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Research Article

In Vitro and In Vivo Evaluation of Desogestrel-Loaded Poly(D,L-lactic Acid) Nanoparticles

Hui Lin,[1] Guoyong Jia,[2] Peng Sun,[1] Liqiao Zhu,[1] Jinna Chen,[3] Qiyue Wan,[3] Lingyun Xiao,[4] and Xianghong Liu[3]

[1]*School of Pharmacy, Shandong University of Traditional Chinese Medicine, Jinan 250355, China*
[2]*Department of Neurology, Qilu Hospital Affiliated to Shandong University, Jinan 250012, China*
[3]*Department of Pharmaceutical Sciences, Qilu Hospital affiliated to Shandong University, Jinan 250012, China*
[4]*Joint Laboratory for the Research of Modern Preparation Technology, Huazhong University of Science and Technology, Wuhan 430071, China*

Correspondence should be addressed to Peng Sun; sunpeng369@126.com and Xianghong Liu; liuxianghong666@126.com

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The aim of this study was to explore the synthesis parameters of desogestrel-polylactic acid nanoparticles (DG-PLA-NPs), optimise the preparation technology, and elucidate the in vitro release characteristics. Considering encapsulation efficiency (EE) and drug loading as the main evaluation indexes, DG-PLA-NPs were prepared using the modified emulsion solvent diffusion method and single factor and orthogonal design tests were performed to investigate the influencing factors and optimise the preparation method. Morphology of the nanoparticles was observed using transmission electron microscopy (TEM), average particle diameter and distribution were determined using dynamic laser particle size analysis, and the EE and drug loading were measured using reversed-phase high-performance liquid chromatography. Among the eight factors, the drug-to-material ratio, water-to-organic phase ratio, and polyvinyl alcohol (PVA) concentration significantly affected the NP EE. In the optimised formulation, the PLA/DG ratio, PVA concentration, and oil-to-water phase ratio were 5, 0.5%, and 5, respectively. The DG-PLA-NPs prepared with the optimised formulation were round or spherical with an average diameter of 209 nm, 79.60% EE, and 6.81% drug loading capacity. The polydispersity index was 0.181, and the zeta potential was −27.37 mV. The in vitro releases of both DG and DG-PLA-NPs conformed to the Weibull equation. The DG-PLA-NPs released desogestrel rapidly in the early stages but slowly at later stages, indicating that compared to DG, the DG-PLA-NPs had obvious sustained-release effects. The DG-PLA-NPs prepared by the modified emulsion solvent diffusion method were small, simple to prepare, and had high drug loading with promising application prospects.

1. Introduction

Desogestrel (DG) is a contraceptive agent that is two and nineteen times as efficient as norethisterone and norethindrone, respectively [1, 2]. DG does not affect androgen, which distinguishes it from other contraceptive drugs [1, 3, 4]. Moreover, it improves the high-density lipoprotein (HDL) and oestrogen antagonistic activity, significantly inhibits ovulation, changes cervical mucus concentration, and suppresses endometrium development [5]. The affinity of DG and its major metabolite, 3-ketone, for the progesterone receptor is much higher than its affinity for progesterone, norethindrone, and linezolid progesterone [6, 7].

Although DG has few side effects such as headache, nausea, breast tenderness, and breakthrough bleeding, it exhibits good contraception and pregnancy can occur after DG withdrawal. DG does not increase the incidence of foetus malformation and has no side effects on the growth of the offspring [8, 9]. However, as the main effective oral progestogen currently in the market, DG resembles most oral medicines that fail to maintain a stable blood drug level during the dynamic process of drug dissolution in the gastrointestinal tract.

Although a certain concentration can be achieved in the later stages by taking dose pills, it still wavers and may become either too high or low. When the concentration of DG is too low, it is relatively ineffective, while high concentrations lead to many side effects [10].

In addition, oral contraceptive pills should be strictly taken as prescribed. Missing doses could lead to contraceptive failure, causing considerable inconvenience to the woman requiring contraception and the actual effectiveness of oral contraception pills would be lower than the expected performance. Thus, it is necessary to improve medication compliance [11–14]. Recently, the use of biodegradable materials as drug carriers for sustained-release contraception drug delivery systems has become a hot research topic in the scientific community because of their high effectiveness, good adherence, few side effects, and good tolerability [15].

Controlled drug delivery technology has progressed tremendously over the last six decades [16]. Sustained contraceptive injections refer to contraception preparations releasing low doses in a stable manner into the human body via a subcutaneous or intramuscular injection to achieve sustained drug release. An injection can achieve sustained release of drugs for a few days, months, or even longer [17–20]. Sustained contraceptive injections can resolve the contraception continuation rates and compliance issues of current clinical preparations to provide a safe, efficient, and convenient contraception method for women of childbearing age.

Nanoparticles (NPs) are solid particles with diameters in the range of 10–1000 nm. They can be used to encapsulate drugs with polymer materials as a carrier or produce globose or spherical colloidal solid granules by adsorbing drugs. NPs can pass through tissues, capillaries, the blood-brain barrier, and reticuloendothelial cells in the human body to arrive at target areas to release drugs [21–26]. As a new sustained-release drug delivery system, biodegradable polymer NPs can control drug release, maintain the effective concentration for a long time, avoid burst release after injection, and reduce dose-related side effects. In addition, they significantly enhance the drug half-life, reduce drug dosage, and subsequently degrade automatically and gradually, causing no harm to humans [27–32]. Polylactic acid (PLA), which has been approved by the US Food and Drug Administration (FDA) in recent years, has good biodegradability and biological compatibility and generates carbon dioxide and water in the body after metabolism. The intermediate product is lactic acid, which is a product of normal glucose metabolism in the human body with no toxic side effects [33–36]. In this study, DG-PLA-NPs were prepared from DG using the modified emulsion solvent diffusion method [37–41] to maintain a constant blood drug concentration and control the sustained release of drug for several weeks or even months. This strategy could greatly reduce the drug delivery frequency and solve the current clinical contraceptive preparation issues, such as continuation rate and compliance issues, to provide a safe, efficient, and convenient contraception method for women of childbearing age.

2. Materials and Methods

2.1. Drugs and Chemicals. DG was purchased from Dalian Meilun Co. Ltd. (Dalian, China), and PLA (molecular weight [MW] = 25,000) was procured from Shandong Medical Instruments Co. Ltd. (Jinan, China). Polyvinyl alcohol (PVA), carbinol, acetone, and other common reagents were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

2.2. Animals. Ten two-month-old female Sprague-Dawley (S-D) rats weighing 180–220 g were randomly divided into two groups, DG and DG-PLA-NP. Normal sexual cycles were confirmed by vaginal smears. The animals were provided by the Laboratory Animal Center of Shandong Traditional Chinese Medicine University (license number: SCXK (LU) 2011-0003) and were cared for according to the protocol of *The Care and Use of Laboratory Animals by the Laboratory Animal Center of Shandong University of Traditional Chinese Medicine*.

