulation is adapted for subcutaneous administration and sustained release of diethylstilbestrol from the particles.

18. A method of treating cancer in a subject in need thereof, comprising:

administering to the subject a formulation, wherein the formulation comprises:

- a particle loaded with diethylstilbestrol; and
- a pharmaceutically acceptable carrier.

19. The method of claim 18, wherein the subject is a human being.

20. The method of claim 18, wherein the cancer is prostate cancer.

21. The method of claim 18, wherein the administering occurs in a manner that bypasses or minimizes first pass metabolism.

22. The method of claim 18, wherein the administering occurs in a manner that minimizes hepatic exposure of diethylstilbestrol.

23. The method of claim 22, wherein the administering minimizes hepatic exposure of diethylstilbestrol by maintaining the liver-to-plasma diethylstilbestrol concentration ratio to less than 2 after 24 hours of administration.

24. The method of claim 22, wherein the administering minimizes hepatic exposure of diethylstilbestrol by maintaining the liver-to-plasma diethylstilbestrol concentration ratio to less than 0.5 after 24 hours of administration.

25. The method of claim 18, wherein the administering occurs through non-oral routes of administration.

26. The method of claim 25, wherein the non-oral routes of administration are selected from the group consisting of inhalation, subcutaneous administration, intravenous administration, intraperitoneal administration, intramuscular administration, intrathecal injection, topical administration, central administration, peripheral administration, and combinations thereof.

27. The method of claim 25, wherein the administering comprises subcutaneous administration.

28. The method of claim **18**, wherein the particle encapsulates diethylstilbestrol.

29. The method of claim **18**, wherein the particle is selected from the group consisting of glass-based particles, metal-based particles, lipid-based particles, liposomes, carbon-based particles, polymer-based particles, silica-based particles, and combinations thereof.

30. The method of claim **18**, wherein the particle has a size of less than about 1,000 nm in diameter.

31. The method of claim **18**, wherein the particle has a size ranging from about 100 nm to about 500 nm in diameter.

32. The method of claim **18**, wherein the pharmaceutically acceptable carrier is loaded into the particle.

33. The method of claim **18**, wherein the pharmaceutically acceptable carrier is selected from the group consisting of excipients, solvents, fillers, binders, disintegrants, lubricants, buffers, oils, and combinations thereof.

34. The method of claim **18**, wherein the formulation further comprises a stabilizer.

35. The method of claim **34**, wherein the stabilizer is loaded into the particle.

36. The method of claim **34**, wherein the stabilizer is present in a concentration ranging from about 5 wt % to about 15 wt %.

37. The method of claim **18**, wherein the formulation further comprises one or more additional therapeutic agents, wherein the one or more additional therapeutic agents are suitable for the treatment of cancer.

38. The method of claim **18**, wherein the particle is dispersed in an aqueous solution.

39. A method of making a formulation for cancer treatment comprising:

loading a particle with diethylstilbestrol.

40. The method of claim **39**, wherein the loading occurs by a method selected from the group consisting of media milling, wet milling, stirring, high pressure homogenization, and combinations thereof.

41. The method of claim **39**, wherein the loading enhances the solubility of diethylstilbestrol.

42. The method of claim **39**, wherein the loading occurs in the presence of a stabilizer, and wherein the stabilizer gets loaded into the particle.

43. The method of claim **39**, wherein the loading occurs in the presence of a pharmaceutically acceptable carrier, and wherein the pharmaceutically acceptable carrier gets loaded into the particle.

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