

Supplementary information

PREPARATION AND CHARACTERIZATION OF 15-DEOXY- $\Delta^{12,14}$ -PROSTAGLANDIN J_2 LOADED POLY(D,L-LACTIDE-CO-GLYCOLIDE) NANOCAPSULES

Preparation of poly(D,L-lactide-co-glycolide) nanocapsules with 15-Deoxy- $\Delta^{12,14}$ -prostaglandin J_2

The poly(D,L-lactide-co-glycolide) (PLGA) nanocapsules were prepared as described previously.^[15] The method involved mixing an organic phase with an aqueous phase. The organic phase was composed of PLGA polymer (50:50, m.w. 50,000 g/mol) (100 mg, Sigma, Sigma Aldrich Chem. Co., USA), acetone (30 ml), 15d-PGJ₂ (100 μ g, Sigma, Sigma-Aldrich Chem. Co., USA), sorbitan monostearate (40 mg), and capric acid triglyceride (200 mg). The aqueous phase consisted of polysorbate 80 (60 mg) and deionized water (30 ml). Until the components of both phases were fully dissolved, the organic phase was slowly added to the aqueous phase. The suspension was agitated for 10 min, followed by concentration using a rotary evaporator to obtain a suspension of 15d-PGJ₂ with a final concentration of 100 μ g/ml. Unloaded PLGA nanocapsules (without 15d-PGJ₂) were also prepared, following the methodology described above.

Size and polydispersion measurements

Parameters such as particle size (hydrodynamic diameter) and polydispersion measurements of 15d-PGJ₂ in the PLGA nanocapsules were determined using the dynamic light scattering technique. The nanocapsule suspensions (with or without 15d-PGJ₂) were diluted $\times 100$ (v/v) in Milli-Q water. Analyses were carried out using a ZetaPlus particle analyzer (Brookhaven, NY, USA), and the results were calculated as the mean of three measurements.

Transmission electron microscopy

To observe the morphology and structure of the PLGA nanocapsules with 15d-PGJ₂, transmission electron microscopy (TEM) observations were performed using a JEOL 1200EX II microscope (JEOL, Japan). Before observation, 15d-PGJ₂-NC was first diluted in water. One drop of the mixture was deposited on a standard copper grid, and the excess liquid was removed. Thereafter, the sample was stained with a 1% phosphotungstic acid solution (w/v). After being dried at ambient temperature, samples were observed by TEM.

Efficiency of incorporation of 15-Deoxy- $\Delta^{12,14}$ -prostaglandin J_2 in poly(D,L-lactide-co-glycolide) nanocapsules and *in vitro* release

The efficiency of incorporation of 15d-PGJ₂ in the nanocapsules was measured by the centrifugation method. Samples of nanocapsules containing 15d-PGJ₂ were

centrifuged, and the supernatant was quantified by high-performance liquid chromatography (HPLC, Waters Co., Milford, USA). The 15d-PGJ₂ incorporation efficiency was calculated from the difference between the drug concentration measured in the filtrate and its total concentration (100%) in the nanocapsule suspension.

For *in vitro* release assays, a 1-ml suspension of nanocapsules containing 15d-PGJ₂ in a centrifuge tube filled with PBS (pH = 7.2) was dialyzed using a cellulose membrane with a molecular exclusion pore size of 1,000 Da and a constant temperature oscillation incubator (37°C, 70 r/min). Aliquots were withdrawn from the centrifuge tube at intervals of 15, 30, 60, 120, 240, and 360 min and analyzed by HPLC.

The chromatographic conditions were optimized using a C18 column (Phenomenex Gemini reversed phase, 5 μ m, 110Å, 150 mm \times 4.60 mm). The mobile phase consisted of 0.02 mol/L sodium monobasic phosphate/acetonitrile at a ratio of 90:10 (v/v) and a flow rate of 1.0 ml/min. The wavelength monitored was 205 nm, and the injection volume was 20 μ l. An analytical curve, reflecting the relationship between concentrations and areas obtained from HPLC, was made using standard solutions of free 15d-PGJ₂ (concentration range, 0.5–10 μ g/ml), and then the percentage of drug released from the 15d-PGJ₂-NC was calculated. The total concentration of 15d-PGJ₂ (100%) in the PLGA nanocapsule suspension was determined after the suspension was diluted in acetonitrile.