2.3. Preparation of DG-PLA-NPs. First, 25 mg polymeric material PLA and 5 mg DG were dissolved in 5 mL acetone to prepare the oil phase [42, 43]. Then, 0.4 g PVA was added to 40 mL water to prepare the aqueous phase. The oil phase was then injected into the aqueous phase containing PVA at a constant speed under magnetic stirring. The solvent diffused into the aqueous phase rapidly under continuous stirring, which disordered the interface between the two phases forming the NPs. Finally, the organic solvent was volatilised by continuously stirring in a water bath at 50°C. The obtained PLA NP suspension was sealed and stored in a dark place at 4°C for later use after filtering through a 0.45 μm membrane for 4 min.

2.4. Orthogonal Design Experiment. Considering encapsulation efficiency (EE) and drug loading capacity as the main evaluation indexes, the PVA concentration, organic phase, ratio of water to organic phase, drug-to-material ratio, the method of injecting the oil phase into the water phase, stirring speed, evaporation time, and evaporation temperature were optimised using single-factor experiments and the orthogonal design test to optimise the preparation method [44, 45].

2.5. Determination of DG Spectrogram. A specific amount of DG was dissolved in methanol in a volumetric flask to a predetermined volume and scanned using an ultraviolet (UV) spectrophotometer (UNICO (Shanghai) Instruments Co. Ltd, Shanghai, China) at the UV wavelength range of 190 to 400 nm with methanol as the control.

2.6. High-Performance Liquid Chromatography (HPLC). DG samples were analysed using reversed-phase high-performance liquid chromatography (HPLC) as reported previously [46, 47] with an HPLC system (HP1200; Agilent Technologies, Santa Clara, CA, USA) equipped with a UV detector at 205 nm. Chromatography was performed using a 4.6 × 250 mm column packed with 5 μm particles (Venusil

XBP C18; Bonna-Agela Technologies, Tianjin, China) at 25°C.

2.7. Standard Curve of DG Content. DG (5 mg) was weighed precisely, dissolved in methanol in a 50 mL volumetric flask, and kept as a reserve solution. Then, 0.1, 0.5, 1.0, 2.0, 3.0, and 4.0 mL of DG reserve solutions were added to 10 mL bottles, dissolved and diluted with methanol to scale, and shaken uniformly to obtain DG solutions of different concentrations. Finally, the photometric value of a 20 μL aliquot of the DG solution was determine at the maximum absorption wavelength.

2.8. Determination of EE and Drug Loading Capacity. DG-PLA-NP colloidal solution (3 mL) was added to a centrifuge tube and centrifuged at 15,000 rpm for 30 min at 4°C to collect the supernatant. Then, 0.2 mL of the supernatant was added to a 10 mL volumetric flask, swirled, diluted with methanol, and shaken uniformly. Finally, the free drug concentration of a 20 μL aliquot was measured using HPLC analysis. Further, 0.2 mL of the DG-PLA-NP suspension solution was added to a 10 mL volumetric flask, eddied for demulsification, diluted with methanol, and finally shaken uniformly. From this solution, the total drug concentration of a 20 μL aliquot was measured using HPLC. The EE was calculated as $\mathrm{EE\%} = (W_{\mathrm{total}} - W_{\mathrm{free}})/(W_{\mathrm{total}}) \times 100$, and the drug loading capacity (DL) was calculated as $\mathrm{DL\%} = (W_{\mathrm{total}} - W_{\mathrm{free}})/(W_{\mathrm{PLA}}) \times 100$, where W_{total} denotes the weight of PLA added to the NPs, W_{free} refers to the weight of PLA remaining in the solution, and W_{PLA} is the weight of PLA [48–50].

2.9. Evaluation of Stability of NPs. In this experiment, we observed the status of the colloid prepared using the optimised preparation method and tested the DL after 0, 10, 20, and 30 days. Three samples ($n = 3$) were evaluated and stored at 4°C.

2.10. Determination of DG-PLA-NP Diameter. A cuvette was filled with 3 mL DG-PLA-NP solution, and the particle size and zeta potential of sorafenib-LNS were determined at 25°C using dynamic light scattering and electrophoretic mobility to determine the diameter, polydispersity coefficient (polydispersity index (PDI)), and zeta potential using a Zetasizer 3100 granulometer (Zetasizer Nano ZS 3000 SH, Malvern Instruments Ltd., Malvern, UK).

2.11. Transmission Electron Microscopy (TEM). The external morphology of the DG-PLA-NPs was determined using transmission electron microscopy (TEM) with the TEM, JEM-1200EX (JEOL Ltd., Tokyo, Japan). A drop of freshly prepared sorafenib-LNS was placed on the surface of a copper grid and air-dried. Owing to the poor conductivity of the organic samples, the particles were negatively stained with a drop of 2% aqueous sodium phosphotungstate for contrast enhancement for 2 min before TEM measurements.

2.12. In Vitro Drug Delivery Test. A phosphate buffer solution with pH 7.4 [50, 51] and the RPMI 1640 medium containing 10% foetal calf serum (Gibco/Life Technologies, Carlsbad, CA, USA) were chosen as the release medium according to common practice. DG is insoluble in water, so to increase its compatibilization effect and retain the "sink state," 0.5% Tween 80 was added to both release media. The dialysis bag was pretreated, and 1 mL DG-PLA-NP solution was added. Both ends of the dialysis bag were clamped, and then it was placed in 5 mL release medium under sink conditions at 37 ± 0.5°C with constant stirring at a speed of 100 rpm at a constant temperature. Then, 1 mL aliquots were withdrawn at 0, 0.5, 1, 2, 3, 4, 8, 12, 24, 36, and 48 h and replaced with equal amounts of the solution to make up for the loss caused by sampling. Meanwhile, the release characteristics of raw DG were determined in the same manner. The sample was filtered through a 0.45 μm filter membrane, and the cumulative release rate was determined using HPLC.

2.13. Pharmacokinetics. Radioimmunoassay was performed to determine the DG concentration in rat serum according to the principle that ^{125}I-labelled antigen (*Ag) competes with unlabelled antigen (Ag) for the binding site of a limited antibody (Ab). The Ag concentration negatively relates to the *Ag-Ab complex, as shown in the dose response curve. The sample could be quantified based on the curve. A separation agent was used to separate rational and binding phases. A Y-counter was used to measure the radioactivity intensity of the precipitate and calculate the binding rate, B/B_0. The concentration of [^{125}IAg-Ab] was negatively correlated to the Ag dose. In the standard logit function, logit B/B_0 was taken as the ordinate and log10X as the abscissa to obtain a standard logit-log curve to determine the unknown antigen concentration.

Specifically, S-D rats with a normal sexual cycle were selected for castration (OVX, ovariectomy), and the vaginal resistance was observed for 5 consecutive days starting from 1 week after castration to verify that there was no physiological cycle change in the rats. The rats were then randomly divided into two groups of five rats each. DG and DG-PLA-NP suspensions were injected into the thigh muscles of the rats, after 12 h of starvation with free access to drinking water before the injections (DG: 34 μg•kg^{-1}). Then, 0.5 mL of blood was withdrawn from the jugular sinus vein at different times (1, 2, 3, 4, 8, 12, 24, 36, 48, 60, 72, 84, and 96 h) after drug administration and placed in prepared dry EP tubes. After analysis, the serum was stored at −20°C. The rats were allowed to eat freely for 4 h after drug administration. A calibration curve was drawn based on a range of progesterone concentrations (0, 0.1, 0.5, 2, 10, 30, and 100 ng/mL) to determine the logit B/B_0 value. The progesterone concentrations of the samples were calculated according to the standard curve.

2.14. Data Analysis and Statistics. The B/B_0 values of the samples at each time point were used in the standard curve equation to calculate the sample concentration. The result of the blood drug concentration was analysed using Drug and Statistics for Windows (DAS Version 2.0, Mathematical Pharmacology Professional Committee of China, Shanghai, China) to obtain the drug dynamic parameters. All

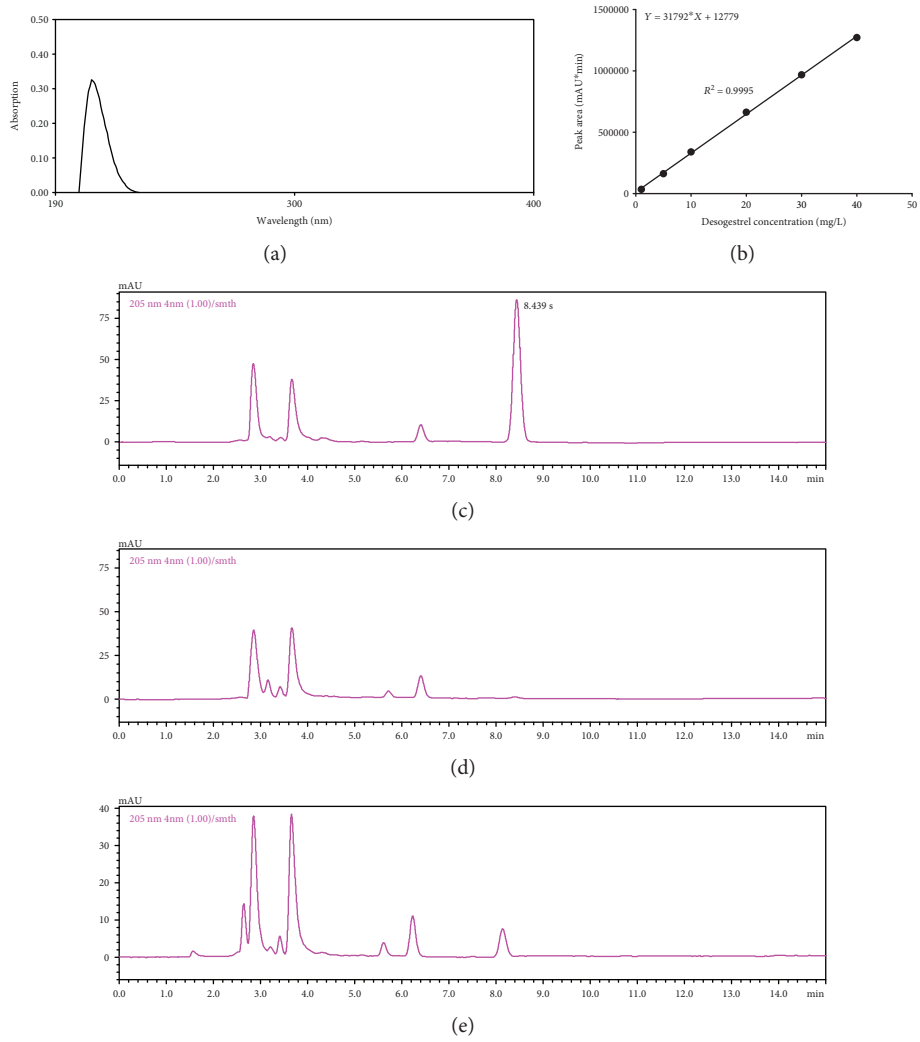

Figure 1: (a) DG spectrogram from 190 nm to 400 nm. (b) Linear return calculation with DG concentration and HPLC peak area. (c-d) HPLC experiments were performed with standard DG (c), polymeric material PLA (d), and DG-PLA-NP samples (e).

experiments were repeated at least three times, and the data were expressed as mean ± standard division (SD) with the significance level set at $P < 0.05$. Statistical differences of influence factors were evaluated using the Kruskal-Wallis test or unpaired t-test with GraphPad Prism 5.0 (GraphPad Software Inc., CA, USA). The F-test was used for data comparison with statistical level of $\alpha = 0.05$.

3. Results

3.1. DG Content Determination. The maximum absorption wavelength of DG was 205 nm (Figure 1(a)). Standard DG samples were analysed using reversed-phase HPLC with a UV detector at 205 nm (retention time: 8.439 s, Figure 1(c)). The DG concentration (C) was taken as the abscissa and the peak area (A) was taken as the ordinate for linear return calculation with the return equation $A = 31792C + 12{,}779$, $R^2 = 0.9995$. The results showed that the A and C of the methanol solution had a good linear relationship when the concentration of the reserve solution was in the range of 1–40 μg/mL (Figure 1(b)). In the subsequent experiments, DG concentrations were measured using HPLC to identify the factors affecting EE and DL% (Figures 1(d) and 1(e)).

3.2. Effect of PVA Concentration. All experimental parameters other than the PVA concentration in the synthesis of DG-PLA-NPs using the modified emulsion solvent diffusion method were unchanged for this evaluation. Pure water solutions with 0.2, 0.5, 1, and 1.5% PVA concentrations were used to prepare the DG-PLA-NPs. Figure 2(a) shows the effects of different PVA concentrations in the aqueous phase on the EE and DL. According to Figure 2(a), the NP EEs and DLs were 60.79 ± 1.9 and 4.99 ± 0.15 for 0.20% PVA and 67.88 ± 0.94 and 5.78 ± 0.23 for 0.50% PVA, respectively; thus, the DG-PLA-NP EE and DL increased with an increase in the PVA concentration. When the PVA concentration exceeded a certain value, the DG-PLA-NP EE and DL values decreased with increasing PVA concentration. However, the