Results of Preparation and Characterization of 15-Deoxy- $\Delta^{12,14}$ -prostaglandin J_2 -nanocapsules

Both the 15d-PGJ₂-NC and unloaded nanocapsule samples appeared as a homogeneous opalescent bluish-white liquid. The diameters and poly index for polydispersions of the 15d-PGJ₂-NC suspensions on day 0, 30, and 60 were 115.20 ± 0.65 nm and 0.033 ± 0.00 , 132.70 ± 0.87 nm and 0.07 ± 0.00 , and 134.10 ± 1.61 nm and 0.13 ± 0.01 , respectively. The suspensions were relatively stable as the average particle size on days 0, 30, and 60 did not show any statistically significant difference. The polydispersion values indicated a narrow range of the particle size distribution [Figure 1a]. The TEM images showed that 15d-PGJ₂-NC were spherical and homogeneously distributed [Figure 1b]. The efficiency of 15d-PGJ₂ incorporation in the PLGA nanocapsules was 64%. In the *in vitro* release assay, using a cellulose membrane with a molecular mass of 1000 Da, only 15d-PGJ₂ could pass through the pores of the membrane. The average accumulated amounts of released 15d-PGJ₂ at 15, 30, 60, 120, 240, and 360 min were 21.4%, 53.1%, 64.8%, 86.1%, and 99.8%, respectively [Figure 1c].

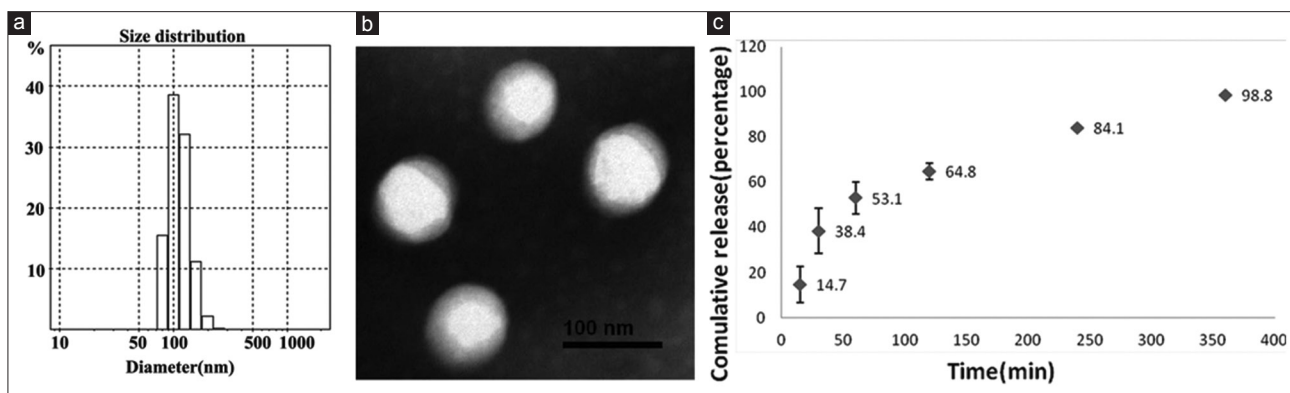


Figure 1: Characterization of 15d-PGJ₂-NC. (a) Distribution of diameter (nm) by intensity (%) of the 15d-PGJ₂-NC suspension on day 0. (b) Images of 15d-PGJ₂-NC observed by TEM. (c) Cumulative release (%) of PLGA nanocapsules containing 15d-PGJ₂ in 360 min. 15d-PGJ₂-NC: 15-Deoxy- $\Delta^{12,14}$ -prostaglandin J₂ nanocapsules; TEM: Transmission electron microscopy; PLGA: Poly(D,L-lactide-co-glycolide).