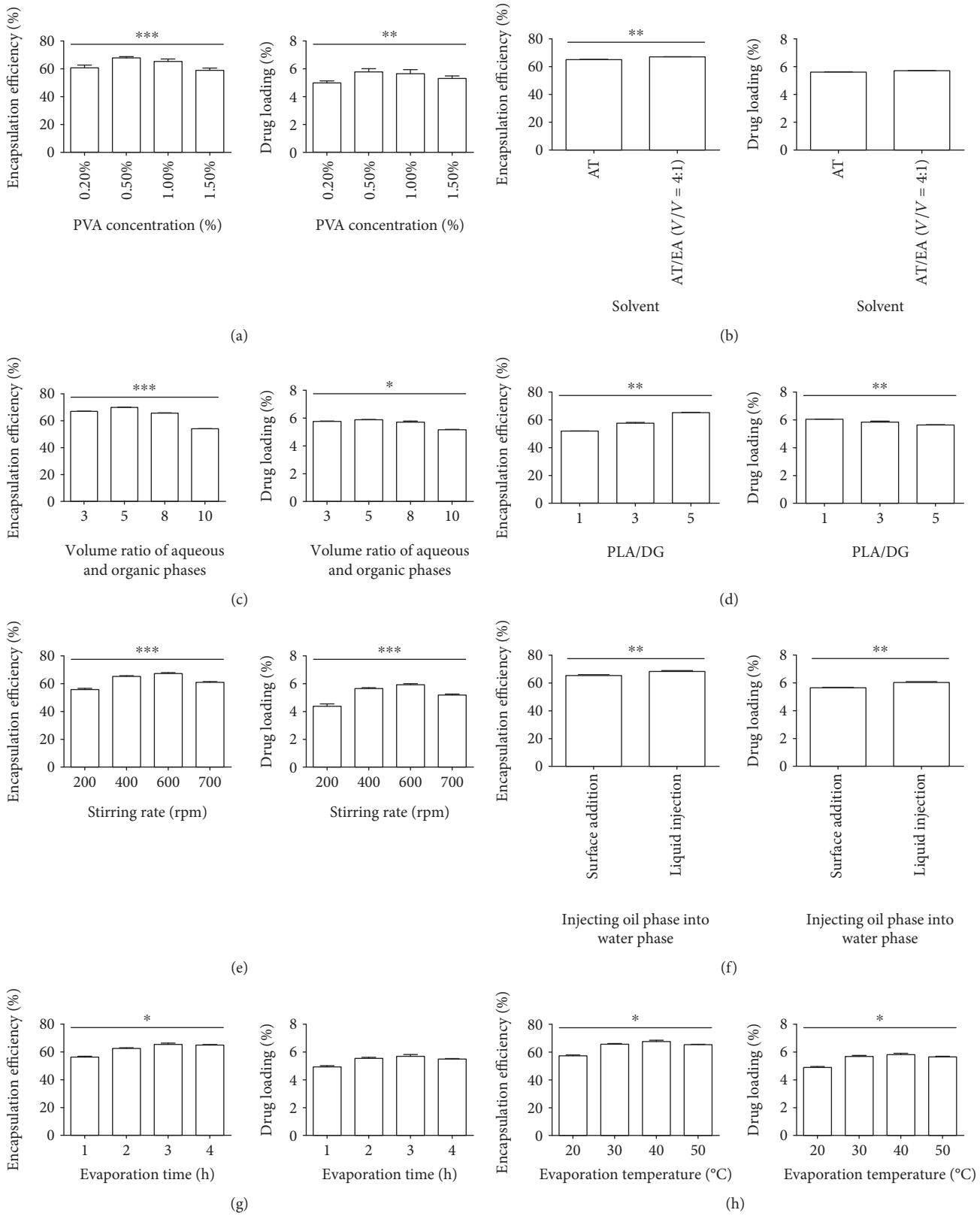

Figure 2: Encapsulation efficiency and drug loading were influenced by factors including PVA concentration (a), organic solvent (b), the ratio of water phase and organic phase (c), the ratio of drug and material (d), stirring speed (e), the method injecting oil into aqueous phase (f), evaporation time (g), and temperature (h). $n = 3$, $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

NP EEs and DLs were 65.35 ± 1.77 and 5.65 ± 0.29 for 1.00% PVA and 58.95 ± 1.54 and 5.3 ± 0.19 for 1.50% PVA (with $P = 0.0003$ for EE and $P = 0.0073$ for DL), respectively.

3.3. Effect of Organic Solvent. All experimental parameters other than the organic solvent in the synthesis of DG-PLA-NPs using the modified emulsion solvent diffusion method were unchanged for this assessment. Acetone and acetone/ethanol (4 : 1, *v*/*v*) solutions were used as the organic solvent to prepare the DG-PLA-NPs. Figure 2(b) shows the effect of different organic solvents on the EE and DL of the NPs. The NP EEs and DLs were 65.09 ± 0.62 and 5.61 ± 0.06 when acetone was used as a solvent and 67.11 ± 0.15 and 5.71 ± 0.03 with acetone/ethanol (4 : 1, *v*/*v*, $P = 0.0053$ for EE and $P = 0.059$ for DL), respectively. The DG-PLA-NP EE in the mixed acetone and ethanol organic phase was higher than that in the single acetone organic phase was.

3.4. Effect of Different Water-to-Organic Phase Volume Ratios. All experimental parameters other than the water phase-to-organic phase volume ratio in the synthesis of DG-PLA-NPs using the modified emulsion solvent diffusion method were unchanged for this evaluation. Solutions with water-to-organic phase volume ratios of 3, 5, 8, and 10 were used to prepare the DG-PLA-NPs to investigate the effect of different aqueous-to-organic phase ratios on the EE and DL of the NPs. The results are shown in Figure 2(c). When the aqueous phase/oil phase (*v*/*v*) volume ratio ranged from 3 to 5, the EE and DL increased with the increasing proportion of water to the organic phase. The NP EEs and DLs were 67.30 ± 0.36 and 5.76 ± 0.1 for a volume ratio of 3, 67.88 ± 0.94 and 5.88 ± 0.06 for a volume ratio of 5, and 65.66 ± 1.15 and 5.72 ± 0.15 for a volume ratio of 8, respectively. However, these parameters decreased when the proportion of the aqueous to organic phase increased continuously and exceeded a certain value. When the volume ratio was 10, the NP EE and DL were 54.07 ± 0.33 and 5.16 ± 0.07 ($P = 0.0006$ for EE and $P = 0.0162$ for DL), respectively.

3.5. Effect of the PLA/DG Ratio. For this analysis, all experimental parameters other than the PLA/DG ratio in the synthesis of DG-PLA-NPs by the modified emulsion solvent diffusion method were unchanged. DG-PLA-NPs were prepared with different PLA/DG ratios of 1, 3, and 5. Figure 2(d) shows the effect of different PLA/DG ratios on the EE and DL. As shown in Figure 2(d), the PLA/DG ratio had a significant effect on both the EE and DL; at PLA/DG ratios of 1, 3, and 5, the EEs were 51.87 ± 0.12, 57.24 ± 1.11, and 65.15 ± 1.04 ($P = 0.0036$) and the DLs were 6.04 ± 0.04, 5.83 ± 0.14, and 5.63 ± 0.07 ($P = 0.0036$), respectively. Thus, increasing the PLA/DG ratio improved the EE but lowered the DL.

3.6. Effect of Stirring Speed. For this assessment, all experimental parameters other than the stirring speed in the synthesis of DG-PLA-NPs using the modified emulsion solvent diffusion method remained unchanged. The DG-PLA-NPs were prepared at stirring speeds of 200, 400, 600, and 800 rpm. Figure 2(e) shows the effect of the different stirring speeds on the DL and EE. For stirring speeds of 200, 400, 600, and 800 rpm, the corresponding EEs were 55.81 ± 1.92, 65.23 ± 1.49, 67.33 ± 1.65, and 60.97 ± 1.39 ($P = 0.0006$) and the DLs were 4.38 ± 0.3, 5.65 ± 0.19, 5.93 ± 0.20, and 5.19 ± 0.19 ($P = 0.0006$), respectively. The EE and DL increased as the stirring speed was increased up to a certain extent, beyond which they slightly decreased.

3.7. Injecting Oil Phase into Water Phase. DG-PLA-NPs were prepared by surface addition and liquid injection to investigate the effect of evaporation time on the DL and EE. All other experimental parameters were unchanged. Figure 2(f) shows that the EEs were 65.37 ± 0.92 and 68.27 ± 0.6 ($P = 0.0097$) and the DLs were 5.65 ± 0.02 and 6.01 ± 0.1 ($P = 0.003$) when the oil phase was added to the water phase by liquid injection and surface addition. The EE and DL of the NPs prepared using liquid injection were higher than those of the NPs prepared using surface addition.

3.8. Effect of Evaporation Time. All experimental parameters other than the evaporation time in the synthesis of DG-PLA-NPs using the modified emulsion solvent diffusion method remained unchanged. DG-PLA-NPs were prepared with different evaporation times of 1, 2, 3, and 4 h to observe the influence of different evaporation times on the DL and EE of the NPs. The results are shown in Figure 2(g). At evaporation times of 1, 2, 3, and 4 h, the EEs were 56.29 ± 1.61, 62.46 ± 1.6, 65.45 ± 2.6, and 64.88 ± 1.29 ($P = 0.0152$), and the DLs were 4.93 ± 0.21, 5.55 ± 0.21, 5.69 ± 0.32, and 5.49 ± 0.13 ($P = 0.0502$), respectively. Although the evaporation time had little effect on DL, a too high or too low evaporation time decreased the DL. Considering the results comprehensively, the appropriate evaporation time was determined to be 3 h.

3.9. Effect of Evaporation Temperature. All experimental parameters other than the evaporation temperature in the synthesis of DG-PLA-NPs using the modified emulsion solvent diffusion method were unchanged for this analysis. DG-PLA-NPs were prepared at different evaporation temperatures of 20, 30, 40, and 50°C to observe their influence on the DL and EE of the NPs. As shown in Figure 2(h), at evaporation temperatures of 20, 30, 40, and 50°C, the EEs were 57.35 ± 1.66, 65.62 ± 1.86, 67.62 ± 2.27, and 65.38 ± 1.26 ($P = 0.0382$), and the DLs were 4.88 ± 0.23, 5.68 ± 0.23, 5.82 ± 0.27, and 5.63 ± 0.16 ($P = 0.0412$), respectively. The results show that when the temperature exceeded a certain degree, the EE decreased, and 40°C was the most suitable evaporation temperature.

3.10. Orthogonal Design Test. The results of single-factor experiments showed that the EE of the NPs was greatly affected by the feed ratio, aqueous phase-to-organic phase ratio, and PVA concentration. To optimise the preparation methods, these parameters were selected for an orthogonal design experiment. The factors were set as three levels and arranged according to the orthogonal design Lg (3^3) table. The NPs were prepared according to the procedures and experimental conditions described in the previous sections. Moreover, the DL and EE were measured as indices to optimise the DG-PLA-NP preparation process. Tables 1–3 show

Table 1: Factor-level in orthogonal-design experiments of Lg (3^3) ($n = 3$).

Level	Factors		
	The ratio of PLA and DG (A)	PVA concentration % (C)	The ratio of aqueous phase and oil phase
1	3	0.2	4
2	4	0.5	5
3	5	1	6

the factor levels including the DG/PLA ratio (3.4 and 5), PVA concentration (0.2, 0.5, and 1.0%), and aqueous-to-oil phase ratio (3.4 and 5 [v/v]). To determine the order of the primary and secondary factors by the range of the absolute difference, the encapsulation rate was directly analysed. The order was PLA/DG > PVA% > water/oil, wherein in PLA/DG, K3 > K2 > K1; in PVA%, K2 > K3 > K1; and in water/oil phase, K2 > K1 > K3. Considering the drug quantity as the measurement index, the order was PVA% > PLA/DG > water/oil, wherein in PLA/DG, K3 > K2 > K1; in PVA%, K2 > K3 > K1; and in water/oil phase, K2 > K3 > K1. The ANOVA result is shown in Tables 4 and 5. The order of the ANOVA result of effects on the NP EE was PLA/DG > PVA% > water/oil phase, which showed that the PLA/DG ratio significantly affected the encapsulation rate of the NPs. The order of the ANOVA results of the effects on the DL is PLA% > PLA/DG > water/oil phase, which indicated that PVA concentration had an important effect on the DL of the NPs.

3.11. Confirmation. Three batch samples were prepared according to the optimised formulations and process to determine the EE and DL and investigate the reproducibility of the method. As seen from the results in Table 6, the average EE and DL of the NPs prepared under the optimised conditions were 79.60% and 6.81%, respectively, which were within the predicted range and showed the good repeatability of the optimised formulations.

3.12. Evaluation of NP Stability. The samples to be tested were stored at 4°C before the analysis. Thirty days after the NPs were prepared, all three samples maintained the same pale blue opalescent suspension of the freshly prepared NPs. The DL values of the NPs were $6.81 \pm 0.12\%$, $6.73 \pm 0.08\%$, $6.64 \pm 0.13\%$, and $6.49 \pm 0.13\%$ after 0, 10, 20, and 30 days, respectively. Although a slight reduction in the DL to $95.24 \pm 1.94\%$ of that of the freshly prepared sample was observed after 30 days (Table 7), we concluded that the NPs were quite stable.

3.13. NP Diameter and Morphology. The NP sizes and PDI were affected by factors such as PVA concentration, organic solvent, ratio of the water phase to the organic phase, ratio of the drug to the material, stirring speed, method of injecting oil into the aqueous phase, evaporation time, and temperature (Table 8). The stirring speed was a major factor affecting the NP size (Table 8).

Although we did not include the sizes of the NPs as a main evaluation index in the orthogonal design experiment, the optimised formulation process created an ideal particle size. Specifically, a certain amount of PLA NPs prepared according to the optimised formulations were analysed using the Zetasizer 3100 particle size analyser to determine the diameter and PDI, which are shown in Figure 3.

The diameter, PDI, and zeta potential of the DG-PLA-NPs were 209.0 ± 2.64 nm, 0.181 ± 0.01, and -27.37 ± 0.59 mV, respectively. The negative charge on the DG-PLA-NP surface stabilised the suspension system. Figure 4 shows the morphology of the NPs, which were spherical, uniform in size, well dispersed, and nonaggregated.

3.14. In Vitro Release Test. Figure 5 shows the release curves of the DG-PLA-NPs and DG in a pH 7.4 phosphate buffer solution and RPMI 1640 medium containing 10% foetal calf serum. It shows that compared to DG, the DG-PLA-NPs had an obvious sustained-release effect.

3.15. Pharmacokinetics. The mathematical models of radioimmunoassay are logit $(Y) = A + B^* \mathrm{Lg}(x)$, NSB/$T$ (%): 2.441%, B_0/T (%): 64.163%, ED75 = 0.3385, ED50 = 2.9657, ED25 = 25.9869, $A = 0.55$, and $B = -1.17$. The standard curve equation of progesterone in serum is logit $B/B_0 = 0.55 - 1.17\mathrm{LgX}$, $R = 0.99800$. After injecting the DG and DG-PLA-NP suspensions, the average blood drug concentration-time curves of the rats are shown in Figure 6. Table 9 shows the main pharmacokinetic parameters of the fitted data.

4. Discussion

In this study, DG-PLA-NPs were prepared using the modified emulsion solvent diffusion method, which is an effective method for the preparation of PLA NPs of insoluble drugs without using toxic organic solvents (dichloromethane) used in traditional synthetic methods. The procedure is simple, convenient, and easy [52, 53]. The use of PLA biodegradable material can overcome the shortcomings of low drug utilization, poor safety, short-term effects, high doses, and blood drug concentration fluctuations caused by frequent use and can reduce the side effects of the target drug. Moreover, PLA NPs have attracted considerable attention owing to good surface modification, targeting ability, stability, sustained release, and simple and convenient preparation [54, 55]. Thus, with a small size, high loading capacity, and simple operation, the DG-PLA-NPs have promising prospects in practical applications.

EE and drug loading were the main evaluation indexes, and single-factor and orthogonal experiments were designed to investigate the effects of PVA concentration, organic solvent, aqueous-to-organic phase ratio, drug/material ratio, the method of injecting the oil phase into the aqueous phase, stirring speed, evaporation time, and evaporation temperature to optimise the preparation process. A mixture of acetone and ethanol was used as the organic solvent because ethanol diffuses faster than acetone does in water. In this

Table 2: Orthogonal experimental results of entrapment efficiency ($n = 3$).

Order	Factors			Entrapment efficiency (%)
	The ratio of PLA and DG (A)	PVA concentration (%)	The ratio of aqueous phase and oil phase	
1	3	0.2	4	64.05
2	3	0.5	5	73.38
3	3	1	6	66.40
4	4	0.2	5	70.03
5	4	0.5	6	74.05
6	4	1	4	70.96
7	5	0.2	6	72.89
8	5	0.5	4	79.94
9	5	1	5	77.95
K1	203.83	206.97	214.95	
K2	215.04	227.37	221.36	
K3	230.78	215.31	213.34	
R	26.95	20.4	8.02	

Table 3: Orthogonal experimental drug loading results ($n = 3$).

Order	Factors			Drug loading (%)
	The ratio of PLA and DG	PVA concentration (%)	The ratio of aqueous phase and oil phase	
1	3	0.2	4	5.92
2	3	0.5	5	6.66
3	3	1	6	6.37
4	4	0.2	5	6.11
5	4	0.5	6	6.82
6	4	1	4	6.48
7	5	0.2	6	6.18
8	5	0.5	4	6.89
9	5	1	5	6.78
K1	18.95	18.21	19.29	
K2	19.41	20.37	19.55	
K3	19.85	19.63	19.37	
R	0.9	2.16	0.26	

Table 4: Analysis of variance of entrapment efficiency.

Factors	Square deviation sum	Degree of freedom (n)	F value	Significance (a)
A	122.190	2	1.794	0.05
B	70.129	2	1.030	0.05
C	12.000	2	0.176	0.05
Difference	204.32	6		0.05

Table 5: Analysis of variance of drug loading.

Factors	Square deviation sum	Degree of freedom (n)	F value	Significance (a)
A	0.135	2	0.426	0.05
B	0.803	2	2.536	0.05
C	0.012	2	0.038	0.05
Difference	0.95	6		0.05

Table 6: Optimization of preparation of NPs ($n = 3$).

Batch number	Entrapment efficiency (%)	Drug loading capacity (%)
1	80.28	6.80
2	78.73	6.69
3	79.81	6.94

Table 7: Drug loading (DL, %) values of NPs after 0, 10, 20, and 30 days ($n = 3$).

Time points (day)	Drug loading capacity (%)	Relative DL (%) to the freshly prepared sample
0	6.81 ± 0.12	100.00 ± 1.76
10	6.73 ± 0.08	98.71 ± 1.14
20	6.64 ± 0.13	97.44 ± 1.90
30	6.49 ± 0.13	95.24 ± 1.94

Table 8: Sizes of the nanoparticles and PDI were influenced by multiple factors.

Factors		Sizes	PDI
PVA concentration	0.20%	266.3 ± 3.06	0.261 ± 0.009
	0.50%	251.0 ± 2.65	0.253 ± 0.004
	1.00%	245.7 ± 4.04	0.249 ± 0.005
	1.50%	228.3 ± 5.03	0.262 ± 0.004
Organic solvent	Acetone	248.7 ± 3.51	0.244 ± 006
	Acetone/ethanol (4 : 1, *v*/*v*)	240.3 ± 3.05	0.228 ± 0.003
Ratio of water phase and organic phase	3	272.3 ± 5.86	0.271 ± 0.011
	5	253.7 ± 3.79	0.263 ± 0.014
	8	249.0 ± 4.01	0.240 ± 0.013
	10	225.7 ± 5.51	0.223 ± 0.012
PLA/DG	1	202.3 ± 4.16	0.205 ± 0.012
	3	220.7 ± 4.04	0.224 ± 0.010
	5	242.0 ± 3.61	0.245 ± 0.015
Stirring speed	200 rpm	275.3 ± 6.03	0.216 ± 0.018
	400 rpm	243.7 ± 4.16	0.242 ± 0.018
	600 rpm	222.7 ± 3.51	0.257 ± 0.012
	800 rpm	189.3 ± 2.52	0.266 ± 0.015
The method injecting oil into aqueous phase	Surface addition	245.7 ± 4.93	0.244 ± 0.008
	Liquid injection	240.0 ± 4.58	0.226 ± 0.011
Evaporation time (h)	1	263.7 ± 3.79	0.225 ± 0.008
	2	255.0 ± 3.46	0.232 ± 0.005
	3	243.3 ± 5.52	0.244 ± 0.011
	4	262.7 ± 6.43	0.262 ± 0.013
Temperature (°C)	20	187.7 ± 4.04	0.181 ± 0.009
	30	203.3 ± 5.03	0.206 ± 0.010
	40	224.3 ± 4.51	0.222 ± 0.011
	50	244.7 ± 5.52	0.247 ± 0.015

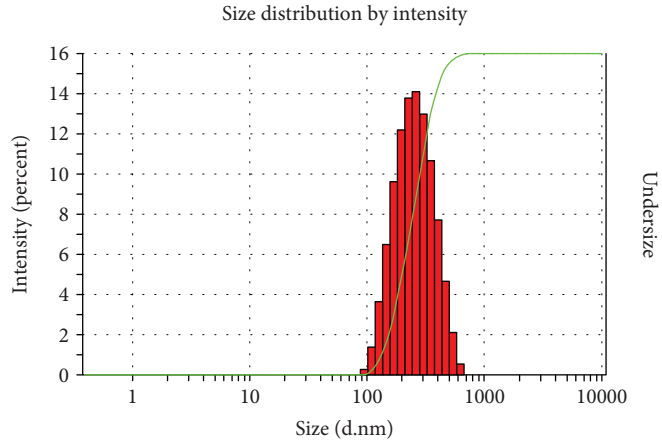

Figure 3: Particle size distribution of desogestrel nanoparticles.

solvent mixture, PLA dissolved in acetone and encapsulated the drugs slowly to form the DG-PLA-NPs with high EE [52].

In the single-factor experiment, the increase in PVA concentration reduced the surface tension, thus promoting the dispersion of the organic phase in the aqueous phase to form NPs with a smaller particle diameter and high specific surface area. Additionally, the rate of dispersion of DG into the continuous phase increased while the EE of the DG-PLA-NPs decreased in this experiment (Figure 2(a)). Within a specific range, the EE and DL increased with the increasing aqueous-

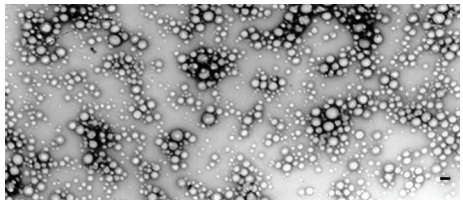

Figure 4: Electron microscopic photograph of desogestrel nanoparticles. Scale bar: 200 nm.

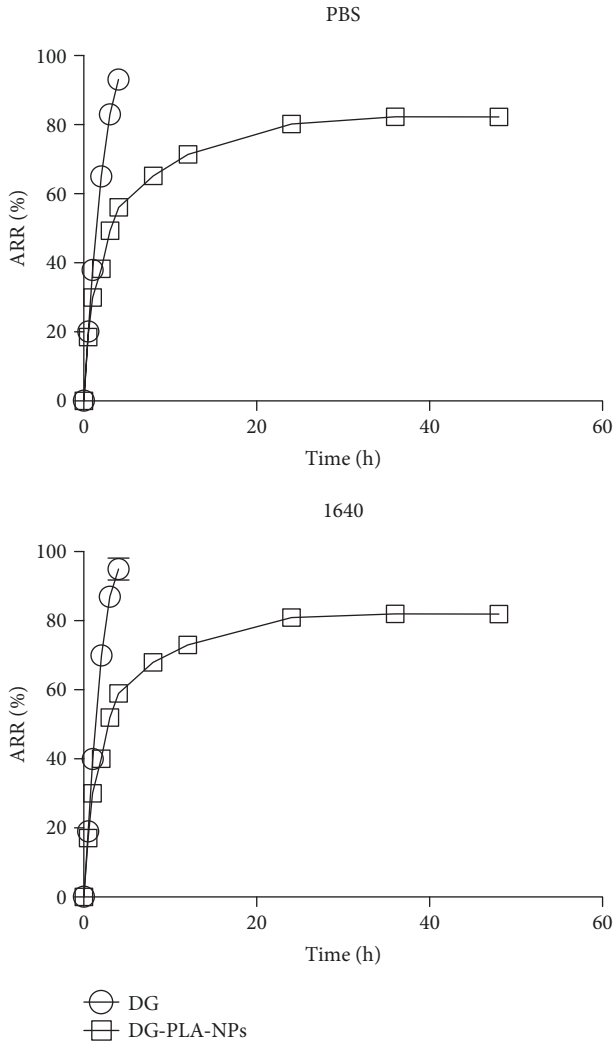

Figure 5: DG and DG-PLA-NPs in vitro release profile in phosphate buffer solution and 1640 medium containing 10% foetal calf serum ($n = 3$). ARR: accumulative release rate; PBS: phosphate buffer solution; 1640: 1640 medium containing 10% foetal calf serum.

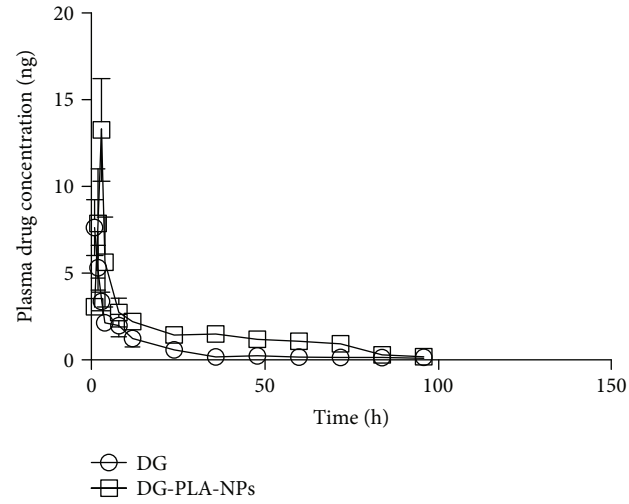

Figure 6: Average plasma concentration-time curves of DG after administration of DG suspension and DG-PLA-NPs ($n = 5$).

Table 9: Main pharmacokinetic parameters of DG suspension and DG-PLA-NPs in rats ($n = 5$).

Parameters	DG	DG-PLA-NPs	Probability of t-test
$t_{1/2}\alpha$ (h)	1.205	7.152	$P < 0.01$
$t_{1/2}\beta$ (h)	10.544	69.315	$P < 0.01$
$K10$/h	0.171	0.050	$P < 0.05$
$K12$/h	0.380	0.037	$P < 0.01$
$AUC_{0-\infty}$ (μg/L*h)	79.057	174.483	$P < 0.01$
T_{max} (h)	1	3	$P < 0.01$
C_{max} (μg/L)	7.642	13.27	$P < 0.01$
CL (L/h/kg)	0.43	0.195	$P < 0.01$

to-organic phase ratio. However, when the aqueous-to-organic phase ratio exceeded a certain value, the EE and DL decreased (Figure 2(c)). On the one hand, the increase in the aqueous phase volume facilitated the diffusion of the organic phase into the aqueous phase to form NPs with smaller particle diameters, thus reducing the EE and DL accordingly. In contrast, the smaller the NP size, the larger the specific surface area was, which increased the possibility that DG would be deposited directly during its dispersion in the aqueous phase. A small aqueous-to-organic phase ratio led to poor NP dispersion, whereas a large ratio resulted in the formation of NPs with smaller diameters, which increased the drug deposition in the aqueous phase and, thus, affected the NP quality. The DG/PLA ratio significantly affected the EE and DL (Figure 2(d)). Increasing the ratio of PLA improves the EE but decreased the DL. In addition, as water was a poor solvent for PLA, an excessively high PLA amount could not exist stably in the solvent and PLA formed a layer of precipitation polymer film on the surface [56]. The stirring speed, evaporation time, and evaporation temperature had little effect on the DL.

The optimised formulation process parameters were a PLA/DG ratio of 5, PVA concentration of 0.5%, and an aqueous phase-to-oil phase ratio of 5. The prepared DG-PLA-NPs were spheroid and oblatoid with an average diameter of 209 nm, 79.60% EE, and 6.81% DL. In addition, factors affecting the particle diameter were also evaluated during the optimization process using the single-factor analysis

(Table 8). However, the NP was not considered a primary evaluation index in the orthogonal design experiment. Among the various influencing factors, stirring speed was the most important. Fortunately, the optimised formulation process presented an ideal nanoparticle size.

Next, the physicochemical properties, including the morphology, particle diameter, particle size distribution, EE, DL, and release characteristics of the DG-PLA-NPs prepared according to the optimal formulation were evaluated in vitro. The results revealed that the prepared DG-PLA-NPs were spherical with a uniform size and demonstrated good dispersion and low aggregation.

As reported, the NPs released in vitro by dialysis had a high recycle rate and good reproducibility, which allowed an accurate evaluation of the DG-PLA-NP release in vitro [57–59]. DG is insoluble in water, so the experiments were performed using phosphate buffer solution and RPMI 1640 medium containing 0.5% Tween 80 as the release medium, which attained the sink-leaching condition.

The results showed that DG was rapidly released in phosphate buffer solution, with an accumulative release rate (ARR) of approximately 93% in 4 h. The DG-PLA-NPs had a sustained-release effect and were released rapidly with a 56% ARR during the first 4 h and subsequent steady and slow release. Using RPMI 1640 medium as the release medium, the ARR was approximately 95% for DG and 59% for DG-PLA-NPs during the first 4 h. Equation models, such as the zero-order kinetics, first-order kinetics, Higuchi equation, Weibull equation, and Ritger-Peppas equation, were used to fit the accumulative release data of DG and the DG-PLA-NPs. The in vitro release of DG and DG-PLA-NPs conformed to the Weibull equation. Compared to DG, the DG-PLA-NPs demonstrated an obvious sustained-release effect in vitro.

Radioimmunoassay and gas chromatography-mass spectrometry (GC-MS) are the main methods used to determine the DG serum concentration [60–62]. However, as GC-MS has a rather complicated operation [63], we used radioimmunoassay for the analysis, which is a new technology that combines radioactive isotope and immunohistochemical techniques to determine ultratrace substances. It has the advantages of both technologies, namely, the high sensitivity and accuracy of the isotope technique and the specificity of the antigen-antibody reaction.

From the specific response of the binding antigen and antibody, desogestrel could be measured accurately and simply. From the pharmacokinetic results shown in Table 9, the DG-PLA-NP distribution half-life ($t_{1/2}\alpha$) and the elimination half-life ($t_{1/2}\beta$) were calculated as 7.152 and 69.315 h, respectively, which were 5.94 and 6.57 times that of the solution, respectively ($P < 0.01$). In addition, the in vivo maximum drug concentration (C_{max}) of the NPs was 13.27 ng/mL and the area under the concentration curve AUC value was 174.483 μg/L·h, which were 1.74 times and 2.21 times that of the solution group, respectively ($P < 0.01$).

As previously reported, the solvent evaporation method was used to prepare progesterone-encapsulated poly(L-lactide)–poly (ethylene glycol)–poly (L-lactide) (PLA-PEG-PLA) nanoparticles [64]. Sustained and slow drug release was achieved by adjusting the PEG concentration. The same method has also been used to encapsulate oestradiol into poly(lactic-*co*-glycolic acid) (PLGA) NPs [65]. In this study, the oral drug bioavailability was improved. Other materials such as budesonide-loaded PLA have also been developed and used for the preparation of small-sized particles with a narrow diameter distribution that are round with a smooth surface using the emulsification/solvent evaporation method [66].

In this study, the polymer particle shape was characterised using scanning electron microscopy to improve the NP characteristics. Thus, using NPs encapsulated drugs, researchers have succeeded in controlling the speed of drug release, extending the drug effect, avoiding sudden release, lowering the dosages, and reducing the toxicity and side effects. Using levonorgestrel- (LNG-) encapsulated PLGA microspheres [67] or a mixture of gestodene- and ethinyloestradiol-encapsulated PLGA microspheres [68], researchers have developed long-acting contraceptives. DG has effect on androgen, which distinguishes it from other contraceptive drugs [1, 3, 4] and makes it attractive for administration.

In this study, for the first time, DG was encapsulated into polylactic acid nanoparticles, which were then administered via injection for controlled release of DG to achieve a long-term effectiveness of the drug. This research suggests that administering DG encapsulated in PLA NPs could directly reduce the drug elimination rate because the slow degradation of PLA in vivo prolonged the DG release time in rats, resulting in sustained drug release. This improved the bioavailability and maintained a high drug concentration in the body, which reduced the dosage and improved the therapeutic effect.

A limitation of the present study is that in the orthogonal design experiment, only the EE and drug loading capacity were considered as the main evaluation indexes, including other factors, such as the size of NPs, zeta potential, and PDI in the orthogonal design experiment might provide a more accurate assessment. However, despite these study design shortcomings, our study clearly demonstrated the potential usefulness of the developed NP drug delivery system, which is worth further investigation for possible future clinical development.

Data Availability

Drs. Peng Sun and Xianghong Liu are responsible for presenting data supporting the results reported in current study when required.

Ethical Approval

This manuscript has not been published or presented elsewhere in part or in entirety and is not under consideration by another journal.

Conflicts of Interest

There are no conflicts of interest to declare.

Authors' Contributions

Hui Lin and Guoyong Jia contributed equally to this work.

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